UNIVERSITY OF CAGLIARI

DOCTORATE IN GEOENGINEERING AND ENVIRONMENTAL TECHNOLOGIES

USE OF MEMBRANE BIOREACTORS FOR THE BIOREMEDIATION OF GROUNDWATER POLLUTED BY CHLORINATED COMPOUNDS

Ph.D. Thesis of Luisa Manigas

February 2008

Ma come, sono già passati? sono già passati tre anni? mi avevano detto che il tempo del dottorato sarebbe corso… ma non credevo tanto velocemente!
E così, arrivata alla fine di questa ricca, ricchissima avventura mi guardo indietro e vedo tante persone che di questo dottorato sono state piacevoli compagne e a che sento di voler ringraziare.

Il primo ringraziamento va di sicuro alla Professoressa Alessandra Carucci, che mi ha consentito di vivere questa bellissima esperienza culturale e formativa, dimostrandomi sempre grande fiducia e guidandomi nella ricerca con grande professionalità e competenza.
Ringrazio il Prof. Aldo Muntoni per gli efficaci suggerimenti nella parte sperimentale sui clorofenoli.

Il mio pensiero corre poi a tutte le persone con cui ho condiviso tempo, fatiche, esperienze scientifiche e ottimistiche considerazioni nelle lunghe ore passate in laboratorio, e che hanno reso quel tempo molto più leggero. Grazie a Martina e alla sua preziosa assistenza in campo analitico e chimico; grazie all'Ingegner Zucca, al suo Panchina Fan Club e al suo innato buonumore: si può sempre lavorare seriamente, ma farlo con il sorriso sulle labbra è molto più bello.
Voglio sicuramente ringraziare tutti i miei tesisti: Cristina, Rossana, Lucio, Esther, Elisabetta, Martina, Rino e Francesca: vederli appassionarsi alla ricerca giorno dopo giorno, mostrando dedizione e interesse e dedicando generosamente tempo e sforzi è stata una soddisfazione immensa. Tra tutti loro un grandissimo grazie va a Paola che con ammirevole solerzia ha condotto la sperimentazione dandomi modo di vivere quell'esperienza stupenda che sono stati i sei mesi passati a Berlino.
To my sweet German friends: if this thesis is in English is just to tell you once again "Thanks": thanks for all the times that you spoke English just to make me understand you and being able to take part to your discussions. Communication is the basis of a boundless culture.
Nel bene e nel male ogni parola, ogni spazio, ogni pausa di questa tesi è stata scritta sotto la costante influenza di uno specialissimo e preziosissimo Supporto, di tipo informatico, morale e caratteriale; dalla battitura della prima parola alla realizzazione dell'ultimo grafico, questo lavoro è nato sotto il suo influsso e ne vive profondamente l'essenza.
E infine il mio grazie più grande va sicuramente a tutti i ragazzi che sono stati anima e cuore di ogni giorno passato al Digita: i ragazzi della sala "Dottorandi G. Cappai".
Ringrazio Isa, che all'inizio della ricerca mi mostrò (con mio immenso stupore) come un semplice vaso di vetro potesse essere accessoriato sotto sapienti mani e dare vita a un reattore biologico (quasi) perfettamente funzionante ed efficiente.
Grazie alla mia compagna di gomito Anna, per il suo sarcasmo intelligente e la sua saggezza incomparabile. Grazie a Walter, a Claudio, a Tiziana e ad Alessia, e grazie a Stefano, anima e ideatore del Digita F.C. nonché socio in affari universitari sin dai tempi dell'immatricolazione: è stato davvero un piacere fare una parte del dottorato in sua compagnia.
E dulcis in fundo, il mio ringraziamento più sentito va sicuramente a due persone stupende che di questi tre anni sono state assolutamente il dono più bello: Gio&Gio, Signora Giovanna e la Dottoressa Giorgia. Con loro ho condiviso di tutto, dalla sperimentazione ai pranzi, dalle giornate che valgono una vita ai momenti di stanca, con loro ho condiviso Freezer e Fenicotteri (e numerosi altri esponenti del mondo animale), sono state preziosissime confidenti di chiacchiere leggere e di riflessioni profonde quando ero a 1300 km di distanza nella fredda Berlino, quando ero a 2 km nel luminoso ufficio RAS o a 2 metri nel mattonato ufficio Dottorandi, ascoltandomi, sostenendomi e incoraggiandomi sempre; con loro abbiamo tirato fuori preziose e inconfutabili verità su questa vita sempre strana, e il loro ottimismo, la loro fiducia incrollabile, o semplicemente la loro presenza positiva mi hanno aiutato a ritrovare il sorriso nei periodi bui, quando credevo di averlo perso, e l'hanno invece fatto diventare più luminoso nei periodi più positivi.
Grazie alla mia famiglia, che silenziosamente ma assiduamente mi ha sempre sostenuta in ogni esigenza; senza di loro tutto questo non sarebbe stato possibile.

Grazie a tutti voi, splendidi protagonisti di questa bellissima esperienza.

Se ho visto più lontano, è perché stavo sulle spalle di giganti
(I.Newton)

Al non mollare, mai.

Index

Introduction

Chlorinated compounds are widely used both in industrial and agricultural applications, because they are the main components of pesticides and are frequently employed as intermediate agents in plastic, wax, resin and many other synthetic goods production processes. The widespread use of chlorinated compounds, which are directly applied on the soil and have been often stored in inappropriate ways, has led in the last decades to a strong and diffuse contamination of many soils and groundwater areas.
Therefore, many scientific experimentations have been performed in order to find out and optimize biological and physical treatments for the remediation of waters polluted by chlorinated compounds. Due to their xenobiotic nature and to their high toxicity, they are hardly degraded by non acclimated bacterial populations naturally present in the environment; therefore, they can be biologically degraded only by specifically acclimated bacterial strains.

The aim of this experimental work has been the application of a new polluted waters treatment technology for the selection of a bacterial population capable of bioremediating a synthetic groundwater polluted by four different chlorinated compounds. The composition of the synthetic water used in the experimentation simulated the one of different polluted groundwaters found in many industrial and agricultural areas, which are often polluted by the chlorinated compounds studied in this research.
The experimental part of the study was performed in the laboratories of the Department of Geoengineering and Environmental Technologies (DIGITA) of the University of Cagliari (Italy), and was carried out for around 600 days. The whole study lasted three years, and for two years it was part of the Ministerial Project *PRIN 2004* (Programma di Ricerca Scientifica di Rilevante Interesse Nazionale – Programme of Relevant National Interest Scientific Research) titled "*Advanced Processes for Remediation of Contaminated Groundwater*", financed by the Italian Ministry of Education, of University and of Research. The programme involved six Operating Units sited in different Italian universities, each of them carrying on a different treatment (either biological or physical-chemical) for remediation of waters polluted by chlorinated compounds.

The innovative technology applied in this study was the biological treatment system known as **MSBR** (Membrane Sequencing Bioreactor), which consists of a *Sequencing Batch Reactor* (SBR) system coupled to a *membrane module* for the filtration of the biological sludge that degrades the chlorinated compounds present in the polluted water fed to the system. SBRs have been widely used for several decades to acclimate biological sludge to particular substances, because they allow the regular alternation of operational parameters and of metabolic conditions that favour the selection of specific bacterial strains. The MSBR couples to the SBR system a membrane module which allows the perfect filtration of the mixed liquor present in the reactor, thus ensuring an effluent totally lacking of all the suspended and of many dissolved solids, as well as of a good deal of coliforms and of some organic pollutant macromolecules.
The synthetic groundwater used in this experimentation was constituted by distilled water in which four chlorinated compounds were solved: 1,2-dichloroethane, 1,2-dichlorobenzene, 2-chlorophenol and trichloroethylene. The effects of some other substances on the degradation of the four main compounds were checked.
The selection of the specialized bacterial strains was achieved starting by a fresh sludge, coming from the oxidation tank of a municipal wastewater treatment plant (Is Arenas,

Cagliari). The fresh sludge was first checked for its capability of degrading the chlorinated compounds, and then, since it showed a minimum degradative capability, it was acclimated in SBR modality in two different reactors, one working according to the traditional SBR system, and the other working in MSBR modality.
During the development of the research, the chlorinated compounds were fed in two different ways:

- each compound was fed *alone* in order to define the maximum removal rate of the different substances, which gave a measure of sludge acclimation to the compounds;
- the compounds were fed in *different mixtures* in order to identify possible effects of each substrate on the degradability of the others.

Degradation of the single substrates was repeated in different periods of the experimentation, in order to check if a sludge acclimated for a longer time could degrade the chlorinated compounds with higher efficiency.
The possibility of implementing the system at a larger scale was investigated by observing the effects of some operational parameters variation on the treatment efficiency.
Differing from the other compounds fed, trichloroethylene was not expected to be degraded, but it was fed to the system in order to check if it could totally or partially inhibit the degradation of the other chlorinated compounds.

During the experimentation, the selection of a biomass capable of degrading the four chlorinated compounds with specific removal rates comparable to the ones observed in past studies was achieved.
The effects of partial or total inhibition on the degradation capability of some chlorinated substrates caused by the presence of other substrates were identified.
The MSBR system equipped in this study showed to be effective in the acclimation of a sludge to xenobiotic compounds which are usually hardly biodegradable.

In order to improve the know-how concerning Membrane Bioreactors management, six months of this study were spent at the Department of Chemical Engineering of the Technical University of Berlin (Germany). In this study, a cascade MBR operating nutrients removal from a synthetic municipal wastewater was studied. Recirculation sludge flow rates and metabolic conditions alternations were varied, and the subsequent effects on nutrients removal efficiency, with particular focus on phosphorus removal, were studied.

Chapter 1.
European and National Legislation on Water Protection Policy

1.1 European Legislation

For several decades, the emissions of dangerous and hazardous substances have been subjected to regulation by European legislation, in order to reduce the pollution of groundwater and surface water bodies caused by these substances.

1.1.1. Surface Waters

1.1.1.1 Directive 76/464/EEC

One of the first directive in water policy field to be adopted at Community level was the *Directive 76/464/EEC - Water pollution by discharges of certain dangerous substances*, which had the main objective of regulating potential aquatic pollution by thousands of chemicals already produced in Europe at that time. The Directive focused on *inland surface waters, territorial waters, inland coastal waters and groundwaters*.

In 1980 another directive, the *Council Directive 80/68/EEC on the protection of groundwater against pollution caused by certain dangerous substances*, regulated groundwater protection policy on a Community level, thus substituting in this field to the guidelines concerning groundwater given by the Directive 76/464/EEC.

The purpose of the Directive 76/464/EEC was to individuate the main polluting substances, and to list them in two separate lists, called **list I** and **list II**, which were included in the Annex to the Directive. The substances listed in list I should have been completely eliminated from the environment, while the ones present in list II should have been reduced in terms of concentration in the environment.

List I and "Candidate List I"

In **List I** a number of groups and families of pollutants were present; they were selected on the basis of their *persistence*, *toxicity* and *bioaccumulation*.

The Commission elaborated a list that was communicated to the Council in 1982 (OJ C 176 of 14 July 1982, p. 3). It included 129 "**candidate list I substances**", and three more substances were subsequently added to the list to bring the total up to 132.

Specific Directives for list I substances

Eighteen individual substances present in "Candidate List I" were then regulated in **five** specific Directives (known as "daughter" directives), that set emission limit values and quality objectives on the Community level. These five Directives contained the first mandatory minimum requirements, individuated with the approach of the use of the best technical means (also called Best Available Techniques, BAT).

The other "candidate list I substances" should also have been regulated, but the regulation procedure was suspended in the beginning of the 1990s, because a more comprehensive and integrated permitting system for industrial installations was being elaborated.

The *IPPC Directive (96/61/EC)* on integrated pollution prevention and control, promulgated in 1996, included in its Annexes the emission limit values for the 18 list I substances of the specific directives.

This topic is better analyzed in a report of 1996 by EC: *"Impact of the Directive 76/464/EEC and its daughter Directives on the most important surface waters of the Community"*.

List II - pollution reduction programmes (Article 7 of Directive 76/464/EEC)

The List II was created in order to list groups and families of substances that could be dangerous for the aquatic environment. Also all the individual List I substances that had not been regulated at Community level yet were present in the List II. Since there were only 18 substances really included in List I, all the other 114 substances of the "candidate list I" and the groups of substances listed in List I must be considered as List II substances.
The Directive set that for the relevant pollutants present in List II, Member States must establish programmes for pollution reduction; in these programmes water quality objectives should have been included, according to Article 7 of the Directive 76/464/EEC.
Proper implementation of List II substances regulated under Article 7 of the Directive was very slow. In the early 1990s, the Commission started infringement procedures against most of the Member States. Most of the cases are still under the attention of the European Court of Justice and there have been already several rulings against Member States.
A Community initial report on pollution reduction programmes in Member States, titled "*Assessment of programmes under Article 7 of Council Directive 76/464/EEC*" was prepared in 2001.

1.1.1.2 Transition to the Water Framework Directive (2000/60/EC)

The Directive 76/464/EEC has been integrated in the *Water Framework Directive (2000/60/EC)*.
The new directive, in Articles 16 and 22, set out the transitional provisions for the existing Directive (76/464/EEC) on discharges of certain dangerous substances. In summary, the provisions are the following:

- article 6 of 76/464/EEC (concerning List I substances) was repealed with the entry into force of Directive 2000/60/EC;
- the list of priority substances of Water Framework Directive replaced the "candidate list I" of 1982;
- the other parts of 76/464/EEC, including the emission reduction programmes, will be still in place until 2013 (there is a transition period);
- the review of "specific Directives" within 2 years after entry into force of Directive 2000/60/EC should have been done.

1.1.1.3 Water Framework Directive (2000/60/EC)

General principles of WFD

The most recent Community Directive on water protection is the European Water Framework Directive 2000/60/EC (also known as WFD), that establishes a framework for Community action in the field of water policy.
The WFD presents a breakthrough in EU water policy, since it concerns both the definition of water protection and its development and implementation. The Directive establishes a framework for the protection of inland surface, transitional and ground water, and aims to achieve good ecological potential and chemical condition of surface water by 15 years from the date of the entry into force of the Directive (22 December, 2000).
The main aims of the Water Framework Directive are the following:

- expanding the scope of water protection to all waters, surface waters and groundwater;
- achieving "good status" for all waters by a set deadline;
- water management based on river basins;

- "combined approach" of emission limit values and quality standards;
- getting the water prices right;
- getting the citizen involved more closely in water protection;
- streamlining legislation.

River basin management
WFD establishes that water policy must be dealt at river basin level; management by **river basin** instead of administrative or political boundaries is the most appropriate model for a single system of water management, because it includes the natural geographical and hydrological units. The WFD underlines that for each river basin district - some of which will cross national frontiers - a *river basin management plan* will need to be established and updated every six years.

Coordination of objectives – good status for all waters
There are several objectives in respect of which the quality of water is protected. The key ones at European level are general protection of the aquatic ecology, specific protection of unique and valuable habitats, protection of drinking water resources, and protection of bathing water. All these objectives must be integrated for each river basin.

Surface water
The concepts of *good ecological status* (a general requirement for ecological protection) and *good chemical status* (a general minimum chemical standard) were introduced by the Directive in order to ensure the protection of all surface waters.

Ecological protection
Good ecological status is defined in terms of the quality of the biological community, the hydrological characteristics and the chemical characteristics. Since no absolute standards for biological quality is applicable across the whole Community, because of ecological variability, the standards are specified considering a slight departure from the situation that would be expected in conditions of minimal anthropogenic impact.

Chemical protection
Good chemical status is defined in terms of compliance with all the quality standards established for chemical substances at European level. The Directive also explains a calculation procedure for renewing these standards and establishing new ones by giving priority to hazardous chemicals. This ensures at least a minimum chemical quality, particularly for very toxic substances, everywhere in the Community.

Other uses
The other uses or objectives for which water is protected are not defined for the whole Community extent, but for specific areas. Therefore, the obvious way to incorporate them is to individuate specific protection zones within the river basin which must meet these different objectives. The overall plan of objectives for the river basin will then require ecological and chemical protection in all the basin as a minimum, but if there are areas that require more stringent requirements, they will be specifically defined.

Groundwater

Chemical status

The case of groundwater is slightly different, since it is presupposed that groundwater should not be polluted at all. For this reason, setting chemical quality standards for groundwater may not be the best approach, as it would be like allowing a certain level of pollution. A very few such standards have been established at European level for particular substances (nitrates, pesticides and biocides), and these must always be respected. But for general protection WFD approach is essentially a precautionary one: it prohibits direct discharges to groundwater, and prescribes (to cover indirect discharges) to monitor groundwater bodies so as to detect changes in chemical composition, and to reverse any anthropogenically induced upward pollution trend. These provisions should ensure the protection of groundwater from all contamination, according to the principle of minimum anthropogenic impact.

Quantitative status

Water quantity is also an important issue for groundwater protection. There is only a certain amount of water recharge into a groundwater each year, and of this water, some is needed to support connected ecosystems (surface water bodies or terrestrial systems such as wetlands). For good management, only that portion of the overall recharge not needed by the ecosystems connected to the groundwater can be extracted: this is the sustainable resource, and the Directive limits extraction to that quantity.

One of the innovations of the Directive is that it provides a framework for integrated management of groundwater and surface water for the first time at European level.

1.1.1.4 Priority substances under the Water Framework Directive

Commission Proposal (COM(2006)397)

In 2006, the Commission proposed a Directive setting environmental quality standards for the priority substances, that must be achieved by Member States by 2015, to ensure "good chemical surface water status". The proposal of Directive also requires progressive reduction of emissions, losses and discharges of all priority substances, and cessation of emissions, losses and discharges of priority hazardous substances within 20 years.

Requirements of the Water Framework Directive

Directive 2000/60/EC sets the EU strategy for water pollution control, and gives a list of 33 priority substances (PS), which represent a significant risk to the European aquatic environment.

Guidelines on water protection set by Council Directive 76/464/EEC are being replaced, harmonised and developed by Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 (Directive 76/464/EEC will be fully repealed in 2013), thus establishing a framework for water policy on a Community level. According to Directive 2000/60/EC, specific measures must be adopted at Community level against pollution of water by individual pollutants or groups of pollutants that present a significant risk to the aquatic environment. These provisions aim at the progressive reduction and, according to point 30 of Article 2 of the Directive, at the cessation of priority hazardous substances discharges, emissions and losses within 20 years after their adoption at Community level. Finally they aim, in order to achieve the objectives of relevant international agreements, to achieve concentrations in the marine environment at background values for natural substances, and close to zero for man-made synthetic substances.

As a consequence of the adoption of these measures, it has been established, in the Annex X to Directive 2000/60/EC, the **list of priority substances**, including the priority hazardous

substances. The list has been made considering the recommendations reported in Article 16(5) of WFD. The Directive takes into account that for naturally produced substances it is impossible to reach the zero concentration in the environment, which, on the other side, must be achieved for substances produced by human activities.
Directive 2000/60/EC introduces in Article 16(2) a scientific methodology for individuating priority substances on the basis of their significant risk to the aquatic environment. This methodology is based on some specific principles which consider the following points:

- evidence regarding the intrinsic hazard of the substance concerned;
- evidence from monitoring of widespread environmental contamination;
- other proven factors which may indicate the possibility of widespread environmental contamination, such as production, use volume and use pattern of the substance concerned.

(European Commission, 2001. Decision No 2455/2001/EC of the european parliament and of the council of 20 November 2001 establishing the list of priority substances in the field of water policy and amending Directive 2000/60/EC).

First list of priority substances

The first step of the strategy was the definition of a **list of priority substances** that were included in the Annex X of the Directive.
Preparation of the priority list included a procedure called **COMMPS** (Combined Monitoring-based and Modelling-based Priority Setting) which was developed to identify the substances of highest concern at Community level. In the constitution of the COMMPS procedure, experts from the Scientific Committee for Toxicity, Ecotoxicity and the Environment, from Member States, from EFTA countries, from the European Environment Agency, from European business associations including those representing small and medium-sized enterprises, and from European environmental organizations have been involved. In order to underline the international character of the Directive, in the COMMPS procedure also the countries which were candidates for membership of the European Union were involved; priority was assigned to those Member States in which some watercourses passing through or flowing into the territory of another Member State were present.
In the COMMPS procedure about 820000 monitoring data from water and sediments from all the Member States were evaluated, and data for more than 310 substances produced, used and distributed in the environment were used for developing models where the monitoring information available was insufficient.
The COMMPS procedure was designed as a dynamic instrument for individuating dangerous and hazardous substances, and is open to **continuous improvement and development**. This has been achieved also by means of the adaptation of the first list of priority substances, that must be done at the latest four years after the entry into force of WFD and at least every four years thereafter. In order to avoid that some priority substances can be excluded from the selection process, no substances are excluded when any selection is done; besides, the best available techniques are taken into account, and all substances identified as 'hazardous' by OSPAR (Organization for the Protection of Marine Environment of the North-East Atlantic)and all chemicals and pesticides on the Community market are included in any selection process (*Decision 2455/2001/EC)*. In accordance with Article 1(c) of WFD, in the future reviews of the list of priority substances further substances will be added to the list, in order to contribute to the cessation of emissions, discharges and losses of all hazardous substances by 2020.
A proposal for a Decision made by both European Parliament and Council, that established the list of priority substances was first presented by the Commission on 7 February 2000

(COM (2000) 47 final). However, this proposal was revised because the final agreement on the Water Framework Directive in the conciliation of 28/29 June 2000 introduced a new requirement for the Commission to identify the priority hazardous substances. The final *Decision (2455/2001/EC)* was adopted on 20 November 2001.
With the provisions of Water Framework Directive, Member States will determine quality standards for pollutants at a river basin level, identified in accordance with that Directive, and will take action to meet those quality standards by 2015 as part of ecological status (Article 4, 11 and Annex V, WFD). For this purpose a programme of measures shall be in place by 2009, and become operational by 2012. This procedure will then replace Directive 76/464/EEC upon its repeal.

1.1.1.5 Decision 2455/2001/EC

Up to 2001, the major instrument at Community level for the control of point and diffuse discharges of dangerous substances was represented by the Council Directive 76/464/EEC on pollution caused by certain dangerous substances discharged into the aquatic environment of the Community, and by the Water Framework Directive.
Article 16 of the Water Framework Directive sets out the largest part of Community water policy in terms of pollution prevention for surface waters; this article lays down new procedures for the identification of substances and development of control measures.
In 2001 the European Parliament and Council adopted the **Decision No 2455/2001/EC**, that established the list of priority substances and amended Directive 2000/60/EC; this list was then integrated by the one present in the proposed Directive "COM(2006)397 final".

List of Priority Substances and Other Pollutants - COM(2006)397 final

On 17 July 2006, a **proposal** for a new Directive that would set guidelines to prevent pollution in surface water was adopted by the European Commission (COM(2006)397 final). The proposed Directive, which is intended to support the Water Framework Directive, defines concentration limits in surface waters for 41 dangerous chemical substances (including **33 priority substances and 8 other pollutants**), giving particular attention to human health and to animal and vegetal life in the aquatic environment.
The list of priority substances includes 33 substances; among them there are some selected existing chemicals, plant protection products, biocides, metals and other groups like Polyaromatic Hydrocarbons (PAH), that are mainly incineration by-products, and Polybrominated Biphenylethers (PBDE) that are used as flame retardants. The complete list is given in Table 1.1.
The other eight pollutants, which fall under the scope of Directive 86/280/EEC and which are included in List I of the Annex to Directive 76/464/EEC, are not in the priority substances list. Environmental quality standards for these substances, that are reported in Table 1.2, are included in the Commission proposal to maintain the regulation of the substances at Community level.
The identification of the priority hazardous substances that should belong to this list has taken into account the selection of substances of concern made in relevant Community legislation regarding hazardous substances or relevant international agreements.
Hazardous substances are defined in the WFD as "*substances or groups of substances that are toxic, persistent and liable to bio-accumulate, and other substances or groups of substances which give rise to an equivalent level of concern*".
Within this list of hazardous substances, 11 substances have been identified as *priority hazardous* substances which are of particular concern for the inland, transitional, coastal and territorial waters. At the article 2(30), the WFD defines the ***priority substances*** as those

"*substances identified in accordance with Article 16(2) and Listed in Annex X. Among these substances there are "**priority hazardous substances**" which means substances identified in accordance with Article 16(3) and (6) for which measures have to be taken in accordance with Article 16(1) and (8).*".

Emissions, losses and discharges of these substances will be stopped within an appropriate timetable that shall not exceed 20 years. A further 14 substances were identified as being subject to review for identification as possible *priority hazardous substances*.

Table 1.1. List of priority substances in the field of water policy indicated by the WFD.

CAS number	EU number	Name of priority substance	Identified as priority hazardous substance
15972-60-8	240-110-8	Alachlor	
120-12-7	204-371-1	Anthracene	X
1912-24-9	217-617-8	Atrazine	
71-43-2	200-753-7	Benzene	
n.a.	n.a.	Brominated diphenylethers	X(***)
7440-43-9	231-152-8	Cadmium and its compounds	X
85535-84-8	287-476-5	C_{10-13}-chloroalkanes (**)	X
470-90-6	207-432-0	Chlorfenvinphos	
2921-88-2	220-864-4	Chlorpyrifos	
107-06-2	203-458-1	1,2-Dichloroethane	
75-09-2	200-838-9	Dichloromethane	
117-81-7	204-211-0	Di(2-ethylhexyl)phthalate	
330-54-1	206-354-4	Diuron	
115-29-7	204-079-4	Endosulfan	X
959-98-8	n.a.	(alpha-endosulfan)	
206-44-0	205-912-4	Fluoranthene (****)	
118-74-1	204-273-9	Hexachlorobenzene	X
87-68-3	201-765-5	Hexachlorobutadiene	X
608-73-1	210-158-9	Hexachlorocyclohexane	X
58-89-9	200-401-2	(gamma-isomer, Lindane)	
34123-59-6	251-835-4	Isoproturon	
7439-92-1	231-100-4	Lead and its compounds	
7439-97-6	231-106-7	Mercury and its compounds	X
91-20-3	202-049-5	Naphthalene	
7440-02-0	231-111-4	Nickel and its compounds	
25154-52-3	246-672-0	Nonylphenols	X
104-40-5	203-199-4	(4-(para)-nonylphenol)	
1806-26-4	217-302-5	Octylphenols	
140-66-9	n.a.	(para-tert-octylphenol)	
608-93-5	210-172-5	Pentachlorobenzene	X
87-86-5	201-778-6	Pentachlorophenol	
n.a.	n.a.	Polyaromatic hydrocarbons	X
50-32-8	200-028-5	(Benzo(a)pyrene),	
205-99-2	205-911-9	(Benzo(b)fluoranthene),	
191-24-2	205-883-8	(Benzo(g,h,i)perylene),	
207-08-9	205-916-6	(Benzo(k)fluoranthene),	
193-39-5	205-893-2	(Indeno(1,2,3-cd)pyrene)	
122-34-9	204-535-2	Simazine	
688-73-3	211-704-4	Tributyltin compounds	X
36643-28-4	n.a.	(Tributyltin-cation)	
12002-48-1	234-413-4	Trichlorobenzenes	
120-82-1	204-428-0	(1,2,4-Trichlorobenzene)	
67-66-3	200-663-8	Trichloromethane (	
1582-09-8	216-428-8	Trifluralin	

Where groups of substances have been selected, typical individual representatives are listed as indicative parameters (in brackets and without number).

These groups of substances normally include a considerable number of individual compounds. At present, appropriate indicative parameters cannot be given.

Fluoranthene is on the list as an indicator of other, more dangerous Polyaromatic Hydrocarbons

Table 1.2. List of the other pollutant substances indicated by the Water Framework Directive.

	CAS number	**Name of other pollutant**
(1)	*not applicable*	DDT total[1]
	50-29-3	para-para-DDT
(2)	309-00-2	Aldrin
(3)	60-57-1	Dieldrin
(4)	72-20-8	Endrin
(5)	465-73-6	Isodrin
(6)	56-23-5	Carbontetrachloride
(7)	127-18-4	Tetrachloroethylene
(8)	79-01-6	Trichloroethylene

[1] DDT total comprises the sum of the isomers 1,1,1-trichloro-2,2 bis (*p*-chlorophenyl) ethane (CAS number 50-29-3); 1,1,1-trichloro-2 (*o*-chlorophenyl)-2-(*p*-chlorophenyl) ethane (CAS number 789-02-6); 1,1-dichloro-2,2 bis (*p*-chlorophenyl) ethylene (CAS number 72-55-9); and 1,1-dichloro-2,2 bis (*p*-chlorophenyl) ethane (CAS number 72-54-8).

The 33 substances (or groups of substances) listed in Table 1 have been demonstrated to be of major concern for European Waters. This list of priority substances has replaced the list of substances in the Commission Communication to the Council of 22 June 1982 on dangerous substances, which was included in List I of Council Directive 76/464/EEC.
The proposal has replaced the **five older directives**, called "daughter" directives, promulgated by the Council, which set specific emission limit values and quality objectives for **18 list I substances.** The specific directives were:

- Council Directive of 22 March 1982 on limit values and quality objectives for **mercury** discharges by the **chlor-alkali electrolysis industry** (82/176/EEC) (OJ L 081, 27.03.1982, p. 29).
- Council Directive of 26 September 1983 on limit values and quality objectives for **cadmium** discharges (83/513/EEC) (OJ L 291, 24.10.1983, p. 1).
- Council Directive of 08 March 1984 on limit values and quality objectives for **mercury** discharges by sectors other than the chlor-alkali electrolysis industry (84/156/EEC) (OJ L 074, 17.03.1984, p. 49).
- Council Directive of 09 October 1984 on limit values and quality objectives for the discharges of **hexachlorocyclohexane** (84/491/EEC) (OJ L 274, 17.10.1984, p. 11).
- Council Directive of 12 June 1986 on limit values and quality objectives for discharges of **certain dangerous substances** in List I of the Annex to Directive 76/464/EEC (86/280/EEC as amended by 88/347/EEC and 90/415/EEC (OJ L 181, 04.07.1986, p. 16 (amended OJ L 158, 25.06.1988, p. 35 and OJ L 219, 14.08.1990, p. 49)).

Stakeholder consultation

As required under WFD article 16(5), a stakeholder consultation process with an Expert Advisory Forum (EAF) on Priority Substances set up in the framework of the implementation of the Water Framework Directive, was engaged by the Commission during the preparation of the proposal on environmental quality standards for priority substances. This consultation grouped Member States, Candidate countries and main stakeholders such as European industry associations and environmental NGOs (Non Governative Organizations).
In March 2004 a consultation of the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) regarding the environmental quality standards was launched.

1.1.2 Groundwater

1.1.2.1 The first directive

At the end of the 1970s a directive on protection of groundwater from pollution caused by dangerous substances was adopted. This directive (80/68/EEC) prescribed that for groundwater protection, direct or indirect introduction of high priority pollutants into groundwater must be prevented and that introduction into groundwater of other pollutants must be limited so as to avoid pollution of this water by these substances (this directive will be repealed by 2013 under the Water Framework Directive).

1.1.2.2 Assessment (1982)

In 1982, the European Community (with the Directorate-General for the Environment, Consumer Protection and Nuclear Safety) carried out a large evaluation of all groundwater resources within its (then) nine Member States. Finally, a general survey (*Groundwater Resources of the European Community: synthetic report*) and individual reports from each Member State were produced. This assessment focused mainly on groundwater **quantity**. Since it was published, attention has turned in Europe (and the United States) to **quality** more than to quantity, so that many groundwater quality monitoring programmes have been expanded and many groundwater protection schemes have been put into place.

1.1.2.3 A groundwater action programme (1996)

In 1991 at the Hague a declaration of the Ministerial Seminar on groundwater was held; it recognised that further action was needed if term deterioration of the quality and quantity of freshwater resources wanted to be avoided. Besides, it called for a programme of actions to be implemented in Member States by the year 2000, aiming at sustainable management and protection of freshwater resources. Due to the requests made by the Council in 1992 and 1995 in the form of resolutions, an action programme and a revision of the 80/68/EEC Directive was elaborated. This was followed-up by the Commission presentation of a proposal for a Decision of the European Parliament and of the Council on an action programme for Integrated Protection and Management of Groundwater, which was adopted on 25th November 1996. In this proposal the Commission underlined the need of establishing procedures for the regulation of abstraction of freshwater and for the monitoring of freshwater quality and quantity (http://ec.europa.eu/environment/water/water-framework/groundwater/policy).

Contemporarily, European institutions requested the Commission to formulate a proposal for a Directive establishing a framework for a European water policy; this finally resulted in the adoption of the Water Framework Directive 2000/60/EC.

Until the WFD adoption, attention on groundwater mainly concerned its use as drinking water (e.g. about 75% of EU inhabitants depend on groundwater for their water supply), ignoring that groundwater is also an important resource for industry (e.g. cooling waters) and agriculture (irrigation). However it became more and more obvious that groundwater should also be protected for its environmental value, and not only for its importance as a drinking water reservoir. In this context, the Water Framework Directive establishes that "good status" on environmental level must be achieved for all waters – surface, coastal, transitional, and **groundwaters** – by the end of 2015. The directive fixes clear objectives but leaves flexibility to Member States on the means to be used to achieve them. It is based on milestones such as risk evaluation of anthropogenic pressures and impacts, monitoring programmes, development of river basin management plans (the first one to be published in 2009) and design and operation of programmes of measures. Groundwater protection is achieved,

according to WFD directives, by fixing both **quantitative** and **chemical** status objectives (while the objectives for surface waters concern ecological and chemical status).
Quantitative status objectives are clearly explained in the WFD, aiming at ensuring a balance between abstraction and recharge of groundwater; on the other hand, chemical status criteria were more complex to be defined at the time of the adoption of the WFD. Therefore it was decided to request the Commission to propose a "daughter" directive clarifying good chemical status criteria and specifications related to the identification and reversal of pollution trends. This new Groundwater Directive has been adopted on 12 December 2006.

1.1.2.4 Current legislative framework

Groundwater in the Water Framework Directive

Water Framework Directive prescribes several different steps for achieving good (quantitative and chemical) status for groundwater by 2015. They require Member States to:

- Define groundwater bodies within River Basin Districts to be designated and reported to the European Commission. When classifying groundwater bodies, pressures and impacts of human activity on the quality of groundwater must be analyzed, and groundwater bodies presenting a risk of not achieving WFD environmental objective must be identified. Member States were obliged to carry out this classification between 2004 and 2005 and report the results back to the European Commission.
- For groundwater areas or habitats and species directly dependent on water, Member States have to establish registers of protected areas within each river basin districts. Besides all bodies of water used for the extraction of drinking water, these registers must include all protected areas covered under the following directives: the Bathing Water Directive 76/160/EEC, the vulnerable zones under the Nitrates Directive 91/676/EEC, and the sensitive areas under the Urban Wastewater Directive 91/271/EEC, as well as areas designated for the protection of habitats and species including relevant Natura 2000 sites designated under Directives 92/43/EEC and 79/409/EEC. Registers shall be reviewed contemporarily with the River Basin Management Plan updates.
- Establish groundwater monitoring networks based on the results of the classification analysis so as to provide a comprehensive overview of groundwater chemical and quantitative status. Member States were also obliged to design a monitoring programme that had to be operational by the end of 2006.
- Set up a *river basin management plan* (RBMP) for each river basin district; in this plan a summary of pressures and impacts of human activity on groundwater status, a presentation in map form of monitoring results, a summary of the economic analysis of water use, a summary of protection programmes and control or remediation measures must be included. The first RBPM will be published at the end of 2009, and a first review is then planned by the end of 2015; every six years thereafter there will be another review.
- Take into account by 2010 the principle of recovery of costs for water services, including environmental and resource costs in accordance with the polluter pays principle.
- In order to achieve environmental objectives, Member States must establish by the end of 2009 a programme of measures; in this programme such measures as abstraction control, prevent or control pollution will be included, and will be operational by the end of 2012. Basic measures include, in particular, controls of groundwater extraction, controls (with prior authorisation) of artificial recharge or augmentation of groundwater bodies (providing that it does not compromise the achievement of

environmental objectives), and point source discharges and diffuse sources liable to cause pollution. Direct discharges of pollutants into groundwater are prohibited and subject to a range of provisions listed in the Article 11. The programme of measures has to be reviewed and if necessary updated by 2015 and every six years thereafter.

1.1.2.5 The new directive on protection of groundwater from pollution and deterioration

The Commission proposal

On 19 September 2003 the European Commission adopted a proposal for a new directive to protect groundwater from pollution. Similarly to the approach followed for WFD editing, this directive proposal introduced for the first time **quality objectives** which oblige Member States to monitor and assess groundwater quality on the basis of common criteria and identify and reverse trends in groundwater pollution.
The requirement set in Article 17 of WFD prescribing of establishing technical specifications to complement the overall groundwater regulatory regime in place was thus fulfilled with the adoption of the directive proposal by the Commission. These specifications concern several key elements, such as characterisation, analyses of pressures and impacts, monitoring, and programme of measures. These elements are a consequence of development and implementation of River Basin Management plans which prescribes that "good environmental status" must be achieved by 2015. The Water Framework Directive required a daughter directive in which detailed provisions on chemical status and other measures to identify and reverse pollution trends were indicated.

The new Groundwater Directive (2006/118/CE)

Directive 2006/118/CE set underground water quality standards and introduced provisions to prevent or limit inputs of pollutants into groundwater.
In establishing quality criteria, the Directive considered local characteristics and allowed possible further improvements based on monitoring data and new scientific knowledge. The Groundwater Directive, as required by WFD guidelines, was based on a scientific approach, since it related to objective evaluations on chemical status of groundwater and on the identification and reversal of significant and sustained upward trends in pollutant concentrations. Directive also established that States will have to set the standards at the most appropriate level and take into account local or regional conditions.

The groundwater directive complements the Water Framework Directive. It requires:

- groundwater quality standards to be established by Member States by the end of 2008;
- pollution trend studies to be carried out by using existing data and data which are mandatory as required by the Water Framework Directive (referred to as "baseline level" data obtained in 2007-2008);
- in order to allow the achievement of the environmental objectives by 2015, pollution trends must be reversed;
- also the provisions set to prevent or limit inputs of pollutants into groundwater must be operational so that WFD environmental objectives can be achieved by 2015;
- reviews of technical provisions of the directive must be carried out in 2013 and every six years thereafter;
- good chemical status criteria must be achieved, on the basis of both EU standards of nitrates and pesticides and threshold values established by Member States.

Groundwater Directive 2006/118/CE establishes specific measures according to what provided for in Article 17(1) and (2) of WFD for control and prevention of groundwater pollution. In particular, these measures consist of criteria for the assessment of good groundwater chemical status, and criteria for the identification and reversal of significant and sustained upward trends and for the definition of starting points for trend reversal. The Directive also complements the provisions preventing or limiting inputs of pollutants into groundwater already contained in Directive 2000/60/EC, and aims to prevent the deterioration of the status of all bodies of groundwater.
The Directive, in Article 3, gives the following criteria that Member State must use in order to define the assessment of the chemical status of a groundwater body pursuant to Section 2.3 of Annex V to Directive 2000/60/EC:

- groundwater quality standards as referred to in Annex I;
- Member States will establish threshold values in accordance with the procedure set out in Part A of Annex II for the pollutants, groups of pollutants and indicators of pollution which, within the territory of a Member State, have been identified as contributing to the characterisation of bodies or groups of bodies of groundwater as being at risk, taking into account at least the list contained in Part B of Annex II.

When setting the threshold values applicable to good chemical status, guidelines given in Part A, points 1, 2 and 3 of Annex II of the directive will be followed, and a particular regard to its impact on correlated surface waters, ecosystems and wetlands will be given. Besides, human toxicology and ecotoxicology knowledge will be taken into account in threshold values definition procedure.
According to the directive, threshold values will be established at the national level, at the level of the river basin district, or at the level of a body or a group of bodies of groundwater. Member States shall establish threshold values pursuant to paragraph 1(b) for the first time by 22 December 2008. The established threshold values shall be published in the river basin management plans that will be submitted in accordance with Article 13 of Directive 2000/60/EC, and will include a summary of the information set out in Part C of Annex II to Directive 2006/118/CE. If a groundwater body is no longer concerned by the presence of a pollutant, its threshold value can be removed from the list.
When assessing the **chemical status** of a groundwater body, Member States will apply the procedure described in paragraph 2 of Article 4 of the directive. According to the definition given by the directive, a good chemical quality status will be reached when the values for the groundwater quality standards listed in Annex I and the relevant threshold values established in accordance with Article 3 and Annex II are not exceeded at any monitoring point in that body or group of bodies of groundwater. If, after an evaluation of risk assessment that takes into account also the extent of the affected groundwater body, some polluting substances are not considered a significant environmental risk, their threshold values can also be exceeded at one or more monitoring point, according to what referred to in paragraph 3 of Annex III. In this case, Member States, in accordance with Article 11 of Directive 2000/60/EC, must take the appropriate measures that may be necessary to protect aquatic ecosystems, terrestrial ecosystems and human uses of groundwater dependent on the part of the body of groundwater represented by the monitoring point or points at which the value for a groundwater quality standard or the threshold value has been exceeded.
In Article 5, the directive establishes that Member states must identify the upward trends in pollutant concentration in groundwater, and find the policy to limit and reverse these trends.

The way in which the trend has been identified and the strategy adopted to reverse the trend will be explained in the river basin management plan laid down by the Member State.
In the sixth article, measures to prevent or limit inputs of pollutants into groundwater are explained. It is here stated that when defining these limits, Member States must pay high attention to the possible presence in the groundwater body of **hazardous substances**. In particular, they will take account of hazardous substances that belong to the pollutants referred to in points 1 to 6 of Annex VIII to Directive 2000/ 60/EC; they will also consider substances belonging to groups of pollutants analyzed in points 7 to 9 of that Annex, if these substances are considered to be hazardous. Besides, Member States will take into account also those pollutants listed in Annex VIII to Directive 2000/60/EC which are not considered hazardous, and any other non hazardous pollutants that are not listed in that Annex, but that are considered by Member States to present an existing or potential risk of pollution.
In *Annex I* to the directive, it is stated that more stringent threshold values must be established for a given groundwater body, if it is considered that the groundwater quality standards could not be sufficient to achieve the environmental objectives specified in Directive 2000/60/EC for associated bodies of surface water, or if they may allow significant diminutions of the ecological or chemical quality of such bodies, or if they can cause any significant damage to terrestrial ecosystems directly dependent on the body of groundwater.
In *Annex II*, the **guidelines for the establishment of the threshold values** by Member States are given. Member States will establish threshold values for all pollutants and indicators of pollution which characterise groundwater at risk of failing to achieve good groundwater chemical status. For threshold determination procedure, Member States will take into account elevated background levels that may naturally occur in a given groundwater body. In the same Annex, a list of substances which must be considered by Member States when establishing threshold values is given; among all the other pollutants, **Trichloroethylene** is also indicated as one of these substances.
In *Annex III* of the directive, the criteria on which Member State will base their **assessment of groundwater chemical status** are given, while in *Annex IV* the identification and reversal of significant and sustained upward trends that must be done by Member States is regulated.

1.2 Italian Legislation

1.2.1 Decreto Legislativo n. 152 of 3/4/2006 (Italian Decree Law n. 152 of 3/4/2006)

In Italy, all the legislation concerning water protection and management policy emitted before 2006 has been amended by the D.Lgs, 152/2006 bringing regulation on all the environmental fields.
This law, with its 318 articles and 45 Annexes, is articulated into *six parts*, each of them concerning a single separate field:

- in the *first part* common rules, such as application field and aims of the law and the criteria for the adoption of the following measures are disciplined;
- in the *second part*, the procedures for Strategic Impact Assessment, Environmental Impact Assessment, and for *Integrated Pollution Prevention and Contro*l (IPPC) are determined;
- in the *third part*, soil protection and prevention against desertification, water protection and water resources management are disciplined;
- in the fourth part, wastes management and polluted sites remediation are regulated;

- in the fifth part, air quality protection and the reduction of gaseous emissions in atmosphere are disciplined;
- in the sixth part, indemnification for environmental damages is regulated.

D.Lgs. 152/2006 enacts European WFD (60/2000/CE) on water policy guidelines, and amends several previously existing Italian laws on water protection and management policy:

- Law 319/76, concerning "Rules on protection of waters from pollution";
- D.P.R. 515/82, on "Implementation of European Directive 75/440/CEE concerning quality of surface waters destined to production of drinking water";
- D.P.R. 236/88 regarding "Implementation of European Directive 80/778 concerning quality of waters destined to human use";
- D.Lgs. 130/92 titled "Implementation of the European Directive 78/659/CEE concerning quality of waters which require protection or improvement to be suitable for fish life";
- D.Lgs. 131/92 titled "Implementation of the European Directive 79/923/CEE concerning quality requirements of waters destined to shellfish";
- D.Lgs. 132/92 concerning "Implementation of the European Directive 80/68/CEE concerning protection of groundwater from pollution caused by some hazardous substances";
- L. 5/94, on "Directives on water resources policy";
- D.Lgs. 152/99, titled "Directives on protection of waters from pollution and implementation of the European directive 91/271/CEE concerning treatment of urban wastewaters and of directive 91/676/CEE concerning protection of waters from pollution caused by nitrates coming from agricultural sources.

The most important section of the D.Lgs. 152/2006 for the development of this work is the **third part**, concerning soil protection and prevention against desertification, water protection and water resources management.
In Article 74, some definitions are given:

- hazardous substances: persistent, bio-cumulable and toxic substances, or other substances which give rise to the doubt of these properties;
- priority substances and priority hazardous substances: substances individuated according to Article 16 of the WFD.

In Article 76, the law states that, in order to achieve the protection and bioremediation of water bodies, two criteria are defined:

- Target of **environmental quality**, defined depending on the capability of water bodies to keep natural autodepuration processes and of supporting animal and vegetal large and diversified communities;
- Target of specific destination quality, which individuates water bodies status suitable to a particular use for human consumption or for fish and shellfish life.

The law establishes that by 22nd December 2015 for all significant surface water and groundwater bodies the status of "**good**" for environmental quality must be achieved, and, where it's already present, the status of "**high**" environmental quality must be kept.
In Article 77, it is stated that by 31st December 2008 every surface water body that has been classified for its level of environmental quality, must have achieved the status of "**sufficient**".

1.2.1.1 Annex I to the third part

Criteria for **determination of status of environmental quality and of specific destination quality** of water bodies are explained in Annex I of the law. In the same Annex, also criteria for determining if a water body can be considered as "significant" are explained.
Besides, the first part of Annex I states that, in addition to significant water bodies, also water bodies important for their naturalistic impact and water bodies that can cause pollution of significant water bodies must be classified and monitored.

Surface water bodies

Status of environmental quality of surface water bodies is defined by taking into account both *ecological status* and *chemical status*.
Ecological status of surface waters is defined according to *qualitative* elements, such as biological elements, morphologic elements of the water body structure, chemical and physical-chemical elements, salinity and pollution from priority substances and substances present in the water body at high concentrations. All these elements are listed in the Annex I with differentiation for rivers, lakes, transition waters and coastal waters.
Chemical status is defined on the basis of average value (on annual basis) of the concentration of hazardous substances in water.
A first classification of the chemical status of a surface water body can be done according to the Table 1/A of the Annex I; anyway, local authorities can also plan the detection of other polluting substances, which are listed in Table 1/B of the same Annex, in order to define the chemical status of a water body. Tables 1/A and 1/B of the Annex I are here reported, for some compounds of interest in this research, in Table 1.3 and 1.4.

Table 1.3. Extract of Table 1/A of the Annex I to the third part of the D.Lgs. 152/2006: standards for quality in surface waters to be achieved by 31st December 2008.

Substance	Concentration [µg/l]
PAH (Polycyclic Aromatic Hydrocarbons)	0.2
Benzene	1
Trichlorobenzenes	0.4
1,2-dichloroethane	10
Trichloroethylene	10

The presence of pollutants with higher concentration than the one indicated in the table determines the classification of the water body as "bad" or "very bad".

Table 1.4. Extract of Table 1/B of the Annex I to the third part of the D.Lgs. 152/2006: further parameters to monitor in surface water bodies.

Substance
Chlorobenzene
1,2-dichlorobenzene
1,3-dichlorobenzene
1,4-dichlorobenzene
1,2,4-trichlorobenzene
1,1-dicloroethane
1,1,2,2-tetracloroethane
1,1,1-tricloroethane
1,1,2-tricloroethane
2-chlorophenol
3-chlorophenol
4-chlorophenol

Groundwater bodies

Status of environmental quality of groundwater bodies is defined by taking into account both *quantitative status* and *chemical status*.

Quantitative status is defined according to *water level* in the groundwater; the status will be "good" when level of groundwater is such as to allow the water body not to get empty on annual basis. Groundwater must not be polluted by sea waters, and must not be affected by human activity in such a way as to damage the connected surface waters and the ecosystems depending on them.

Chemical status of the groundwater is defined taking into account *conductivity of water* and *pollutants concentration*. The substances which must be monitored in groundwaters are at least the following:

- oxygen concentration;
- pH value;
- conductivity;
- nitrates;
- ammonium ion.

A "good" chemical status will be reached in a groundwater body when the concentration of these substances does not overcome the quality standards defined at European level, and are in such concentration as not prevent the connected surface waters to achieve the environmental quality standards predefined.

Differing from surface waters, the variation of conductivity in the groundwater does not mean intrusion in the water body of sea water.

1.2.1.2 Annex II to the third part

In Annex II to the law, at Section B, the quality standards for waters used for culture of salmons and carps are given. In Table 1/B of the Annex II, the maximum concentration of several substances in these waters are listed; among them, also the maximum concentration of phenolic compounds is given, and is set at the value of 0.01 μg/l.

In the same section, Table 3/B, the value of 2-chlorophenol concentration limit prescribed by U.S. E.P.A. is reported (Ambient Water Quality Criteria, 1978); it is the maximum concentration which does not alter the taste of fish, and is set at 60 μg/l.

In Section C of the same Annex, the maximum admitted concentration values for the culture of shellfish are given; for halogenated substances it is recommended that the concentration of each substance in the shellfish meat must be in such a concentration as to ensure a good quality of the products of the culture. Besides, the concentration of the substances in the water must be such as not have dangerous effects on shellfish life.

1.2.1.3 Annex V to the third part

As established in Article 101, all drains in water body are subjected to the limits of concentration indicated in the Annex V to the third part of the law.
Point 1.2 of this Annex prescribes that drains of industrial wastewaters which are discharged in surface water bodies must be in agreement with the limits indicated in Table 3. For production cycles indicated in Table 3/A of the Annex, the limits are given in terms of weight of pollutant emitted for weight of good produced or of raw material employed in the production process. For the same production cycles also concentration limits indicated in Table 3 at final discharge must be considered.
An extract of Table 3 and of Table 3/A of the Annex for some substances of interest in this work are reported in Table 1.5 and 1.6.

Table 1.5. Extract from Table 3 of Annex V to the third part of the law. Limit emission values in surface waters and in sewage system.

Substance	**Limit of emission in sewage system [mg/l]**	**Limit of emission in surface waters [mg/l]**
Phenols	1	0.5
Aromatic Organic Solvents	0.4	0.2
Chlorinated Solvents	2	1

Table 1.6: Extract from Table 3/A of Annex V to the third part of the law. Limit emission values for unity of product, referred to specific production processes.

Production of 1,2-dichloroethane			
Process	**Emission quantity on product unit**	**Monthly average**	**Daily average**
Only production of 1,2-DCA	g/t	2.5	5
Production of 1,2-DCA and transformation or use of the same 1,2-DCA in the same plant, with the exception of use in heat excangher.	g/t	5	10
Use of 1,2-DCA for metal degreasing in industrial plants (different from the ones of the previous point)	*	*	*
Transformation of 1,2-DCA in substances differing from vinylchloride	g/t	2.5	5

to be continued

follows from the previous pgae

Production of Trichloroethylene			
Process	**Emission quantity on product unit**	**Monthly average**	**Daily average**
Production of Trichloroethylene and of perchloroethylene	g/t	2.5	5
Use of Trichloroethylene for metal degreasing	*	*	*

*: for these substances no limits are indicated, but the following limits, together with the ones indicated in Table 3 of the Annex (here reported in Table 1.5) must be respected:

	Daily average [mg/l]	**Monthly average [mg/l]**
1,2-DCA in its use for metals degreasing in production plant differing from the ones in which 1,2-DCA is produced	0.2	0.1
Trichloroethylene in processes of trichloroethylene and perchloroethylene production	0.5	1
Use of Trichloroethylene for metal degreasing	0.2	0.2

1.2.1.4 Fourth Part

In the fourth part of the law, rules concerning wastes management and polluted sites remediation are given.

In this section, 5 Annexes refer to the remediation of polluted sites; Annex 5 reports the concentration limit values for pollutant agents in soils, sub-soils and groundwater. These values are given in Table 1 of the Annex (limit values for soils) and in Table 2 of the Annex (limit values for groundwater). Extracts of these two tables, referring to some substances of concern for this research, are reported in Table 1.7 and 1.8.

Table 1.7. Extract from Table 1 of the Annex V to the fourth part of the law. Concentration limit values for pollutant agents in soils and subsoils.

Substance	**Sites destined to vegetation cultures both for public and private use [mg/kg]**	**Sites destined to commercial and industrial use [mg/kg]**
1,2-dichloroethane	0.2	5
1,2-dichlorobenzene	1	50
2-chlorophenol	0.5	25
Phenol	1	60
Trichloroethylene	1	10

Table 1.8. Extract from Table 2 of the Annex V to the fourth part of the law. Concentration limit values for pollutant agents in groundwater.

Substance	**Concentration [µg/l]**
1,2-dichloroethane	3
1,2-dichlorobenzene	270
2-chlorophenol	180
Trichloroethylene	1.5

Chapter 2.
Chemical and Physical Properties of the Compounds Studied

The experimentation carried out in this study focused on the degradation of four chlorinated compounds: 1,2-dichloroethane (1,2-DCA), 1,2-dichlorobenzene (1,2-DCB), 2-chlorophenol (2-CP) and trichloroethylene (TCE).

2.1 Chemical and physical properties of the compounds

2.1.1 1,2-dichloroethane

1,2-DCA is a synthetic, volatile and flammable liquid; in Table 2.1 its main chemical and physical properties are shown.

Table 2.1. Main chemical and physical properties of 1,2-dichloroethane.

Physical status	Liquid
Formula	$ClCH_2ClCH_2$
CAS number	107-06-2
Colour	Colourless
Odour	Similar to chloroform
Molecular weight	98.96 g/mol
Freezing point	-35°C
Boiling point	83°C
Solubility in water	8.69 g/l at 20°C
Log K_{ow}	1.47
Log K_{oc}	1.28
Relative density	1.26 at 20°C
Vapour pressure	8.53 kPa at 20°C
Flammability limit	0.25-0.64 g/l

2.1.1.1 Production processes

1,2-DCA is industrially produced through two main processes, which are often combined.
The first process is a reaction in liquid or vapour phase, in which chlorine reacts with ethylene at a temperature of about 200°C in presence of a catalyst, usually 1,2-dibromoethane or metallic chlorides. The stoichometric reaction of this process is the following:

$$H_2C{=}CH_2 \rightarrow Cl{-}CH_2{-}CH_2{-}Cl$$

The second process is a reaction of ethylene with oxygen and HCl, in presence of a catalyst such as $Cu^{(II)}Cl$ (Drury and Hammons, 1979).
Commercial 1,2-DCA is pure at 97% and contains around 0.1% (in weight) of alchilammines that have the purpose of inhibiting its decomposition. Some impurities, such as polychlorinated ethanes, can be present, and the non-inhibited product can also contain Cl or HCl (Drury and Hammons, 1979; International Association Research in Cancer, www.aicr.org.uk).

According to the data produced by EPA, world production of 1,2-DCA in 1989 has been estimated in 422 millions of tons.

2.1.1.2 Use of 1,2-dichloroethane

1,2-DCA is used for 80-84% as an intermediate reagent in production of vinyl chloride, the monomer of PVC (polyvinylchloride), with subsequent formation of hydrochloric acid:

$$Cl–CH_2–CH_2–Cl \rightarrow H_2C=CH–Cl + HCl$$

1,2-DCA is also used in the production of chlorinated solvents, such as trichloroethylene, tetrachloroethylene and trichloroethane. Traces of 1,2-DCA have been found in industrial products for cleaning clothes and metals, for production of synthetic fibres and for removal of oils, greases, resins and gums. The compound is used for manufacture of acetyl cellulose and tobacco extract; it is a fumigant for grain, upholstery and carpets, and is employed for agricultural use in the USA for postharvest fumigation of grain and for use in orchards, agricultural premise and mushroom houses. It is used in leather cleaning, rubber goods fabrication, drum filling, degreaser compounds, rubber cement, acrylic adhesives and metal cleaning industries (Spectrum Laboratories, www.speclab.com).
1,2-dichloroethane was used in past years as a component of domestic products for cleaning solution and pesticides, of some adhesives and of some colorants and dyes.

2.1.1.3 Wastes disposal

Disposal of wastes deriving from industrial processes that involve the use of 1,2-DCA is usually done by means of inhertization and subsequent disposal in landfill, or by incineration.

2.1.1.4 Effects of 1,2-DCA on human health

1,2-dichloroethane is harmful if swallowed, inhaled or absorbed through skin. According to data on animal exposition, 1,2-DCA may cause cancer; risk of cancer depends on duration and level of exposure.
Inhalation of vapours irritates the respiratory tract, and may cause headache, weakness, cyanosis, nausea, vomiting, and diarrhoea. These symptoms may be followed by central nervous system effects, liver damage, kidney damage, adrenal gland damage, cyanosis, weak and rapid pulse and unconsciousness. Death can occur from respiratory and circulatory failure (Wirtschafter and Schwartz, 1939; Hadengue and Martin, 1953; Menschick, 1957; Troisi and Cavallazzi, 1961; Suveev and Babichenko, 1969).
Ingestion of 1,2-DCA causes irritation to the gastrointestinal tract; symptoms may include nausea, vomiting and diarrhoea. Doses of 0.5-1.0 g/kg can be fatal. Several major syndromes can be identified including central nervous system depression, gastroenteritis, and disorders of the liver and kidneys. Frequently-observed cardiovascular insufficiency and haemorrhagic diathesis may be related to changes in oxygenation and effects on the liver (Weiss, 1957; Morozov, 1958; Hinkel, 1965; Bogoyavlenski et al., 1968; Martin et al., 1968; Schönborn et al., 1970; Yodaiken & Babcock, 1973; Dorndorf et al., 1975; Andriukin, 1979).
Skin contact causes irritation, rash and blister formation. Prolonged contact can cause skin burns; 1,2-DCA can be absorbed through skin with toxic effects.
Eye contact through vapours causes eye irritation. Splashes cause severe irritation, possible corneal burns and eye damage.

Repeated or prolonged exposure may cause weight loss, low blood pressure, jaundice, reduced urinary output, dermatitis, eye damage and anaemia (Safety Data Sheet by Mallinckrodt www.mallchem.com).

2.1.2 1,2-dichlorobenzene

1,2-DCB is chemically stable, but it is combustible in presence of oxidizing agents. Hydroxilation occurs only at high temperatures and in alkaline conditions. In Table 2.2 the main chemical and physical properties of 1,2-dichlorobenzene are shown.

Table 2.2. Main chemical and physical properties of 1,2-dichlorobenzene.

Physical status	Liquid
Formula	$C_6H_4Cl_2$
CAS number	95-50-1
Colour	Colourless
Odour	Pleasant aromatic odour
Molecular weight	147.01 g/mol
Freezing point	-17°C
Boiling point	180°C
Solubility in water	0.14 g/l at 25°C
Log K_{ow}	3.38
Log K_{oc}	2.51
Relative density	1.305 at 20°C
Vapour pressure	0.133 kPa at 20°C
Flammability limit	2.2-9.2% (in volume
Flammability point	66°C

2.1.2.1 Production processes

1,2-DCB is the main by-product of the process of solvents and pesticides production, which takes place by means of reactions between gaseous chlorine and benzene. 1,2-dichlorobenzene is released to the environment also as subsequence of inappropriate disposal procedures and of use of tri- and tetra-chlorobenzene, employed as solvents, flame retardants and pesticides.
Chlorobenzenes are also synthesized during processes of waters disinfection with chlorine, and during wastes incineration processes.

2.1.2.2 Use of 1,2-dichlorobenzene

1,2-DCB is used as cleaning agent, in herbicides, in insecticides, as solvents for gums, glues, resins, oils and asphalts, as additive for combustible oils and paints, as heat transfer medium, as degreasing agent for metals and clothes, as wood protector agent, as deodorizing agent for sludge.

2.1.2.3 Wastes disposal

Wastes deriving from use of 1,2-dichlorobenzene are disposed of in landfill or incinerator.

2.1.2.4 Effects of 1,2-dichlorobenzene on human health

Available data on the effects of exposure to 1,2-dichlorobenzene in humans are restricted to case reports and two epidemiological studies; no clinical investigations on the effects of exposure in human volunteers were identified (Canadian Environmental Protection Act: Priority substances list report,www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/).
Case reports of adverse effects associated with exposure to 1,2-dichlorobenzene or mixtures containing 1,2-dichlorobenzene are confined to haematological disorders, including anaemia and leukaemia (Girard et al., 1969; Tolot et al., 1969). In the only identified cross-sectional epidemiological study of workers exposed to 1,2-dichlorobenzene, there was no evidence of "organic injury or untoward haematological effects" in an unspecified number of workers exposed to mean levels of 15 ppm 1,2-dichlorobenzene (Hollingsworth et al., 1958); however, little information on study design was presented in the published account of this investigation.
There was an increase in the total number of chromosomal aberrations (primarily single and double breaks) in the peripheral leucocytes of 26 laboratory workers exposed for 4 days, 8 hours per day to 1,2-dichlorobenzene vapour compared to a control group of 11 non-exposed laboratory personnel (Zapata-Gayon et al., 1982). No quantitative information on exposure was provided in the account of this study.

2.1.3 2-chlorophenol

2-CP derives from phenol after the substitution of a hydrogen atom with a chlorine; it is semi-volatile, combustible, unsuitable with oxidizing agents, corrosive, photosensitive and hygroscopic. In Table 2.3 some main chemical and physical properties of 2-chlorophenol are shown.

Table 2.3. Main chemical and physical properties of 2-chlorophenol.

Physical status	Liquid
Formula	ClC_6H_4OH
CAS number	95-57-8
Colour	Amber-like
Odour	Unpleasant, penetrating
Molecular weight	128.56 g/mol
Freezing point	7°C
Boiling point	174°C
Solubility in water	28.5 g/l at 20°C
Log K_{ow}	2.15
Log K_{oc}	3.65
Relative density	1.26 at 20°C
Vapour pressure	0.029 kPa at 20°C
Flammability point	72°C

2.1.3.1 Production processes

2-CP is synthesized in industrial processes by means of the substitution of a hydrogen atom with a chlorine one, in presence of NaCl, during the production processes of phenossialiphatic compounds as herbicides.
Synthesis of 2-CP occurs incidentally also during disinfection with hypochloride of waters containing phenols. In Italy, chlorination is nowadays the most used treatment for disinfection of waters, but the possible presence of phenols gives place to the formation of other compounds, often toxic, such as 2-CP.

2-chlorophenol can be produced also after degradation of pesticides (OMS, 2003).

2.1.3.2 Use of 2-chlorophenol

2-CP is used in the preparation of deodorants, cosmetic products, solvents, pesticides, paints, resins and disinfectants. As a 1-5% emulsion it is very effective in killing action of ascarid eggs and larvae on soil, brick, concrete, metal, and wood surfaces. Used as a disinfectant, bactericide, germicide for animal pathogenic bacteria (gram negative and gram positive vegetative) in household premises, sickroom equipment, sickroom premises, commercial premises, industrial premises, bathroom premises, on swimming pool related surfaces, urinals, water closets and garbage containers. (Spectrum Laboratories, www.speclab.com). It is also used in the process of extraction of nitrogen and sulphur from coal and as bleaching agent in the production of paper.

2.1.3.3 Wastes disposal

Wastes containing 2-chlorophenol are disposed of in landfill or incinerated.

2.1.3.4 Effects of 2-chlorophenol on human health

2-chlorophenol is potentially carcinogenic for humans. LD_{50} value for man is 2000 $mg/kg_{(body\ weight)}$.

Ingestion of 2-CP causes increment of respiration and blood pressure, fever, increased bowel action, motor weakness, collapse with convulsions and death. It causes lung, liver and kidney damage and contact dermatitis (Spectrum Laboratories, www.speclab.com).

Acute exposures by all routes may cause muscular weakness, gastroenteric disturbances, severe depression, collapse; although effects are primarily on central nervous system, oedema of the lung and injury of pancreas and spleen also may occur. Orally 8 g or more produce rapid circulatory collapse and death.

Chronic poisoning from oral or percutaneous absorption may produce digestive disturbances, nervous disorders with faintness, vertigo, mental changes, and skin eruptions (Spectrum Laboratories, www.speclab.com).

Giving 2-CP at 500 ppm to rats for five weeks causes increase of red blood cells and of haemoglobin. Experiments on rabbits have shown that 0.15 ml of a 1% solution of 2-CP cause eyes irritation, oedema and cornea opacization (Spectrum Laboratories, www.speclab.com).

Giving 2-CP on rats has caused tumours; 2-CP is also a possible toxic agent on aquatic organisms and on those living in marine sediments.

2.1.4 Trichloroethylene

TCE is an halogen organic carbon compound, with the structure of an ethylene molecule in which the three hydrogen atoms are substituted by three chlorine atoms. It is a synthetic compound and at environmental temperature is liquid; it's possible carcinogenic. In Table 2.4 the main chemical and physical properties of trichloroethylene are shown.

Table 2.4. Main chemical and physical properties of trichloroethylene.

Physical status	Liquid
Formula	C_2HCl_3
CAS number	79-01-6
Colour	Colourless
Odour	Sweet
Molecular weight	131.79 g/mol
Fusion point	-84.8 °C
Boiling point	87°C
Solubility in water	1 g/l at 20°C
Log K_{ow}	2.42
Relative density	1.5 at 20°C
Vapour pressure	8 kPa at 20°C
Explosivity point	8-10.5% in volume

2.1.4.1 Production processes

Up to the early '70s, TCE was mainly produced through a two-phases process starting from acetylene: first an acetylene conversion to 1,1,2,2-Tetrachloroethane with a reaction at the temperature of 90°C with chlorine, in presence of iron chloride as catalyst:

$$H\text{-}C\equiv C\text{-}H + 2\ Cl_2 \rightarrow Cl_2CH\text{-}CHCl_2$$

followed by dehydroalogenation by treatment with aqueous basis (NaOH) or in gas phase at 300-500°C in presence of barium chloride or calcium chloride as catalyst:

$$Cl_2CH\text{-}CHCl_2 + Ca(OH)_2 \rightarrow ClCH{=}CCl_2 + CaCl_2$$

$$Cl_2CH\text{-}CHCl_2 \rightarrow ClCH{=}CCl_2 + HCl$$

Nowadays most of TCE production starts from ethylene, first chlorized to 1,2-DCA in presence of a catalyst (iron chloride):

$$CH_2{=}CH_2 + Cl2 \rightarrow ClCH_2\text{-}CH2Cl$$

and then heated at around 400°C in presence of further gaseous chlorine:

$$ClCH_2CH_2Cl + 2\ Cl_2 \rightarrow ClCH{=}CCl_2 + 3\ HCl.$$

This last reaction is catalyzed by several substances: the most largely employed catalyst is a mixture of potassium chloride and aluminium chloride, but also porous coal can be used. A by-product of this reaction is tetrachloroethylene, which can also become its main by-product, if the amount of chlorine added in the second reaction is appropriately dosed. Trichloroethylene and tetrachloroethlyene are subsequently purified by distillation.

2.1.4.2 Use of trichloroethylene

TCE is used mainly as a solvent for substances insoluble in water. Nowadays it is used almost exclusively for metals degreasing, while in past it was used as solvent for dry cleaning, both in domestic and commercial applications; it was also used as an extractor for food (such as for

caffeine from coffee), as a discolourer and even as an anaesthetic. For obvious reasons of safety and environmental protection, these employments have been nowadays rejected.

2.1.4.3 Wastes disposal

Wastes coming from the use of TCE must be disposed of in landfill or incinerated.

2.1.4.4 Effects of trichloroethylene on human health

Studies indicate that 40 to 70% of TCE is absorbed by inhalation exposure. In an oral exposure study with rats, absorption of about 90% was observed. Dermal absorption is also known to occur, but the fraction absorbed has not been measured.
Human body eliminates most absorbed TCE within two days. The small amount remaining may be released slowly from fat cells over several days to a few weeks.
Inhalation and ingestion exposures to high levels of TCE such as with poisonings and its former use as an anesthetic have resulted in disturbances of heart rhythm. This observation is supported by results in some animal studies. High TCE exposure levels by inhalation that occurred in industrial accidents caused central nervous system symptoms such as headache, sleepiness, dizziness, blurred vision and loss of facial sensation. High acute exposures have resulted in nerves damage, causing facial numbness and jaw weakness that lasted several months.
Chronic inhalation exposure to low levels of TCE in occupational settings has produced central nervous system effects including memory loss, mood swings, and facial nerve damage. Another study of long-term exposure reported such effects as eye irritation, cough, drowsiness, weakness, dizziness and heart palpitations.
Abnormalities of the immune system were detected in a study of humans exposed to TCE-contaminated drinking water. Investigators who conducted an animal study concluded that the immune system was sensitive to TCE.
Studies of humans exposed to high levels of TCE in an occupational setting indicate that this substance can induce damage to the liver and kidney.
A large study of cancer registry data from New Jersey found associations between exposure to TCE in drinking water and elevated rates of leukemia and lymphoma. Several human studies have detected an association between kidney cancer and TCE exposure.
Some studies of humans exposed to TCE in drinking water reported increases in developmental effects involving the nervous system, heart, and hearing. These are tentative findings and cannot as yet be definitely associated with TCE exposure. Some animal studies supported the effects seen in humans, with TCE exposure causing developmental effects, especially those associated with the heart. Other animal studies indicate that TCE can affect the ability of males and females to reproduce and can also decrease foetal growth (National Research Council, Board on Environmental Studies and Toxicology, 2006; Agency for Toxic Substances and Disease Registry (ATSDR), 2001 and 1997).

2.2 Environmental fate of the compounds studied

Environmental pollution caused by 1,2-dichloroethane, 1,2-dichlorobenzene, 2-chlorophenol and trichloroethylene is caused by their strong use in civil and industrial field (metals degreasing, clothes cleaning, production of plastic goods, gums, resins, adhesives and paints) and by inappropriate storage and disposal techniques. Due to the scarce biodegradability of these substances by non-acclimated microorganisms naturally present in the environment, the effects of the compounds emitted in past decades are nowadays still present.

These compounds are detectable in surface waters and in groundwater, in soils and in atmosphere, frequently close to the emission points.
The high density, high viscosity and low solubility of the compounds makes them easily detectable in groundwaters. Due to their density, which is higher than water, these compounds are classified as DNAPL (Dense Non Aqueous Liquid Phase); when they penetrate into the groundwater, tend to accumulate at the bottom of the groundwater from where they are hardly extractable. Thus, their dispersion in water, due to their low solubility, occurs very slowly and can cause groundwater pollution for decades. In many cases, pollution of groundwaters by these compounds has made them unusable for human purposes.
Adsorption of organic contaminants on solid matrix of soil occurs as a consequence of the competition between water and contaminants, or among the contaminants themselves.
In an inorganic soil and in presence of water, **adsorption** of water is the most frequent process, due to its similar polarity with minerals. This gives as a consequence that organic contaminants (which are non-ionic substances) are not easily adsorbed on soil minerals.
Thus, the interactions between organic compounds and soil are ruled by chemical properties of the exposed solid surface, by granulometric distribution, by the nature of compounds, by the fact that they are in free or aggregate shape, by exposition time, by competition among different compounds and by transport flow rate.
Affinity of an organic compound for solid matrix is represented by the *distribution coefficient* K_d:

$$K_d = K_{oc} \cdot f_{oc}$$

where f_{oc} is the *organic carbon fraction in soil*, and K_{oc} the *organic carbon repartition coefficient*.
There is another coefficient to be considered when predicting the affinity of the organic compound for water or for the organic components of soil. This coefficient is the *octanol/water repartition coefficient* K_{ow}; the highest is the value of K_{ow} of a substance, the highest is the affinity of the substance for the organic fraction of the soil rather than for water, and the adsorption of the substance in the organic fraction of the soil increases.
For chlorinated benzenes, the increase of chlorine atoms number causes the increase of K_{ow} and K_{oc} values, and lowers solubility and vapour pressure.

2.2.1 1,2-dichloroethane

The majority of the 1,2-dichloroethane released into the environment enters the atmosphere mostly as a consequence of its production and use as a chemical intermediate, solvent, and use as a lead scavenger in gasoline.
Once in the atmosphere, it may be transported for long distances and is primarily removed by photo-oxidation (half-life approx. 1 month). Releases to water are primarily removed by evaporation (half-life of several hours to 10 days). Releases on land are dissipated by volatilization to air and by percolation into groundwater where it is likely to persist for a very long time.
1,2-Dichloroethane is not expected to bioconcentrate in the food chain; its presence in some food products is probably due to its use as an extractant. Major human exposure is from urban air, drinking water from contaminated aquifers and occupational atmospheres (Spectrum Laboratories, www.speclab.com).

2.2.1.1 1,2-DCA in water

Pollution of waters by 1,2-DCA is mainly due to incidental events or to inappropriate storage techniques.

When 1,2-dichloroethane is released to surface water, its primary loss is by evaporation. The half-life for evaporation depends on wind and mixing conditions and is of the order of hours in laboratory (Dilling et al., 1975). However a modelling study using the EXAMS model for a eutrophic lake gave a half-life of 10 days (Spectrum Laboratories, www.speclab.com). The half-life for evaporation is much less in a river or stream: in an experiment performed on site, half-life for 1,2-DCA in a river has been 1.4 hours (Scherb, 1978). Chemical and biological degradation is very slow, while adsorption to sediment is not expected.

2.2.1.2 1,2-DCA in atmosphere

In the troposphere, rain-out and adsorption on atmospheric aerosols are unlikely because of the high vapour pressure and the low solubility of the compound (Cupitt, 1980). The major part of 1,2-dichloroethane is removed from the atmosphere via oxidation by hydroxyl radicals. On the basis of experimentally-derived rate constants, and hydroxyl radical concentrations of $4.8 \cdot 10^6$ and $1.0 \cdot 10^6$ radicals/ml, respectively, half-lives for this reaction have been calculated of 10 days (Radding et al., 1977) and 36 days (Howard and Evenson, 1976). A lifetime of 53 days was predicted, which would preclude accumulation in the troposphere and transport to the stratosphere (Howard and Evenson, 1976). The reported degradation products are formyl chloride, hydrogen chloride, carbon dioxide, carbon monoxide, and monochloroacety chloride (Pearson and McConnell, 1975; Spence and Hanst, 1978). Since 1,2-dichloroethane absorbs light within the solar spectral region, photolytic transformation is possible (Cupitt, 1980). However, the extent of this reaction has not been verified experimentally.

2.2.1.3 1,2-DCA in soil

Small releases on land evaporate fairly rapidly because of 1,2-dichlorethane's moderately high vapour pressure. Larger releases may leach rapidly through sandy soil into groundwater, especially because of its low K_{ow} coefficient and its good solubility in water (Spectrum Laboratories, www.speclab.com; OMS, 1995); once in groundwater, due to its DNAPL nature, it can pollute the water body for a long time, and can result very difficult to extract.

In soil, 1,2-DCA adsorbs selectively to bentonite clay and peat moss, but not to dolomitic limestone and silica (Dilling et al., 1975).

Microorganisms naturally present in soil can transform it in less hazardous compounds. 1,2-DCA is not reduced by iron, the most commonly used reactive agent employed in Permeable Reactive Barriers often used in polluted soils remediation.

2.2.2 1,2-dichlorobenzene

1,2-DCB has been included among the major pollutants in polluting list laid down by EPA (1985).

Since chlorinated benzenes are chemically stable in nature (Ambient Water Quality Criteria for Dichlorobenzenes, EPA; www.epa.gov/ged/publica/gokey10.htm), biodegradation is the only process for the decrease of chlorinated benzenes level in the environment. This stability can cause bioaccumulation and pollution of the environmental matrix for long time.

Mainly, pollution of environment by 1,2-DCB is caused by use of solvents (25% of total emissions of 1,2-DCB in environment come from solvents use, according to Howard (1989), by landfill leachate, and from industries emissions.
1,2-dichlorobenzene present in wastewaters during their treatment can volatilize in atmosphere, or it can be biodegraded or adsorbed by biomass, and is then found in the sludge that must be disposed (Genevini, 1996).

2.2.2.1 1,2-DCB in water

According to extensive monitoring data and to K_{oc} values, when 1,2-DCB is released to water, adsorption to sediment is a major environmental fate process.
Aquatic hydrolysis, oxidation and direct photolysis are not expected to be important.
Since 1,2-dichlorobenzene (1,2-DCB) has a high affinity for lypophilic materials and is reported to have a relatively low vapour pressure and low aqueous solubility at ambient temperatures, sorption, bioaccumulation, and volatilization are expected to be competing processes. The rate at which each of these competing processes occurs determines which fate is predominant for 1,2-DCB in the aquatic environment. Should volatilization occur at a more rapid rate than sorption or bioaccumulation, then atmospheric processes would be expected to regulate the fate of 1,2-DCB. On the other hand should sorption and bioaccumulation occur more rapidly than volatilization, biodegradation of 1,2-DCB by aquatic microorganisms would be anticipated to regulate the fate of this compound.
1,2-Dichlorobenzene may be biodegraded in water in aerobic conditions after microbial adaptation; however, it is not expected to be biodegraded under anaerobic conditions which may exist in lake sediments or various groundwaters.
The persistence half-life of 1,2-dichlorobenzene in water column has been estimated to be 0.3-3 days in rivers, 3-30 days in lakes and 30-300 days in groundwaters; adsorption to sediment attenuates volatilization. In a model river (one meter deep, flowing 1 m/sec with a wind velocity of 3 m/sec at 20 °C) estimated half-life has been of 4.4 hours (Spectrum Laboratories, www.speclab.com).

2.2.2.2 1,2-DCB in atmosphere

The major source of 1,2-dichlorobenzene emission to the atmosphere has been reported to be solvent applications which may emit 25% of annual production to the atmosphere. If released to air, 1,2-dichlorobenzene exists predominantly in the vapour-phase and reacts with photochemically produced hydroxyl radicals at an estimated half-life rate of 24 days in a typical atmosphere. Direct photolysis in the troposphere is not expected to be important. The detection of 1,2-dichlorobenzene in rainwater suggests that atmospheric removal via wash-out is possible.
General population exposure to 1,2-dichlorobenzene may occur through inhalation of contaminated air, since 1,2-dichlorobenzene has been detected in widespread ambient air (Spectrum Laboratories, www.speclab.com).

2.2.2.3 1,2-DCB in soil

If released on soil, 1,2-dichlorobenzene can be moderately to tightly adsorbed. Volatilization from soil surfaces may be an important transport mechanism, even if it may be attenuated by tight adsorption to soil particles or by leaching. Chemical transformations by hydrolysis, oxidation or direct photolysis are not expected to occur in soil. Leaching from hazardous waste disposal areas adjacent to surface waters has been reported and the detection of 1,2-dichlorobenzene in various groundwaters indicates that leaching can occur. 1,2-

dichlorobenzene is slowly biodegraded in soil under aerobic conditions (Spectrum Laboratories, www.speclab.com).

2.2.3 2-chlorophenol

Release of 2-chlorophenol to the environment occurs through its use as a synthetic intermediate primarily for dyes and higher chlorinated phenols. 2-chlorophenol is a synthetic organic compound and has no known natural sources.

2.2.3.1 2-chlorophenol in water

According to experimental K_{oc}'s value, if 2-chlorophenol is released to water it may be adsorbed on sediments, although this estimated value predicts that this adsorption will be low or moderate. 2-CP is not expected to bioconcentrate in aquatic organisms and does not chemically hydrolyze.
2-chlorophenol is susceptible to photolysis near the surface of waters and biodegradation is an important fate.
Evaporation from water may be an important transport process with a half-life of 3.3 days estimated for evaporation from a river 1 m deep, flowing at 1 m/sec with a wind velocity of 3 m/sec.
Since the pK_a of 2-chlorophenol is 8.52, it exists in water and sediment in a partially dissociated state, and pH may affect its transport and reactivity in the environment (Spectrum Laboratories, www.speclab.com).

2.2.3.2 2-chlorophenol in atmosphere

If 2-chlorophenol is released to the atmosphere it may be susceptible to photolysis and reaction with NO_x in polluted air. The estimated vapour phase half-life in the atmosphere is 1.96 days mainly as a result of addition of ozone to the aromatic ring. Washout may be an important transport removal process (Spectrum Laboratories, www.speclab.com).

2.2.3.3 2-chlorophenol in soil

Since the pK_a (acid dissociation constant, expressed in logarithmic scale on base 10) of 2-chlorophenol is 8.52, it exists in moist soils in a partially dissociated state, and pH may affect its transport and reactivity in the environment. According to K_{oc}'s values, when released to the soil it is expected to show low or moderate adsorption to the soil, and may leach to the groundwater. Hydrolysis in soil is not important. Biodegradation in soils may be important with loss of 94% reported for 2-chlorophenol incubated in non-sterile clay loam soil at 4 °C in 6.5 hours, while 1% loss was observed in sterile soil in 12 days (Spectrum Laboratories, www.speclab.com).

2.2.4 Trichloroethylene

Trichloroethylene (TCE) is a synthetic liquid substance with a sweet odour. It was once used as a general anaesthetic. Its current main use is as a solvent and degreaser for metal compounds in the metal parts manufacturing, electronics and automotive industries. It is also found in many consumer products including paints, paint strippers, adhesives, varnishes and spot removers. TCE may be buried in landfills or dumped into the ground or sewers when mixed with grease or oil where it readily migrates to groundwater.

2.2.4.1 Trichloroethylene in water

TCE is the most common organic contaminant detected in U.S. *groundwater*. Although TCE in surface soil or surface water readily volatilizes into the air as a gas, it generally remains stable in groundwater for months to years. Under low oxygen conditions in groundwater, TCE eventually degrades to other toxic chlorinated chemicals such as 1,1-dichloroethene and vinyl chloride. While the odour threshold for TCE in air is reported as 100 parts per million (ppm), there is no information on its odour threshold in water.

The high Henry's Law Constant indicates rapid evaporation from water. Actually, field studies also support rapid evaporation from water; once trichloroethylene is in water, much evaporates into the air; again, about half will break down within a week, while it takes days to weeks to break down in surface water. In groundwater the breakdown is much slower because of the much slower evaporation rate. Very little trichloroethylene breaks down in the soil, and it can pass through the soil into underground water (Agency for toxic substances and disease registry; www.atsdr.cdc.gov/toxprofiles/phs19.html).

Half-lives of evaporation have been reported to be on the order of several minutes to hours, depending upon the turbulence. Trichloroethylene is not hydrolyzed by water under normal conditions. It does not adsorb light of less than 290 nm and therefore should not directly photodegrade. However, slow (half-life -l0.7 months) photooxidation in water has been noted (EPA, 2006; www.epa.gov/OGWDW/dwh/t-voc/trichlor.html).

Marine monitoring data only suggest moderate bioconcentration (2-25 times). Bioconcentration factors of 17 to 39 have been reported in bluegill sunfish and rainbow trout (EPA, 2006; www.epa.gov/OGWDW/dwh/t-voc/trichlor.html).

2.2.4.2 Trichloroethylene in atmosphere

So far, the biggest source of trichloroethylene in the environment is evaporation from factories that use it to remove grease from metals. It can also enter the air and water when it is disposed of at chemical waste sites. It evaporates easily but can stay in the soil and in groundwater. Once it is in the air, about the half will be broken down within a week. When trichloroethylene is broken down in the air, phosgene, a lung irritant, can be formed. Trichloroethylene can break down under high heat and alkaline conditions to form dichloroacetylene and phosgene (Agency for toxic substances and disease registry; www.atsdr.cdc.gov/toxprofiles/phs19.html).

Trichloroethylene is relatively reactive under smog conditions with 60% degradation in 140 min and 50% degradation in 1 to 3.5 hours reported. Atmospheric residence time based upon reaction with hydroxyl radical is 5 days (6-8) with production of phosgene, dichloroacetyl chloride, and formyl chloride (EPA, 2006; www.epa.gov/OGWDW/dwh/t-voc/trichlor.html).

2.2.4.3 Trichloroethylene in soil

Relatively high vapour pressure and low adsorption coefficient to a number of soil types indicates ready transport through soil and low potential for adsorption to sediments. The mobility in soil is confirmed in soil column studies and river bank infiltration studies. According to experimental observations, 4-6% of environmental concentrations of trichloroethylene adsorbed to two silty clay loams (Koc = 87 and 150), while no adsorption to Ca-saturated montmorillonite and 17% adsorption to Al-saturated montmorillonite was observed (EPA, 2006; www.epa.gov/OGWDW/dwh/t-voc/trichlor.html).

Chapter 3.
Advanced Oxidation Processes for Chlorinated Compounds Polluted waters

3.1 Generalities on polluted water and soil remediation techniques

Processes employed for the decontamination of polluted groundwaters and soils can be divided into two main categories: *in situ* and *ex situ* processes.
Ex situ decontamination processes are made by extracting the polluted soil or water from their original site and then by treating it in a proper treatment plant; the decontaminated matrix can be then replaced in its original place or disposed of in proper sites.
In situ decontamination processes take place directly in the polluted site, and can be operated both in *on site* or *off site* modality: *on site* processes are operated directly in the polluted ground, while *off site* processes are operated by extracting the polluted matrix and decontaminating it in a treatment structure properly equipped in the nearing of the polluted site.
As can be easily understood, ***ex situ*** processes are more effective than the *in situ* ones, in terms of treatment efficiency and decontamination kinetic rates, because the treatment process can be carried out with the optimal operational parameters (such as temperature, pH, humidity, time length of the process). Furthermore, these treatments are much better controllable, and some process by-products can be detected and eliminated before the replacement of the matrix into its original site.
On the other hand, ***in situ*** processes are more cost-effective, because there are no costs for the extraction and transport of the polluted matrix to the treatment plant, and for the subsequent replacement of the decontaminated matrix in the soil. Nevertheless, *in situ* processes are more difficultly controllable than *ex situ* processes, and their degradation rates are often slower because of the difficulty of controlling the operative parameters of the process.
The recent environmental legislation trend is that of indicating *in situ* processes as preferable, because of the minimum impact on the environment and the lower risk of alteration of the natural properties of soil and water and of the ecosystem equilibrium.
Besides, with *in situ* processes it is possible to avoid the extraction of the polluted matrix from its site, thus avoiding the risk of producing the contaminants dispersion in the environment by volatilization.

3.2 Non biological treatment processes

In the last decades, European and local law regulations have focused with increasing attention on the problem of treatment and remediation of surface waters and groundwaters polluted by organo-chlorinated solvents.
European Water Framework Directive 60/2000/CE introduced the concept of "Priority substances" referring to those substances whose elimination from waters must be obtained in all the member states within 15 years from the entry into force of the Directive. Most of the substances detected as Priority Substances usually belong to the class of *xenobiotics*, which means that they are produced by human activity and are not naturally present in the environment. The xenobiotic nature of these substances makes them hardly metabolizable by non-acclimated bacterial populations, with the consequence that remediation of waters polluted by these substances is hardly achieved by means of biological treatments. Besides,

sometimes only an incomplete biodegradation of these substances can be achieved even with acclimated bacterial populations, with the subsequent production of secondary by-products which can be even more toxic than the original compounds.
This, together with the more and more stringent emission limits allowed by recent environmental laws, explains why scientific research makes nowadays a strong effort for the development of chemical-physical methods capable to decontaminate with high efficiency waters polluted by xenobiotics.

3.3 Conventional chemical-physical treatments

Conventional chemical-physical water treatments have been so far used for decades, and they can be classified into two big categories.
One class is constituted by treatments based on **phase separation** of the contaminants, from the polluted water to other matrixes: adsorption and stripping techniques belong to this category.
The other treatments class is based on **chemical oxidation** of the contaminants, in order to completely decompose them into water, carbon dioxide and inorganic or at least non-toxic products.
It is quite evident that the second treatments class is preferable to the other one, because it allows the complete destruction of the pollutant agents, thus eliminating the problem of the disposal of matrixes polluted by some by-products, such as process sludge rich of pollutants.
Unfortunately, it has been observed in several studies (Fedorak et al., 1984; Barreiro et al., 1992; Reemtsma et al., 1997), that substances which are hardly biodegraded, are also difficultly degraded by conventional non-biological treatments. That is why a new generation of more powerful and effective oxidation techniques is being developed nowadays; this class of liquid and gaseous streams decontamination treatments is called **Advanced Oxidation Processes** (**AOPs**).

3.4 Advanced Oxidation Processes

Several typologies of AOP have been developed so far; they use different reagents and catalyzing substances, but fundamentally they are all based on the production of OH radicals (usually indicated with OH·), which are extremely reactive and attack with high kinetic rates most part of organic substances (Farhataziz and Ross, 1977; Hoignè and Bader, 1983). Their high reactivity makes the AOP suitable for the degradation of a wide range of pollutants present in water, but at the same time it makes them very expensive if used for the whole decontamination treatment: that is why they are often used coupled with biological treatments (before and/or after it), in order to act on a smaller part of pollutant substance and to allow lower costs of reagents and catalyzers. For the same reason it is usually recommended to employ AOP processes when COD concentration in polluted water is below 5 g/l (Andreozzi et al., 1999).
A list of some AOPs, together with the reagents involved in the oxidation, is given in Table 3.1.

Table 3.1. AOP and reactants involved (Andreozzi et al., 1999)

Process Denomination	**Reactants involved**
Fenton	H_2O_2/Fe^{2+}
Fenton-like	H_2O_2/Fe^{3+}

to be continued

follows from the previous page

Photo-Fenton	$H_2O_2/Fe^{2+}(Fe^{3+})/UV$
Photo-Fenton with Oxalate	H_2O_2/Fe^{3+} - Oxalate
Photocatalysis	$TiO_2/h\nu/O_2$
Ozone and hydrogen peroxide	O_3/H_2O_2
Ozone catalysis	O_3/UV
Hydrogen peroxide catalysis	H_2O_2/UV
Thermal oxidation	High temperature/oxygen
Catalytic oxidation	O_2/metal

Nowadays the most widely employed AOP treatments are O_3/H_2O_2, H_2O_2/UV and O_3/UV.

3.4.1 Typologies of AOPs

3.4.1.1 Fenton Process

This is one of the oldest decontamination techniques for water treatment: it was developed in 1894 by Fenton (Fenton et al., 1894), and in the last decades it has been revaluated for its high efficiency in removal of many toxic substances (such as phenolic pesticides) from waters.
In Fenton process, OH radicals are produced by means of reaction of iron salts (Fe^{2+}) with H_2O_2 (Haber et al., 1934). The whole Fenton process owes it success to the fact that high treatment efficiency is reached through a very simple reaction, which requires common reagents and a simple configuration of the reactor; besides, iron and H_2O_2 are not toxic and easily collectable.

3.4.1.2 Fenton-like Process

This process is very similar to the basic Fenton; the difference lies in the pH value that must be kept during the reaction around the value of 2.7-2.8, because this leads to the reduction of Fe^{3+} to Fe^{2+}, thus increasing the amount of iron salts available for the production of OH radicals (Pignatello et al. 1992).

3.4.1.3 Photo-Fenton Process

OH radicals production rate can be highly increased if UV-VIS light (in a wavelength higher than 300 nm) is irradiated during the process (Kiwi et al., 1993; Pulgarin et al., 1996). Actually, UV-VIS light causes the photolysis of Fe^{3+} complexes, with the subsequent production of Fe^{2+} salts that react with the H_2O_2 present, giving way to the pathway reaction explained for Fenton process.

3.4.1.4 Photo-Fenton with Oxalate Process

Safarzadehet-Amiri et al. (1996) have discovered that the utilization of ferrioxalate in the Photo-Phenton process can increase its efficiency in removal of organic pollutants, since ferrioxalate in acidic solution generates carbon dioxide and ferrous ions Fe^{2+} (free or linked to ferrioxalate) which gives a continuous source of reagent for Fenton process.
The effectiveness of this process lies in the low energy requirement, which is just 20% of the one necessary to complete the traditional Photo-Fenton process (Safarzadehet-Amiri et al., 1997).

3.4.1.5 Photocatalysis

In photocatalythic processes, oxygen is employed as an oxidizing agent, while a semiconductor metal oxide is used as a catalyst (Ollis, 1993). Among all the catalyzing agents

tested so far, TiO_2 has shown to have the best properties in terms of stability, performance and costs (Zhang et al., 1994; Rajeshwar, 1995). Reaction starts when the catalyst agent is irradiated with UV light, which makes the material create an electron hole pair as a result of exposure to ultraviolet radiation (www.titaniumart.com/photocatalysis-ti02.html). Photocatalytic activity in TiO_2 has been extensively studied because of its potential use in sterilization, sanitation, and remediation applications, and nowadays the possibility of employing wavelengths of solar spectrum in order to activate the reaction of the process and reduce process costs is subject of experimentation. However, despite the great efforts spent in research, photocatalysis is still seldom used in industrial applications.

3.4.1.6 Ozone and Hydrogen Peroxide reaction (H_2O_2/O_3)

This process lies on the decomposition of ozone in water, which starts with the presence of OH^- ion and proceeds with the production of OH radicals; during the process, also H_2O_2 is produced. It is thus clear that the addition of hydrogen peroxide to the ozone aqueous solution enhances the ozone decomposition rates with further formation of OH radicals. The H_2O_2/O_3 process does not require very different reactor design in comparison to the one required for the simple ozonization process, because it is necessary only to add an apparatus for supplying the H_2O_2.

3.4.1.7 Hydrogen Peroxide photolysis (H_2O_2/UV)

In H_2O_2 photolysis, the reaction is started by irradiating the aqueous solution containing the pollutants and the hydrogen peroxide with UV lights with wavelengths shorter than 280 nm; this leads to the homolysis of the H_2O_2 molecule, with the subsequent production of two free radicals (Baxendale et al., 1957).

3.4.1.8 Ozone catalysis (O_3/UV)

For this process, the polluted water is saturated with ozone and then irradiated with UV light of 254 nm of wavelength. The production of radicals comes both from ozone and from the hydrogen peroxide produced during the reaction; thus the system has the chemical behaviour of both the H_2O_2/O_3 and H_2O_2/UV processes (Peyton et al., 1988).

3.4.1.9 Thermal oxidation

Oxidation equipment (thermal or catalytic) is used for destroying contaminants in the exhaust gas from air strippers and SVE (Soil Vapour Extraction) systems. Thermal oxidation units are typically single chamber, refractory-lined oxidizers equipped with a propane or natural gas burner and a stack. Lightweight ceramic blanket refractory is used because many of these units are mounted on skids or trailers. Flame arrestors are always installed between the vapour source and the thermal oxidizer. Operating temperatures range from 760 to 870 °C, and gas residence times are typically 1 second or less (http://www.frtr.gov/matrix2/section4/4_63.html).

3.4.1.10 Catalytic oxidation

For the degradation of chlorinated volatile organic compounds (VOCs), one of the most effective treatment technique is the catalytic oxidation.

The addition of a catalyst to a thermal oxidation system accelerates the rate of oxidation by adsorbing the oxygen and the contaminant on the catalyst surface, where they react to form carbon dioxide, water and hydrochloric gas. VOCs are thermally destroyed at temperatures typically ranging from 320° to 540° C. In the catalytic oxidation, first the contaminated air is directly preheated (electrically or, more frequently, using natural gas or propane) to reach a

temperature necessary to initiate the catalytic oxidation (around 370 °C) of the VOCs. Then the preheated VOC-laden air is passed through a bed of solid catalysts where the VOCs are rapidly oxidized. Catalysts used to oxidize VOCs are generally metal oxides such as nickel oxide, copper oxide, manganese dioxide, or chromium oxide; noble metals such as platinum and palladium may also be used, since they exhibit the highest activity for the oxidation of volatile organic compounds. Most commercially available catalysts are proprietary (http://www.frtr.gov/matrix2/section4/4_63.html). Alternatively, the use of transition metal oxides has been proposed because of their resistance to deactivation.
Other types of catalysts, such as zeolites, pillared clays, molten salt-based systems and perovkites have also been considered for chlorinated volatile compounds emission control (Aranzabal et al., 2006). An experimentation carried out by González-Velasco et al. (2000a,b) showed similar activity in chlorinated volatile compounds degradation while using zeolites and noble-metal as catalysts in catalytic oxidation. However, very few studies have been performed to investigate the reaction mechanism of the chlorinated VOC deep catalytic oxidation. The reaction mechanism is believed to involve a reaction between adsorbed oxygen and the adsorbed reactant molecule as a Langmuir–Hinshelwood mechanism (Rossin and Farris, 1993; Papenmeier and Rossin, 1994) or a reaction between adsorbed oxygen and the gas-phase reactant molecule as an Eley–Rideal mechanism (Wang et al., 1992; Yu et al., 1992).

3.4.2 General remarks for AOP use

In all the processes, a fundamental role is played by the **pH value**, which, if kept in the optimal range, can improve the whole process efficiency. The proper pH value is investigated for each treatment by means of experimental studies; during photooxidation processes the pH of reaction medium decreases due to formation of acidic species, thus a constant automatic control of pH value must be equipped in the reactors where this process takes place.
When treating polluted waters with UV radiations, particular care must be given to the presence of **suspended solids and particulates** which can strongly inhibit the process and thus should be eliminated before the treatment.
In all AOPs it is also very important to limit as much as possible the presence in water of **carbonate**, which acts as radicals scavenger, since carbonates and bicarbonates (HCO^{3-} and CO_3^{2-}) react with OH radicals producing CO_3 radicals, which are much less reactive than the other ones.
Besides, in all AOP reactors, in order to reduce the block of the system, the concentrations of **fats, oils and metallic ions** entering the reactors must be kept very low (below 10 mg/l).
When treating very volatile substances with a O_3/UV process, attention must be paid to the **volatilization** of the substances, and a vapour treatment system must be equipped.
As far as the **costs** are concerned, AOP treatments have similar overall costs of the traditional chemical-physical treatments (air stripping, GAC adsorption and similar processes), i.e. around 0.3-3 €/m^3 of treated water.

3.4.3 Application of AOP to degradation of different pollutants

Aqueous streams contaminated with different solvents are nowadays often treated in full scale plant by means of the processes previously described, and many researches at laboratory and

pilot plant scale are still in progress in order to individuate the optimal operating conditions and the most efficient reagents and catalyzers for the different treatment processes.

In an experimentation carried out by Chatterjee et al. (2006), several halocarbons (chlorophenols, trichloroethylene, 1,2-dichloroethane and 1,4-dichlorobenzene) have been photodegraded with visible light; the reaction took place on the surface of a dye-modified **TiO_2** semiconductor. In this study, the system involved excitation of surface adsorbed dye, followed by charge injection into the TiO_2 conduction band and formation of reactive $O_2^{\bullet}/H_2O_2^{\bullet}$ radicals; after 5 h of irradiation with a 150 W Xenon lamp, over 55-72% degradation of pollutants has been achieved.
In another study, **Photo-Fenton** was compared with Fenton for the degradation of gaseous dichlorometane (Feitz et al., 2002). Fenton showed to be no more effective in dichloromethane degradation than was the simply Milli-Q water, because Fe(II) quickly converted to Fe(III) but was unable to regenerate; thus, after a short period, the degradation process stopped. However, by using UV light and increasing the hydraulic retention time of the contaminated gaseous flow in the reactor, the degradation of the chlorinated substance started again, and reached a removal percentage of 65%.
Degradation of trichloroethylene (TCE) in natural sand systems was studied by Yeh et al. (2003), who tested the possibility of applying **Fenton** technique to the sand using the iron salts present in it. Two kinds of sands were employed in the study: one was natural silica sand, while the other, with a higher iron concentration, was aquifer sand. Column experiments were performed, and the reactivity of H_2O_2 at a certain concentration in the two systems was measured. Results showed that the aquifer sand, containing more iron, allowed a higher reactivity of hydrogen peroxide and 4 times more TCE was removed in comparison to the natural sand system.
In the experimentation carried out by Hincapiè et al. (2005), the degradability of several pesticides classified by the water framework directive as *Priority Substances* (PS) was tested by the use of **photo-Fenton** and of **photo-catalysis** with TiO_2, both driven by solar energy; the tests were performed in a large pilot plant. Two different iron concentrations were tested in the photo-Fenton process (2 and 55 mg iron/l), and the results showed that the lowest amount of iron was enough to perform a good degradation process. Photo-Fenton was more effective in pesticides removal than photo-catalysis with TiO_2. Degradation of all the substances studied was possible without mineralization with both processes, and was achieved in a quite short time (around 5 hours).

3.4.4 Advanced Oxidation Processes for removal of 1,2-dichloroethane

Rodriguez et al. (2005) carried out an experimental study for comparing the effectiveness of **photo-Fenton** process with the one of **TiO_2 photocatalysis** under aerobic and anaerobic conditions, for the treatment of water polluted by several chlorinated solvents (dichloroethane, dichloromethane and trichloromethane). The experimentation showed that photo-Fenton was the most appropriate for the decontamination of water polluted with these substances.
The use of ultraviolet irradiation and hydrogen peroxide (**H_2O_2/UV**) for the control of solvent contamination in small water utilities was investigated by Symons et al. (1989). In this study the degradation of benzene, 1,2-dichloroethane, 1,1-dichloroethylene, 1,1,1-trichloroethane, trichloroethylene, carbon tetrachloride, and 1,4-dichlorobenzene with UV irradiation and

H_2O_2 has been compared with the decrease of the concentration of these substances stored at the dark.
An experimentation on the degradation of 1,2-dichloroethane and of some other chlorinated compounds was carried out by Stepnowski et al. (2002), with the use of an enhanced photo-degradation system (**H_2O_2/UV**). Several concentrations of hydrogen peroxide were tested, and, except for the lowest one (1.17 mM), complete degradation of 1,2-dichloroethane was always reached in 24 hours. The increase of UV radiation slightly accelerated the process due to the increased formation of hydroxyl radicals.

3.4.4.1 Catalytic Oxidation Treatments for 1,2-dichloroethane

Due to the **highly volatile** nature of 1,2-dichloroethane, **catalytic oxidation treatments** are often used for its destruction from gaseous flows.
Aranzabal et al. (2006) achieved the complete degradation of 1,2-dichloroethane by means of **catalytic oxidation over palladium supported on alumina**. The kinetics of catalytic oxidation of 1,2-DCA were checked for different values of temperature and inlet concentrations. 1,2-DCA was first dehydrochlorinated and formed vinyl chloride (VC), followed by direct oxidation of VC to CO, which was finally oxidized to CO_2.
The **catalytic oxidation** of chlorobenzene and 1,2-dichloroethane oxidized alone and in two-components mixtures with selected non-chlorinated volatile organic compounds was investigated by Musialik-Piotrowska and Mendyka (2004). The tests were carried out over Platinum-based catalyst on a monolithic metallic support. Dichloroethane conversion was inhibited in the presence of each non-chlorinated mixture. Both chlorinated compounds lowered the catalytic reactivity of non-chlorinated solvents and increased the concentration of incomplete oxidation products.
The **oxidation** of 1,2-DCA over H-type zeolites was evaluated by Lopez-Fonseca et al. (2000). The activity of the zeolites was reduced in the presence of water vapour. The presence of water in the 1,2-DCA decomposition changed significantly the reaction product distribution.

3.4.5 Advanced Oxidation Processes for removal of 1,2-dichlorobenzene

Different advanced oxidation processes have been tested experimentally by Munoz et al. (2006) to remove the organic carbon content of a paper mill effluent containing also 1,2-DCB. The considered AOPs were: **TiO_2-mediated photocatalysis**, **TiO_2-mediated photocatalysis assisted with H_2O_2**, **TiO_2-mediated photocatalysis coupled with Fenton**, **photo-Fenton**, **ozonation and ozonation with UV-A light irradiation**. The application of the selected AOPs all resulted in a considerable decrease in dissolved organic carbon content with variable treatment efficiencies depending upon the nature and type of the applied AOP. A **Life Cycle Assessment** (LCA) study was used as a tool to compare the different AOPs in terms of their environmental impact. Heterogeneous photocatalysis coupled with the Fenton's reagents proved to have the lowest environmental impact accompanied with a moderate dissolved organic carbon removal rate. On the other hand, heterogeneous photocatalysis appeared to be the worst AOP both in terms of dissolved organic carbon abatement rate and environmental impact. For the studied AOPs, LCA has indicated that the environmental impact was attributable to the high electrical energy (power) consumption necessary to run a UV-A lamp or to produce ozone.
The oxidation of 1,2-dichlorobenzene in aqueous solutions by means of **Fe(III) homogeneous photocatalysis under UV lamp and sunlight irradiations** was studied by

Andreozzi et al. (2006). The study individuated the optimal oxygen concentration and pH value for having the best working conditions.

3.4.5.1 Catalytic Oxidation Treatments for dichlorobenzene

As for dichloroethane, the high volatility of dichlorobenzene makes catalytic oxidation one of the most used treatment for the gaseous streams polluted by this substance.
In a study by Choi et al. (2006), **oxidative destruction** of gas-phase 1,2-dichlorobenzene was conducted over **vanadia–titania aerogel catalysts**. Vanadia–titania catalysts prepared by a sol–gel method and by supercritical drying showed a very high activity and thermal stability in degradation of the chlorinated solvent. In the gas phase catalytic oxidation of 1,2-DCB over vanadia–titania aerogel, carbon oxides are almost the sole products and the selectivity exceeds 95% over all the range of temperatures, while conversion exceeds 90% at above 350°C.
Several types of **perovskite oxides** have been investigated by Poplawski et al. (2000) as catalysts for the oxidation of 1,2-dichlorobenzene. Perovskites containing Cr were more active than perovskites containing other transition metals. The presence of water appeared to enhance the catalytic activity of some perovskites.
Three studies performed by Krishnamoorty et al. (1998, 1999, 2000) showed that Vanadium Oxide $\mathbf{V_2O_5}$ supported on either $\mathbf{TiO_2}$ **or** $\mathbf{Al_2O_3}$ is active for the oxidation of 1,2-dichlorobenzene.
1,2-dichlorobenzene present in wet air was degraded by means of **catalytic oxidation over protonic zeolites**, which are active to oxidize 1,2-DCB in the range of 350–400 °C. However, the addition of platinum increased activity of zeolites but also favoured by-products formation such as polychlorinated compounds (Taralunga et al., 2006).

3.4.6 Advanced Oxidation Processes for removal of chlorophenols

In an experimentation carried on by Tang and Huang (1996), **Fenton** process was applied to the degradation of 2-chlorophenol, 2,4-dichlorophenol and 2,4,6-trichlorophenol. The study showed that Fenton treatment efficiency was affected by the level of chlorination of phenol molecule, and its efficiency depended also on the position of the chlorinated substitute in the molecule. In particular, it was observed that the reactivity of chlorophenols decreases with the increase of the amount of chlorinated substitutes in the aromatic ring.
In another study by the same authors (Tang and Huang, 1995) it was found that the dechlorination rate obtained with **Fenton** process was faster for 3-chlorophenol than for 4-chlorophenol, which was faster than for 2-chlorophenol. This was explained assuming that in the monochlorophenols, OH and Cl groups are in ortho or para directions in the aromatic ring, and so they have a directory effect which influences the hydroxylation (and the subsequent degradation) of the whole molecule.
In an experimentation by Barbeni et al. (1987), **Fenton** process was tested for its efficiency in degrading different chlorophenols, such as 2-chlorophenol, 3-chlorophenol, 4-chlorophenol, 3,4-dichlorophenol and 2,4,5-trichlorophenol. The results showed that increasing the concentration of Fe^{2+} enhanced the decomposition process, and the presence of Fe^{3+} alone (without Fe^{2+}) with H_2O_2 had no effect on the degradation of the chlorophenols.
Degradation of 2-chlorophenol by the application of both **Fenton** and **photo-Fenton** processes was studied by Perez-Moya et al. (2007). Results showed that hydrogen peroxide concentration had a main direct effect on the favourable reduction of chlorinated compounds. Temperature had also an important effect in the 2-chlorophenol degradation, especially when the ratio of Fenton reagents was not correctly chosen. More than 90% chlorinated compounds reduction could be achieved in only 30 min of treatment.

In some studies (Huang at el., 2001; Lu et al., 2002) **catalysts** such as goethite, ferrihydrate and hematite were studied in addition to **H_2O_2 and iron oxides** for the degradation of 2-chlorophenol. The studies showed that the degradation rate of 2-chlorophenol depended on catalysts concentration, and found out the more active catalysts, among the ones tested, in 2-chlorophenol degradation. Besides, the structure of the catalysts in terms of porosity was found to be of high importance in the development of the chlorophenol degradation process.
Wet air oxidation over different catalysts has also been widely studied as possible treatment for the degradation of 2-chlorophenol from aqueous solutions.
Li et al. (2007a, 2007b) used a series of **$Ce_xZr_{1\_x}O_2$ mixed-oxide-supported Ru catalysts** in order to degrade a 2-chlorophenol solution of 2 g/l. This technique showed to have a high efficiency and to be more effective in terms of higher activity than the corresponding CeO_2 or ZrO_2-supported catalysts.

Chapter 4.
Biological Treatments of Waters Polluted by Chlorinated Solvents

4.1 Generalities on biological treatments

4.1.1 Abiotic and biological degradation processes

The most part of chlorinated compounds present in the environment are not degraded; nevertheless often they are partly subjected to *natural attenuation*, which means that they are partly removed by particular processes occurring naturally in the environment. The degradation processes which take place spontaneously in the environment can follow two reaction pathways:

- reactions without electron exchange;
- reactions with electron transfer.

Both kinds of reaction can be dealt by microorganisms (*biological degradation*), or can occur in absence of microorganisms (*abiotic degradation*).

Usually, **abiotic reactions** do not involve the transport of electrons, and take place in water with substitution of the chlorinated substitute of the chlorinated molecule with an oxidrilic group, and the subsequent production of an alcohol. Otherwise, the process occurs with the elimination of a chlorine and a hydrogen atom linked to two adjacent carbon atoms, with the subsequent formation of a double bond between them. Both reactions have quite low kinetic rates; the half life times of some of them are reported in Table 4.1.

Table 4.1. Half life time for some of the most commonly used chlorinated solvents at 20°C. (Nocentini et al., 2005)

Chlorinated substance	Half life time for abiotic reactions [years]
Carbon tetrachloride	7000
Chloroform	1.3
Dichloromethane	1.5
1,1,1-Trichloroethane	0.5-2.5
1,1,2-Trichloroethane	170
1,2-dichloroethane	50
Chloroethane	0.12
Tetrachloroethylene	0.7-6
Trichloroethylene	0.9-2.5

The reactions which involve electron transport are usually **biological reactions**. Removal of pollutants from water is based on the natural metabolic processes made by some bacterial and fungi strains, which use the pollutant agents as source of carbon and/or of energy for their natural processes of growth. Microorganisms operating biological degradation of polluting substances can follow different metabolic pathways and can operate in different metabolic conditions, but they are all based on **oxidation and reduction processes**.
These kind of processes imply the transfer of electrons from a source of electrons (the substance with the most electronegative element, which is usually the water pollutant in case

of a water treatment) to an electron acceptor; this transfer liberates a certain amount of energy that is used by the microorganisms for their growth processes.
As far as the biological degradation of chlorinated solvents is concerned, if their chlorination level is not very high (1 or 2 chlorinated substitutes in the molecule) they act in the metabolic process as electron donors, while the role of electron acceptors is usually played by oxygen: in this case the chlorinated compounds are oxidised and the metabolism is **aerobic**.
More chlorinated molecules have a higher electronegativity due to the presence of a higher amount of chlorinated substitutes. The electronegativity of these chlorinated substances is higher than the one of oxygen, and so the chlorinated molecules act as electron acceptors; in this case the chlorinated compounds are degraded with a reduction reaction, and **anaerobic** metabolic conditions are established.
Kinetic rates of degradation of pollutants agents in **biological treatments** are usually very low if compared with the ones observed in chemical-physical treatments. Moreover, biological treatments are easily affected by such factors as temperature, pH, oxygen concentration, presence of nutrients, presence of toxic substances and, in case of soil bioremediation, optimal content of water in the polluted matrix.
Nevertheless, the use of biological treatments is nowadays growing fast, for several reasons.
First of all the **low costs** required, due to the fact that usually no supplementary reagent must be added in the process, and to the fact that if the treatment is dealt in the proper way, the degradation of the polluting substance is complete and there are no costs for the disposal of treatment by-products (such as, for instance, the sludge rich of pollutants produced during the traditional chemical-physical processes).
Besides, if the biodegradative process is properly dealt, **the complete degradation of different** contaminants can be achieved in the same process.
Finally, in case of a biological *in situ* treatment, a proper **bacterial population can be inoculated** in the soil, so to allow the growth of the strains capable of degrading the polluting agents with a minimum direct impact on the soil; otherwise the bacterial population existing in the soil can be acclimated to the polluting substances, thus **enhancing the natural remediation capacity** of the soil.
Depending on the nature of the contaminant agent which is degraded, different organisms are involved (fungi, bacteria or plants), and different metabolic processes are used for the assimilation of the substances.

4.1.2 Direct metabolism and cometabolism

Metabolic processes carried out by microorganisms can be divided into two main categories: **aerobic** and **anaerobic** processes.
Both kinds of processes can require just a single substrate (***direct metabolism***); in this case, the microorganisms are capable of metabolizing the polluting substances without requiring any further substrate.
However, for the most part of chlorinated compounds, bacteria need another substrate to deal the metabolic processes; in this case the biodegradative process is called ***cometabolism***.
Cometabolism occurs because most microorganisms are not capable of gaining any energetic advantage from the metabolism of the chlorinated substances, and this implies as a consequence that the process of accumulation of energy in the form of ATP, used by the microorganisms for their life and growth, does not occur.
In this case, in order to perform the biological degradation of chlorinated compounds, stimulation of the biodegradative process is required. Therefore it is necessary to add in the

treatment system a so called *primary substrate*, which is degraded by the microorganisms in order to take the energy required for their vital needs. The degradation of the primary substrate makes the microorganisms produce some enzymes which are "incidentally" used for the degradation of the other substrates present in the biological system, such as, for instance, the chlorinated compounds. The term "incidentally" refers to the fact that no energetic benefit is gained by the microorganism from the transformation of that substance (Nocentini et al., 2005).

4.1.2.1 Aerobic metabolism

Cometabolic aerobic biodegradation

In aerobic metabolic processes, chlorinated compounds are used as electrons donors, while oxygen acts as the electrons acceptor; in this way the chlorinated compounds are oxidized and give place to unstable compounds, such as epoxides, which are decomposed and form chlorides and organic non-chlorinated compounds, that are easily biodegradable (Nocentini et al., 2005).

Aerobic cometabolic biodegradation of chlorinated solvents usually starts in presence of a **monoxigenase enzyme** with maximum specificity toward the primary substrate; monoxygenase enzyme is produced by the microorganisms during the metabolism of an organic compound from which the microorganism takes carbon and energy for its growth.

In order to explain why in a cometabolic process the oxidation of a chlorinated compound does not produce the energy necessary for the metabolism, a scheme of the reaction and of the substances involved is shown in Figure 4.1 (oxidation of the primary substrate, for instance a hydrocarbon) and in Figure 4.2 (oxidation of a chlorinated compound in a cometabolic process).

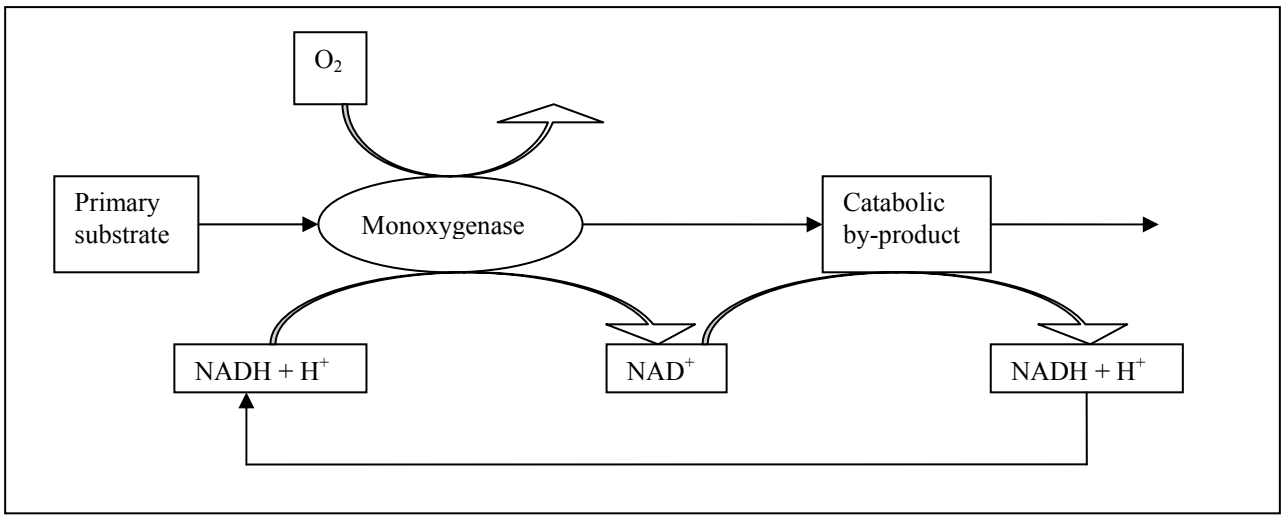

Figure 4.1. Monoxigenase-mediated oxidation of a primary substrate

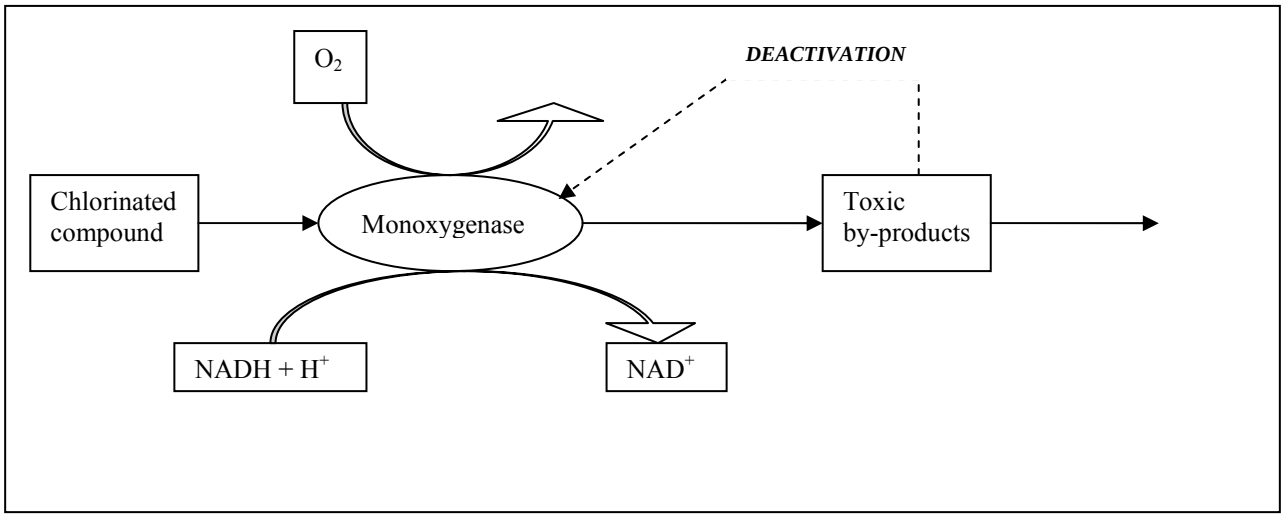

Figure 4.2. Monoxigenase-mediated oxidation of a cometabolic substrate (e.g. a chlorinated compound).

Many chlorinated compounds are biodegradable only through cometabolism, while some others are degradable through direct metabolism.
Frequently used *primary substrates* are phenol and hydrocarbons such as methane, butane, propane, toluene (Shim et al., 2001; Zannoni et al., 2003, Chang et al., 1995); also some chlorinated compounds (such as chloroethane and vinilchloride) can be used as primary substrates for the cometabolic biodegradation of other more chlorinated compounds. Due to the large variety of possibly employable primary susbstrates, it is clear how important it is to choose the most proper co-substrate for the chlorinated solvent to degrade. An important role in this choice is played by research performed to test how different primary substrates act on the biodegradation of different chlorinated compounds.

Direct aerobic biodegradation

In the last decades, some microorganisms have been detected, which are capable of degrading several chlorinated compounds using them in their metabolic processes as electrons donors, and thus as unique source of carbon and energy; in this case the process is not inhibited, because final products of the reaction are not toxic for the specific microorganisms. According to different authors (Verce et al., 2002; Coleman et al., 2002; Hartmans et al., 1992) direct aerobic oxidation is mostly effective for the degradation of low chlorinated compounds, with one or two chlorinated substitutes.

4.1.2.2 Anaerobic metabolism

Cometabolic anaerobic biodegradation

In anaerobic conditions, degradation of chlorinated compounds occurs following a **reduction** reaction; these compounds act as electrons acceptors in the metabolic pathway, whilst the electrons donor is usually an organic compound (such as methane or methyl alcohol) or an inorganic compound (such as H_2).
Anaerobic degradation of a chlorinated compound can be seen as a progressive dechlorination of the compound molecule; this process can be schematically assumed to occur through two main steps.
In the *first* phase, an electron is transferred from the reducing agent to the chlorinated aliphatic substance, with the subsequent elimination of a chlorine atom (in the form of a

chloride ion) and the formation of an alchilic radical. The formation of the alchilic radical is usually the limiting step of the whole reaction.
In the *second* phase, a hydrogen atom is placed on the radicalic carbon; this is possible due to the liberation of another electron in the metabolism, and to the presence of another hydrogen ion coming from the aqueous medium in which the reaction takes place.
Thus, in anaerobic conditions, the previously described process (which is known as Reductive Dechlorination, RD, or more generally as **Reductive Dealogenation**) operates the degradation of a chlorinated aliphatic compound by means of the *sequential progressive substitution of any chlorine atom in the molecule with hydrogen atoms* (Vogel, 1987).
In most cases, the anaerobic RD of a chlorinated compound is possible only through a cometabolic pathway. Microorganisms operating the RD use HCO_3^-, SO_4^- or other organic and inorganic compounds as electrons acceptors, and the chlorinated compound is used "incidentally" as further electron acceptor. Obviously, there can be a competition between the chlorinated compounds and the other electrons acceptors, which can result in an interruption of the dechlorination process if the primary substrate becomes preferable for microorganisms to chlorinated compound; thus, while dealing with the process, a great attention must be paid to ensure the right proportions of the substances acting as electron acceptors.

Direct aerobic biodegradation

Recently it has been discovered that some bacterial strains are capable of operating reductive dechlorination without the need of another cometabolite; in this case the process is called "***direct reductive dechlorination***" (Semprini, 1997) and in this process, bacterial strains use the aliphatic chlorinated compound as electron acceptor in the oxidation of the primary substrate, thus taking an energetic advantage from it.
In the anaerobic direct reductive dechlorination, microorganisms take energy from the substitution of any chlorine ion of the chlorinated molecule with an hydrogen atom. The hydrogen used in this reaction usually comes from the fermentation of organic substrates present in the system, operated by some other bacterial strains.
If the compound is highly chlorinated (with three or four chlorinated substitutes) its electronegativity is so high to attract with high rates the electrons involved in the metabolic process; as the molecule becomes progressively less chlorinated, its lower electronegativity makes the process kinetics slow down. At this point of the reaction, oxygen becomes more electronegative than the chlorinated solvent, and the process becomes more effective, in terms of dechlorination, in aerobic conditions.
This is the reason why, often, highly chlorinated compounds are degraded with **an alternation of anaerobic/aerobic conditions**; in the anaerobic phase the chlorinated compounds act as electron acceptors in the metabolic pathway, while, when their chlorination level becomes too low to continue with high efficiency, the presence of oxygen makes the process progress in aerobic conditions with good rates. For the same reasons, it is also easily understandable how, when the chlorinated compound must act as electron acceptor in the metabolic process (i.e. in anaerobic conditions), the presence of oxygen in free or linked form (O_2, CO_2, SO_4^{2-}, etc.) must be avoided, in order to avoid competition in terms of electron attraction.

4.2 Application of biological treatments to degradation of chlorinated compounds

Biological treatments are nowadays more and more frequently used for the decontamination of water streams polluted by chlorinated solvents; actually, differing from many chemical-physical treatments, biological treatments, if dealt in the proper way, offer the high advantage of the complete degradation of the chlorinated compounds, with the final formation of water and carbon dioxide. Thus, no dangerous process sludge must be disposed or decontaminated after the treatment, and, if the biological process is successful, no toxic by-products are formed. This is clearly extremely effective in terms of **costs** and of **treatment simplicity**. Obviously, in order to carry out the biological process with the maximum efficiency, the optimal operational parameters must be selected and maintained; that is why many experimental studies are dealt, with different chlorinated solvents to degrade, in different operational conditions.

4.2.1 Biological degradation of 1,2-dichloroethane

4.2.1.1 Aerobic treatments

The largest part of biological treatments of water streams polluted by 1,2-dichloroethane (1,2-DCA) is based on **aerobic** metabolism.

Specific aerobic bacterial strains degrading 1,2-DCA

Many experimentations have been carried out in order to detect new bacterial strains capable of using 1,2-DCA as only source of carbon and energy for the metabolic processes.

For many years, until 1992, the most important study performed on a specific bacterial strain capable of degrading 1,2-DCA was the one from Janssen et al. (1984, 1985). They developed a culture of *Xanthobacter autotrophicus GJ10* that was isolated on 1,2-DCA as the sole carbon and energy source. This bacterium produced a hydrolytic haloalkane dehalogenase that converted 1,2-DCA to 2-chloroethanol (Van den wijngaard et al., 1992,) with no indication of chloroacetaldehyde formation. The latter compound was further degraded via chloroacetaldehyde to chloroacetic acid by the subsequent action of two dehalogenase enzymes. Chloroacetic acid was dechlorinated and converted to glycolate (Janssen et al., 1984). This process of conversion of 2-chloroethanol to glycolate was also found in a *Pseudomonas sp.*, in a study by Stucki and Leisinger (1983).
Janssen et al. (1985) investigated with high detail this degradation pathway: they discovered that of the two dehalogenase enzymes produced by the *Xanthobacter autotrophicus GJ10*, one is specific for halogenated alkanes, while the other is specific for halogenated carboxylic acids. In their experimentation, with the degradation of 1,2-DCA, stoichiometric amounts of chlorides produced were found as final reaction products, and during the degradative reaction the formation and disappearance of stoichiometric amounts of 2-chloroethanol was observed. The organism isolated, able to use 1,2-dichloroethane, also utilizes a number of other haloalkanes for growth and is a nitrogen fixing hydrogen bacterium. Before this study, the capability to degrade halogenated aliphatic compounds was mainly observed among members of the *Pseudomonas* (Hardman et al., 1981; Motosugi et al., 1983) and had not been described for chemolithoautotrophic bacteria.
The degradation of haloalkanes was due to a haloalkane dehalogenase with broad substrate specificity. The haloalkane dehalogenase catalyzes the hydrolytic release of halide from

haloalkanes, resulting in the replacement of the halogen substituent by a hydroxyl group. This was shown for the degradation of 1,2-dichloroethane, 1-chlorobutane, 1-chloropropane, and bromoethane. Further conversion of the compounds produced proceeded via oxidation to carboxylic acids. Ammonia-stimulated 2-chloroethanol dehydrogenase activity could be detected in 1,2-dichloroethane- and methanol-grown cells, suggesting the route for 1,2-dichloroethane degradation given in Fig. 4.3.

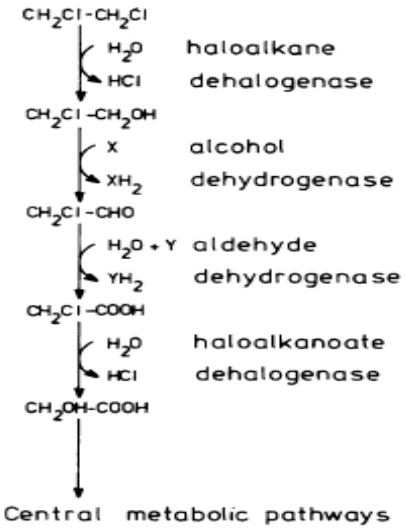

Figure 4.3. Proposed degradative pathway for the metabolism of 1,2-dichloroethane

The results of this study show that *Xanthobacter Autotrophicus* is capable of playing an important role in the degradation of organochlorinated compounds, and that hydrolytic dehalogenases are involved in the microbial metabolism of short-chain halogenated hydrocarbons.
In a following study, carried out by van den Wijngard et al. (1992) four new bacterial strains (identified as strains of *Ancylobacter Aquaticus*) capable of growing on 1,2-DCA as only source of carbon and energy were discovered. In all strains tested, 1,2-DCA was degraded by initial hydrolytic dehalogenation to 2-chloroethanol, followed by oxidation by a two dehydrogenase enzymes. The resulting chloroacetic acid was converted to glycolate by chloroacetate dehalogenase. Analyzed for the identification of their nucleotide sequences of the haloalkane dehalogenase genes, the strains AD20 and AD25 showed to have the same sequences of *Xanthobacter autotrophicus* GJ10 and GJ11.

In a following study performed by Hage and Hartmans (1999), a bacterial strain, that was called *Pseudomonas sp. strain DCA1*, was isolated from a 1,2-dichloroethane-degrading biofilm. Strain DCA1 utilized 1,2-DCA as the sole carbon and energy source and did not require additional organic nutrients, such as vitamins, for optimal growth. The affinity of strain DCA1 for 1,2-DCA was very high, with a K_m value below the detection limit of 0.5 mM. While other 1,2-DCA utilizers start degradation of the compound with a hydrolytic dehalogenation, the first step in 1,2-DCA degradation by strain DCA1 is an oxidation reaction with a monooxygenase enzyme. Probably, oxidation of 1,2-DCA resulted in the formation of the unstable intermediate 1,2-dichloroethanol, which spontaneously releases chloride, with the subsequent formation of chloroacetaldehyde. The DCA degradation pathway in strain DCA1 proceeds from chloroacetaldehyde via chloroacetic acid and presumably glycolic acid; this

pathway is similar to the ones observed in 1,2-DCA degradation by other DCA-utilizing bacteria.
In the same study, two different pathways of degradation of 1,2-DCA by *Xanthobacter Autotrophicus* and *Ancylobacter aquaticus*, and *Pseudomonas sp. DCA1* were proposed; they are shown in Figure 4.4.

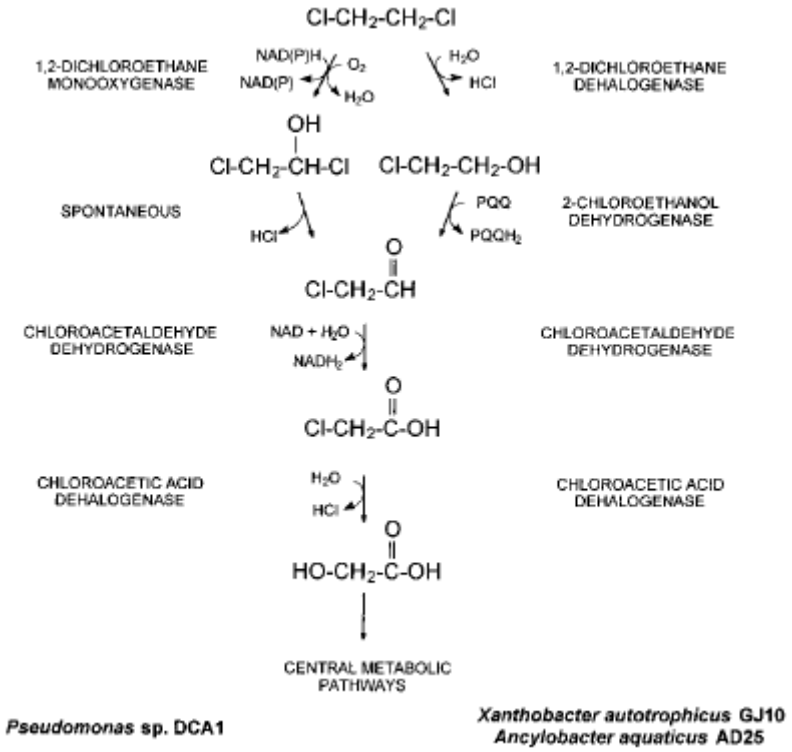

Figure 4.4. Different pathways of DCA degradation by *Pseudomonas* sp. Strain DCA1 (proposed), *X. autotrophicus* GJ10, and *A. aquaticus* AD25.

Reactor design

Due to the particular nature of the 1,2-DCA, which is a very volatile substance, aerobic metabolism necessary for the removal of the substance from a wastewater requires particular reactors design. Actually, in a traditional bioreactor in which aerobic metabolic processes must be sustained, the necessary oxygen is usually supplied by means of continuous aeration. Nevertheless, direct aeration can cause in few hours the complete loss of 1,2-DCA by volatilization (**stripping phenomenon**); that is why several innovative configuration and designs schemes of the reactors have been proposed for the aerobic biodegradation of 1,2-DCA.

In an experimentation carried out by Freitas Dos Santos and Livingston (1995) a novel **extractive membrane bioreactor** has been ideated for overcoming the usual problem of stripping. In this study it was previously observed that the operation of a conventional air-lift bioreactor at a hydraulic residence time of 11.6 hours, led to the loss of the 25-34% of the total 1,2-DCA supplied, which was lost in the gaseous effluent of the system. In contrast, the use of the new reactor equipped in this study implied the loss of just 1.5% of 1,2-DCA for stripping, with the same residence time. The reactor operated by separating the 1,2-DCA containing wastewater from the aerated biomedium. This was achieved by means of a silicone

rubber membrane coiled around a Perspex draft tube. 1,2-DCA diffused across the silicone rubber membrane and into a biofilm growing attached to membrane surface, while oxygen diffused into the biofilm from the biomedium side. Oxygen and 1,2-DCA met in the biofilm and degradation occurred without the exposition of 1,2-DCA to the direct aerating gas flow. The removal of 1,2-DCA obtained in this study was quite good, with a process efficiency of 94.5%. The system had also another advantage: the limitation of the flux of 1,2-DCA across the membrane with consequent accumulation of 1,2-DCA at the membrane-biofilm interface, which reduces the mass transfer driving force for 1,2-DCA extraction from the wastewater.

In another experimentation by Freitas Dos Santos et al. (1997), the efficiency of 1,2-DCA removal in a **membrane attached biofilm** (MAB) system was studied with the use of *Xanthobacter Aquaticus GJ10* growing on 1,2-DCA. The advantage given by the use of MAB systems is to avoid the loss of 1,2-DCA (and, more in general, of all volatile organic compounds) by stripping. The system proved to have a problem because of the excess biofilm growth on the membrane surface, which resulted in reduced flux of organic substrate across the membrane. Then, it was concluded that an optimal biofilm thickness could be individuated from a compromise between the level of air stripping and flux of pollutant across the membrane, and that biofilm thickness could be controlled by manipulating cell endogenous decay. The addition of sodium chloride to the biological system showed to be effective to control excessive biofilm development.

A **fluidized bed reactor without direct aeration** for the aerobic degradation of 1,2-DCA and dichloromethane was ideated and tested by Herbst and Wiesmann (1996). In this study, oxygen was supplied by diffusion through a synthetic membrane. High bacteria concentrations and high 1,2-DCA degradation rates were obtained.

In a study by Inguva and Shreve (1999), a method to determine the kinetics of degradation of trichloroethylene by *Burkholderia cepacia PR1* and of 1,2-dichloroethane by *Xanthobacter Autotrophicus GJ10* was optimized. A first order linear rate law applied to 1,2-DCA biodegradation by *X. Autotrophicus GJ10*, while a non-linear rate law applied to TCE biodegradation by *B. cepacia* PR1. The half saturation constant was determined to be 0.026 nM (3.47 ppm).

4.2.1.2 Anaerobic treatments

Specific anaerobic bacterial strains degrading 1,2-DCA

Anaerobic treatments for biodegradation of 1,2-DCA are less applied than the aerobic ones. A study in which methanogenic bacteria, operating in anaerobic conditions, were employed to degrade 1,2-DCA was dealt by Holliger et al. (1990). In this study, methanogenic bacteria reductively dechlorinated 1,2-dichloroethane via two reaction-mechanisms: a dihalo-elimination yielding ethylene and two hydrogenolysis reactions yielding chloroethane and ethane, consecutively. The importance of the role of acclimation in the selection of specific bacterial strains was underlined by the observation that cells of *Methanosarcina barkeri* grown on H_2/CO_2 converted 1,2-dichloroethane and chloroethane at higher rates than acetate or methanol grown cells.

While many microorganisms capable of degrading 1,2-DCA as only source of carbon and energy in aerobic conditions, and some bacterial strains in anaerobic conditions have been identified, only one microorganism growing on 1,2-DCA in **anoxic**, nitrate-reducing conditions has been individuated, in a study by Dinglasan-Panlilio et al. (2006). 1,2-DCA at

500 μM/d was degraded with nitrate as the terminal electron acceptor; complete reduction of nitrate via nitrite to nitrogen gas was also observed. The bacterial strain Betaproteobacteria (affiliated with the genus *Thauera*) was individuated as responsible of this degradative activity; complete mineralization of 1,2-DCA to CO_2 was observed.

An anaerobic process for the biodegradation of 1,2-DCA from a polluted groundwater has been studied in an experimentation performed by Dyer et al. (2003). An aqueous solution of methanol, ammonium chloride and sodium chloride was injected into a confined aquifer, in order to test the feasibility of treating the dissolved phase of 1,2-DCA via reductive dechlorination. Biodegradation of 1,2-DCA was localised, probably because of the limited mixing of the carbon substrate within the test zone.

In another study performed by Egli et al. (1987) two bacterial strains capable of anaerobically degrading 1,2-DCA in the low μM range have been tested. *M. thermoautotrophicum* transformed a portion of 1,2-dichloroethane and ethene was identified as the product.

Reactor design

In an experimentation performed by De Wildeman et al. (2001), dechlorination of 1,2-dichloroethane (1,2-DCA) in an **upflow anaerobic sludge blanket (UASB)** reactor with anaerobic granular sludge was examined. With a 1,2-DCA volumetric loading rate of 87.6 mg/(l·d) its average removal efficiency was 82%; no chlorinated intermediates or residues were found. 1,2-DCA was converted mainly to ethane (65-80%) and ethene (1%). The effluent was subjected to a Nitrox test, and showed not to be toxic for nitrifying bacteria, indicating that such UASB treatments can protect a subsequent aerobic nitrifying system.

Natural anaerobic conditions were employed in the study performed by Nobre and Nobre (2004), who observed the natural anaerobic attenuation of 1,2-DCA in a polluted aquifer. Results of field investigations have provided evidence that under the natural anaerobic-reducing conditions 1,2-DCA was naturally biodegraded into vinyl chloride and ethene. Although the results are favourable, the natural degradation of the 1,2-DCA does not guarantee acceptable removal efficiency. Therefore, a pilot test to evaluate the enhancement of these processes is being carried out through the use of a biosparging system near the source to achieve sequential aerobic–anaerobic treatment zones.

Another study on the natural attenuation of 1,2-dichloroethane, in this case in a landfill, was performed by Sanin et al. (2000). The tests consisted of filling multiple 8 litres simulated landfill reactors that were operated with and without water addition, in order to simulate the absence and presence of an engineered cover in the landfill. Reductive dehalogenation of 1,2-DCA to ethylene was measured in all reactors. The study suggested that moisture in the reactor played a fundamental role to achieve the removal of 1,2-DCA; besides, the association with humic matter in decomposed refuse may represent an alternative mechanism for contaminant sequestration.

4.2.1.3 Anaerobic/aerobic treatments

Specific bacterial strains degrading 1,2-DCA

Klecka et al. (1998) tested the ability of different bacterial strains to degrade 1,2-DCA both in anaerobic and in aerobic conditions, both in soil-water microcosms and in aquifer samples. Biodegradation of 1,2-dichloroethane was observed under methanogenic or sulphate reducing conditions. 1,2-DCA was transformed to ethylene in a single step via reductive

dehalogenation, and no other metabolites were detected in the reaction mixtures. Acclimation of the microorganisms was necessary for achieving the biodegradation of 1,2-DCA; actually, lag periods ranging from 7 to 8 weeks preceded degradation in microcosms. Aerobic biodegradation of 1,2-dichloroethane to carbon dioxide was also observed after 13 weeks in aquifer material. The ability of naturally occurring microorganisms to degrade 1,2-dichloroethane should be taken into account when evaluating the fate and lifetime of the compound in the environment, and has a potential application in the remediation of contaminated groundwater.

Reactor design

Some experimentations have been carried out in order to check the effectiveness of an alternated anaerobic/aerobic treatment. Actually, the anaerobic treatments are the most suitable for highly chlorinated substances, but, on the other hand, when the chlorination level of the molecule lowers down, an aerobic treatment becomes more effective.
In an experimentation performed by Cacciatore et al. (2005), a pilot scale test was conducted to evaluate the feasibility for in situ bioremediation of a chlorinated organics and phenol plume in groundwater. Chlorinated organics studied were tetrachloroethylene, trichloroethylene, 1,2- and 1,4-dichlorobenzene and 1,2-dichloroethane. 1,2-DCA, 1,2-dichloroethylene, vinyl chloride, chlorobenzene, and ethylene were found among the degradation products. The pilot stage was conducted in two stages; an anaerobic stage to treat the chlorinated organics followed by an aerobic stage to treat reduced chlorinated organics and phenols.

4.2.2 Biological degradation of 1,2-dichlorobenzene and other chlorobenzenes

4.2.2.1 Aerobic treatments

Almost all the treatments studied up to now for the bioremediation of waters polluted by different isomers of dichlorobenzene are based on aerobic processes.

Specific bacterial strains degrading various di-and monochlorobenzenes

Many experimentations have been performed in order to identify new bacterial strains capable of using 1,2-DCB and other di- and mono-chlorobenzenes as the only source of carbon and energy for their metabolic processes.
In a study carried out by Schraa et al. (1986) an organism, identified as an *Alcaligenes sp.*, was capable of using 1,4-dichlorobenzene as the sole carbon and energy source. During growth with 1,4-dichlorobenzene in pure culture, stoichiometric amounts of chlorides were released. It was proposed that dioxygenase enzymes are involved in the initial steps of 1,4-dichlorobenzene degradation, while ring opening proceeds via ortho cleavage.

In another experimentation dealt in order to find out a bacterial strain capable of using 1,4-DCB as the only source of carbon and energy, Spain and Nishino (1987) isolated a *Pseudomonas* by selective enrichment from activated sludge. The organism also showed to grow well on chlorobenzene and benzene.

In a study performed in 1995, Spiess et al. isolated the strain *Xanthobacter flavus 14p1* from a river sludge selective enrichment with 1,4-dichlorobenzene as the sole source of carbon and energy. The selected bacterium did not use other aromatic or chloroaromatic compounds as growth substrates. During growth on 1,4-dichlorobenzene, stoichiometric amounts of chloride

ions were released. It was observed that 1,4-DCB degradation is initiated by dioxygenation by means of dihydrodiol dehydrogenase and catechol 1,2-dioxygenase, and that ring opening proceeds via ortho cleavage.

De Bont et al. (1986) isolated a gram-negative bacterium, probably an *Alcaligenes sp*., from a mixture of soil and water samples by using 1,3-dichlorobenzene as the sole carbon and energy source. During growth on 1,3-dichlorobenzene, almost stoichiometric amounts of chlorides were released.

In a study on chlorobenzene degradation performed by Reineke and Knackmuss (1984), a chlorobenzene-degrading bacterium was isolated by continuous enrichment from a mixture of soil and sewage samples. This organism belonged to the *strain WR1306*. During growth on chlorobenzene, stoichiometric amounts of chlorides were released.

The degradation of chlorobenzene was investigated also by Jechorek et al. (2003) with the specially chosen strain *Methylocystis sp. GB 14 DSM 12955*, in a soil column filled with aquifer material from a depth of 20 m. Groundwater polluted by chlorobenzene was continuously fed to the column, and a mixture comprising 4% CH_4 and 96% air was inflated into the column. Chlorobenzene was completely degraded under the mixed culture conditions of the column experiments.

In an experimentation carried out by Sander et al. (1991), two *Pseudomonas sp.* strains, capable of growth on chlorinated benzenes as the sole source of carbon and energy, were isolated by selective enrichment from soil samples of an industrial waste deposit. The strains grew on monochlorobenzene, all three isomeric dichlorobenzenes, and 1,2,4-trichlorobenzene (1,2,4-TCB). During growth on these compounds both strains released stoichiometric amounts of chloride ions. The first steps of the catabolism of the chlorobenzenes proceeded via dioxygenation of the aromatic molecule.

The capability of a bacterial strain of biodegrading several chlorobenzenes was tested in a study by Rapp and Timmis (1999). The utilization of 1,2,4,5-tetrachloro-, 1,2,4-trichloro-, the three isomeric dichlorobenzenes and fructose as the sole carbon and energy sources at nanomolar concentrations was studied in batch experiments with *Burkholderia sp. strain PS14*. Initial concentration of all the chlorobenzenes was 500 nM; the compounds were metabolized within 1 h to a concentration below their detection limits of 7.5 nM. Therefore, it could be supposed that Burkholderia sp. strain PS14 exhibits a very high affinity for chlorobenzenes at nanomolar concentrations.

The aerobic degradation of di- and trichlorobenzenes by two bacteria isolated from polluted soils was studied by Adebusoye et al. (2007). The isolated bacteria were identified as *Enterobacter sp. SA-2* and *Pseudomonas sp. SA-6*. The strains were able to grow extensively on dichloro- and trichlorobenzenes. Approximately, 80–90% of these xenobiotics were degraded in 200 h.

In an experimentation performed by Bosma et al. (1988), all three isomers of trichlorobenzene were reductively dechlorinated to monochlorobenzene via dichlorobenzenes in anaerobic sediment columns. Since monochlorobenzene and dichlorobenzene are mineralized by bacteria in the presence of oxygen, the anaerobic degradation could be favourably coupled to

an aerobic treatment in order to obtain complete mineralization of otherwise recalcitrant trichlorobenzenes.

Specific bacterial strains growing on 1,2-DCB as source of carbon and energy

In 1988 Haigler et al. (1988) selected from an activated sludge a *Pseudomonas sp.* that was capable of growth on 1,2-dichlorobenzene (o-DCB) or chlorobenzene as a sole source of carbon and energy.
In a following study performed by Seignez et al. (2001), production of biomass capable to degrade a mixture of chlorobenzene (CB) and 1,2-dichlorobenzene was investigated in a batch culture with substrates supplied by pulses. CB and 1,2-DCB concentrations which gave the best adapted biomass productivity were 150 and 30 µg/l, respectively; the biomass yield was 0.38 g of biomass dry weight per gram of substrate. The pulses of 200 µl CB and 40µl 1,2-DCB inhibited the degradation. Among the metabolites, muconic acid was found in large quantities.

Rehfuss and Urban (2005) isolated from a wastewater treatment bioreactor a microorganism with the ability to aerobically degrade chlorobenzene, dichlorobenzene and phenol as sole carbon sources. The 16S rRNA gene sequence of the microorganisms involved in the biodegradative process shares the greatest similarity with members of the *Rhodococcus* genus.

In a study by Monferràn et al. (2005), a subsurface microbial community was isolated from a polluted site and acclimated in 15 days in aerobic conditions using 1,2-dichlorobenzene as the sole carbon source. From this acclimated community, the authors isolated a strain of *Acidovorax avenae*, which was able to perform the complete biodegradation of 1,2-DCB at 0.12 mM in two days; the reaction produced stoichiometric amounts of chlorides. This pure strain was also tested for biodegradation of chlorobenzene, 1,3-DCB and 1,4-DCB, giving similar results than with 1,2-DCB.

Reactor design

In order to optimize reactor efficiency in treatments of water streams polluted by dichlorobenzenes, several experimentations were carried out to test different reactor configurations.
In a study by Albrechtsen et al. (1996) the effect of **solids as support for the biomass** degrading several aromatic hydrocarbons (among which 1,2-DCB) was evaluated. In this experimentation, several biomass support materials as quartz sand, rock wool and crushed tiles on the degradation of 8 aromatic hydrocarbons was investigated; the water stream to be decontaminated was a polluted groundwater collected from two aerobic aquifers. In order to have a comparison on the system effectiveness, experiments with only groundwater as well as groundwater suspensions with aquifer sediment were run. Presence of rock wool supported growth of bacteria and increased the degradation (in terms of rates and number of compounds degraded) compared with experiments with groundwater only. Quartz sand gave the most promising results with respect to growth of bacteria, and the degradation patterns of most of the compounds were similar to those obtained in experiments including aquifer sediments, although the latter showed the most substantial degradation. This study suggests that in case aquifer sediment is not available, quartz sand can be added as biomass support material in studies on degradation of organic xenobiotics present in groundwater.

4.2.2.2 Anaerobic treatments

Very few examples of anaerobic treatment of chlorobenzenes have been found in specialized literature.

A study on anaerobic chlorobenzene biodegradation was performed by Kao et al. (1999), who studied the bioremediation of a groundwater polluted with trichloroethylene and chlorobenzene (CB). Results showed that TCE and CB were degraded, and by-products of TCE degradation (such as dichloroethylene isomers and vinyl chloride) were found. Methanogenesis was the dominant biodegradation pattern within the source and mid-plume areas, while the aerobic biodegradation process dominated the downgradient area. CB was used in the degradation process as the primary substrate electron donor, and enhanced TCE biodegradation under both aerobic cometabolism and reductive dechlorination conditions.

4.2.3 Biological degradation of 2-chlorophenol and other chlorophenols

Chlorophenolic compounds are toxic and recalcitrant to biodegradation; however, their slow degradation in natural environment under aerobic and anaerobic conditions has been observed. Aerobic biodegradation proceeds through the formation of catechols, while under anaerobic conditions reductive dehalogenation is the preferred metabolic pathway. Number and position of chlorinated substitutes on the phenolic ring has influence on the rate and extent of the degradation of chlorophenols. In engineered systems, acclimation of biomass to chlorophenols strongly enhances the biodegradation process efficiency, because it helps in reducing the initial lag-phase and in increasing the degradation rate. Biological removal of chlorophenols can be also enhanced by the alternation of anaerobic and aerobic conditions (Annachhatre et al., 1996).

Several studies on the biodegradation of chlorophenols from polluted water have been performed, both in anaerobic and in aerobic conditions; also systems based on the alternation of both conditions have been studied.

4.2.3.1 Aerobic treatments

Specific bacterial strains degrading several chlorophenols as only source of carbon and energy

Many experimentations have been performed in order to detect new bacterial strains capable of using 2-CP and other di- and mono-chlorophenols in aerobic conditions as the only source of carbon and energy for their metabolic processes.

In an experimentation dealt by Lu et al. (1996), four bacterial strains (*Pseudomonas putida*, *Pseudomonas testosteroni*, *Pseudomonas aeruginosa* and *Agrobacterium radiobacter*) were used for the biodegradation of phenol and different chlorophenols. All four species degraded the chlorophenols following the ortho-cleavage pathway. *P. testosterone*, *P. putida* and *P. aeruginosa* effectively removed phenol at 200 mg/l, and *P. testosteroni* could remove 2-chlorophenol at 10 mg/l, while the other three species could not remove 2-chlorophenol. *P. testosteroni* also could rapidly metabolize 3-CP at 10 mg/l, and removed 98% of 4-CP at 10 mg/l within one day. *P. aeruginosa* and *A. radiobacter* also metabolized 4-CP after 2 and 7 days of lag period, respectively.

In another study, Kim and Hao (1999) studied the degradation of 3-CP and 4-CP by phenol-induced cells of *Acinetobacter* species. Degradation trend changes were observed at the variation of the rate between phenol and chlorophenol fed.

The kinetics of phenol and chlorophenol utilization by *Acinetobacter* species and the interactions between the two substrates were studied by Kao et al. (2002). The degradation kinetics of growth (phenol) and nongrowth (4-CP) substrates, present individually and in combination, were investigated. Batch experiments were performed using an *Acinetobacter* growing on phenol alone and in combination with 4-CP.

Baker et al. (1980) studied chlorophenol degradation under aerobic conditions in soil, sediment and water. 2-chlorophenol, 4-chlorophenol and 2,4-dichlorophenol were degraded by soil and sediment microorganisms at 0°C and 4°C. The addition of some chlorophenols stimulated aerobic, and to a lesser extent, anaerobic microbial growth.

In a study by Farrel and Quilty (2002), a bacterial strain (*Pseudomonas putida* CP1) was added to a commercial mixed microbial community used to degrade 2-CP (at a concentration of 1,65 mM), in order to check if it could improve the degradation of the chlorinated compound. Degradation of 2-chlorophenol by the commercial mixture was via a meta-cleavage pathway leading to incomplete degradation, while *P. putida CP1* was shown to be capable of the complete degradation of 2-chlorophenol via an ortho-cleavage pathway. The augmented mixed culture displayed increased degradative capabilities, with times of degradation and lag periods reduced when compared to those achieved by *P. putida CP1* in isolation.

Degradation and detoxification of a mixture of persistent compounds (2-chlorophenol, phenol and m-cresol) were studied by Gallego et al. (2003) using pure and mixed indigenous cultures in aerobic reactors. Individual compounds were degraded by pure bacteria cultures within 27 h. The mixture of 2-clorophenol (100 mg/l), phenol (50 mg/l) and m-cresol (50 mg/l) was degraded by mixed bacteria cultures within 36 h: 99.8% of total phenols was removed, both in batch and in continuous flow reactor.

4.2.3.2 Anaerobic and anoxic treatments

Bacterial strains degrading chlorophenols as only source of carbon and energy

In a study performed by Schmidt et al. (1983) the anaerobic degradation of several chlorophenols by a defined mixed microbial community has been observed. The chlorophenols studied were phenol, acetone and alkanols plus 4-chlorophenol or a mixture of isomeric chlorophenols; they were completely degraded by a defined mixed culture with Pseudomonas sp. strain B13. Total degradation of the chlorinated compounds was indicated by release of stoichiometric amounts of chlorides. In the fully acclimated culture, hybrid strains such as *Alcaligenes sp. strain A7-2*, which are more competitive than Pseudomonas sp. strain B13 in chlorophenol degradation were detected.

In an experimentation performed by Madsen and Licht (1992), an anaerobic bacterium which degrades several chlorinated phenols was isolated. Dechlorination of the ortho substitutes of the phenolic OH group was preferred. The isolated bacterium was related to the genus *Clostridium*, and showed the highest growth rate when fed with a medium containing pyruvate and yeast extract.

Cole et al. (1994) isolated a bacterium capable of anaerobic growth via reductive dehalogenation of 2-chlorophenol. The organism, designated strain *2CP-1*, is a gram-negative rod and is a facultative. The organism grew in reduced anaerobic mineral medium supplemented with 2-chlorophenol, acetate and vitamins, and did not grow when either 2-chlorophenol or acetate was omitted; phenol was produced in the degradation process. Only the ortho position of 2-CP was dehalogenated, and additional chlorines at other positions decreased or blocked ortho dechlorination. Analysis of the organism's 16S rRNA sequence revealed that the selected microorganism was a member of the *delta proteobacteria*, more closely related to the myxobacteria than to the sulfidogenic bacteria.

Another bacterial culture capable of growing with only chlorophenols as source of carbon and energy was selected in a study by Haggblom and Young (1995). In this experimentation, sulfidogenic consortia enriched from an estuarine sediment were maintained on either 2-, 3-, or 4-chlorophenol as the only source of carbon and energy for over 5 years. Degradation of 4-chlorophenol was coupled to sulfate reduction, since substrate utilization was dependent on sulfidogenesis and chlorophenol loss did not proceed in the absence of sulfate.

In a study by Basu et al. (1996), the effects of seed material from different sludges (anaerobically digested sewage sludge; sediment from a bleached kraft mill (BKM) aerated lagoon; anaerobic bioreactor biomass treating BKM wastewater) were investigated for their potential of 2-CP dehalogenation, at a concentration of 0.38 mM. In batch cultures using a mineral medium containing yeast extract, dehalogenation was observed after lag periods of 97 to 250 days; phenol was identified as an intermediate. The dehalogenation rates increased significantly in all cultures after re-spiking with 2-CP and yeast extract medium.

In a study by Sun et al. (2000), strain *SF3*, a gram-negative, anaerobic, motile, short curved rod that grows by coupling the reductive dechlorination of 2-chlorophenol (2-CP) to the oxidation of acetate, was isolated from a sediment. The isolate used acetate, fumarate, lactate, propionate, pyruvate, alanine, and ethanol as electron donors for growth coupled to reductive dechlorination. Among the halogenated aromatic compounds tested, only the ortho position of chlorophenols was reductively dechlorinated, and additional chlorines at other positions blocked ortho dechlorination. The optimal temperature for growth was 30°C, and no growth or dechlorination activity was observed at 37°C. The physiological features and 16S ribosomal DNA sequence suggest that the organism is a novel species of the genus Desulfovibrio, that was designated as *Desulfovibrio dechloracetivorans*.

Degradation of several chlorophenols (2-CP, 3-CP, 4-CP, 2,4-dichlorophenol (DCP) and 2,6-DCP) in anoxic denitrifying conditions has been studied in batch cultures inoculated with activated sludge in an experimentation by Bae et al. (2002). Although 3-CP, 4-CP, 2,4-DCP and 2,6-DCP were not stably degraded, 2- CP was degraded and its degradation capability was sustained in a subculture. In 2-CP-degrading cultures, nitrate was consumed stoichometrically and concomitantly during 2-CP degradation, and a dechlorination intermediate was not detected, suggesting that 2-CP degradation was coupled with nitrate reduction. 2-CP was degraded in presence of nitrate, but not in the absence of nitrate or in presence of sulfate. This suggests that the enrichment culture strictly requires nitrate for degradation of 2-CP.
Effects of the presence of **heavy metals** on the biodegradability of chlorinated phenols have been evaluated in two studies.

One of the study was performed by Kuo et al. (1996), who studied the effect of the addition of Cd(II), Cu(II), Cr(VI), or Hg(II) at 0.01 to 100 ppm on metabolism of 2-chlorophenol (2CP), 3-chlorobenzoate (3CB), phenol, and benzoate by anaerobic bacterial consortia. The main effects observed were long acclimation periods, reduced dechlorination or biodegradation rates, and failure to biodegrade the target compound. With Hg(II) at 1.0 to 2.0 ppm, 2CP and 3CB were biodegraded 133 to 154% faster after an acclimation period, suggesting adaptation to Hg(II). Dehalogenation and aromatic degradation in the anaerobic consortia showed differential sensitivities to the heavy metal ions added. The study suggests also that potentially proper combinations of anaerobic bacterial species to bioremediate sites contaminated with both heavy metals and aromatic pollutants can be elaborated.

In another study, Kong (1998) analyzed the biotransformation of 2- and 3-chlorophenol in anaerobic fresh and acclimated sediments in the presence and absence of $CuCl_2$, $CdCl_2$ and $K_2Cr_2O_7$ at concentrations from 10 to 200 mg/1. Different inhibitory effects of specific metals were observed on dechlorination of chlorophenols. In case of fresh unacclimated slurry, addition of 20 mg_{Cu}/l resulted in no inhibition of chlorophenols dechlorination. In contrast, 20 mg/l of Cd and Cr moderately affected lag periods prior to dechlorination. In case of acclimated slurry, the dechlorination activity of 2- and 3-CP was inhibited in the presence of 20 mg/l of three tested metals.

A proposal of 2-CP degradative pathway has been made by Becker et al. (1999); in their study, biotransformation of 2-chlorophenol by a methanogenic sediment community resulted in the accumulation of phenol and benzoate. 3-chlorobenzoate was the most persistent product of the degradation. The anaerobic biodegradation of phenol to benzoate occurred via para-carboxylation and dehydroxylation reactions, which may also explain the observed conversion of 2-chlorophenol to 3-chlorobenzoate.

Reactor design

In order to optimize the treatment processes of water streams polluted by chlorinated phenols, several experimentations on possible reactor designs have been performed.

In two similar studies, Chang et al. (2003 and 2004) studied the efficiency of a system composed of a silicone gas-permeable membrane bioreactor in reductive dehalogenation of 2-CP. After acclimating a sludge coming from a swine wastewater treatment plant for about 4 months, the bioreactor showed a high dechlorinating performance, and 2-CP was removed with an efficiency of 92.8%. H_2 was used as electron donor for 2-CP dechlorination, and phenol was produced as reaction intermediation. Both nitrate and sulfate inhibited 2-CP dechlorination: nitrate competed with 2-CP as the electron acceptor, while sulfate retarded the activity of hydrogen-dechlorinating bacteria and thus inhibited the 2-CP dechlorination.

In the other study by the same authors, the hydrogenotrophic biofilm was cultivated in three silicone-tube membrane bioreactors under the conditions of denitrification (DN), sulfate-reduction (DS) and dechlorination (DC). Experimental results showed that after acclimation for more than four months with 2-CP, the respective 2-CP removal efficiency was 95% in DN, 94% in DS and 95% in DC reactors, under the condition of 25 $mg_{2\text{-}CP}$/l influent with hydraulic retention time (HRT) of 15 h. The metabolic pathway of 2-CP was different in different reactors. The 2-CP was thought to be utilized as carbon and energy source in DN and DS reactors, while the dechlorination occurred in the DC reactor in lack of nitrate and sulfate.

4.2.3.3 Anaerobic/aerobic treatments

In some studies, the efficiency of processes based on alternating anaerobic and aerobic conditions has been evaluated.
One of these studies was performed by Armenante et al. (1994); in their study 2,4,6-trichlorophenol (2,4,6-TCP) was successfully and completely degraded in a two-stage anaerobic/aerobic biological process in which the initial step was conducted anaerobically, resulting in the reductive complete dechlorination of the target compound to 2,4-dichlorophenol (2,4-DCP), and then 4-chlorophenol (4-CP). 4-CP was then attacked and completely degraded aerobically in a second stage.

In another study, Majumder et al. (2007) studied the removal of 2-CP from wastewaters using sequential upflow anaerobic sludge blanket (UASB) and rotating biological contactor (RBC) reactors. Sodium formate, sodium propionate, glucose and methanol were used as different carbon sources in the feed as co-substrate. Methanol was the best carbon source for UASB reactors, and allowed a 2-CP removal of 95%. The performance of sequential reactors was also evaluated at five different chlorophenolic shock loadings. During shock loading study the concentration of 2-CP in the wastewaters was increased to 45, 60, 75, 90 and 105 mg/l as compared to the normal feed containing 30 mg/l 2-CP. During shock loadings, complete removal of 2-CP was observed in the sequential reactors.

4.2.4 Biological degradation of trichloroethylene

Several studies on the biodegradation of trichloroethylene from polluted water streams have been performed, both in anaerobic and in aerobic conditions; also systems based on the alternation of both metabolic conditions have been studied.

4.2.4.1 Aerobic treatments

Bacterial strains degrading trichloethylene together with other substrates

Some experimentations have been performed in order to detect new bacterial strains capable of using TCE as source of carbon and energy for co-metabolic processes alone or together with other substances as co-substrates.

In an experimentation by Frascari et al. (2006) vinyl chloride-, methane- and propane-utilizing bacterial strains have been used for the aerobic cometabolic biodegradation of a chlorinated solvents mixture of vinyl chloride (VC), aliphatic hydrocarbons (CAHs), cis- and trans-1,2-dichloroethylene (cis-DCE, trans-DCE), trichloroethylene, 1,1,2-trichloroethane (1,1,2-TCA) and 1,1,2,2-tetrachloroethane (1,1,2,2- TeCA). Tests were performed both on bioaugmented and non-bioaugmented sediment-groundwater slurry microcosm. VC, methane and propane were utilized as growth substrates. The low-chlorinated solvents of the mixture were characterized by high depletion rates.

In another study by Han et al. (2007), a semicontinuous slurry-microcosm method was applied to trichloroethylene cometabolic biodegradation. TCE was aerobically degraded in the microcosm study with cometabolism using toluene as the primary substrate. Based on the nucleotide sequence of 16S rRNA genes, the toluene-oxidizing bacteria in microcosms were identified to be *Ralstonia sp. P-10* and *Pseudomonas putida*. According to the results obtained in this experimentation, it can be stated that with a given amount of toluene

injection, it is recommended to maximize the effective time duration of toluene presence in reactor design for TCE cometabolic degradation.

The presence of **other substances** can be determinant in the biodegradability of TCE in aerobic conditions, since these substances can act as **co-substrates** or as inhibitory agents. The effect of the interactions among several substances and TCE has been checked in many experimentations.

In an experimentation carried out by Pries et al. (1994), the role of the production of different enzymes by acclimated biomass degrading mixtures of halogenated aliphatic substances (among which trichloroethylene) has been studied. In this study, it was found that a limited number of halogenated aliphatic compounds can serve as a growth substrate for aerobic microorganisms, that specifically develop a variety of enzyme systems to degrade these compounds.

The effect of **propane, methane and butane** on the degradation of TCE has been studied by Tovanabootr et al. (1998). Initial lag periods of 2 weeks for methane and 3 weeks for propane and butane degradation were observed. Methane- and propane-utilizers were active toward TCE cometabolism, whereas butane-utilizers showed no ability to transform TCE. TCE was transformed most rapidly during active methane utilization, and continued to be degraded at a slower rate for approximately 1 week after methane was consumed. Both methane- and propane-utilizers showed positive correlations between TCE transformation rates and primary substrate utilization rates.

In another study, performed by Kuo et al. (2004), the effect of **toluene** as co-substrate in the aerobic biodegradation of TCE has been studied. In-situ pilot studies of aerobic cometabolism were conducted to evaluate the injection of toluene-vapour and air into TCE-contaminated aquifer. Delivery of primary substrate (toluene) in a vapour state with air enhanced the growth of indigenous toluene-utilizing bacteria that would degrade TCE by aerobic cometabolism. Meanwhile, delivering toluene in a vapour state effectively reduced potential clogging near the injection points due to excessive microbial growth, which was observed in the field when the injection of neat toluene was employed. Over 90% removal of TCE was achieved, and primary substrate (toluene) was degraded to a concentration below 10 mg/L.

The effect of **phenol** on aerobic biodegradation of TCE was studied by Volcik et al. (2005); in this study TCE was removed in a single-pulse bioreactor under aerobic conditions. The TCE-removal capacity of the heterotrophic culture was dependent on pulsed phenol injection and on cyclic addition of phenol and TCE. It was found that the addition of cycloheximide (an antibiotic against propagation and growth of fungi and yeast) increased the TCE degradation activity of the mixed microbial suspension. A certain residual amount of TCE remained in some of the experiments.

Reactor design

In order to optimize the treatment of water streams polluted by TCE, several experimentations were performed to determine the most advantageous configurations of the reactor to develop the treatment process.

In a study by Misra et al. (2001) a hybrid reactor combining **trickling filter** (TF) and **activated sludge process** (ASP) for the degradation of TCE from a wastewater has been

studied. The biofilm acclimation was achieved in 55±60 days with gradual increase in TCE concentration from 1 mg/l to 100 mg/l. The removal efficiency increased with decreasing flow rate, and the TCE removal was found to be 99.99% at 6 l/d of flow rate; this corresponded to an HRT of 28 h (TF 18 h+ASP 10 h).

Kao et al. (2001) equipped a system of laboratory column studies for evaluating a **barrier system** for TCE biodegradation. The oxygen-organic material of the biobarrier system was able to release oxygen and primary substrates continuously upon contact with water. Aerobic acclimated sludge was inoculated in the columns to provide microbial consortia for TCE degradation. Simulated TCE contaminated groundwater was pumped in this system with a flow rate of 0.25 l/day. Decreases in TCE concentrations were observed over a 4-months operating period, and TCE was removed with an efficiency of 99%.

4.2.4.2 Anaerobic degradation

Natural attenuation of TCE concentration often occurs in polluted soils due to the instauration of anaerobic conditions. Several studies on the **natural attenuation of TCE** and its "in-situ" degradation have been performed, both by replying real soil conditions in laboratory experiments and by direct field observations.

In an experimentation performed by Lorah et al. (2001), real polluted soil conditions have been reproduced in laboratory by equipping soil microcosms prepared under methanogenic, sulfate-reducing, and aerobic conditions using sediment and groundwater from a freshwater wetland polluted by TCE. Under methanogenic conditions, biodegradation rates of TCE were extremely rapid, showing a half-life of about 2 days. TCE biodegradation rate was slower under sulfate-reducing conditions than under methanogenic conditions, while in the aerobic microcosm experiments, biodegradation occurred only if methane consumption occurred, indicating that some methanotrophic bacteria were involved.

In a study by Lenczewski et al. (2003), an example of natural attenuation of TCE in groundwater in fractured shale bedrock was studied. Distribution of different chlorinated compounds in the aquifer, whose concentration decreased with distance downgradient towards the seep, suggested anaerobic biodegradation. Measurements of redox potential at the site indicated that iron-reduction, sulfate reduction, and potentially methanogenesis were occurring and conduced to dechlorination of TCE. Groundwater samples analysis revealed the presence of methanotrophs, methanogens, iron-reducing bacteria and sulfate-reducing bacteria, all of which have previously been implicated in anaerobic biodegradation of TCE. 16S rDNA sequence from DNA extracted from two wells were similar to sequences of organisms previously implicated in the anaerobic biodegradation of chlorinated solvents. The combined data strongly suggest that anaerobic biodegradation of the highly chlorinated compounds is occurring.

Another study dealing with the natural attenuation of TCE contamination in soils has been performed by Brigmon et al. (1998), who studied the extensive trichloroethylene (TCE) groundwater contamination. This study was undertaken to estimate the potential of four rhizosphere soils along the seep line to naturally attenuate TCE. Laboratory microcosms were setup to evaluate both biotic and abiotic attenuation of TCE. Results demonstrated that **sorption to soil** was the dominant mechanism during the first week of incubation, with a 90% TCE removal from the aqueous phase. A limited amount of TCE biodegradation was observed, and attempts to stimulate TCE biodegradation by either methanotrophic or

methanogenic activity through amendments with methane, oxygen, and methanol were unsuccessful.

The potential for in situ anaerobic bioremediation of trichloroethylene (TCE) and dichloromethane (DCM) was studied by DeWeerd et al. (1998) with soil column and serum bottle microcosm experiments. Soil columns with continuous groundwater recycle were used to evaluate treatment with complex nutrients (casamino acids, methanol, lactate, sulfate) and with benzoate and sulphate, and methanol. When the complex nutrients were fed, microbial dechlorination of TCE to ethane was observed, whereas the benzoate/sulfate and methanol supported microbial dechlorination of TCE only to *cis*-1,2-dichloroethylene.

In an experimentation by Johnston et al. (1996) the anaerobic biodegradability of benzene, toluene, ethylbenzene, ortho-, meta- and para-xylene (BTEX) and trichloroethylene (TCE) in aquifer sediment downgradient of an unlined landfill was evaluated. The biodegradative potential of the contaminated aquifer was measured in laboratory microcosms equipped using anaerobic aquifer sediment from the downgradient of the landfill. TCE was degraded in microcosms with aquifer material from all three boreholes. The addition of calcium carbonate stimulated TCE biodegradation which was not further stimulated by nutrient addition. TCE was converted to ethylene, a harmless byproduct, in all tests.

Cometabolic degradation of TCE

Several experimentation have been performed in order to find out the best **growth substrates in cometabolic degradative** processes of TCE.

In an experimentation by Yu and Semprini (2002) microcosm studies were conducted to demonstrate the effectiveness of tetrabutoxysilane (TBOS) as a slow-release anaerobic substrate to promote reductive dehalogenation of trichloroethylene (TCE). Hydrogen consumption was correlated with TCE reductive dehalogenation, indicating that it served as an electron donor in the process. TBOS was found to be a slow-release anaerobic substrate to support long-term dechlorination of TCE to ethylene.

In another study the effect of **other growth substrates** on the anaerobic degradation of TCE has been tested. In this study by Wu et al. (1998), reductive dechlorination of trichloroethylene (TCE) to ethylene was supported by complex organic matter such as cane molasses and ligno-cellulosic materials as a substrate and electron donor under methanogenic conditions. The studies were performed with an anaerobic dechlorinating, methanogenic microbial consortium. Three ligno-cellulosic materials (corn cobs, hard wood chips and newsprint) supported dechlorination of TCE to
ethylene.

The efficiency of alternating anaerobic and aerobic conditions in a granular biofilm reactor for the degradation of TCE was studied in an experimentation by Tartakovsky et al. (2005). The coexistence of aerobic methanotrophic and anaerobic methanogenic bacteria in this reactor was expected to allow for a combination of reductive and oxidative pathways of TCE degradation and result in mineralization of TCE. The experiment confirmed degradation of TCE due to the co-existence of methanogenic and methanotrophic populations in a single reactor.

Chapter 5.
Sequencing Batch Reactors

5.1 Description of the system

Sequencing batch reactors (SBRs) are activated sludge systems for wastewater treatment, operated in "Fill-and-draw" modality. Wastewater treatment is operated in **batch** modality, which means that during the degradation process operated by activated sludge, no input of fresh wastewater or output of treated supernatant occurs.
All the processes that in a traditional wastewater treatment plant (WWTP) take place in different sections, in a SBR system are operated in a unique tank, in which the various operating conditions necessary for the removal of pollutants from water occur. According to a definition of SBR systems given by U.S. EPA, it can be stated that "the SBR is no more than an activated sludge system which operates in time rather than in space".
"Fill-and-draw" batch processes were used for the first time at the beginning of the 20th century, but the high complexity of the system design and the subsequent high costs caused the pull-out of this technology. However, in the late 1950s, the interest of scientific community for SBRs systems rose again, due to the lower costs ensured by the improvements in WWTP management technology.

5.2 Description of wastewater treatment process in an SBR plant

Before entering the biological treatment section, constituted by the tank of the SBR system, wastewater usually passes through screens and grit for solids removal, and sometimes it is also subjected to a primary sedimentation process, which takes place in a primary clarifier. Due to the configuration of SBR systems, the secondary sedimentation section is not required, since the process of separation between supernatant and activated sludge after the degradative biological process occurs in the same tank in which biological treatment of water takes place.
After passing the section of grit and primary filtration, wastewater enters the tank, where it mixes with the activated sludge already present in the reactor from the previous phase of the process.
Concentration of contaminants present in wastewater is diluted after mixing with the activated sludge; however this dilution is not very high, since the volume of wastewater entering the system is usually several times larger than the volume of activated sludge already present in the tank at the moment of wastewater feeding.
At this point the degradative reaction can start, and it goes on with the same biological processes that occur in the biological treatment section of a traditional WWTP. The only difference is that the degradation takes place in **batch modality**, so that during the whole length of the degradation phase, no influent enters the biological section and no effluent goes out of it.

The previously described operation of an SBR is based on the "Fill-and-Draw" modality, which consists of sequential cycles, each of them composed of the following five steps:

- Idle;
- Fill;
- React;

- Settle;
- Draw.

The length of each of these phases can be set depending on wastewater composition and on the typology of degradative process that is desired, according to the influent characteristics and to the requirements of law emission limits established for the effluent.
Depending on the composition of the wastewater to be treated, it will be necessary to perform some treatability tests in order to define the different phases lengths and to identify the ones that ensure the highest removal efficiency.

Idle phase

Idle phase occurs between Draw phase (in which the effluent is discharged) and Fill Phase (in which wastewater is added to the system). If variable idle times are used in this phase, equalization of the influent is achieved.

Fill phase

In the Fill phase, the addition of fresh wastewater to the system occurs; during this phase, wastewater is added to the settled sludge present in the system. Fill phase can be *static*, *mixed*, or *aerated*.
During *Static Fill* both mixing and aeration are off. Thus, biological activity during Static Fill phase is not stimulated and substrate is not degraded, so that when mixing starts (in the following phase), F:M ratio is quite high. A high F:M ratio favours the growth of floc forming microorganisms versus filamentous microorganisms, thus reducing the problem of bulking that can affect sludge settleability. Besides, a high F:M ratio favours those microorganisms which store substrate for their metabolic processes, such as the heterotrophs which operate phosphorus removal. A Static Fill can then be assimilated to a "selector" compartment of a traditional WWTP for control of the F:M ratio.
Mixed Fill occurs when the addition of wastewater to the sludge present in the system occurs in mixing conditions. Biomass comes rapidly in contact with wastewater and degradation of organic substrates starts. The oxygen present in molecular form is quickly degraded and anoxic conditions are established, so that denitrification processes for nitrates and nitrites removal can take place.
During *Aerated Fill*, both aeration and mixing are on, so that biological degradation processes start immediately in aerobic conditions.

React phase

In React phase, the degradation processes begun in Fill phase are completed. Reaction phase can be operated both in *mixed* and *aerated* conditions.
Mixed React is operated without the inflation of air into the system, so that anoxic conditions are first established, and then, if the process continues and also the sources of oxygen in linked form (nitrates, nitrites, carbon dioxide, sulphurs) are consumed, then anaerobic conditions can take place. This kind of React phase is operated when denitrification (in anoxic conditions) or phosphorus removal (in anaerobic conditions) are desired.
Aerated React is operated by means of air inflation into the system, and aerobic conditions are established; in these conditions, carbon removal and nitrification process can take place.
Obviously, if necessary, different modalities of React phase can succeed: for instance, if complete removal of nitrogen from wastewater must be achieved, React phase can be operated first in aerated modality, so to allow the transformation of ammonia-nitrogen into nitrites and nitrates; React phase can then be operated in mixed modality, so to set anoxic

conditions and allow denitrification processes to take place, with the reduction of nitrates and nitrites and their conversion into gaseous nitrogen. This alternation of metabolic conditions works as the succession of an aerated section and an anoxic section in a traditional WWTP.
In the same way, aerobic and anaerobic conditions can be alternated if phosphorus removal from wastewater must be achieved by means of EBPR (Enhanced Biological Phosphorus Removal).
Extraction of excess sludge can be done at any moment during degradation phase. Sludge age will depend on the volume of excess sludge daily extracted:

$$\theta_c = V_{tot}/Q_{ex}$$

where:
θ_c = sludge age [d]
V_{tot} = total volume of the tank [m^3]
Q_{ex} = volume of excess sludge daily extracted [m^3/d]

Settle phase

In Settle phase, agitation and aeration are stopped, so that sludge can settle; this process can be operated in completely quiet conditions, or by means of a gentle mixing which can improve sludge settleability. The length of this phase can last around 0.5-1.5 hours.

Draw phase

In the last phase of the cycle (the Draw phase), supernatant is extracted from the SBR tank. Supernatant extraction is done by means of a decanter, which can be both *floating* or *fixed* (see section on Tank and Equipment Description).

All these phases can be set for time length and modality, and they sequence each other in cyclic modality. Thus, each SBR cycle is composed by all the previous phases; since any cycle must ensure the complete degradation of pollutants present in wastewater, React Phase will be the one to mostly influence the duration of the whole cycle.

It is clear that a great difference of SBR systems in comparison to traditional WWTP is the fact that no sludge recirculation is required, thus eliminating the need of pumps for the recirculation which is usually operated in a traditional WWTP; also recirculation of aerated mixed liquor, usually done when nitrogen removal must be achieved, is not necessary in a SBR system.
Due to its configuration, SBR system works as an equalization tank, capable of damping possible peak flows and peak loads; this causes the protection of sludge from peak flows, which can wash out the biomass, and from peak loads, which may upset the treatment process.
However, one or more equalization tanks can be necessary after the SBR, in order to avoid the input of high flows of supernatant into the filtration and disinfection sections, and subsequently to reduce the size of these sections.
SBR systems need a quite high level of automation, achieved with the use of valves and timers, and therefore their management and building are more sophisticated than in a traditional WWTP.

5.3 Applicability of SBRs

The small footprint of SBR systems makes them particularly suitable for situations in which small areas are available for wastewater treatment.
The high level of sophistication required for the conduction of an SBR makes these systems cost effective only if applied in relatively small plants; too large plants would be too expensive and difficult to build and manage.
One of the strong point of SBR systems is their **flexibility**. It is very easy to modify the length of the different phases of the process, and it's also very easy to change metabolic conditions in the degradative phase, thus establishing aerobic, anoxic or anaerobic conditions in order to achieve complete removal of carbon, phosphorus and nitrogen present in the influent.

5.4 Design Criteria

Criteria for SBRs sizing are the same that must be considered while sizing traditional WWTPs; thus both physical and chemical parameters concerning influent and effluent of the system must be taken into account.
In particular, design flow, sludge TSS concentration, wastewater temperature, alkalinity, pH, concentration of BOD_5, TKN (Total Kjeldal Nitrogen), ammonia-nitrogen and total phosphorus of the influent, and law emission limits for BOD_5, nitrogen, phosphorus and Fecal Coliform in the effluent must be considered while sizing an SBR system.
Typical design parameters for an SBR treating both municipal and industrial wastewater are reported in Table 5.1 (AquaSBR Design Manual, 1995).

Table 5.1. Key design parameters for an SBR system treating industrial or municipal wastewater (AquaSBR Design Manual, 1995)

Parameter	Municipal wastewater	Industrial wastewater
Food/microorganism (F:M)	0.15-0.4	0.15-0.6
Treatment cycle duration	4 hours	4-24 hours
Typical mixed liquor suspended solids	2-2.5 g/l	2-4 mg/l
Hydraulic Retention Time	6-14 hours	varies

Once the design parameters are determined, the number of cycles per day, number and volume of tanks and the influent and effluent volumes can be calculated; aeration equipment, decanter and the necessary piping can then be sized.

5.5 Construction of an SBR

One of the strong point of an SBR system in comparison with a traditional WWTP is the less footprint: no secondary clarifier is required in a SBR system, and only for wastewater with high concentration of suspended solids a primary clarifier is required. In a SBR, no pumps for sludge and mixed liquor recirculation are required.
The most complex point in the construction of an SBR is the **control system:** automatic switches, automatic valves and instrumentations that sequence and time the different phases of any cycle.
Due to the high sophistication of SBR systems, they are used mainly for small applications.

5.5.1 Tank and Equipment Description

An SBR system is constituted mainly by a tank, the aeration and mixing equipment, a decanter and the control system.
The **tank** is usually made of steel or concrete. For industrial wastewater, SBR tanks are usually made of steel covered with protective layers in order to prevent corrosion deriving from the aggressive nature of industrial wastewaters. For municipal wastewaters, the tanks are usually made of concrete. Depending on the daily wastewater flow, more than one tank can be necessary.
For **mixing** and **aeration**, the most used systems are *jet aeration* systems, since they can ensure mixing action with or without aeration.
Decanter is the part that mostly distinguish different typologies of SBRs; they can be both *floating* or *fixed*.
Floating decanters offer the advantage of maintaining the inlet orifice slightly below the water surface to minimize the removal of solids in the effluent removed during the DRAW step. Floating decanters also offer the operating flexibility to vary fill-and-draw volumes.
Fixed decanters are built into the side of the basin and can be used if the Settle step is extended. Extending the Settle step minimizes the chance that solids in the wastewater will float over the fixed decanter. In some cases, fixed decanters are less expensive and can be designed to allow the operator to lower or raise the level of the decanter. Fixed decanters do not offer the operating flexibility of the floating decanters.

5.6 Performance

Treatment efficiency of SBRs is similar to the one of traditional WWTPs. In an SBR, usually BOD_5 removal efficiency is around 85-95%; SBRs are usually built in order to achieve the effluent concentrations listed below:

- 15 mg/l BOD_5;
- 30 mg/l TSS;
- 5 - 8 mg/l TN;
- 1 - 2 mg/l TP.

5.7 Costs

When predicting costs necessary for building an SBR, general estimations can be done, even if it must be considered that capital and construction cost estimations are site-specific.
In Table 5.2, budget level costs for SBR projects presented between 1995 and 1998 are shown. Budget level costs include blowers, diffusers, electrically operated valves, mixers, sludge pumps, decanter and the control panel.

Table 5.2. SBR Equipment costs based on different projects. (Aqua Aerobics Manufacturer Information, 1998)

Design Flowrate (MGD)	Budget level equipment cost ($)
0.012	94000
0.015	137000
1	339000
1.4	405000
1.46	405000
2.0	564000
4.25	1170000

Obviously, the costs shown in Table 5.2 do not include such costs as sitework, excavation/backfill, installation, contractor's overhead and profit, and legal, administrative, contingency and engineering services. Costs for other possible sections present in the whole treatment plant such as screening, filtration, equalization, disinfection and aerobic digestion of sludge must be included when calculating the whole cost of a treatment plant based on SBR technology. Other costs to consider are the ones due to labor, overhead, supplies, maintenance, operating administration, utilities, chemicals, safety and training, laboratory testing and solids handling.

Operations and Maintenance costs associated with an SBR system may be similar to a conventional WWTP. Lower costs can be achieved in a SBR because clarifiers, clarification equipment and recirculation activated sludge pumps are not necessary. On the other hand, the maintenance requirements for the automatic valves and switches that control the SBR system may be more intensive than for a conventional activated sludge system.

5.8 Advantages and disadvantages of SBR systems

Advantages

- SBRs are very effective when the **selection of a particular bacterial strain** of the activated sludge to degrade some specific substances, and the acclimation of bacterial population to new substrates is required. These processes are favoured in SBR systems because biomass is cyclically subjected to strong variations of substrate amount and environmental conditions, and this allows the selection of the most resistant strains of microorganisms, which are able to deal with the degradation of the new substrates.
- In SBR systems, equalization, primary clarification (in most cases), biological treatment, and secondary clarification can be achieved in a single reactor vessel, so that there is a quite high potential of **cost saving**.
- SBRs have a great operating **flexibility** and control.
- SBRs have a **minimal footprint**.

Disadvantages

- In comparison with traditional WWTPs, SBRs imply a higher level of **sophistication**, especially for larger systems, for timing units and controls.
- SBRs require higher level of **maintenance** (compared to conventional systems) associated with more sophisticated controls, automated switches, and automated valves.
- In SBRs it is possible to have **discharge** of floating or **settled sludge** during the Draw or Settle phase with some SBR configurations.
- In SBRs it is possible to require **equalization** after the biological treatment, depending on the downstream processes.

Chapter 6.
Membrane Biological Reactors

6.1 Generalities on Membrane Biological Reactors

Membrane Biological Reactors (MBR) are constituted by the combination of a traditional activated sludge reactor with a membrane module for the extraction of effluent.
Wastewater that must be treated by a MBR flows into a tank in which biological treatment is carried out, and then sludge flow is channelled through a membrane filtration unit where sludge and water are separated. Filtrate is drained out as effluent and the concentrate is recirculated into the aeration tank; surplus sludge is discharged via a sludge valve (van Dijk and Roncken, 1997).
Membrane modules can be microporous, ultraporous or nanoporous, depending on the pores dimensions.
The strong advantages of these systems lay on the possibility of **getting rid of sedimentation unit** after biological treatment, thus eliminating the problems associated to its management and reducing the footprint of the plant.
The differences of MBR systems with the traditional wastewater treatment plants can be summarized in the following points:

- **High biomass concentration**
  In MBR systems, biological process can be operated at high sludge concentration, because, since the separation of the effluent from mixed liquor is achieved by means of filtration, sludge settleability does not constitute a problem anymore.
  Biomass concentration can be kept at a value of even 35 g/l (National Water Research Institute and Orange County Water District, 1994). However, too high concentrations of biomass give problems with viscosity, membrane fluxes and oxygen transfer.

- **Heat production**
  In a MBR, heat is produced through several processes:
  - the biological processes (carbon oxidation, nitrification, denitrification) are exothermic reactions. Due to high biomass concentration and high bacterial activity the heat production due to biological processes is quite significant in MBRs;
  - 80% of the energy input for membrane filtration is converted into heat. This means a heat production by membrane filtration of 6-8 $kWh/m^3_{treatedww}$.

  Due to the heat production, bacterial processes can take place at temperatures between 35 and 40°C, which is often the optimum for biological processes. In some cases, an additional cooling system is necessary to prevent high temperature in the bioreactors.

- **Oxygen consumption**
  Due to the oxidation processes carried out by the highly concentrated biomass with a high specific activity, in MBRs there is a high oxygen consumption; thus, a high minimum maintenance energy is needed. The high oxygen consumption makes an advanced oxygen supplying system necessary to keep the bioreactor compact.

- **Good effluent quality**

In a MBR, the complete mineralization of influent organic matter is facilitated by the high biomass concentration, and by the retention of high molecular weight compounds operated by the membrane. As a result of membrane separation, sludge retention time is independent from hydraulic retention time. Membrane separation in bioreactors is most attractive for situations in which long solids retention times are required to achieve the complete removal of pollutants. After passing an ultrafiltration membrane, the effluent is also free of suspended solids, bacteria and viruses. This gives possibilities for effluent reuse and fargoing treatment.

- **Low sludge production**
  Sludge production in membrane bioreactors is much lower than in conventional aerobic systems due to the high temperatures and the relatively low F/M ratios.

Implementation of MBR systems, especially because of the substitution of the clarifier with a section of supernatant extraction by filtration, ensures the following advantages in wastewater treatment:

- strong reduction of footprint of the whole treatment plant in comparison to the traditional WWTP, due both to the elimination of the secondary clarifier and to the increase in suspended solids concentration in biological section;
- possibility of managing the biological process completely independently from variations of flow rate, since sludge retention time and hydraulic retention time are completely separated;
- possibility of working with high suspended solids concentration (up to 30 g_{TSS}/l) and high sludge age (>20 days), since sludge settleability does not constitute a problem anymore;
- possibility of working with higher organics loads to the plant, because of the high biomass concentration available;
- decrease of the amount of excess sludge produced, associated to the higher sludge age values;
- selection of specific bacterial population degrading particular substrates, due to the high sludge age which allows the growth of specific bacterial strains;
- possibility of keeping into the reactor large organic molecules (larger than the membrane molecular cut off) until they are degraded and reduced to smaller and simpler molecules. This is the case of hydrocarbons and mineral oils in emulsion (Scholz and Fuchs, 2000);
- improvement of nitrification rates, due to the higher sludge retention times achievable, that allow the growth of nitrifying bacterial populations. Some studies have shown higher ammonification rates of organic nitrogen than the ones observed in traditional systems (Chen et al., 2003);
- strong improvement of qualitative characteristics of the effluent in terms of solids, that are completely absent, compliant with the reuse of water for agricultural purposes.

However, due to the fact that MBR is a quite new technology (it has been used for around 15 years), many management points of MBR systems are still to be improved, for the point of view of both biological processes occurring in membrane bioreactors and of membrane fouling control.

6.2 Theoretical principles of the process

Porosity of membranes used for filtration processes can range from 0.0001 (so to retain also dissolved solids) to 1.0 μm. A membrane works by retaining some constituents of solids in water while leaving some other to cross the membrane.
The influent to the membrane module is called *feeding flow*, while the liquid that passes through the membrane is called *permeate* (or *filtrate*); the liquid that is retained by the membrane, and that contains all the substances present in water, is called *concentrate*.
The rate of the flow of water that passes through the membrane is called *flux* and is measured as a flow rate per surface unit. This is schematically represented in Figure 6.1, where Q indicates the flow rate, C is the concentration, and P indicates pressure, while f, p and c indicate feeding, permeate and concentrate.

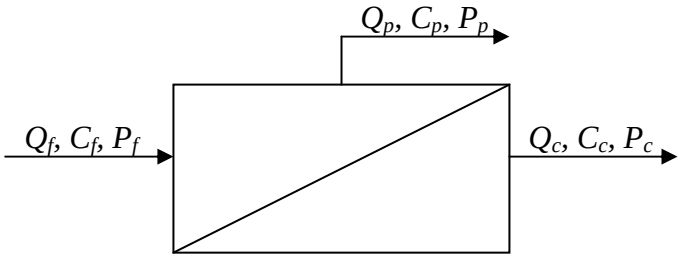

Figure 6.1. Division of the influent when passing through the membrane module.

Filtration systems, which membranes belong to, can be divided primarily into "*depth filters*", in which all the depth of filtering element contributes to retain the particles from the flow, and "*screen filters*", in which particles whose dimensions are larger than pores are retained on a single side of the filter. Membranes belong to this last category, the one of screen filters.

Membranes can be operated in "*dead end*" modality, in which feeding flux is orthogonal to the membrane surface, or in "*cross flow*" modality, in which feeding flux is tangential to the membrane module.

Membrane structures can be classified into *symmetric*, *asymmetric*, *porous* or *dense*; their properties are summed up in Table 6.1.

Table 6.1. Characteristics of membranes structure.

Microporous	Isotropic (constant pore dimension in the depth of the membrane)	Maximum equivalent diameter of the pores
	Anisotropic (variable pore dimension in the depth of the membrane)	
Asymmetric	Filtration layer + support layer	Nominal diameter

6.3 Materials of the membranes

Membranes can be made of *natural* or *synthetic* and of *organic* or *inorganic* materials.
Membranes can also be classified depending on the modality of separation of particles present in filtered water stream: it can occur by *adsorption*, by *diffusion*, by *ionic exchange* or by *osmosis*.
In general, membranes can operate filtration in different physical conditions:

- solid-liquid separation;
- liquid-liquid separation;
- gas-gas separation;
- liquid-gas separation.

Membranes are also capable of reacting with the substances present in the permeate: selective permeability or semi-permeability derive from pores characteristics and from the presence of ionic groups in membrane surface.

6.4 Geometry of membrane modules

Geometric structure of a membrane is valuable if it is capable of *minimizing fouling during filtration* process, and, contemporarily, of having a *good specific surface of the module*, where, for "specific surface" the filtrating surface per unity of occupied volume is intended.
Also *structural simplicity*, *management flexibility* and *modularity* are important factors while deciding if a membrane module is valuable.
The main typologies of filtration modules are the following:

- with spiral wrapping;
- hollow fibres;
- plate frame modules;
- tubular modules;
- wrinkle sheets.

A very important parameter often taken into account while considering the membrane filtration process is the molecular weight of the compound that can be retained by the membrane. This is called ***Molecular Weight Cut Off*** (MWCO); it refers to molecular cut off of the solute at 90%, and is expressed in Dalton.

6.5 Typologies of membrane processes

Membrane filtration processes, as can be observed in Table 6.2, can be divided into **microfiltration** (MF), **ultrafiltration** (UF), **nanofiltration** (NF), **reverse osmosis** (RO), **dialysis** and **electrodialisis** (ED).

Table 6.2. Typologies of membrane processes and their power force (Cheryan, 1998)

Process	Power force	Concentrate	Permeate
Osmosis	Concentration gradient	Solutes	Water
Dialysis	Concentration gradient	Large molecules	Small molecules
Microfiltration	Pressure gradient	Suspended particles	Dissolved solids
Ultrafiltration	Pressure gradient	Large molecules	Small molecules
Nanofiltration	Pressure gradient	Bivalent ions	Monovalent ions
Reverse osmosis	Pressure gradient	Monovalent ions	Water
Electrodialysis	Electromotive force	Non ionic solutes	Non ionic solutes
Pervaporation	Pressure gradient	Non volatile molecules	Volatile small molecules

6.5.1 Microfiltration

Microfiltration membranes have pores size down to 0.1-0.2 microns (Fane and Chang, 2002). Microfiltration is used for removal of inorganic particles, microorganisms, colloids and oils from water streams. The electromotive force that governs the process is the pressure gradient applied between the feeding side and the permeate side of membrane. Typically the process takes place at pressures of 1 bar, or slightly higher. Practically it is not recommended to work at pressures higher than 0.7-0.8 bar, both for physical resistance of membrane and for hydraulic reasons. Actually, due to the relatively large dimensions of the pores, higher pressure values would imply an initial increase of membrane productivity, in terms of extracted permeate, but, in a short time, it would cause an abrupt flux decrease caused by the accumulation of solute on the liquid-membrane interface, by pores occlusion and by filter surface dirtiness.

6.5.2 Ultrafiltration

Ultrafiltration allows separation of soluble macromolecules and lipophylic polymers from water streams; besides, with ultrafiltration it is also possible to achieve removal of proteins, virus and bacteria. Pores size ranging from 0.1 microns down to 5 nanometres (Fane and Chang, 2003), and pressure applied during the process is between 1 and 10 bar. Ultrafiltration is used both in industrial productive cycles and in wastewater treatment; it can also be used for oily emulsions and chemical reagents recovery.
In order to achieve high water quality requirements, ultrafiltration is often coupled to other treatments: if coupled to activated carbons or to nanofiltration and reverse osmosis, ultrafiltration can allow the production of drinking water from low quality surface waters (Durnand-Bourlier et al., 2000; Doyen et al., 2000).

6.5.3 Nanofiltration

Nanofiltration is characterized by pore dimension of around 1-2 nm, and a MWCO of around 500 Dalton. It allows separation of toxins, bivalent dissolved ions and other compounds such as pesticides and herbicides. Separation takes place not only for physical reasons but also for electrostaticity of the membrane surface.
Nanofiltration membranes retain bivalent ions, and allow monovalent ions to pass through; subsequently, osmotic pressure associated to the difference of saline concentration between the two sides of the membrane is relatively low, so that it is not necessary to apply high pressure values.
The main applications of nanofiltration process are:

- colour removal;
- disinfection;
- treatment of some industrial effluents;
- potabilization of waters: in this use efficiency of nanofiltration is comparable to the one of reverse osmosis, but with lower operative pressures and higher permeate fluxes (Antonelli and Rozzi, 2000)

6.5.4 Reverse osmosis

Reverse osmosis, also known as hyperfiltration, is a separation process that uses pressure to force a solvent through a membrane that retains the solute on one side and allows the pure solvent to pass to the other side. More formally, it is the process of forcing a solvent from a region of high solute concentration through a membrane to a region of low solute concentration by applying a pressure in excess of the osmotic pressure. It is the reverse of the normal osmosis process, which is the natural movement of solvent from an area of low solute concentration, through a membrane, to an area of high solute concentration when no external pressure is applied. The membranes used in this process are semi-permeable, meaning that they allow the passage of solvent but not of solute.
Membranes used for reverse osmosis have a dense barrier layer in the polymer matrix where most separation occurs. This process requires that a high pressure is applied on the high concentration side of the membrane, usually 2–17 bar (30–250 psi) for fresh and brackish water, and 40–70 bar (600–1000 psi) for seawater, which has around 24 bar (350 psi) natural osmotic pressure to overcome.
Reverse osmosis process ensures that all the ions present in solution are retained, included the monovalent ones. Pores dimension is included between 0.1 and 1 nm. In the process, pressure reaches and sometimes overcomes 70 bar, due to the high values of osmotic pressure difference that takes place between the two sides of the membrane. Energy consumption is thus quite high in comparison to the other membrane processes. Reverse osmosis is mainly used in marine water desalination.

6.5.5 Electrodialysis

Electrodialysis process is based on an electromotive force which comes from the application of an electrical field between two electrodes. Membranes used for electrodialysis are built so to have the property of selective permeability; therefore they can be permeable to cations (cationic membranes) or to anions (anionic membranes). An electrodialysis filtering module is constituted by several membranes of different selectivity close to each other, so to form divisions in which the difference of potential causes the formation of low electrolytic content; water depurated in this way is subsequently removed from the module.
The main limit of electrodialysis lays in its capability of removing only ions, without any action towards organic molecules. Besides, the colloidal fouling trend typical of anionic membranes constitutes another problem that obstacles the diffusion on large scale of this technology. On the other hand, the possibility of inverting polarities inside the module, improves the easiness of ordinary membrane cleaning procedures.

6.6 Factors affecting membranes performance

A membrane filtration process based on pressure gradient is affected by the following factors:
- intrinsic resistance of the membrane;
- difference of applied pressure (TMP, Trans Membrane Pressure);
- hydrodynamic regime at the interface between the membrane and the solution to be filtered.

6.6.1 Intrinsic resistance

Intrinsic resistance of a membrane is the physical resistance opposed by the clean membrane to the filtration of pure water. It is function of pore dimension, of surface porosity of the membrane, of the ratio between surface and volume of porous surface ($m^2 \cdot m^{-3}$) and of membrane depth.

6.6.2 Transmembrane pressare

With the term Transmembrane pressure (TMP) it is intended the pressure difference applied between upstream and downstream the membrane in order to determine the permeate cross through the membrane. TMP is thus defined in this way:
$TMP = P_{feeding} - P_{permeate}$

where:
$P_{feeding}$ = pressure on the side of the solution to be filtered [bar];
$P_{permeate}$ = pressure on the side of the permeate [bar].

For submerged membranes, pressure upstream the membrane is calculated as average hydrostatic charge between upper and lower extremity of the module:

$$P_{feeding} = \frac{\rho \bullet g \bullet h_1 + \rho \bullet g \bullet h_2}{2}$$

where:
ρ = feeding density [$kg \cdot m^{-3}$]
g = gravity acceleration [$m \cdot s^{-2}$]
h_1 = immersion depth of the upper extreme of the filtering surface [m];
h_2 = immersion depth of the lower extreme of the filtering surface [m].
$P_{permeate}$ is the suction charge applied by means of a suction pump.

6.6.3 Permeate flux

Permeate flux is the ratio between extracted flow rate and the filtering surface:

$$J = \frac{Q}{S}$$

where:
J = extracted permeate flux [$L \cdot m^{-2} \cdot h^{-1}$];
Q = extracted permeate flow rate [$L \cdot h^{-1}$];
S = filtering surface of the membrane [m^2].

6.6.4 Permeability

Permeability is the ratio between flux and applied TMP:

$$P = \frac{J}{TMP}$$

Permeability (P) is usually expressed as [$L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$].

6.6.5 Hydraulic regime

Usually, turbulence obtained by means of increase of feeding flow rate can be helpful in decreasing membrane fouling. However, overcoming a certain feeding flow rate can cause membrane fouling, because while turbulence cleans away the bigger molecules from the surface of the membrane, the small molecules enter into the pores of the membrane causing its irreversible fouling.
A membrane system can be operated in two ways:

- at *constant TMP*, with progressive flux decrease, caused by the gradual membrane fouling;
- at *constant flux*, with progressive increases of TMP, due to the gradual membrane pores blocking.

Conventionally, operational parameters of a membrane are referred to standard conditions of temperature. *Temperature* can strongly affect dynamic viscosity of the permeate, thus determining relevant variations of permeability. The relation between dynamic viscosity and temperature is exponential:

$$\eta_T = e^{\left(A + \frac{B}{T} + C \cdot T + D \cdot T^2\right)}$$

where:

η_T = dynamic viscosity of the permeate at the process temperature [mPa·s];
T = temperature [K];
A, B, C and D are specific values for the liquid constituting the permeate. For water, these values are:
A = -24.71;
B = 4209;
C = 4.52710^{-2}
D = -3.410^{-5}.

Usually, the calculations are referred to water at 20°C; at this temperature, viscosity is 1.018 mPa·s.
Flux at actual temperature can be determined with the following formula, function of flux and viscosity at 20°C:

$$J_T = \frac{\eta_{20} \cdot J_{20}}{\eta_T}$$

The maximum achievable flux depends on membrane and influent characteristics and on hydrodynamic conditions hold in the system.

6.7 Physical phenomena affecting membranes efficiency

Accumulation of solids on membrane surface causes resistance to flux, due to three mechanisms:

- reduction and blocking of the pores;
- fouling;
- formation of gel or of solids accumulation, due to polarization and concentration mechanisms.

The phenomenon of *pore volume reduction* or of their complete blocking takes place only when the solids present in the influent have smaller dimensions than the membrane pores or than its molecular cut off.

6.7.1 Membrane pores blocking

Membrane pores blocking occurs when solid particles of the dimension of the pores are blocked into them.
Instead, pore volume reduction occurs because solid particles adhere to the inner surface of membrane pores, causing their constriction.

6.7.2 Fouling

The term **fouling** is used to describe the phenomena of fouling of membrane surface, caused by accumulation of the particles present in the feeding. Prediction of fouling extent is particularly relevant for membrane module sizing and its management, since it determines if pretreatments are necessary and allow to define operative conditions and forecast costs and filtration process efficiency. Some substances affect process efficiency through fouling formation; they are listed in Table 6.3.

Table 6.3. Typologies of fouling and substances that cause it.

Fouling type	**Substances causing fouling**	**Notes**
Fouling (formation of deposit, sometimes known as *biofilm* formation)	Metallic oxides, organic and inorganic colloids, bacteria, microorganisms, polarization for concentration.	Extent of membrane damage can be reduced by controlling the concentration of these substances in the feeding (for instance by using a microfiltration process upstream of reverse osmosis).
Precipitation (formation of deposits)	Calcium sulphate, calcium carbonate, calcium fluoride, barium sulphate, formation of metal oxides.	Extent of precipitation phenomena can be reduced by limiting the content of salts, by adding acids and other compounds to reduce the formation of calcium carbonate and of other precipitates.
Membrane damage	Acids, bases, extreme conditions of pH, free chlorine, bacteria, free oxygen.	Extent of membrane damage can be reduced by controlling the concentration of these substances in the feeding.

In order to reduce the extent of fouling phenomena, three different approaches can be adopted:

- pre-treatment of the liquid to be treated;
- membrane backwash;
- membrane chemical cleaning.

Pre-treatment has the purpose of reducing TSS and bacteria concentration in the liquid fed to the process. Frequently the liquid constituting the feeding is conditioned in order to limit chemical precipitation phenomena in the filtration unit.
However, the most common method used to eliminate the solid material accumulated on membrane surface is **membrane backwash**, which is operated by driving water and/or air streams in the opposite direction than the feeding.
Membrane chemical cleaning is applied in order to remove the constituents that are not removed by conventional backwash.
However, usually phenomena of membrane damage due to the presence of some substances are irreversible and damage the membrane without the possibility of recovering it.

6.7.3 Polarization by concentration

Polarization by concentration is the accumulation of solute at the interface liquid/membrane. This accumulation causes the formation of a limit concentration layer (the polarized layer), in which the concentration of solute retained by the membrane is higher than it is in the bulk liquid. Thus, a concentration gradient derives from this process, because the hydrodynamic conditions of the system, close to the quietness, govern the mechanisms of Brownian transport which are back-diffusive, and that contrast the convective transport through the membrane. Physically, it implies that a further resistance is added to the intrinsic resistance of the membrane.
Taking into account the scheme reported in Figure 6.2, it can be observed that solute concentration profile in proximity of the membrane has a progressive increase in comparison to the value into the liquid bulk kept in continuous mixing, which is constant; this value increases when it reaches the *gel layer* that is formed near the membrane surface.
This gel layer has a constant solute concentration; it acts as a real dynamic membrane, capable of exerting a further filtrating action.

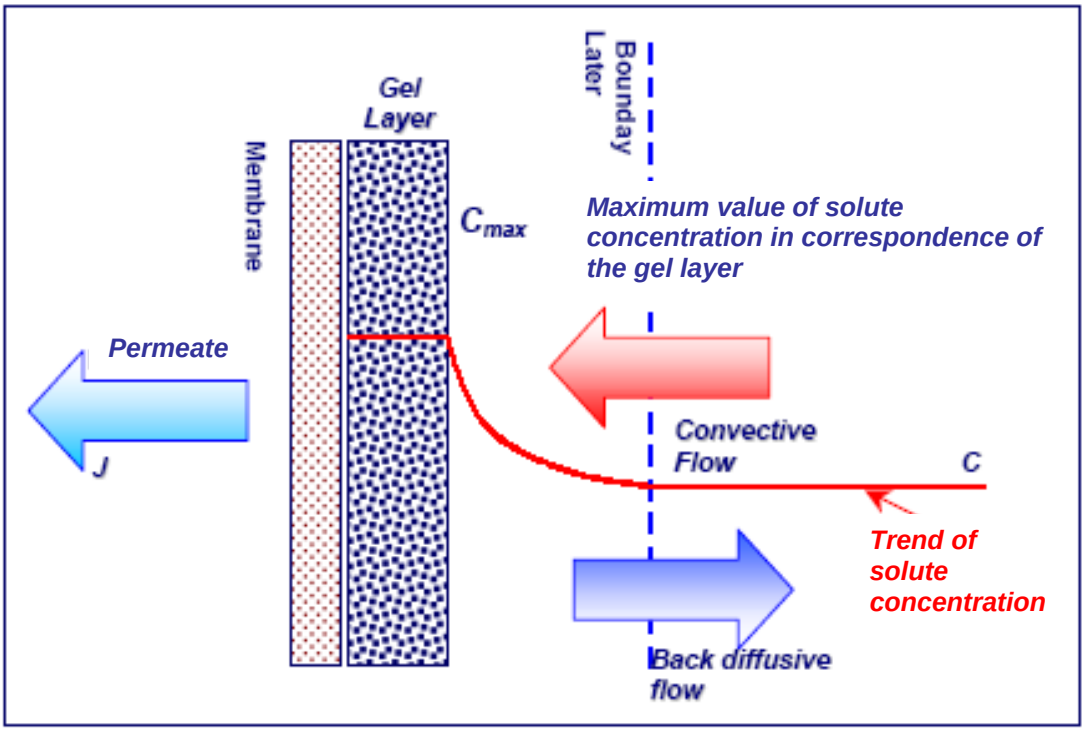

Figure 6.2. Mechanism of polarization by concentration.

If the process is microfiltration, gel layer will mainly consists of suspended solids; if the process is nanofiltration, the constituents of gel layer will be mainly dissolved macromolecules.
Polarization favours the precipitation of scarcely soluble solids on membrane surface and causes a progressive increase of the colloidal substances and suspended particles concentration close to the membrane.
Due to the discovery of the gel layer formation, it has been possible to experimentally verify the existence of two separate zones in the system:

- in the first part there is a linear proportional relation between applied pressure and achievable flux;
- once the maximum flux is achieved, any further increase of TMP does not cause any increase in permeate flux but it gives place only to higher energetic consumption.

6.8 Physical factors causing membrane fouling

Physical factors causing membrane fouling can be due to different reasons:

1. **Membrane characteristics**:

- hydrophilicity;
- surface configuration;
- pores dimension;
- surface charge of the membrane.

2. **Characteristics of the solute in the feeding**:

- proteins;
- pH and saline content;
- lipids, oils, greases;

- anti-foaming agents.

3. **Operational conditions**:
- temperature;
- influent flow rate and turbulence conditions.

6.9 MBR systems

MBR systems are based on the combination of activated sludge biological processes with membrane filtration processes. In several experimental studies two main different MBR systems have been developed:
- MBR for separation of solids present in aqueous solution;
- MBR for oxygen transfer into bulk liquid without air bubble inflation;
- MBR for the extraction of organic pollutants from particularly strong wastewaters.

The only typology so far implemented at plant scale is the first one. Even if the earliest cases of biological processes coupled to micro-ultrafiltration processes on membranes were applied in the 1970s, MBR systems in their modern version have been developed in the late 1980s, when many small scale plants for municipal and industrial wastewater were built. However, the high management costs, associated to the high energetic consumption and to the necessity of substituting the damaged membrane modules, prevented MBR technology from its large diffusion.
The development of submerged membranes, which took place in Canada and Japan in the early 1990s, and the subsequent decrease of energetic consumption favoured MBR market: a large increase of MBR installations was observed in many areas. Around 66% of MBR are nowadays operative in Japan; the rest of MBR plants are in North America, Europe and, less, in Australia. In almost all the cases (98%), the metabolic process adopted is the aerobic one.

6.9.1 Process schemes

The three main possible MBR configurations are the following:
- configuration with membranes **submerged** inside the oxidation/nitrification section;
- configuration with membranes submerged into a reactor **external** to the oxidation/nitrification section.

So far, the most largely used configurations is the first one. The major advantage of submerged membrane systems in comparison to those with external membranes, lays in the possibility of *reducing energetic costs* associated to mechanical cleaning of membrane. If filtrating unit is submerged into a tank external to the biological reactor, it is necessary to take into account the progressive accumulation of solids into this tank, which can determine TSS concentration differences between this compartment and the biological reactor.
MBR systems can be operated both at constant TMP and at constant flux; the modality at constant TMP is more effective from the energetic point of view and can be achieved by determining that the hydraulic level in filtration compartment is set at a certain value. However, usually constant flux processes are preferred, since they allow to prevent influent flow rate peaks.

6.9.2 Fouling in MBR systems

Fouling formation is strongly influenced by the characteristics of the liquid stream to be filtered, by hydrodynamic conditions, by membrane material and by module geometry. It is possible to define fouling as *the consequence of all the factors that give place to a deterioration of membrane performance* (Fane et al., 1989). Some of the previously quoted elements are particularly important in MBRs, since wastewater to be treated is highly heterogeneous and it is necessary, more than in other treatment techniques, to control energetic costs linked to the necessity of keeping the required hydrodynamic conditions.
Usually, reversibility of the fouling status is defined according to the possibility of recovering membrane performance. Most part of operators in MBR field states that *irreversible blocking situation is the one in which fouling is eliminable only with chemical treatment of the membrane*. Intrinsic heterogeneity characteristics of activated sludge present in MBRs make it very difficult to exactly define the effects that each element present in the feeding has on fouling. Several recent researches have shown that total suspended solids, colloids and dissolved macromolecules affect fouling.
In micro-ultrafiltration coupled to biological processes, fouling is formed for the following reasons:

- scaling;
- biofouling;
- adsorption/organic fouling;
- pores blocking;
- *cake layer* formation on membrane surface;
- accumulation of debris present in feeding stream.

Scaling is the precipitation of inorganic salts on membrane surface or inside the pores. This phenomenon takes place when solubility index of a salt present in feeding is overcome, and is favoured by polarization by concentration; for this reason salts precipitation is more likely to occur in nanofiltration and reverse osmosis processes. In MBR systems, scaling can occur in presence of processes of chemical phosphorus removal through simultaneous precipitation in presence of iron or manganese salts.

Biofouling is caused by the growth of biological films on membrane surface (Ridgway and Flemming, 1996). Usually it develops together with the other fouling mechanisms due to dissolved or particulate substances. Negative consequences on membrane processes can be summed up in the following points:

- increase of hydrodynamic resistance to filtration, with is correlated to increases of management costs associated to energetic consumptions, to chemical cleaning interventions and to the employers of the plant;
- increase of polarization by concentration. Biofilm produced on a membrane forms a gel layer that retains the particles rejected by the membrane, thus intensifying the back-diffusive transport;
- reduction of membrane life.

Interactions between sludge and membrane cause several solute adsorption phenomena on the membrane, both on the surface and inside the pores; this causes inevitably permeability reduction. This phenomenon is very evident on organic new generation membranes, constituted by the combination of more polymers, most of all in presence of wastewaters

containing particular solvents in aqueous solution. Membrane pore occlusion by particles smaller than pores diameter occurs very frequently.

The formation of a further filtering layer (*cake layer*) composed by dirtying material occurs when colloids, macromolecules, and other high molecular weight solutes are filtered. The main causes of cake layer formation are suspended solids and colloidal particles. A porous layer is formed on membrane surface and if TMP is too high, cake layer depth increases, thus causing the contemporary increase of resistance to filtration. At this point, in order to achieve the required permeate flux, TMP increases thus accelerating the formation of the dirtying layer. The accumulation of debris on filtering surface occurs when wastewater is not appropriately pre-treated before biological treatment in MBR. The consequences of the accumulation can be very serious and sometimes require the substitution of the damaged module.

6.9.3 Pre-treatments

High installation costs of the membranes suggest the opportunity of evaluating different available **pre-treatment technologies**, both for new plants and for developing existent plants. Actually, the presence of appropriate mechanical treatments ensures the removal of coarse material capable of damaging the membrane surface, and the elimination of colloidal compounds, of oils and of greases involved in fouling mechanisms. While designing the plant, pre-treatments typology choice is strongly influenced by the following parameters:

- available surface;
- chosen process scheme;
- typology of installed membrane;
- micro-grit spacing;
- energetic costs;
- disposal of sludge produced during the pre-treatment.

6.9.4 Membrane cleaning procedures

Ordinary membrane cleaning is done by inducing turbulent hydrodynamic conditions capable of slowing down phenomena of pores occlusion and polarization by concentration. These conditions are achieved in different ways, depending on plant configuration.
For *non-submerged membranes* it is necessary to prefigure the installation of a recirculation pump for feeding the filtration unit with the sludge taken from the biological section. Cleaning is thus achieved by imposing high recirculation rates on the filtering surface (1-5 m/s).
In *submerged membranes* applications, where modules are submerged inside the same biological compartment, the optimal hydrodynamic regime is obtained by inflating coarse air bubbles, so to determine membrane shaking and sludge movement close to the membrane/liquid interface. Stream speed achievable in this way are around 0-1 m/s, for air flow rates between 0.2 and 1.5 $m^3_{air}/m^2_{membrane} \cdot h$.
A possible alternative to these two systems is constituted by the combination of cleaning action deriving from sludge recirculation flow with the one coming from air inflation (Chua et al., 2002); both applications have been implemented with external and submerged membranes.

An accurate choice of membrane operating conditions can favour delay in fouling formation, thus overdrawing the mechanical cleaning action operated by sludge recirculation (MBR with external membrane) or by air inflation (MBR with submerged membrane). By stopping permeate extraction for a limited time length it is possible to reverse the TMP, which is the driving force responsible of filtration process. A subsequent membrane relaxation occurs, with reduction of the forces that retain the dirtying substances on the membrane surface; in this way removal of the compounds obtained with turbulence is very higher.
Periodical chemical cleaning procedures can contribute to removal of organic and inorganic compounds responsible of fouling. Duration, frequency and concentrations of chemical reagents to adopt for the cleaning procedure are strictly dependent on:

- typology of membrane (geometry, structure and material);
- characteristics of dirtying compound to be removed;
- qualitative characteristics required for the effluent;
- disposal of cleaning products.

It is convenient to calibrate chemical cleanings frequency, depending on specific characteristics of the system considered. An accurate optimization of cleaning operations favours a better conservation of membrane characteristics, thus prolonging its life time. Typically, organic compounds removal is made with sodium hypochlorite at chlorine concentration that can vary depending on membrane typology. High reagents concentrations make it necessary to carefully isolate filtration section subjected to cleaning, in order to avoid toxic effects on biomass. Chemical cleanings are not always able to restore original permeability. This is due to the fact that not all the dirtying compounds are really eliminated by means of chemical cleaning. In the case of biofouling, the use of hypochlorite determines exclusively the inactivation of the biomass responsible of fouling, but it does not imply its removal from membrane surface.

6.10 Experimentations performed on membrane fouling

Two kinds of fouling have been individuated by Ognier et al. (2002a), who stated that **fouling can be both reversible or irreversible**, depending on the possibility of eliminating it with chemical cleaning of membrane. They performed an experimentation in which a lab-scale MBR was equipped, and tests of different length were performed in order to check the effects of flux on fouling formation. The study showed that during short-term experiments, the transmembrane pressure increase was constant, and that an exponential relationship existed between the fouling rate and the flux. On the other hand, during long-term experiments, the transmembrane pressure increase could be considered as constant during the first 550 hours, but after that became suddenly unstable, with a d(TMP)/dt value of approximately 100 times higher than that of the first period. It was then concluded that the reversible nature of the fouling was different in the studied cases: while the fouling was *reversible* during the short-term experiments, in long-term experiments it proved to be *irreversible* during the first period, whereas the major part of the fouling that took place in the second period was reversible. This can be explained assuming that the fouling formation mechanism that occurs during the long-term experiments is different from the one that occurs in the short-term experiments, because long contact time causes the deposit of some compounds on membrane surface and thus an irreversible membrane pore blockage.
The **critical flux** concept, introduced by Field et al. (1995), can be used to control filtration operations because, according to this theory, no deposition occurs if the permeation flux is

under a critical value (usually called *sub-critical* flux, J_c). However, if this method can be adapted to suspensions of solid particles, it is more difficult to apply to macromolecular solutions.
According to Fane and Chang (2002), **critical flux** is the one at which deposition starts to begin. At this flux the forces of convection are just balanced by removal mechanisms, such as shear-induced diffusion. The measurement of J_c is most conveniently done by stepping flux and observing the TMP history: once the TMP starts to increase perceptibly with time, deposition has begun and the J_c is exceeded.
A deeper study on the **critical flux** concept was carried out by Defrance et al. (1999), who studied the difference of filtration conduced at fixed TMP and filtration conduced at fixed permeate flux. They performed tests with fixed permeate flux by progressively increasing permeate flow rate; they observed that for each fixed value of permeate flow rate, TMP increased gradually and stabilized at a certain value after around 15 minutes. This was observed until the flux was kept under a critical value (the sub-critical flux); above this critical flux value, the TMP increased rapidly and did not stabilize. The critical flux increased linearly with the velocity of permeate extracted. The effect of reaching the critical flux was the same both if it was obtained at constant TMP conditions and at constant flux conditions.
Another interesting study on **critical flux** application was performed by Le-Clech et al. (2003), who tested the effects of such parameters as membrane pore size and TSS concentration on the instauration of critical flux. They observed that the impact of membrane pore size on J_c is observed only at low pore size and/or at low TSS levels; besides, there was no significant difference for a shift from 4 to 8 g_{TSS}/l , but a significant increase of J_c was observed for a TSS increase to 12 g_{TSS}/l. It was also observed that the TSS effect on J_c was generally around twice that of the aeration effect. The calculation of mean sub-critical values for a range of pressure-related critical parameters revealed reduced short-term fouling at larger-pore sizes, but that this may be coupled with internal membrane fouling.
The effect of **adsorption** on membrane fouling in a MBR was studied by Ognier et al. (2002b). A particular group of protein solution (beta-lactoglobulins solutions) was tested in order to check how it could influence fouling formation, and the fouling formed after filtration of this solution was compared to the one observed after filtration of raw suspension and settled suspension. Before the study, it was assumed that *fouling formed due to the adsorption phenomenon is irreversible*, and that it remains also after back-washing. It was also observed that fouling caused by adsorption takes place at the beginning of the filtration, and that it causes specific hydraulic resistance of the same order of magnitude as the clean membrane resistance. Besides, it was observed that other phenomena, such as progressive pore clogging, can take place even if subcritical conditions are kept.
The theory stating that even if filtration flux is kept under **sub-critical value** irreversible fouling formation occurs, is confirmed by another experimentation by Ognier et al. (2004). It was observed that despite the choice of sub-critical flux, irreversible fouling formed on membrane surface. It was then hypothesized that open surface was reduced due to a progressive blocking of membrane pores. It could be then concluded that despite operating conditions held at constant flux, an increase occurs in the rate of circulation in the pores which remain open; this increase may reach critical local filtration conditions, resulting in the formation of deposits which are reversible but induce an abrupt change in transmembrane pressure.
Also **hydrodynamic conditions** hold in the system are particularly important for fouling formation: Liu et al. (2003) performed an experimentation in order to investigate these effects. They set up a series of experiments in which the varied operational parameters were suspended solids concentration (variable between 2 and 20 g/l), air flow (varied between 10

and 100 $m^3/(m^2 \cdot h)$), and filtration flux, which was varied between 4.5 and 27 $l/(m^2 \cdot h)$. Water cross-flow velocity was measured after the variation of each parameter, and membrane resistance to filtration was monitored. Filtration resistance was observed to increase, and this was deduced as a power function of the cross-flow velocity, filtration fluxes and SS concentrations. It was also observed that membrane filtration resistance sharply increased at a flux over the critical flux or at an aeration intensity below the critical aeration intensity.

Shear stresses caused by recirculation of sludge are often assumed as very important in order to slow down fouling formation process. In an experimentation performed by Wisniewski and Grasmick (1998), the effect of **shear stresses caused by recirculation of sludge** in a MBR system on fouling formation and on size distribution of the biological suspension was studied. During sludge recirculation inside the MBR, different linear velocities of recirculation were set, and samples of sludge under different velocities were taken, in order to carry out granulometric analyses and dead-end filtration tests. The results showed that variations of recirculation velocity induces changes of the particle size; this change causes the destruction of the floc, and the formation of many non-settleable micro-flocs, which modify sludge filterability. In the study, the effect of different classes of organic particles (soluble, colloidal, settleable) on membrane fouling was also quantified, and it was observed that **soluble fraction** gives the most important contribution to fouling formation.

Importance of **colloids and solutes** on fouling formation was studied also by Bouhabila et al. (2001); they concluded that air bubbling can be partially effective for fouling removal, because it's efficient only for limiting particle deposition and polarisation phenomena, but not for internal fouling prevention.

Mercier-Bonin et al. (2001) tested the effect on fouling formation of a **higher air flow inflation** and of the conditions of **flow unsteadiness**, which increase the turbulence in the system and allow the extraction of more permeate. This showed to be effective except for microfiltration, in which the phenomenon of particle debris on membrane pores was more evident, with the consequence of reducing permeate flux. Besides, the study showed that turbulence created by air inflation and by flow unsteadiness was effective in terms of gel layer reduction only if it was started at the very beginning of the filtration process.

Another study on the effect of **aeration on removal of cake layer and, subsequently, on suction pressure** was performed by Ueda et al. (1997). They observed that cake-removing efficiency of the uplifting air was influenced by the turbulence of the flow. This efficiency was improved either by augmenting air flow rate, or by augmenting aeration intensity (air flow rate per surface area) by compressing membrane modules over a smaller area.

Choo et al. (1996) observed that struvite precipitation on membrane surface due to filtration of an alcohol-distillery wastewater could cause the formation of a gel layer. Struvite is an **inorganic foulant**, whose precipitation on membrane surface together with microbial cells of the activated sludge strongly limit membrane permeation capability.

The effect of dissolved oxygen (DO) concentration and of mixing intensity on fouling formation in a MSBR (membrane sequence bioreactor) was studied by Kang et al. (2003). Sludge filterability at any phase was tested, and it was observed that **DO concentration and mixing intensity** were effective in any phases in terms of fouling reduction.

Magara and Itoh (1991) have experimentally verified that in a system working at constant TMP, fouling depends on **TSS concentration**. Also other authors (Ueda et al., 1996; Van Bentem et al., 2001; Wilkes et al., 1999) have verified that the increase in TSS concentration can determine a consistent increase of sludge viscosity, with subsequent permeability decrease. It was observed by Gui et al. (2002) that TMP increases if TSS concentration increases from 1 to 10 g/l, and they also observed that aeration can be helpful in TMP reduction mostly for high sludge concentration.

Besides, it is important to take into account the role of **Extracellular Polymeric Substances** (EPS) on fouling formation. EPS derive from cellular metabolism, and have a protective effect towards the cell, constituting a source of carbon substrate when external carbon sources are not available (Morgan et al., 1990). EPS are constituted by proteins, carbohydrates, humic substances and DNA residuals released by cellular metabolism.
Nagaoka et al. (1996, 1998, 1999, 2000) have demonstrated that EPS production rate tends to increase in correspondence of organic load increases.
Cho and Fane (2002) have demonstrated that the presence of high EPS concentration in MBR systems can cause sudden permeability decreases due to the establishment of local flux conditions that are very different from the ones set by the operator for the whole process.
According to Drews et al. (2005), there is a linear relationship between fouling rate (intended as the increase of filtration resistance in time), and **polysaccharide concentration**.
A further affecting factor is given by the **non-stationarity** of the processes, such as the intermittent feeding.
Membrane geometric shape exerts a strong influence on blocking dynamics, since it is able to influence deposit pathways of substances causing fouling.
Experimentations performed in the past years have been mostly focused on hollow fibres MBR systems, that have a high specific surface per occupied volume.
Carrol and Booker (2000) have proposed a physical model for fouling; in particular, they have individuated three phases for fouling development:

- a first phase in which fouling rate is independent from fibres length and from membrane surface;
- a second phase in which fibres length influences the phenomenon (longer fibres are associated to lower fouling rates);
- a third phase in which flux has already decreased in comparison to the initial value, axial fouling characteristics are less relevant and thus the achieved stationary state depends more on module surface than on fibres length.

Chang and Fane (2001) have evaluated the effect of **diameter and fibres length** on a submerged fibres MBR performance. Tests performed at constant pressure have shown that fibres diameter effect is as higher as the turbulence induced by air inflation. An excessive decrease of fibres diameter determines a non-homogeneous flux distribution, with subsequent formation of high local fluxes which are harmful for the global filtering process. Besides, the use of a fibre with too small inner diameter causes a higher pressure gap in vertical direction, thus making it necessary to apply a higher TMP in order to achieve the same permeate flux.

6.10.1 Use of MBRs for the degradation of particular typologies of wastewater

One of the most interesting use of MBR systems is the degradation of compounds with high molecular weight; retention of large molecules in the biological system operated by membrane pores make it possible to keep on their degradation until they are reduced to smaller and simpler molecules, which are more easily degradable.
One of the study performed on degradation of **high molecular weight compounds** was the one by Cicek et al. (1998). They observed that the membrane was effective in retaining **heterotrophic microorganisms and MS-2 viruses**, thus eliminating the need of effluent disinfection. Casein was chosen as high molecular weight compound to be degraded, and by means of respirometric tests, it was shown that with high influent casein concentration, its

degradation was slow and nitrification did not occur; instead, if influent casein concentration was low, its complete degradation occurred very fast, and nitrification took place.
Retention of MS2-viruses by membrane was also observed by Winnen et al. (1996).
Several applications of MBR have been used for treating **industrial wastewater**.
A high-strength **food industry** wastewater has been efficiently treated by Scott et al. (1998), who used the membrane both for air inflation into the biological system and for filtration of the mixed liquor. Actually the ceramic micro-porous membrane utilized ensured a 20-70% higher aeration efficiency than the traditional ring-spargers. COD influent value of 9600 mg/l and BOD_5 influent value of 5300 mg/l were reduced by 95%, while suspended solids concentration in the effluent was kept under 25 mg/l.
Oily wastewater from an automobile engine manufacturing plant was treated with an MBR in a study by Seo et al. (1997). The plant was operated at different HRT and COD removal of more than 90% was obtained with HRT higher than 10d, thus indicating that this kind of wastewater was degradable with an MBR system equipped with an ultrafiltration polysulfone hollow fibre membrane.
One of the strong point of MBR system is the possibility of dealing with biological degradation at high sludge concentration, that allows the treatment of **high organic load wastewater**. This was studied in an experimentation performed by Holler et al. (2001); they operated treatment of high organic load municipal wastewater in an MBR with HRT of 1.5 hours. The organic load rate was varied from 6 to 13 kg/(m^3·d) and biomass concentration varied between 10 to 22 g/l.

6.10.2 Experimental studies on optimization of MBR systems design

Many studies have been performed in order to identify the most effective configurations of MBR systems, both in terms of removal efficiency and of installation and management costs.
Judd et al. (2002) evaluated advantages of both **plate frame and hollow fibre** MBR systems, by analyzing two real MBR plants, each of them using one of the two membrane module configurations; the hollow fibres were by Zenon Inc., while the plate frame was by Kubota. Apart from the membranes configurations, the two systems were very similar for influent flow rate, COD removal efficiency and therefore for effluent quality. Thus, the comparison between the two plants was done in terms of fouling formation and membrane cleaning costs. Hollow fibres systems show to be less expensive to be produced than plate frame, and are also backwashable, while plate frame are not. On the other hand, due to the easiness of membrane cleaning by means of turbulence created on membrane surface, plate frame modules are less subjected to membrane fouling than hollow fibres systems. Because of the lack of possibility of backwash in plate frame module, it is necessary to operate them in strict sub-critical flux conditions. Due to the higher easiness of hollow fibre to be blocked by small particles, often MBR plants with this typology of membrane module are equipped with a fine screen upstream the biological section. Both studied systems ensured the retention of 5-log of Faecal Coliform, and both operated at high TSS concentration (around 15 g/l), associated to high SRT (>20 d) and low HRT (<4 hours). Also in terms of odours emissions, both systems were equal, and no significant odour was detectable near the plant.
Membrane modules cost must be considered: plate frame modules are more expensive than hollow fibres ones, but are simple to manage, since no backwash is required. Besides, for both systems, the highest cost is due to aeration, which requires an effort of 0.3-2 $kWh/m^3_{treatedww}$.
Fane and Chang (2002) individuated some **physical properties of membranes** that favour their use in MBR systems:

- *hydrophilicity* of polymers constituting the membrane, that prevent fouling caused by biosolids and biosolutes;
- *robustness* of the material to chemical cleaning agents, and ability to cyclic stresses, particularly if air-blubbing and backwash are operated;
- *modest costs* of the components;
- *ease of fabrications*, because some materials are more suitable to be extruded in hollow fibres or processed into micropores.

Also **design** of the MBR must be considered if management costs and simplicity want to be achieved. The design influences such factors as energy demand, ease of cleaning and replacement of damaged membrane modules. The importance of these factors make flat sheet systems preferable than tubular, hollow fibres and spiral wounded modules.
Fibre orientation is also very important for MBR performance: in the same study, Fane and Chang (2002) observed that the performance of the submerged hollow fibre membrane varies in complex ways, depending on **fibre size** and **with or without air-bubbling**.

- Without bubbling: for small fibres (id/od = 0.39/0.65) transverse is better than axial, and performance is sensitive to liquid crossflow, while for larger fibres (id/od = 1.8/2.7) the benefit of transverse flow decreases with crossflow, and at a moderate crossflow axial becomes better than transverse;
- With bubbling (typical for MBRs): axial orientation is better than transverse. There is evidence of trapped bubbles in the transverse orientation. This finding supports the use of vertical axial fibres in submerged MBRs.

In an evaluation of **MBR management costs** depending on operational parameters, Yoon et al. (2004) have individuated for municipal wastewater an economically optimum HRT of 16 hours and TSS concentration of 11 g/l; with these parameters, aeration for biodegradation of organic compounds would be 13.3 m^3/(air·min) if the influent rate was 1000 m^3/d.
In order to define the most effective MBR system configuration, a **high density packed hollow fibres system and a low density packed ceramic tubular membrane system** were compared in a study by Shimizu et al. (1996). The best operational parameters for the high density packed hollow fibres system were individuated, and it was observed that the critical flux had the same values for both the ceramic and the hollow fibres systems.
A MBR system operated with filtration by **gravity** (instead of filtration by suction charge) was equipped and studied by Ueda and Hata (1999); in this system a good treatment efficiency of a domestic wastewater was achieved, and MBR could be operated for more than 350 days without interruption for chemical cleaning of membrane.
The effect of the use of a **rotary pump** for sludge recirculation on MBR performance was studied by Kim et al. (2001). They observed that rotary pump caused high shear stresses to microbial particles, causing floc breakage, with subsequent liberation of colloids and organic particles that caused the formation of a cake layer on membrane surface, thus reducing its filtration efficiency.
Energetic costs associated to a MBR management were studied by Zhang et al. (2003), who found out that filtration is the most energy consuming process of a MBR system: pumps, aeration, pipe system and sludge return velocity consume only the 37.66-52.20% of the total energy of the system.

Chapter 7.
Materials and Methods

7.1 Analytical procedures

The substances studied in this research were 1,2-dichloroethane (1,2-DCA), 1,2-dichlorobenzene (1,2-DCB), trichloroethylene (TCE), 2-chlorophenol (2-CP) and phenol. Nitrates and chlorides were also measured in order to verify the complete mineralization of chlorinated compounds, and the possible instauration of anoxic conditions.
Due to their high volatility nature, 1,2-DCA, 1,2-DCB and TCE have been analyzed by means of gas-chromatography (GC), while 2-CP and phenol have been analyzed by means of High Performance Liquid Chromatography (HPLC). Nitrates and chlorides were analyzed by means of an Ion-Chromatograph (IC).

7.1.1 Analytical procedures for 1,2-DCA, 1,2-DCB and TCE

7.1.1.1 Gas-chromatographic analysis

In this experimentation, a **GC Agilent 6890N** has been used. It was equipped with two detectors, a FID (Flame Ionization Detector) and an ECD (Electron Capture Detector).
The column for chromatographic separation was a capillary one; this kind of columns is specifically indicated for the analysis of chlorinated volatile compounds. Stationary phase of the column was a gel layer inside the column, while mobile phase was the carrier gas inflated into the column, which carried the volatilized analyte. As for any gas-chromatograph, when exposed to high temperatures, the column must always have carrier gas inside itself in order to avoid damages to stationary phase.
Capillary columns are becoming more and more widely used for precise and reliable measurements of chlorinated compounds, due to their good capability of separating the substances to be analyzed independently from possible impurities present in the sample.
In this experimentation, the gas-chromatographic analysis was performed by means of a GC coupled to an autosampler; more details on the GC characteristics are listed in Table 7.1.
When the GC is connected to an autosampler, the sample in gaseous form is injected into the GC by means of the autosampler needle, which is threaded into the GC injector.
After injection, the gaseous sample is driven into a *liner*, which is a glass small tube of about 10 cm length, of variable inner shape, depending on the properties of the compound to be analyzed. In this experimentation, two kinds of liners were tested: one with inner conical section, and another one with inner regular cylindrical section. After a comparison between the two liners, more reliable results were observed to be achieved with the second liner, the one with inner cylindrical constant section, and this was chosen for all the analysis performed in the experimentation.
The gaseous sample, containing the substance to be analyzed (the so called *analyte*) after its injection into the liner, is dragged out of it by the carrier gas flow, which is usually nitrogen or helium.
Carrier gas with the analyte flows into the capillary column, which is installed in the ***oven*** of the GC; the oven is one of the most important sections of the GC, since the separation process which allows the chromatographic analysis is dependent from the temperatures hold in the oven. During the analysis, the oven temperature can be set at a certain temperature, or it can

be varied, thus giving place to the so called *temperature ramps*. Temperature ramps contain the information concerning the variations of temperature in the oven, and the duration of each temperature condition; subsequently, the length of the whole analysis can be calculated.
In this experimentation, oven was kept at a fixed temperature for 1,2-DCA and TCE analysis, while a temperature ramp was arranged for 1,2-DCB analysis. In Table 7.2 more details on temperature ramps used in this experimentation are shown.
The analyte is carried by the carrier gas in the column, where it is retained for a variable time length, depending on the higher or lower affinity of the analyte to the stationary phase. Since the carrier gas flows continuously into the column, the analyte is sooner or later dragged out of the column.
At the end of the column, the carrier gas together with the analyte is forced off the column by a *make-up* gas, which drives the gas toward the detectors.
In a GC, one or more **detectors** are present, whose role is that of giving a measure of the quantity of analyte present in the analyzed sample.
The two detectors used in this experimentation were FID and ECD.
FID (Flame Ionization Detector) is a destructive, non-selective and wide range detector, which is capable of detecting almost all substances, with the exception of water. It works on the principle that a flame is continuously fed into the detector by means of hydrogen and air flow; the external part of the FID is connected to the earth, and acts as a negative electrode, while another electrode (the positive one) is present inside the FID. When only the carrier gas flows through the FID, the gas is burned and decomposed into its ions by the destructive power of the flame present in the FID; the electrons that are liberated in this way give place to an electric flow between positive and negative electrodes, or, in other terms, to a currency signal. Since the electrodes are usually coupled to a signal translator, such as a computer, the currency signal is detected and visualized as a line which represent the stable signal, or *baseline*. When the analyte flows through the FID, carried by the carrier gas, the flame destroys the analyte molecules, causing the liberation of electrons which alters the baseline signal, giving an additive currency, which is detected by the computer and gives place to the chromatographic peaks.
ECD (Electron Capture Detector) is a selective, non-destructive detector, capable of detecting only halogen substances, for which is more sensitive than FID. ECD contains a small radioactive source, which continuously liberates beta-radiations (electrons). The electrons source constitutes a negative electrode, while a positive electrode is placed at a small distance; thus, the emitted electrons generate an electrical currency. When the carrier gas flows through the ECD, the electrons hit the gas carrier molecules, and give place to an electrical currency which corresponds to the *baseline*. When halogen substances flow through the ECD, due to their high electronegativity they capture the free electrons present between the two electrodes of the detector, and give place to a decrease of the electrical currency. The signal decrease is amplified by a proper device and is recorded and converted by a computer, giving place to the chromatographic peaks.
The highest is the analyte quantity, the larger will be the chromatographic peak area.

Thus, in a **chromatogram**, the *time* at which the peak occurs gives indication of the *kind* of substance analyzed, since retention time into the column depends on the substance affinity to the stationary phase, and is typical of each different substance. Instead, the *area* of the chromatographic peak gives measure of the *amount* of the substance detected.
The most used methodology for quantification of the amount of analyte detected is the use of a **standard** of known concentration. The substance to be detected is used in pure form to

prepare a solution of known concentration which is analyzed; by the comparison of its area with the one of the analyte, it is possible to calculate the analyte concentration.

Table 7.1. Characteristics of the gas-chromatograph used in the research.

Column	Capillary, HP-5
Column length	30 meters
Column inner diameter	0.32 mm
Column stationary phase film depth	0.25 μm
Column temperatures interval	-60 - +250 °C
Injector temperature	200°C
Injector pressure	200 psi
Carrier gas	Helium
Carrier gas flow rate	10 ml/min
FID Make up gas	Helium
FID Make up gas flow rate	10 ml/min
ECD Make up gas	Nitrogen
ECD Make up gas flow rate	60 ml/min
FID temperature	250°C
ECD temperature	250°C

Table 7.2. Temperature rates for the analysis of compounds studied in the research.

1,2-DCA	35°C hold for 2 minutes
TCE	35°C hold for 2 minutes
1,2-DCB	35°C for 2 minutes; at 35°C/min up to 70°C; at 45°C/min up to 190°C

7.1.1.2 Headspace Autosampler

In this experimentation, an Agilent 7694E Headspace Sampler operated in combination with the GC.

The Autosampler worked preparing the samples to the injection into the GC, and operating the injection.

Samples to be analyzed were put into a 10 ml volume vial, which were filled with only 5 ml of aqueous sample. Filling the vial with only the half of the volume available had the purpose of leaving an empty space in the vial, which is usually called *headspace*. After being filled with the sample, the vial was sealed with a silicone/PTFE sept, together with an aluminum seal, which ensured the total sealing of the vial, and did not allow the exit of the gaseous phase. In Figure 7.1, a typical 10 ml vial used for sampling the reactor activated sludge is shown.

Figure 7.1. Ten-milliliters vial for the sampling of reactors sludge.

The samples in the vials were then analyzed using the **headspace technique**. Headspace is a reliable analytical technique consisting of heating the vials at a certain temperature (which is usually set at around 10°C less than the evaporation temperature of the substance to be analyzed) for a certain time length. Due to the heating, the substance present in liquid phase of the vial evaporates, until the equilibrium between the substance concentration in liquid and gaseous phase is reached, according to Henry's law. Heating time is tested experimentally, and is set at the time that ensures the achievement of the equilibrium between the two phases.
The autosampler consists of a *carousel* where the vials containing the samples to be analyzed are placed; here they are stirred and heated for a certain time, until a vial is taken and a needle is inserted into it. A gas (usually helium) is then inflated in the vial causing an overpressure; when the gas inflation is stopped, the overpressure generated into the vial headspace causes the release of the gaseous atmosphere into a connector tube, which is isolated and thermically conditioned; it leads the gas from the vial atmosphere to the injector of the connected GC.
More details on the operational parameters set in the autosampler used in this research are shown in Table 7.3.

Table 7.3. Operational parameters of the autosampler used in the research.

Heating time	10 minutes
Stirring time	1 minute
Heating temperature	70°C
Gas inflated	Helium
Connector temperature	80°C
Injection time	1 minute

For each analysis session, in which it was possible to analyze a maximum amount of 12 samples, two standard samples were analyzed. They were prepared in 20 ml volume vials, in which 10 ml of bidistilled water plus a certain amount of the chlorinated compound to be analyzed in pure form were added. Standards were created in order to have a chlorinated compound concentration of the same order of magnitude of the substances to be analyzed.
The software used for the GC management, and for the elaboration of the results, was the Agilent Chemstation.

7.1.2 Analytical procedures for phenol and 2-CP

7.1.2.1 HPLC analysis

In this experimentation a HPLC Dionex (P680 HPLC Pump and UVD170U detector) was used. It was equipped with an UV-Lamp detector and a C_8 column.
A HPLC is schematically constituted by a pump system, a separation column and a detector. An injector allows to inject the sample into the chromatographic column; the sample, which is in liquid form, right after its injection is driven into the column by a liquid eluent, of variable composition. When the sample enters the column, the analyte is retained in the column for a variable time length, depending on its affinity to the stationary phase of the column. Sooner or later, due to the eluent flow, the analyte is flushed off the column and forced into a detector. The detector is sensitive to the amount of analyte, and sends a signal to a recorder (usually a computer), which gives as output a chromatogram. As for gas-chromatography, the time at which the substance is revealed gives an indication of its nature, while the area of the chromatographic peak gives measure of the amount of analyte present in the sample.
The HPLC used in this experimentation worked based on the principle of the so called "RP-HPLC" (Reversed Phase HPLC); this consists of a non-polar stationary phase and an aqueous polar mobile phase. The stationary phase of the column used is a silica which has been treated with RMe_2SiCl, where R is the straight chain alkyl group C_8H_{17}. Due to the attraction between non-polar substances, the retention time is longer for non-polar molecules, while polar molecules are eluted more readily. For the same reason, retention time is increased by the addition of polar solvent to the mobile phase and decreased by the addition of more hydrophobic solvents. Reverse phase HPLC (which is nowadays the most commonly used) operates on the principle of hydrophobic interactions, which result from repulsive forces between a polar eluent, the relatively non-polar analyte, and the non-polar stationary phase. The binding of the analyte to the stationary phase is proportional to the contact surface area around the non-polar segment of the analyte molecule upon association with the ligand in the aqueous eluent; the retention can be decreased by adding less-polar solvents such as methanol and acetonitrile into the mobile phase to reduce the surface tension of water. Another important component is the influence of the pH since this can change the hydrophobicity of the analyte; for this reason in this experimentation a buffering agent constituted by 85% H_3PO_4 at 0.04% w/w in bidistilled water was used as pH control agent.
Eluent can consist of different phases in different percentages, which can vary during the analysis. If the sample to be analyzed is in an aqueous matrix, the eluent must be composed mostly by water. Usually the eluent is acidified by addition of phosphoric acid, because HPLC columns work at fixed pH values, so that sometimes sample pH needs to be conditioned. Eluent contains also an organic matrix, usually methanol or acetonitrile, which give the required polarity for the chromatographic separation in the column.
In Table 7.4, characteristics and operational parameters of the HPLC used in this research are listed, and in Table 7.5 the composition of the eluent used in this experimentation is shown.

Table 7.4. Operational parameters of the HPLC used in the research.

Column type	C_8
Column length	150 mm
Column width	4.6 mm
Film thickness	5 μm
Eluent flow rate	1.5 ml/min
Detector Wavelength	218 nm

Table 7.5. Eluent composition.

Eluent composition	66% solution A; 34% solution B
Solution A composition	85% H_3PO_4 at 0.04% w/w in bidistilled water
Solution B composition	Pure acetonitrile

The HPLC is controlled by the software Dionex Chromeleon; it allows the management of operational parameters of the HPLC and the elaboration of the analytical results, with the subsequent production of chromatograms. Standard solutions of the substances to be analyzed in pure form are periodically injected, and a calibration line is calculated; based on this calibration line, the software gives as an output the concentrations of the substances detected during the analysis. In Figure 7.2 the HPLC used in this experimentation is shown.

Figure 7.2. HPLC used in the experimentation.

7.1.3 Analytical procedures for nitrates and chlorides measurement

7.1.3.1 Ion-chromatographic analysis

Measures of nitrates and chlorides were performed by means of a liquid Ion chromatograph, the Dionex ICS-90, equipped with an electrical conductivity detector.

The main components of an Ion Chromatograph are an injector, a high pressure pump system, a separation column, a liquid eluent, a regenerating solution, a chemical suppressor and a conductimetric cell.

The injector is usually very small, and contains around 10 µl of sample; once the sample is injected, it is flushed away by the eluent and is driven toward the column.

Eluent carries the sample into the system; from its storage bottle, which is pressurized by nitrogen, the eluent is forced into the pump and passes through the pressure transducer. The flow passes also through a *dumper* which minimizes pressure variations and thus the base rumor. Eluent is then forced into the injection cell, where it joins the injected sample, and together they are driven into the system.

The column is made of polymeric resin, and contains the basic functional groups electrically charged, capable of making reversible exchanges of anions with the solution injected. In the column, the stationary phase is solid, while the mobile phase is the liquid constituted by the eluent and the sample, which is forced into the column by the pump pushing action. Separation mechanism is based on the ionic exchange between stationary phase and mobile

phase. Different ions move through the column with different velocities, depending on their affinity and interactions with the ionic exchange sites.
Downstream the column there is the *chemical suppressor*, that selectively detects the ions, reduces the baseline and amplifies the analyte signal. The suppressor uses a combination of ionic exchange and chemical regeneration for neutralizing the electrolytes present in the eluent. Eluent and regenerating solutions react in the suppressor, and eluent conductivity is thus reduced. Regenerating solution is a H_2SO_4 solution diluted 1:200; it flows continuously through the suppressor, regenerating the active sites. The eluent itself flows off the cell, pressurizes the regenerating solution and forces it into the suppressor.
When the sample flows off the suppressor, it enters a conductimetric cell, that measures electrical conductivity of the sample, and produces a signal which is transmitted to the management software. Inside the cell two electrodes and a temperature sensor for a possible compensation are present.
In Table 7.6, the operational parameters of the Ion Chromatograph used in this research are reported.

Table 7.6. Operating parameters of the Ion Chromatograph used in the research.

Eluent composition	Solution AS14 diluted 1:1000
AS14 solution composition	90.7% bidistillated water; 8.5% Na_2CO_3 8mM; 0.8% $NaHCO_3$ 1mM
Eluent pressure	0.55-0.83 MPa (80-120 psi)
Temperature range	4-40°C
Detector conductivity range	0.01 µS-3000 µS

The Ion Chromatograph used in this experimentation was controlled by the software Dionex Chromeleon; it allows the management of operational parameters of the Ion Chromatograph and the elaboration of the analysis results, with the subsequent production of chromatograms. Standard solutions of the substances to be analyzed in pure form are periodically injected, and a calibration line is calculated by the software; based on this calibration line, it gives as an output the concentrations of the substances detected during the analysis.
In Figure 7.3, the Ion Cromatograph is shown.

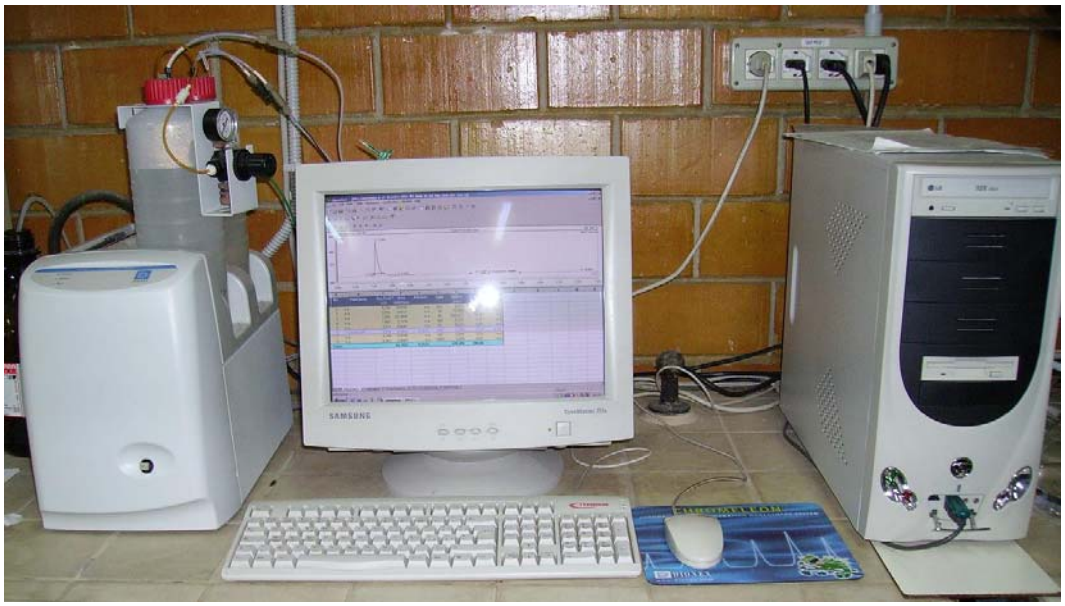

Figure 7.3. The Ion Chromatograph used in this experimentation

7.1.4 Total and volatile suspended solids measurement

Once a week, a certain volume *V* of mixed liquor (usually 50 ml) was taken from the reactor, and was used to measure the concentration of total and volatile suspended solids.
The measure started by weighting a fiber glass filter, of the porosity of 1.2 μm; this weight constituted the measure *A*.
The sludge taken from the reactor was then filtered with the weighted filter, and the filter with the filtered sludge was put into an oven kept at 105°C for at least 24 hours. The weight of the dried sludge and the filter constituted the measure *B*.
The filter with the sludge was then put into the muffles at 560°C for one hour; the weight of the filter and of the inorganic part (non volatile) of the sludge was the measure *C*.
Total suspended solids (TSS) were calculated in this way:

$$TSS = \frac{B - A}{V},$$

while the volatile suspended solids (VSS) measure was calculated with this formula:

$$VSS = \frac{B - C}{V}.$$

7.2 Materials and methods adopted in the experimentation

7.2.1 Equipments used for carrying out biological processes

The experimental activity carried out in this study developed by performing biodegradation tests on synthetic groundwater, i.e. a groundwater realized in laboratory by mixing distilled water and a certain amount of chlorinated compounds.
Biodegradative processes were carried out in three typologies of biological reactors:

- batch tests;
- SBR;
- MSBR.

7.2.1.1 Batch tests

Batch tests had the purpose of checking if acclimated or fresh sludge was capable of degrading the different chlorinated compounds studied in the experimentation.
Batch reactors were consisted of bottles of 1 liter volume, with a proper modification in the entrance, so that they were sealable by means of the same septs and seals used for sealing the vials. Thus, vapors of the volatile substance present in the bottle headspace were hold in the bottle, and any substance loss by volatilization was prevented.
Bottles were partially filled with fresh or acclimated sludge, sealed, and then an aqueous solution of chlorinated compounds was injected. Sludge and chlorinated compounds solution were stirred continuously by means of a magnetic stirrer, and the bottles were usually kept at the constant temperature of 30°C, with the exception of some particular tests made in order to check degradative ability of the biomass at different temperatures.
Batch reactors can be seen in Figure 7.4, were the magnetic stirrer used and the thermostatic chamber in which the bottles are stored are visible.

Figure 7.4. Batch tests used in the experimentation; the magnetic stirrer and the thermostatic chamber are visible.

7.2.1.2 SBR

A biological reactor operated in SBR modality was used in the research for three main purposes:

- the achievement of a primary acclimation of the fresh sludge first to 1,2-DCA, then to 1,2-DCB and 2-CP;
- the SBR was operated in parallel with the MSBR, fed with the same substances, and usually kept at the same operating conditions, in order to compare its degradative efficiency with the one of the MSBR;
- finally the SBR was used in order to check the effect that the variation of some operating parameters had on chlorinated compounds degradation.

The SBR used in this research was a glass vessel, of the total volume of 3 liters, of which only 2.1 liters were filled with mixed liquor. The empty space left in the reactor constituted the so called *headspace*, which ensured the oxygen amount necessary for the completion of oxidative biological processes.

The reactor was sealed, and all inputs and outputs toward the external of the reactor were controlled; gas transfers between inside and outside the reactor were allowed only at the beginning and at the end of the SBR cycle. During the React phase, no connection of the reactor atmosphere to the external was allowed, in order to avoid loss by volatilization of chlorinated compounds; actually, their high volatility would cause their fast loss. Sealing was achieved by means of the particular design of the cap, which was perfectly linked to the glass vessel; also reactor input and output valves and connections were perfectly sealed, due to the use of silicone/PTFE caps, Teflon tape and silicone grease paste.

Mixed liquor inside the reactor was always stirred by means of a stirring engine connected with an axial driveshaft, designed so that no free space that could allow the loss of gas from the reactor atmosphere was present.

Reactor cap was equipped with:

- input for chlorinated compounds aqueous solution feeding;
- input for mineral medium feeding;
- inputs for dosage of acid and base, in order to keep a constant pH value in the mixed liquor;
- support for a pH probe;
- support for a dissolved oxygen measurement probe;

- support for temperature probe;
- output for biological sludge sampling;
- output for extraction of clarified supernatant;
- output equipped with a relief non-return valve.

Mineral medium, chlorinated compounds solution and supernatant inputs and outputs were connected to peristaltic pumps, which were automatically governed by means of a timer, according to the times fixed depending on the SBR cycle duration. The tubes of connection between pumps and reactor were made of Viton®, a particular material which does not adsorbs chlorinated compounds.
Relief non-return valve had the purpose of allowing the gas produced during biological processes to flow off the reactor, so to avoid a possible overpressure inside the reactor; at the same time it prevented external air to enter the reactor, which could cause dilution of the reactor atmosphere.
Oxygen and pH control was achieved by means of connection of the reactor probes to a Biostat MD®, which recorded pH and dissolved oxygen (DO) values and governed peristaltic pumps for the dosage of acid and base into the reactor, in order to keep pH at the value of 7.
Biostat MD® was also used for the control of temperature, which was kept at 30°C (with the exception of particular phases of the experimentation) by means of a water jacket heater external to the vessel, in which warm water flowed.
The reactor was kept in a mobile hood (Asalair Carbo 900 Exhaust), equipped with activated carbon filter for the capture of volatile organic compounds.
The reactor and its complete equipment can be seen in Figure 7.5.

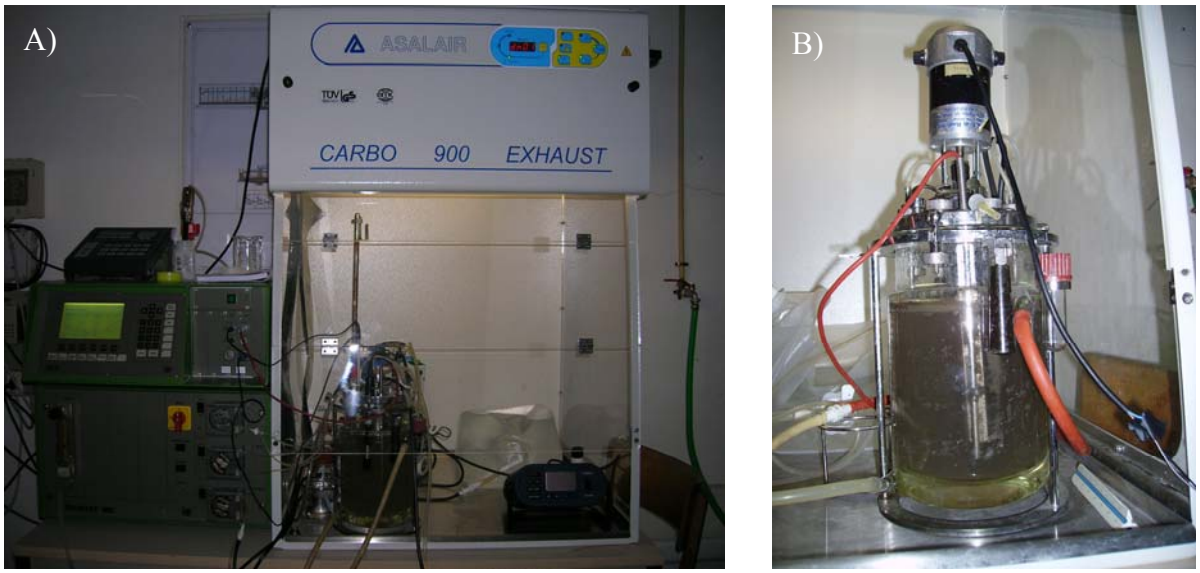

Figure 7.5. A) SBR reactor connected to the Biostat MD, and enclosed into the hood; B) SBR reactor in detail.

7.2.1.3 MSBR

Biomass acclimated to 1,2-DCA and 1,2-DCB was inoculated into a Membrane Sequencing Batch Reactor (MSBR), in order to perform biological degradation of all chlorinated compounds fed both singularly and in various combinations.
The MSBR was equipped like the SBR in order to avoid the volatilization of the compounds fed and to ensure the isolation of the reactor headspace from the external atmosphere. Therefore, the cap of the MSBR had the same shape of the SBR one, with the only difference

that the output for the extraction of clarified supernatant was substituted by an output connected with the membrane module for extraction of supernatant, an input connected with the membrane module for air inflation and an output equipped with a relief non-return valve.
Outputs and inputs for mineral medium and chlorinated compounds feeding, and for air inflation and filtration were connected to peristaltic pumps, which were automatically governed by means of a timer, according to the times fixed depending on the SBR cycle duration. The tubes of connection between pumps and reactor were made of Viton®, a particular material which does not adsorbs chlorinated compounds.
Relief non-return valve had the purpose of allowing the gas produced during biological processes to flow off the reactor, so to avoid a possible overpressure inside the reactor; at the same time it prevented external air to enter the reactor, which could allow dilution of the reactor atmosphere.
Oxygen and pH control was achieved by means of connection of the reactor probes to a Biostat MD®, which recorded pH and dissolved oxygen (DO) values and governed peristaltic pumps for the dosage of acid and base into the reactor, in order to keep pH at the value of 7.
Biostat MD® was also used for the control of temperature, which was kept at 30°C (with the exception of particular phases of the experimentation) by means of a water jacket heater external to the vessel, in which warm water flew.
The reactor was kept in a mobile hood (Asalair Carbo 900 Exhaust), equipped with activated carbon filter for the capture of volatile organic compounds.
The reactor and its complete equipment can be seen in Figure 7.6.

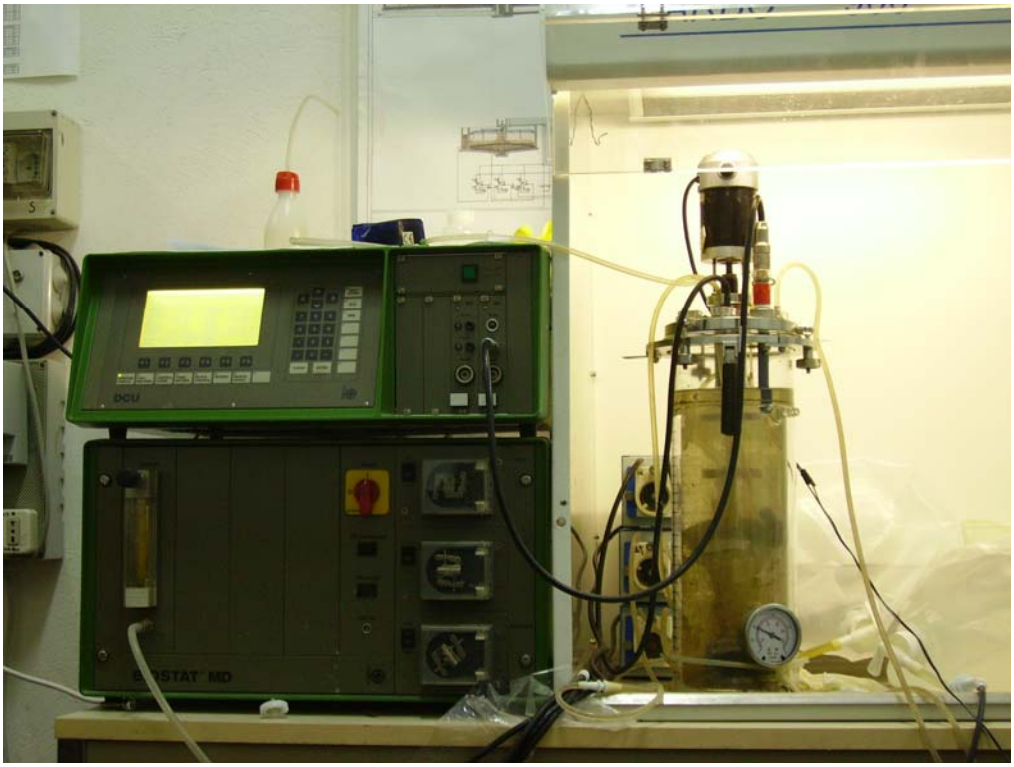

Figure 7.6. The MSBR and its complete equipment

Membrane module

Differing from a traditional SBR system, in the MSBR the extraction of supernatant was achieved by means of filtration of mixed liquor instead of discharge of clarified supernatant.

Thus, inside the MSBR a **membrane module** was placed, whose extraction valve was connected to two peristaltic pumps working in different flow directions: one pump operated filtration by applying a suction charge, while the other operated backwash by pumping fresh water inside the membrane module.
Membrane module was a Zee-Weed® (ZW1) by Zenon Inc. It was an ultrafiltration module, made by polymeric, organic, hydrophilic, non-ionic and chlorine-resistant material; its structure was asymmetric.
Pores diameter was 0.04 μm and membrane surface had an area of 0.047 m^2.
Specific surface was 10000 m^2/m^3. Filtration occurred with a *cross flow* scheme, and the permeate flux followed an outside-inside direction.
Membrane module was **hollow fiber**; many capillary tubes were placed in U-shape in a sealed tube, and their extremities were fixed into two septs of epoxic resin.
Fibers were grouped all together and the permeate extracted from each of them was collected by a central collector, connected to the external pump that applies the suction charge causing the extraction of the permeate.
Another central tube, coaxial with the permeate one and holed at regular distances, allowed the aeration of the membrane module: air inflation from the external by means of a proper valve, connected to an aerator, formed bubbles that caused turbulence in the mixed liquor and helped in removing fouling. For the previously explained reasons, air inflation was active only during filtration phase.
Operational parameters of the ZW1 membrane module are listed in Table 7.7.

Table 7.7. Operational parameters of the ZW1 membrane module by Zenon Inc.

Typical operating TMP	-(1-0.5) bar (-(10-50) kPa)
Maximum applicable suction charge (TMP_{max})	0.62 bar (62 kPa)
Optimal exercise flux	23.7 l/(h·m^2)
Maximum flux	35 l/(h·m^2)
Maximum TMP backpulse pressure	0.55 bar (55 kPa)
Maximum water backwash flux	35.5 l/(h·m^2)
Maximum aeration flux	3.6 l/(h·m^2)
Maximum hypochlorite exposure	1 g/l
Maximum operating and cleaning temperature	40°C
Operating pH range	5-9

7.2.2 Operational methods used in the experimentation

7.2.2.1 Batch tests

Batch tests were performed by inoculating 300 ml of fresh or acclimated sludge (depending on the kind of test that was going to be performed) into the 1 liter volume batch; TSS and VSS concentration of the sludge were measured before the test.
In parallel to the batch inoculated with activated sludge, another batch was always equipped (**blank batch**), which was filled with 300 ml of water and with the proper amount of chlorinated compounds solutions. The blank batch had the purpose of allowing the control of abiotic degradation of the chlorinated compound fed, so that a comparison with the biological degradation observed in the other batch was possible.
Once the sludge was inside the bottle, it was sealed, and 5 ml of aqueous solution of the chlorinated compound to be degraded was injected into the bottle by means of a 5 ml syringe equipped with an hypodermic needle. The aqueous solution of chlorinated compound was

prepared with a certain concentration, so that the desired concentration of chlorinated compound inside the bottle at the beginning of the test was ensured.
Right after the injection of the chlorinated compound solution, the bottle was placed on the magnetic stirrer, and the mixed liquor inside the bottle was stirred for around ten seconds. The first sample of the batch test was then immediately taken, by means of a 5 ml syringe, paying particular attention to avoid an excessive turbulence of the sample, which could cause volatilization of the chlorinated compound present in the sample. The sample was then rapidly but gently moved to a 10 ml vial, which was immediately sealed. Following the same procedure, other samples were taken from the bottle at fixed time intervals (usually one hour) and at the end of the test.
Usually, two 5 ml samples were taken from the bottle, in order to perform a double analysis and get two comparable concentration values; this allowed to have an indication of possible errors made during the analysis.
If the test was to be performed in aerobic conditions, sludge was inflated with air for around ten minutes before the test beginning, in order to supply the oxygen necessary for the removal of the organic substances possibly present in the sludge and of the chlorinated compounds later fed. If the batch test was to be performed in anaerobic conditions, sludge was inflated with nitrogen for around 10 minutes before the test start, in order to strip all the oxygen present in the mixed liquor.
Batch tests performed in this experimentation had variable time length, depending on the sludge, the substrate and the co-metabolite used for the test. Batch test length varied from 1 day to 1 week; at the end of any batch test, the bottle was opened, emptied, washed and refilled with the same sludge. In the following test, the chlorinated compound fed could vary for concentration and composition, and a co-metabolite could also be added in order to check its possible effect on degradation improvement. In order to remove all the non-degraded substances present in the sludge, it was washed for three times with water.

7.2.2.2 SBR

SBR was operated both in fed-batch modality and with daily cycles.

Fed-batch modality

Fresh sludge was acclimated to 1,2-DCA in **fed-batch** modality, which consists in performing cycles of variable length, depending on the time required in react phase for the complete degradation of the substrate fed to the biological reactor.
Also acclimation to 1,2-DCB and 2-CP of the sludge acclimated to 1,2-DCA was achieved in fed-batch modality.
A typical cycle of the SBR used in fed-batch modality in this experimentation was composed of the following phases:

- mineral medium feeding (15 minutes);
- chlorinated compounds feeding (5 minutes);
- react (variable length);
- sedimentation of the mixed liquor (30 minutes);
- extraction of the supernatant (15 minutes).

While react phase had variable length, the other phases had fixed time lengths. In Figure 7.7 a schematization of the phases length is shown.

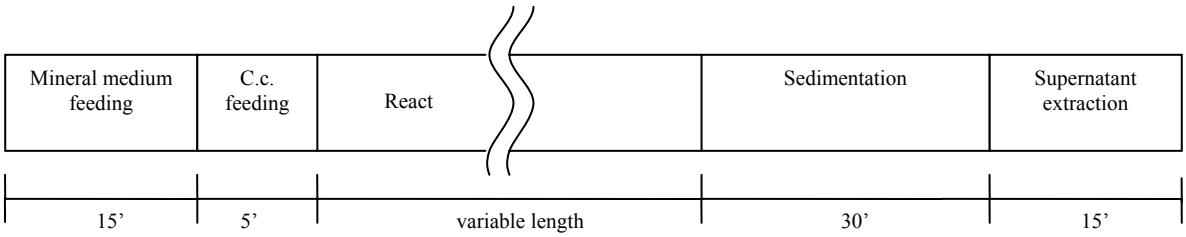

Figure 7.7. Schematization of phases length during a SBR cycle in fed-batch modality.

Mineral medium was fed before the solution of chlorinated compounds, in order to allow the system to start the reaction phase with the maximum chlorinated compounds concentration available. Feeding chlorinated compounds before mineral medium would have caused an initial degradation before the end of the feeding phase, so that their concentration at the beginning of the react phase would have been less that the maximum available, and subsequently, the kinetic rate of their degradation would have been lower. For the same reason, feeding time of chlorinated compounds solution was concentrated in just 5 minutes.
In fed-batch modality, react phase had variable length, and it was stopped when complete degradation of chlorinated compounds was achieved. However, mostly at the beginning of the acclimation of the sludge to new compounds, if no degradation of the fed substrates was observed, react phase was interrupted after one week, and a new cycle was started; in this way, fresh aerated mineral medium could be supplied.
When the sludge gradually acclimated to the chlorinated compounds, it took progressively less time for the substrates to be degraded; thus, react phase length was increasingly reduced of a proper number of days.
Sludge sedimentation took place in conditions of complete quietness, and good sludge settleability was always observed during all the experimentation; no bulking problem was ever observed.

Daily cycles modality

The SBR was operated in daily cycles modality when it was conducted in parallel with the MSBR, fed with the same substances, and usually kept at the same operating conditions, in order to compare the degradative efficiencies of the reactors. Besides, the SBR was used in daily cycles modality in some periods of the research, in order to check the effect that the variation of some operating parameters had on chlorinated compounds degradation.
In daily cycles modality, react phase had the length of $22^h55'$, while the length of the other phases was the same that in the fed-batch modality.
Thus, a schematization of the phases length in daily cycles modality is shown in Figure 7.8.

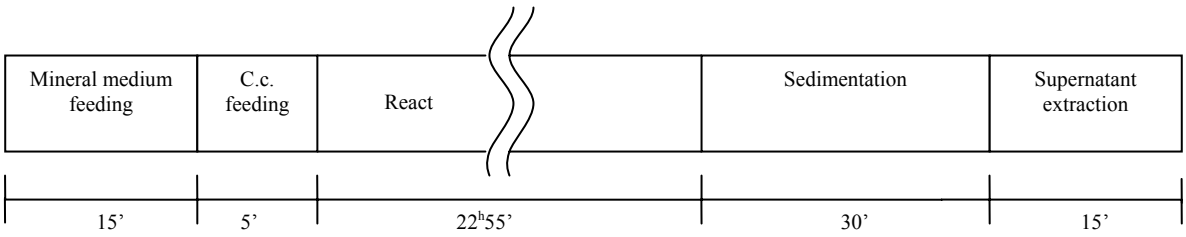

Figure 7.8. Schematization of phases length during a SBR cycle in daily cycles modality.

Volumes

Both in fed-batch and in daily cycles modality, the volumes of liquid fed and extracted were the same. They are detailed in Table 7.8.

Table 7.8. Volumes involved in SBR operation.

Total volume of mixed liquor	2.1 l
Extracted supernatant volume	1.3 l
Sludge residual volume at the end of the cycle	0.8 l
Mineral medium volume fed at any cycle	0.95 l
Chlorinated compounds solution fed at any cycle	0.35 l

With these volumes, hydraulic retention time (HRT) and sludge retention time (SRT) in the SBR operated in daily cycle modality were the following:

HRT = 1.61 d
SRT = 95 d.

Sampling

At the end and at the beginning of each cycle, samples for measuring chlorinated compounds, nitrates and chlorides were taken.
The initial sample was taken right after the feeding of the chlorinated compounds solution.
For *chlorinated compounds analysis*, a 5 ml sample was taken by means of a 5 ml syringe properly connected to the output for biological sludge sampling. The sample was then rapidly but gently moved to a 10 ml vial, which was immediately sealed. Following the same procedure, other samples were taken from the SBR at fixed time intervals and at the end of the cycle.
Usually, two 5 ml samples were taken, in order to perform a double analysis and get two comparable concentration values; this allowed to have an indication of possible errors made during the analysis.
For *phenol and 2-CP analysis*, a 5 ml sample was taken by means of a 5 ml syringe properly connected to the output for biological sludge sampling. The sample was then filtered at 0.45 μm and stored into a screw-cap vial, which was then placed at 4°C until it was analyzed by means of the HPLC.
For *chlorides and nitrates* analysis, a 5 ml sample was taken by means of a 5 ml syringe properly connected to the output for biological sludge sampling. The sample was then filtered at 0.45 μm and stored into a screw-cap vial, which was then placed at 4°C until it was analyzed by means of the Ion-Chromatograph.
For *suspended solids measurements*, once a week a sample of 50 ml of mixed liquor was taken from the reactor at the end of the cycle.

Oxygen supply

According to many past studies, in which degradation of the same chlorinated compounds studied in this research was obtained by means of aerobic metabolism (see Chapter 4 on biological treatments), also for this experimentation it was decided to carry out the biological processes in **aerobic metabolic conditions**.
Usually, in order to ensure aerobic conditions in biological systems, continuous mixed liquor aeration by means of air inflation is operated. However, due to the high volatility of the compounds treated in this research, this method could not be adopted, because air inflation would have caused the stripping of the chlorinated compounds by volatilization, causing their

complete loss in few hours. Besides, there was no external air oxygen input into the system, because the reactor was completely sealed and thus isolated from outside.
Therefore, an **innovative method for supplying the necessary oxygen was developed in this research**; this was achieved in two ways:

a) aeration of the mineral medium for at least 12 hours before its feeding into the reactor;
b) instauration in the reactor of a headspace of around one liter volume.

Oxygen present in the mineral medium ensured the oxygen necessary for the metabolic processes only partially. The rest of the necessary oxygen was ensured by the presence of the headspace in the reactor. The oxygen contained in the atmosphere of the headspace diffused into the bulk liquid, according to Henry's law.
Oxygen present in the mineral medium was consumed quite rapidly by metabolic activity of the biomass, and was completely used in a few hours; the absence of oxygen in the bulk liquid caused the transfer of oxygen from the atmosphere, which was rich in oxygen, to the liquid phase, due to the difference of concentration. A typical trend of oxygen concentration in the reactor can be observed in Figure 7.9.

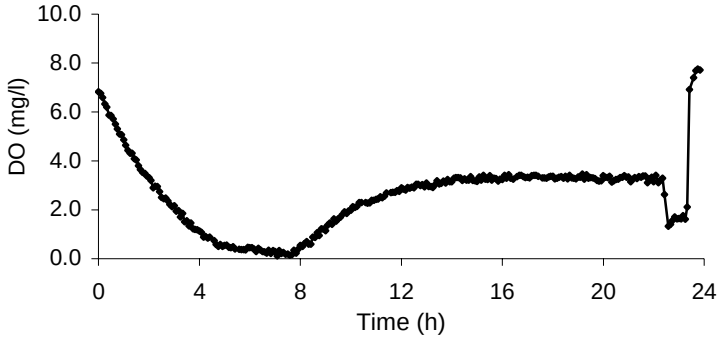

Figure 7.9. Typical oxygen concentration in the reactor during an SBR cycle.

In this figure, the rapid oxygen consumption in the first phase, when the oxygen present in the mineral medium was consumed, can be observed. Afterwards, more slowly, the oxygen present in the headspace diffused into the bulk liquid, as can be observed in the increase of the oxygen concentration line. The oxygen concentration then stabilized at a certain value, which was given by the equilibrium between the oxygen transfer rate from the headspace to the bulk liquid, and the oxygen consumption rate for the microorganisms metabolic processes.
Oxygen supply operated in this way was sufficient for the metabolic processes in almost all phases of the experimentation. Some exceptions were due to the high amount of non-chlorinated compounds fed as substrates in some phases of the research. These non-chlorinated compounds were easily degraded by the biomass, causing the consumption of the oxygen present and the instauration if anoxic conditions, with the subsequent interruption of the degradation of chlorinated compounds.

Mineral Medium

Mineral medium was an aqueous solution of salts and micro-elements necessary for the metabolic requirements of the microorganisms present in the sludge. It was prepared by solving in tap-water a proper amount of different elements, in order to achieve a determined composition of the mineral medium.
The composition of the mineral medium used in this experimentation was especially elaborated in order to be the most suitable for the growth of specific bacterial strains. From

the analysis of past studies, the bacterial strains responsible of degradation of the chlorinated compounds studied in this research were individuated; it was supposed that for this research, the same bacterial strains would have been selected. With the information of the involved bacterial strains, a web-site (www.dsmz.de) specialized in bacterial strains acclimation and selling was consulted. From this web-site, the recipes of the mineral medium suggested for the growth of the same bacterial strains involved in this research were taken. Since these recipes concerned different bacterial strains, they were partially different from each other; thus, they were properly adapted in order to be suitable for all the bacterial strains that were to be selected in this experimentation.
The final composition of the mineral medium used in this research is shown in Table 7.9.

Table 7.9. Composition of the mineral medium used in the experimentation.

MINERAL MEDIUM	
Reagent	**Amount**
K_2HPO_4	85.83 g
KH_2PO_4	41.19 g
$Ca(OH)_2$	0.66
$MgSO_4 \cdot 7H_2O$	5.42 g
$(NH_4)_2SO_4$	1.35 g
Solution A	200 ml
Water	Fill to 20 l
SOLUTION A	
Reagent	**Amount**
$FeSO_4 \cdot 7H_2O$	0.200 g
EDTA	0.500 g
Solution B	100 ml
Distilled water	Fill to 1 l
SOLUTION B	
Reagent	**Amount**
$ZnSO_4 \cdot 7H_2O$	2 g
$MnSO_4 \cdot H_2O$	0.512 g
$Na_2B_4O_7$	0.244 g
$CoCl_2 \cdot 6H_2O$	0.200 g
$CuCl_2 \cdot 2H_2O$	0.010 g
$NiCl_2 \cdot 6H_2O$	0.020 g
$(NH_4)_6MoO_{24} \cdot 4H_2O$	0.153 g
Distilled water	1 l

Chlorinated compounds solutions

Chlorinated compounds used in this experimentation were not easily soluble; therefore, in order to avoid deposits of these substances in the reactor, they were solved in distilled water before their feeding.
Usually, chlorinated compounds solutions were made by solving the proper amount of chlorinated compound into 3 liters of distilled water; this solution was stirred for around one hour, in order to ensure that chlorinated compounds could completely solve in the water.
However, 1,2-DCB was so hardly soluble that it required a stirring action of around 24 hours.

After solubilization, the solution was poured into a LDPE bag, impermeable to chlorinated compounds; the bag was collapsible, and so, before closing it, a manual elimination of every air deposit in the bag was operated. This was done in order to avoid the presence of any minimum headspace; actually, the presence of empty atmospheric spaces would have cause a partial volatilization of the chlorinated compound from the liquid solution, thus reducing its concentration, and altering the concentration of initial cycle.
The bag was equipped with a tap for the connection to the feeding peristaltic pump by means of a Viton® tube.
Due to the high volatilization tendency of 1,2-DCA and 1,2-DCB, the aqueous solutions of these two compounds could not stay in the bags for more than three days. After this time, the concentration of the substances in the aqueous solution would be too low, with the subsequent alteration of their concentration in the reactor at the beginning of the cycle. This is the reason why these solutions could not be prepared in volumes higher than 3 liters.

7.2.2.3 MSBR

MSBR was operated with cycles of variable length, and both with one and with two cycles per day.

Operation with cycles of variable length

When a certain level of acclimation of sludge toward the chlorinated compounds was observed in the SBR, the sludge was inoculated into the MSBR, where degradation of chlorinated compounds took place. When some particular substances (such as phenol or TCE) were fed to the system, partial or total inhibition of the degradation was observed; since complete degradation of the substances fed was not observed in one day, the cycles were carried on for longer time, specifically for 5 to 8 days.
When the cycles carried out in the SBR had variable length, each cycle was constituted by the following phases:

- mineral medium feeding (15');
- chlorinated compounds feeding (5');
- react phase (variable time length);
- permeate extraction and backwash phase ($1^h20'$).

Mineral medium was fed before the solution of chlorinated compounds in order to allow the system to start the reaction phase with the maximum chlorinated compounds concentration available.
Feeding chlorinated compounds before mineral medium would have caused the begin of their degradation before the end of the feeding phase, so that their concentration at the beginning of the react phase would have been less that the maximum available, and subsequently, the kinetic rate of their degradation would have been lower. For the same reason, feeding time of chlorinated compounds solution was concentrated in just 5 minutes.
A schematization of the time length of each phase is shown in Figure 7.10.

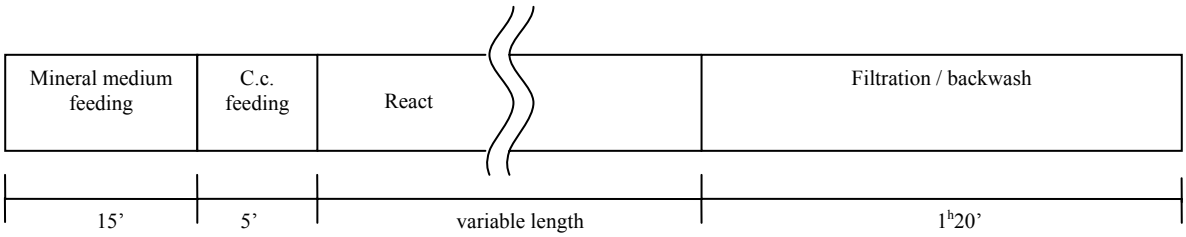

Figure 7.10. Schematization of phases length during a MSBR cycle of variable length.

Operation with one cycle per day

When the MSBR was operated with one cycle per day, chlorinated compounds were fed either singularly or in combination, in order to check if possible interactions of each of them on the degradation of the others could occur.
For most part of the experimentation, MSBR was conducted with one cycle per day. Each cycle consisted of the following phases:

- mineral medium feeding (15');
- chlorinated compounds feeding (5');
- react phase ($22^h20'$);
- permeate extraction and backwash phase ($1^h20'$).

Mineral medium was fed before the solution of chlorinated compounds in order to allow the system to start the reaction phase with the maximum chlorinated compounds concentration available.
Feeding chlorinated compounds before mineral medium would have caused the begin of their degradation before the end of the feeding phase, so that their concentration at the beginning of the react phase would have been less that the maximum available, and subsequently, the kinetic rate of their degradation would have been lower. For the same reason, feeding time of chlorinated compounds solution was concentrated in just 5 minutes.
A schematization of the time length of each phase is shown in Figure 7.11.

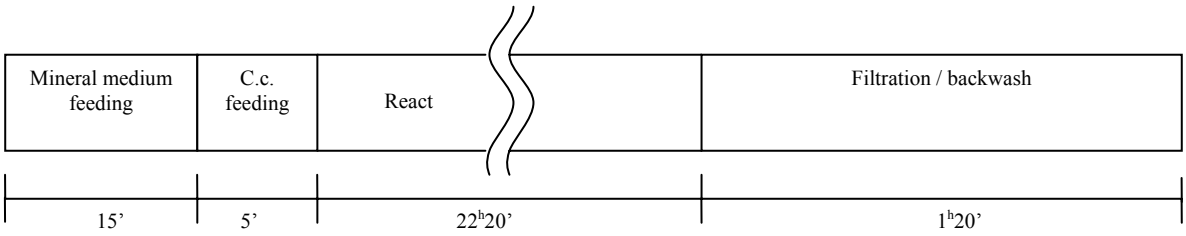

Figure 7.11. Schematization of phases length during a MSBR cycle when one cycle per day was performed.

Operation with two cycles per day

For some months, the MSBR was conducted with two cycles per day, and the chlorinated compounds fed had the same concentration than when they were fed with one cycle per day. Feeding with two cycles per day had the purpose of increasing the total chlorinated compound load to the biological system, so to test its capability of degrading a higher amount of these substances.
The phases that constituted each cycle were the same than in daily cycles, but their length varied:

- mineral medium feeding (15');
- chlorinated compounds feeding (5');

- react phase ($10^h20'$);
- permeate extraction and backwash phase ($1^h20'$).

A schematization of the time length of each phase is shown in Figure 7.12.

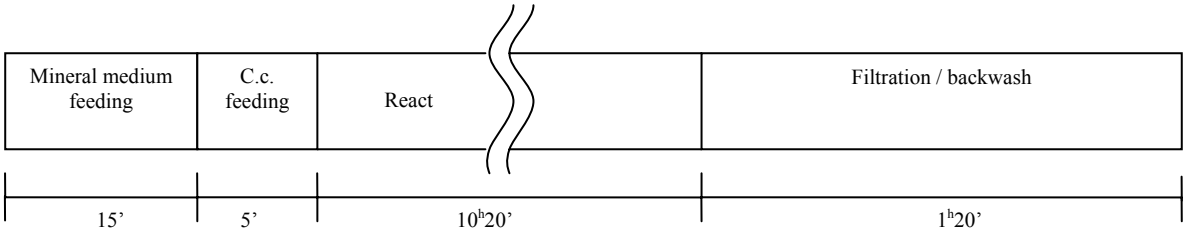

Figure 7.12. Schematization of phases length during a MSBR cycle when two cycles per day were performed.

Permeate extraction and backwash phase

Differing from the SBR, there was no supernatant extraction from the reactor, but permeate extraction was operated by means of sludge filtration.

Filtration took place at the end of the cycle, after the react phase, while backwash was performed when the half of the total permeate was extracted, and filtration was temporarily stopped; when backwash finished, filtration started again.

In Table 7.10 filtration and backwash flow rates and times are listed; also maximum pressure values observed in both processes are reported.

Table 7.10. Operating parameters for filtration and backwash.

Filtrated volume (permeate+backwash volume)	2100 ml
Filtration flow rate	28 ml/min
Filtration time	115'
Backwash volume	100 ml
Backwash flow rate	20 ml/min
Backwash time	5'
Maximum filtration pressure observed	-0.7 bar
Maximum backwash pressure observed	+0.45 bar

At the end of the cycle, when filtration started, also an aerator for air inflation in the membrane was automatically switched on, in order to create turbulence in the reactor, and to favor membrane mechanical cleaning. Actually, filtration was the only phase in which a direct aeration of the biological system was possible, since if the chlorinated compounds had been already degraded no volatilization problem could occur, while if the chlorinated compound was not degraded yet and could volatilize, its concentration had already been measured by taking a sample before the aeration started.

Membrane chemical cleaning

Around every two weeks, and anyway every time that filtration pressure fell down to less than -0.7 bar, chemical membrane cleaning was done.

Chemical cleaning was operated at the end of the cycle, in order to avoid the volatilization of non-degraded chlorinated compounds.

Membrane module was extracted from the reactor, and gently rinsed with tap water, with a careful manual removal of the biggest pieces of sludge.

Subsequently, the membrane module was submerged in an aqueous solution of 2.86 g/l of NaClO with Cl content (w/w) of 7%. Contemporarily, an aerator was connected to the

membrane module, and air inflation was switched on for all the time that the membrane module was submerged in the chlorine solution, so to create inside the vessel turbulent conditions that could help in removing fouling from membrane surface.
Membrane was left in this solution for 2 hours; afterwards it was gently rinsed with tap water, paying particular attention not to leave some NaClO solution in the membrane fibers, because this could cause the death of bacteria once the membrane module was replaced in the reactor.
Membrane module was then submerged in the reactor, and the reactor cap was sealed and connected again to all the pumps and sensors, so that cycles could continue as usual.

Volumes
Both when MSBR was operated with two cycles per day and when it worked with one cycle per day, the volumes of liquid fed and extracted were the same. They are detailed in Table 7.11.

Table 7.11. Volumes involved in SBR operation.

Total volume of mixed liquor	3.1 l
Extracted supernatant volume	2.0 l
Sludge residual volume at the end of the cycle	1.1 l
Mineral medium volume fed at any cycle	1.65 l
Chlorinated compounds solution fed at any cycle	0.35 l

With these volumes, hydraulic retention time (HRT) and sludge retention time (SRT) in the SBR operated in daily cycle modality were the following:

HRT = 1.55 d
SRT = 140 d

With these volumes, hydraulic retention time (HRT) and sludge retention time (SRT) in the SBR operated with two cycles per day were the following:

HRT = 0.78 d
SRT = 140 d.

The SRT were so high because the only sludge extraction from the system occurred by taking the samples, which consisted of around 8 ml of sludge per day for the measurements of chlorinated compounds, chlorides and nitrates, and of 50 ml of sludge per week for the measurement of suspended solids.

Mineral medium and chlorinated compounds solutions were the same that were used for SBR reactor. Also the oxygen supply and the sampling methodologies were the same that were used for the SBR reactor.

7.2.3 Indicators of metabolic processes efficiency

7.2.3.1 Mean specific degradation rate

For all the reactors used in the experimentation, at the end and at the beginning of any cycle, samples for measurement of chlorinated compounds, nitrates and chlorides were taken.
The concentration values of these substances, together with the value of volatile suspended solids (VSS) concentration at the moment of taking the sample, were used for calculating the

so called *mean specific degradation rate* of the chlorinated compound. This index indicated the total amount of chlorinated compound consumed in a cycle, per unit of amount of VSS, normalized per one day.
It was calculated in this way:

$$v_{spmean} = \frac{C_e - C_b}{n_d \cdot C_{VSS}} = \left[\frac{g_{cc}}{d \cdot g_{VSS}}\right]$$

where:

C_e = concentration of the chlorinated compound at the end of the cycle [mg_{cc}/l];
C_b = concentration of the chlorinated compound at the beginning of the cycle [mg_{cc}/l];
n_d = length of the cycle [d];
C_{VSS} = volatile suspended solids concentration [mg_{VSS}/l].

Mean specific degradation rate gives indication of the capability of the activate sludge of degrading all the chlorinated compound fed.

7.2.3.1 Maximum specific removal rate

Mean specific degradation rate gives indications of the capability of the biomass to degrade the substrate fed but, taking into account only the concentrations at the beginning and at the end of the cycle, it does not give specific indications on the maximum degradation rates.
Therefore another indicator has been taken into account in this experimentation, the *maximum specific degradation rate*.
This rate is calculated by measuring the concentration of the chlorinated compounds in the mixed liquor at some specific time intervals, that in this experimentation were set at one hour. With the chlorinated concentration values of any hour, it was possible to identify the time length of the react phase in which the maximum degradation rate was achieved. This time usually lasted around four hours, and from the calculation of the slope of the regression line correlated to these four points, the maximum specific degradation rate was calculated (Figure 7.13).

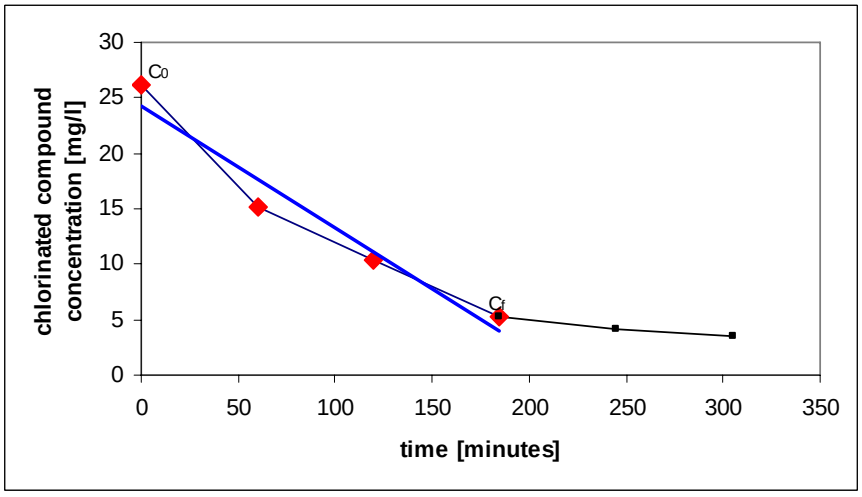

Figure 7.13. Typical trend of the chlorinated concentration during one cycle; in red the important points for the calculation of the maximum specific degradation rate are highlighted.

Therefore, the formula for the calculation of the maximum specific rate, with reference to Figure 7.11, is the following:

$$v_{spmax} = \frac{C_f - C_0}{n_{min} \cdot C_{VSS}} \cdot 60\frac{min}{h} \cdot 24\frac{h}{d} = \left[\frac{g_{cc}}{d \cdot g_{VSS}}\right]$$

where:

n_{min} = time [min]

Usually, daily measures of the chlorinated compounds at the end and at the beginning of the cycle were performed; however, for the calculation of maximum specific rates, it was necessary to take samples for the first 6 hours from the beginning of the cycle. This was not done every day, but only when a *kinetic test* was performed; this occurred around 15 or 20 days after any variations of operating parameters or of reactors design, and only when daily analyses showed a stable trend in chlorinated compound degradation. Kinetic tests were performed usually in double, which means that a test was repeated one or two days after the previous one, in order to confirm the results obtained. The calculation of the maximum degradation specific rate was done with the concentration of suspended solids measured in the most recent solids measurement at the moment of the kinetic test.

7.2.4 Measurement of chlorides

When samples for chlorinated compounds measurement were taken, also samples for chlorides measurement were taken. This allowed to monitor if complete degradation (or *mineralization*) of the chlorinated compound occurred. If the compound was mineralized, only H_2O, CO_2 and chlorides could be found as final by-product; thus, the presence of some other compounds or the lack of the stoichiometric amount of chlorides could indicate that the complete mineralization of the initial chlorinated compound had not been achieved.
According to stoichiometric balances, for the oxidation of one 1,2-DCA mole, 2 chlorides moles should be liberated, as can be seen from the oxidation reaction:

$C_2H_4Cl_2 + 2.5\ O_2 \rightarrow 2\ CO_2 + 2\ HCl + H_2O$

Following the same approach, two moles of chlorides produced per mole of 1,2-DCB oxidized and one mole of chlorides per mole of 2-CP oxidized were expected among the final by-products.
Thus, at the end of any cycle, and also during the kinetic tests, the balance between moles of chlorinated compounds oxidized and moles of chlorides produced was done; alternatively, the ratio between the moles of chlorides theoretically produced and the moles of chlorides really found among the by-products was calculated. This value allowed to evaluate the complete mineralization of the chlorinated compound fed.

7.2.5 Measurement of nitrates

With the same frequency of chlorinated compounds and chlorides measurements, also samples for nitrate measurement were taken. Measures of nitrates had the purpose of verifying if the oxygen present in the system was sufficient for keeping the metabolic processes in aerobic conditions. A decrease of nitrates in the bulk liquid indicated the instauration of anoxic conditions, and could explain a possible decrease in the degradation rate, or the partial or total inhibition of the degradation.

Chapter 8.
Experimental Results

Research development
The research carried out has been developed through three main phases:

1) **Batch tests**, performed to investigate the capability of fresh and of acclimated sludge of degrading the different chlorinated compounds studied in the research.
2) Acclimation of fresh sludge to 1,2-DCA, achieved in a **SBR** operated first in **fed-batch** modality and then with daily cycles.
3) Degradation of all the chlorinated compounds in the **MSBR**; chlorinated compounds were fed both singularly and in different combinations, in order to check the possible interactions of some chlorinated compounds on the degradation of the others.

8.1 Batch tests

8.1.1 Batch tests with 1,2-dichloroethane

8.1.1.1 Batch tests with fresh sludge
At the beginning of the experimentation, some batch tests were performed using **fresh sludge** coming from the oxidation tank of the municipal wastewater treatment plant of Is Arenas (Cagliari, Italy). This sludge was tested for its capability of aerobically and anaerobically degrading 1,2-DCA.

Anaerobic batch tests
Three batch tests were performed in anaerobic conditions, each of them lasting one week; the second one was performed with the sludge used for the first test.
In the anaerobic batch tests, no degradation of 1,2-DCA was observed.

Aerobic batch tests
Five sequential aerobic batch tests were performed; for each test the sludge used was the one coming from the previous test. The first two tests lasted one week, the third one lasted four days, and the last two tests lasted two days. In all the tests, the initial concentration of 1,2-DCA in the batch was around 50 mg/l. An increase in specific removal rate of 1,2-DCA was progressively observed in the tests (Table 8.1 and Figure 8.1).

Table 8.1. Mean specific degradation rates of batch tests performed with fresh sludge and fed with 1,2-DCA.

Batch test n.	Mean specific degradation rate $[g_{DCA}/(d \cdot g_{VSS})]$
1	0
2	0
3	0.01
4	0.04
5	0.04

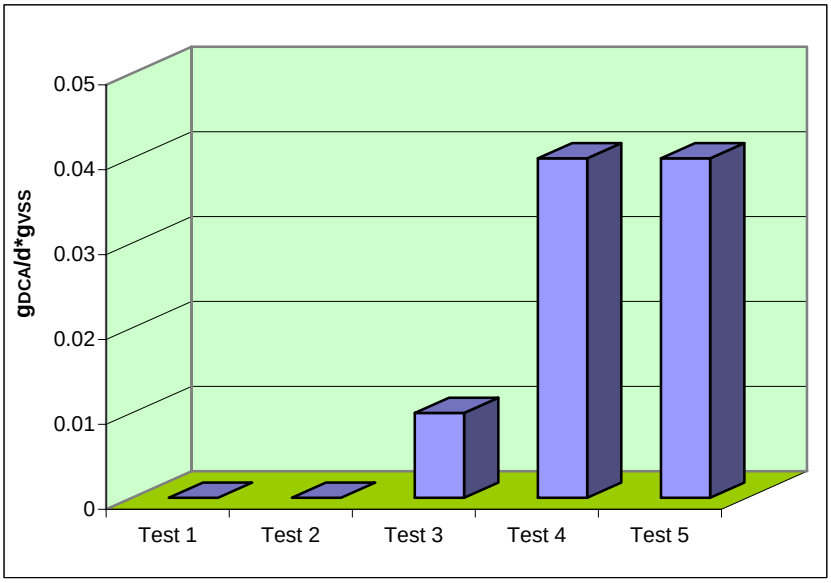

Figure 8.1. Trend of degradation rates obtained in the initial batch tests with fresh sludge fed with 1,2-DCA.

8.1.1.2 Batch tests with sludge acclimated to 1,2-DCA

Comparison of degradation efficiency at 30°C and at 20°C

After 140 days of sludge acclimation to 1,2-DCA achieved in the SBR, a batch test with the acclimated sludge was performed in order to compare 1,2-DCA degradation efficiency at 20°C and at 30°C.

The results are shown in Table 8.2 and Figure 8.2.

Table 8.2. Results of the batch tests on 1,2-DCA degradation at 20°C and 30°C.

Time [h]	1,2-DCA concentration at 20°C [mg/l]	1,2-DCA concentration at 30°C [mg/l]
0	38.6	42.4
1	34.5	29.2
2	31.4	27.2
3	31.1	26.2
4	29.7	26.2
5	29.3	23.5
6.5	26.4	23.6
Maximum specific degradation rate [$g_{DCA}/(d \cdot g_{VSS})$]	0.40	0.60

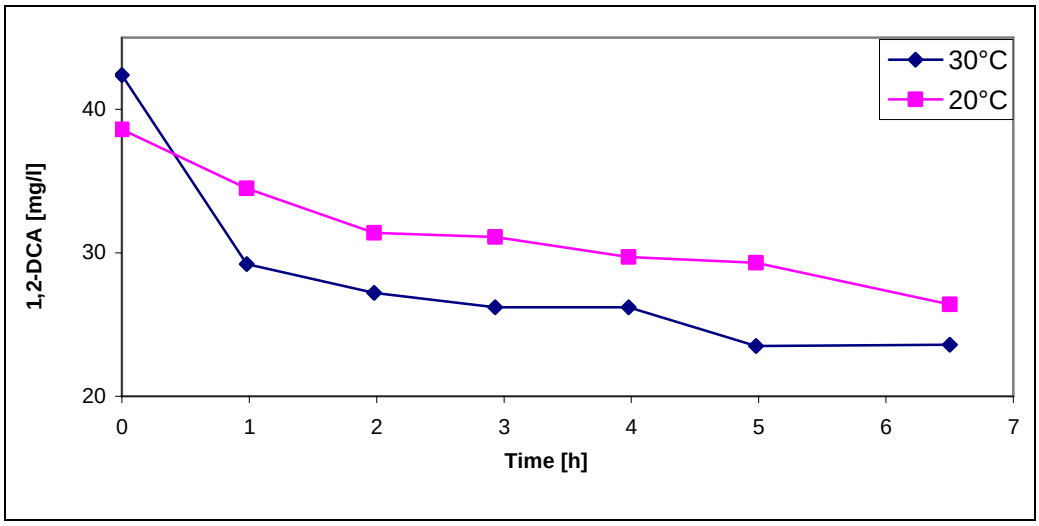

Figure 8.2. Batch tests on 1,2-DCA degradation at 20°C and 30°C.

Effect of different methanol concentrations on 1,2-DCA degradation

After 5 months of sludge acclimation to 1,2-DCA achieved in the SBR, a batch test with the acclimated sludge was performed in order to check the effect of methanol on 1,2-DCA degradation. In this test, three batches were inoculated with the acclimated sludge coming from the SBR, and a proper amount of aqueous solution of 1,2-DCA was added in each batch in order to reach a concentration in the batch of around 50 $mg_{1,2\text{-}DCA}/l$. Also a proper amount of methanol was added, in order to reach three different methanol concentrations in the batches. Methanol was added in order to investigate if it could act as a co-substrate for the degradation of 1,2-DCA. Metabolic conditions kept in the batches were aerobic.
Results of the tests are reported in Table 8.3; in Test A only 1,2-DCA was fed, in Test B 1,2-DCA and methanol at a concentration of 40 mg/l were fed; in Test C 1,2-DCA and methanol at 400 mg/l were fed. All the values reported in the table indicate the progressive 1,2-DCA concentration.

Table 8.3. Batch tests on 1,2-DCA degradation in presence of methanol at different concentrations.

Time [h]	**Test A (no methanol)**	**Test B (methanol at 40 mg/l)**	**Test C (methanol at 400 mg/l)**
	1,2-DCA [mg/l]	**1,2-DCA [mg/l]**	**1,2-DCA [mg/l]**
0	52.14	49.75	46.12
2	38.8	42.6	45.87
4	32.8	38.97	44.13
24	18.1	25.9	38.7
Max specific rate $[g_{1,2\text{-}DCA}/(d \cdot g_{VSS})]$	0.58	0.32	0.06
Mean specific rate $[g_{1,2\text{-}DCA}/(d \cdot g_{VSS})]$	0.17	0.12	0.04

8.1.2 Batch tests with 1,2-dichlorobenzene

8.1.2.1 Batch tests with fresh sludge

Some batch tests were performed in order to investigate the capability of fresh sludge of aerobically degrading 1,2-DCB. Like the sludge used for the batch tests on 1,2-DCA, also this sludge was taken from the oxidation tank of the municipal wastewater treatment plant of Is Arenas (Cagliari, Italy); the initial concentration of 1,2-DCB in the batch was around 10 mg/l.

Anaerobic batch tests

Three sequential tests were performed in anaerobic conditions, and at the end of any test the sludge was washed and inoculated in the following test. The initial concentration of 1,2-DCB in the batch was around 10 mg/l. Each test lasted one week; no degradation of 1,2-DCB was observed during the three tests.
In a following test, methanol at 40 mg/l was added in order to verify if it could act as a co-substrate in the degradation of 1,2-DCB, but no degradation of the chlorinated compound was observed.

Aerobic batch tests

Four sequential tests were performed in aerobic conditions, and at the end of any test the sludge was washed and inoculated in the following test. The initial concentration of 1,2-DCB in the batch was around 10 mg/l. Each test lasted one week; no degradation of 1,2-DCB was observed during the four tests.
In another test, methanol at 20 mg/l was added in order to verify if it could act as a co-substrate in the degradation of 1,2-DCB, but no degradation of the chlorinated compound was observed.

8.1.2.2 Batch tests with sludge acclimated to 1,2-DCA

Since no degradation of 1,2-DCB was observed with fresh sludge, sludge acclimated to 1,2-DCA for around 3 months was used to inoculate batch tests in order to investigate the capability of acclimated sludge of degrading 1,2-DCB. This approach was based on the hypothesis that a sludge already capable of degrading a chlorinated compound could degrade another chlorinated compound more easily than a non-acclimated bacterial population.
All tests with acclimated sludge were performed in aerobic conditions, because the acclimated bacterial population had been grown in aerobic conditions; the initial concentration of 1,2-DCB in the batch was around 10 mg/l.

Batch test for 1,2-DCB degradation

Three separate batch tests, each of them lasting one week, were performed with the acclimated sludge; after any test, new sludge from the SBR was taken and inoculated in the following batch test. In all the three batch tests, 1,2-DCB was degraded even if with a very low mean specific degradation rate (0.002 $g_{1,2\text{-DCB}}/(d \cdot g_{VSS})$).

Effect of different methanol concentrations on 1,2-DCB degradation

Similarly to what done for 1,2-DCA, also for 1,2-DCB some batch tests were performed in order to investigate if methanol could affect the degradation of the chlorinated compound. Three batch tests were conducted in parallel for one week: one batch was fed with only 1,2-

DCB, another with 1,2-DCB and methanol at 40 mg/l, and the last with 1,2-DCB at 400 mg/l. The results shown that specific degradation rate of 1,2-DCB decreased when methanol concentration increased.

Batch test for possible interactions between 1,2-DCB and 1,2-DCA

Due to the low biodegradability of 1,2-DCB, it was supposed that it could negatively affect biomass capability of degrading 1,2-DCA. Thus, in the 190th day of acclimation, a batch test was performed, in which acclimated sludge was fed first with 1,2-DCA, then with 1,2-DCB and finally again with 1,2-DCA.

The first part of the batch test with 1,2-DCA lasted one day, then the sludge was taken from the batch, washed with water and inoculated again into the batch; at this point 1,2-DCB was fed and degraded for one week. Finally, the sludge was extracted from the batch, washed and then inoculated again into the batch, which was fed with 1,2-DCA.

Degradation rate of 1,2-DCA was measured in order to check if feeding 1,2-DCB could have affected the degradability of 1,2-DCA, causing a decrease of 1,2-DCA specific degradation rate. The results are shown in Table 8.4.

Table 8.4. Batch test on the effects of 1,2-DCB feeding on 1,2-DCA degradation.

Time [h]	1,2-DCA concentration before 1,2-DCB feeding [mg/l]	1,2-DCA concentration after 1,2-DCB feeding [mg/l]
0	46.34	52.13
2	39.12	45.65
4	27.13	35.32
24	6.78	19.53
Maximum specific removal rate [$g_{DCA}/(d \cdot g_{VSS})$]	0.58	0.50
Mean specific removal rate [$g_{DCA}/(d \cdot g_{VSS})$]	0.19	0.16

Batch test for effects of methanol on 1,2-DCA and 1,2-DCB degradation

In order to confirm the results on methanol effects on 1,2-DCA and 1,2-DCB degradation already observed in the two previous batch tests, another batch test was performed on the 200th day of acclimation. In this test, three batches were equipped and operated in parallel; Batch A was fed with 1,2-DCA (50 mg/l) and 1,2-DCB (15 mg/l), Batch B was fed with 1,2-DCA (50 mg/l), 1,2-DCB (15 mg/l) and methanol at 40 mg/l and Batch C was fed with 1,2-DCA (50 mg/l), 1,2-DCB (15 mg/l) and methanol at 400 mg/l. Sludge used for this test was taken from the SBR after 307 days of reactor operation. The test lasted one week; mean and maximum specific removal rates obtained in the test are shown in Table 8.5.

Methanol lowered the specific removal rates of both compounds, and inhibited almost completely their degradation when fed at 400 mg/l.

Besides, it was also possible to observe that 1,2-DCB lowered both maximum and mean specific removal rates of 1,2-DCA.

Table 8.5. Batch test for the degradation of 1,2-DCA and 1,2-DCB with methanol at different concentration.

Time [h]	Test A (no methanol)		Test B (methanol at 40 mg/l)		Test C (methanol at 400 mg/l)	
	1,2-DCA [mg/l]	1,2-DCB [mg/l]	1,2-DCA [mg/l]	1,2-DCB [mg/l]	1,2-DCA [mg/l]	1,2-DCB [mg/l]
Mean specific rate [$g_{DCA}/(d \cdot g_{VSS})$]	0.51		0.24		0.04	
Maximum specific rate [$g_{DCA}/(d \cdot g_{VSS})$]	0.07	0.002	0.05	0.001	0.02	0

8.1.3 Batch tests with 2-chlorophenol

8.1.3.1 Batch tests with fresh sludge

Some batch tests were performed both in anaerobic and in aerobic conditions, in order to investigate the capability of fresh sludge of degrading 2-CP. Like the sludge used for the batch tests on 1,2-DCA and 1,2-DCB, also the activated sludge used for these tests was taken from the oxidation tank of the municipal wastewater treatment plant of Is Arenas (Cagliari, Italy).

Anaerobic batch tests

Three sequential tests were performed in anaerobic conditions; batches were initially inoculated with the sludge acclimated to 1,2-DCA and 1,2-DCB and at the end of any test the sludge was washed and inoculated into the following test; batches were fed with **2-CP** at a concentration of around 10 mg/l. Each test lasted one week, and no degradation of 2-CP was observed during the three tests.

A new batch with fresh sludge was equipped, and was fed with **2-CP and phenol**, in order to check if this substrate could act as a cometabolite in the degradation process. The use of phenol as co-metabolite for the degradation of chlorinated phenols was already used in past studies (see Chapter 4 on Biological treatments of waters polluted by chlorinated solvents), because usually the substrate chosen as cometabolite is a substance with a similar structure of the molecule which must be degraded, but without the halogenated substitutes. The test lasted one week and again, in anaerobic conditions, no degradation of 2-CP was observed.

Aerobic batch tests

Three sequential tests were performed in aerobic conditions; batches were initially inoculated with the sludge acclimated to 1,2-DCA and 1,2-DCB and at the end of any test the sludge was washed and inoculated into the following test; batches were fed with **2-CP** at a concentration of around 10 mg/l. Each test lasted one week, and no degradation of 2-CP was observed during the three tests.

A new batch with fresh sludge was equipped, and was fed with **2-CP and phenol**, in order to check if this substrate could act as a cometabolite of the degradation process; the test lasted 13 days. Results can be observed in Figure 8.3 and Table 8.6, maximum specific removal rates are calculated on a daily basis.

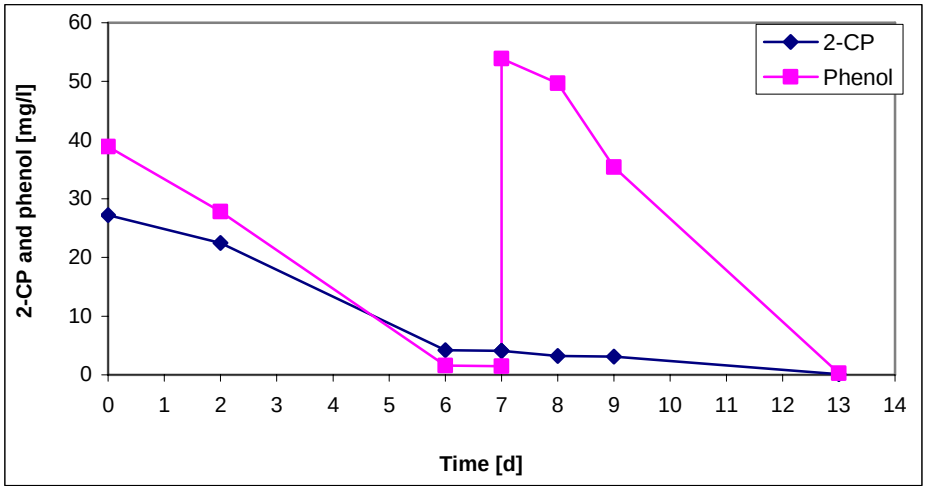

Figure 8.3. Batch test with fresh sludge for the degradation of 2-CP with phenol as co-substrate.

Table 8.6. Batch test of the degradation of 2-CP with phenol as co-substrate.

Time [d]	2-CP [mg/l]	Phenol [mg/l]
0	27.2	38.9
2	22.5	27.8
6	4.2	1.6
7	4.1	1.5
7	4.1	53.9
8	3.2	49.7
9	3.1	35.4
13	0.1	0.3
Maximum specific removal rate [g/(d·g_{VSS})]	0.0013	0.0021

In presence of phenol, after a lag-phase of around 2 days, degradation of 2-CP took place and stopped when phenol concentration decreased under 1.5 mg/l; in parallel also phenol was consumed. When another peak of phenol was added to the system, degradation of 2-CP took place again.

8.1.3.2 Batch tests with acclimated sludge

On the 300th day of sludge acclimation to 1,2-DCA, a series of anaerobic and aerobic batch test with acclimated sludge were started, in order to investigate its capability of degrading 2-CP.

Anaerobic batch tests

Feeding of 2-CP

Three sequential tests were performed in anaerobic conditions; batches were initially inoculated with the sludge acclimated to 1,2-DCA and 1,2-DCB, and at the end of any test the sludge was washed and inoculated into the following test; the batches were fed with **2-CP** at a

concentration of around 10 mg/l. Each test lasted one week and no degradation of 2-CP was observed during the three tests.

Feeding of 2-CP and methanol
A new batch with acclimated sludge was equipped, and was fed with **2-CP and methanol**, in order to check if this substrate could act as a co-substrate in the degradation process. Initial 2-CP concentration was around 10 mg/l, while methanol was fed with an initial concentration of around 40 mg/l; the test lasted one week and no degradation of 2-CP was observed.

Feeding of 2-CP and 1,2-DCA
Three sequential batch tests were equipped, inoculated with sludge acclimated to 1,2-DCA and 1,2-DCB, and fed with **2-CP and 1,2-DCA** in order to check if 1,2-DCA (which was the growth substrate of the acclimated biomass) could be used as cometabolite in the degradation of 2-CP. At the end of any test new acclimated sludge was taken from the SBR and inoculated in the following batch. Initial 2-CP concentration was around 10 mg/l, while 1,2-DCA had an initial concentration of around 30 mg/l; each test lasted one week and no degradation of 2-CP was observed in any of them.

Feeding of 2-CP, 1,2-DCA and methanol
Another batch test was performed, inoculated with new acclimated sludge taken from the SBR, and fed with **2-CP** (10 mg/l), **1,2-DCA** (30 mg/l) and **methanol** (40 mg/l). The test lasted one week and no degradation of 2-CP was observed, while 1,2-DCA was degraded with the usual specific removal rates.

Feeding of 2-CP and phenol
Finally, another test was performed, inoculated with acclimated sludge, and fed with **2-CP** (10 mg/l) and **phenol**. The test lasted one week and degradation of only phenol was observed.

Aerobic batch test

Feeding of 2-CP
Three sequential tests were performed in aerobic conditions; batches were initially inoculated with the sludge acclimated to 1,2-DCA and 1,2-DCB; at the end of any test the sludge was washed and inoculated into the following test; the batches were fed with **2-CP** at a concentration of around 30 mg/l. Each test lasted one week, and no degradation of 2-CP was observed during the three tests.

Feeding of 2-CP and methanol
A new batch with acclimated sludge was equipped, and was fed with **2-CP and methanol**, in order to check if this substrate could act as a co-substrate in the degradation process. Initial 2-CP concentration was around 30 mg/l, while methanol was fed with an initial concentration of around 40 mg/l; the test lasted one week and no degradation of 2-CP was observed.

Feeding of 2-CP and 1,2-DCA
Three sequential batch tests were equipped, inoculated with sludge acclimated to 1,2-DCA and 1,2-DCB, and fed with **2-CP and 1,2-DCA** in order to check if 1,2-DCA (which was the growth substrate of the acclimated biomass) could be used as cometabolite into the degradation of 2-CP. At the end of any test new acclimated sludge was taken from the SBR and inoculated

in the following batch. Initial 2-CP and 1,2-DCA concentration was around 30 mg/l; each test lasted one week and no degradation of 2-CP was observed in any of them.

Feeding of 2-CP, 1,2-DCA and methanol

Another batch test was performed, inoculated with new acclimated sludge taken from the SBR, and fed with **2-CP** (30 mg/l), **1,2-DCA** (30 mg/l) and **methanol** (40 mg/l). The test lasted one week and no degradation of 2-CP was observed, while 1,2-DCA was degraded with the usual specific removal rates.

Feeding of 2-CP and phenol

A new batch with acclimated sludge was equipped, and was fed with **2-CP and phenol**, in order to check if this substrate could act as a cometabolite of the degradation process; the test lasted 20 days. Results can be observed in Figure 8.4 and Table 8.7.

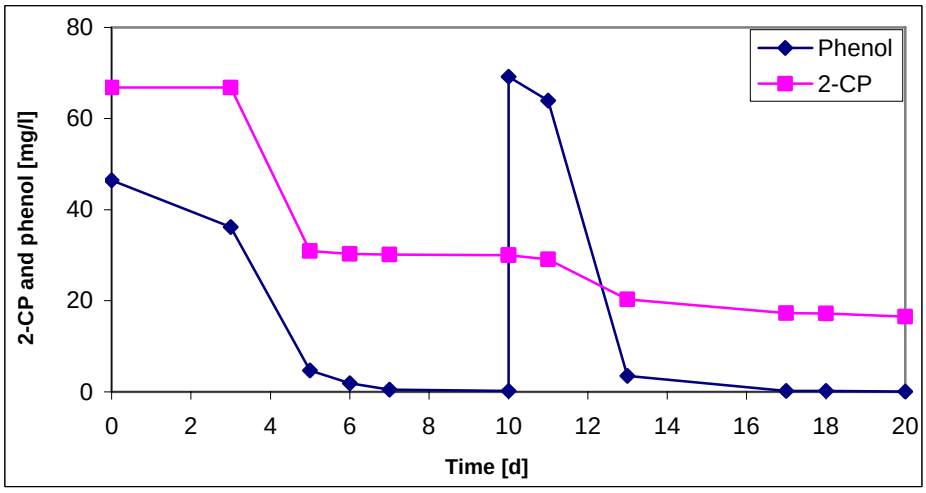

Figure 8.4. Batch test with acclimated sludge for the degradation of 2-CP with phenol as co-substrate.

Table 8.7. Results of batch test inoculated with acclimated sludge and fed with 2-CP and phenol.

Time [d]	2-CP [mg/l]	Phenol [mg/l]
0	66.8	46.4
3	66.8	36.2
5	30.9	4.7
6	30.3	1.9
7	30.1	0.5
10	30	0.2
10	30	69.2
11	29.1	63.9
13	20.3	3.5
17	17.3	0.2
18	17.2	0.2
20	16.5	0.1
Mean specific removal rate [g/(d·g_{VSS})]	0.02	0.02

to be continued

follows from the previous page

Maximum specific removal rate [g/(d·g_{VSS})]	0.08	0.1

In presence of phenol, after a lag-phase of 3 days, degradation of 2-CP took place and stopped when phenol concentration decreased under 1.5 mg/l; in parallel also phenol was consumed. When another peak of phenol was added to the system, degradation of 2-CP took place again.

Feeding of 2-CP, phenol and 1,2-DCA

On the 363rd day, a new batch test was performed, inoculated with acclimated sludge and fed with **2-CP**, **phenol** as co-substrate and **1,2-DCA** as biomass primary substrate. This test was performed in order to check if the presence of phenol and 2-CP could affect the specific removal rate of 1,2-DCA and if the presence of 1,2-DCA could enhance the cometabolic effect of phenol. The test lasted 7 days and phenol was added every time its concentration lowered to down 4 mg/l.

Results are shown in Figure 8.5 and in Table 8.8. Maximum specific removal rates are calculated on a daily basis.

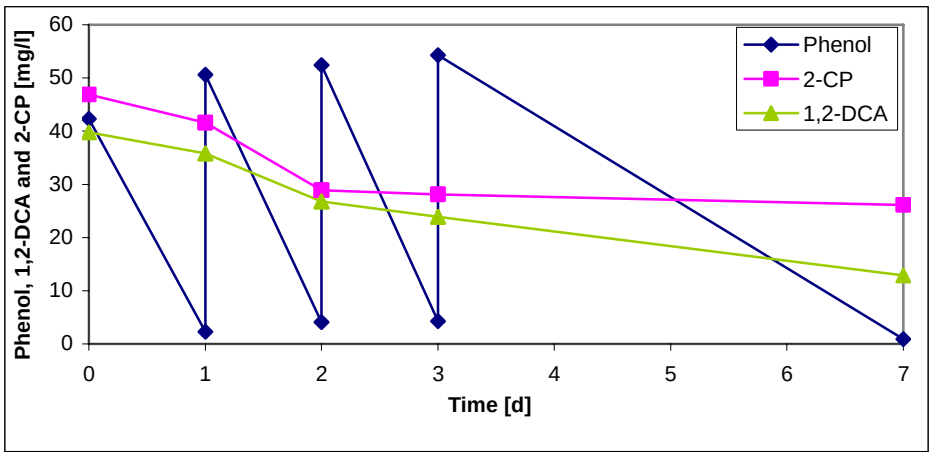

Figure 8.5. Results of batch test with acclimated sludge fed with 1,2-DCA, phenol and 2-CP.

Table 8.8. Results of test batch with acclimated sludge fed with 1,2-DCA, phenol and 2-CP.

Time [d]	Phenol [mg/l]	2-CP [mg/l]	1,2-DCA [mg/l]
0	42.3	46.9	39.8
1	2.3	41.6	35.4
1	50.6	41.6	35.4
2	4.1	28.9	26.8
2	52.4	28.9	26.8
3	4.3	28.1	25.9
3	54.3	28.1	25.3
7	0.9	26.1	12.9
Max. specific removal rate [[g/(d·g_{VSS})]	0.22	0.04	0.06

Feeding of 2-CP, phenol and 1,2-DCB

A new batch test was performed, inoculated with acclimated sludge and fed with **2-CP**, **phenol** as co-substrate and **1,2-DCB**. This test was performed in order to check if the presence of phenol and 2-CP could affect the specific removal rate of 1,2-DCB and if the presence of 1,2-DCB could decrease the degradation rate of 2-CP. The test lasted 7 days and phenol was added every time that its concentration lowered down to 4 mg/l. Results are shown in Figure 8.6 and in Table 8.9. Maximum specific removal rates are calculated on a daily basis.

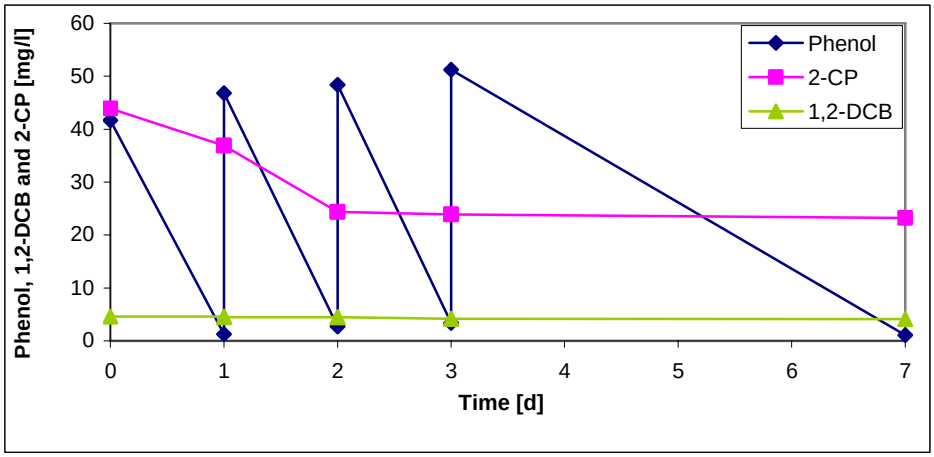

Figure 8.6. Results of test batch with acclimated sludge fed with 1,2-DCB, phenol and 2-CP.

Table 8.9. Results of test batch with acclimated sludge fed with 1,2-DCB, phenol and 2-CP.

Time [d]	Phenol [mg/l]	2-CP [mg/l]	1,2-DCB [mg/l]
0	41.7	43.9	4.6
1	1.3	36.9	3.8
1	46.8	36.9	3.1
2	2.7	24.4	3.1
2	48.4	24.4	3
3	3.4	23.9	3.1
3	51.2	23.9	2.8
7	1.1	23.2	2.7
Maximum specific removal rate [[g/(d·g_{VSS})]	0.23	0.04	0.003

Feeding of 2-CP, phenol, 1,2-DCB and 1,2-DCA

A new batch test was performed, inoculated with acclimated sludge and fed with **2-CP**, **phenol** (as co-substrate), **1,2-DCA** and **1,2-DCB**. This test was performed in order to check if the contemporary presence of all the substrates could affect the removal efficiency of each of

them. The test lasted 7 days and phenol was added every time that its concentration lowered down to 4 mg/l. Maximum specific removal rates are calculated on a daily basis.
Results are shown in Figure 8.7 and Table 8.10.

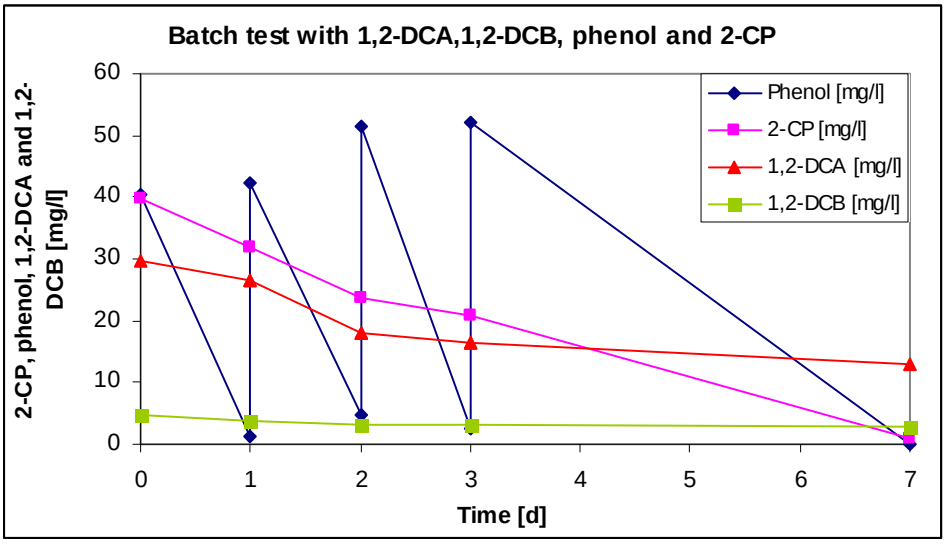

Figure 8.7. Results of batch test with acclimated sludge fed with 1,2-DCA, 1,2-DCB, phenol and 2-CP.

Table 8.10. Results of test batch with acclimated sludge fed with 1,2-DCA, 1,2-DCB, phenol and 2-CP.

Time [d]	Phenol [mg/l]	2-CP [mg/l]	1,2-DCA [mg/l]	1,2-DCB [mg/l]
0	40.3	39.7	29.8	4.7
1	1.2	31.8		
1	42.2	31.8		
2	4.8	23.7	18.1	3.3
2	51.4	23.7		
3	2.4	20.9		
3	52.1	20.9		
7	0.1	0.9	12.8	3
Maximum specific removal rate [[g/(d·g_{VSS})]	0.24	0.04	0.03	0.003

8.2 SBR

8.2.1 SBR operated in fed-batch modality

After checking the capability of the acclimated sludge of degrading the chlorinated compounds, a first acclimation of the sludge to 1,2-DCA and 1,2-DCB was carried on in the SBR operated in fed-batch modality.

8.2.1.1 Acclimation to 1,2-DCA

Sludge coming from the aerobic batch test on 1,2-DCA was inoculated in the SBR and its degradation took place in fed-batch modality. The initial concentration of 1,2-DCA at the beginning of the cycle in the reactor was 50 mg/l. After 30 days of operation, almost complete degradation of 1,2-DCA was observed to be achieved in one day, and thus the length of the following four cycles was set to one day. On the 35th day, 90% of the total sludge of the system was substituted with fresh non-acclimated sludge; this caused a temporary interruption of 1,2-DCA degradation. The total acclimation of the sludge in the SBR lasted around 70 days. Trend of 1,2-DCA concentration in the SBR is shown in Table 8.11 and Figure 8.8.

Table 8.11. Trend of 1,2-DCA, nitrates and chlorides concentration in the SBR operated in fed batch modality.

Time [d]	1,2-DCA [mg/l]	$N\text{-}NO_3$ [mg/l]	Cl^- [mg/l]	mmol 1,2-DCA	mmol Cl	$mmol_{Cl}/mmol_{1,2\text{-}DCA}$
0	58.2	17.3	0.05	0.02	0.05	2.23
1	57.3					
2	58.6					
3	56.9					
4	57.2	19.4				
4	52.1	16.5	2.33	1.08	2.33	2.16
5	47.3					
5	37.2					
6	12.3					
6	5.4					
7	1.1	17.9				
7	66	16.1	2.78	1.31	2.78	2.12
9	4.1	16.9				
9	62.1	17.9	2.42	1.24	2.42	1.95
11	3.7	18.4				
11	59.12	17.2	2.87	1.18	2.87	2.43
14	3.5	18.5				
14	64.45	18.9	3.57	1.31	3.57	2.73
16	2.9	17.9				
16	61	17.3	2.88	1.26	2.88	2.29
18	1.7	18.9				
18	29.16	19.3	1.10	0.61	1.10	1.81
20	0.4	18.2				
20	55.4	17.8	1.84	1.14	1.84	1.61
22	1.6	17.7				
22	57.4	18.5	2.28	1.18	2.28	1.94
24	1.9	16.9				
24	32.6	18.3	1.26	0.69	1.26	1.84

to be continued

follows from the previous page

24	0.3	19.4				
24	32.9	19.8	1.27	0.70	1.27	1.82
26	0.1	18.7				
26	28.16	18.4	1.34	0.60	1.34	2.25
28	0	18.9				
28	19.15	17.4	0.72	0.41	0.72	1.78
30	0	17.1				
30	19.7	16.3	0.98	0.35	0.98	2.78
31	0	19				
31	64.6	18.5	3.09	1.33	3.09	2.32
32	1.8	17.4				
32	26.5	17.9	1.04	0.56	1.04	1.84
33	0	18.4				
33	33.9	18	1.36	0.70	1.36	1.94
34	0.8	18.6				
34	43.5	18.3	2.20	0.92	2.20	2.38
35	0	17.9				
35	76.3	17.6	0.10	0.03	0.10	3.17
35	74.9					
36	73.6					
37	74.2					
38	74.8	18.4				
38	75.1	18.9	0.44	0.43	0.44	1.01
39	69.2					
39	67.6					
42	61.6					
42	59.3					
42	54.9					
43	55.7					
43	54.7	18.6				
44	56.8	18.9	2.69	1.20	2.69	2.23
45	52.5					
46	36.5					
49	0	19				
49	44.4					
50	41.6	18.1	2.22	0.88	2.22	2.54
51	0.3	17.8				
51	42	17.4	1.94	0.89	1.94	2.17
52	23.6					
53	0	18.6				
53	29.6	17	1.87	0.63	1.87	2.97
54	0	18.4				
55	43.7	18.9	1.68	0.93	1.68	1.81
56	0	17.3				
56	49.6	17	1.75	1.05	1.75	1.67
58	0.1	18.3				
58	48.3	18.6	3.05	1.01	3.05	3.01
60	0.5	16.8				
60	47.3	17.1	2.71	0.97	2.71	2.81
61	1.8	18.2				
61	36.4	18.9	2.04	0.77	2.04	2.66

to be continued

follows from the previous page

62	0.2	19.4				
62	42.12	17.2	1.91	0.89	1.91	2.13
63	1.3					
64	0	17.9				
64	26.8	18.5	1.13	0.57	1.13	1.98
65	0	18.9				
65	49.2	17.7	1.69	1.04	1.69	1.64
66	0.4	18.1				
66	47	19	2.01	0.99	2.01	2.03
67	0.3	17.7				
67	52.3	18.1	1.91	1.11	1.91	1.73
68	0.2	17				
68	49.5	17.4	2.32	1.04	2.32	2.24
69	0.7	18.7				
Mean specific removal rate [$g_{DCA}/(d \cdot g_{VSS})$]	0.11					

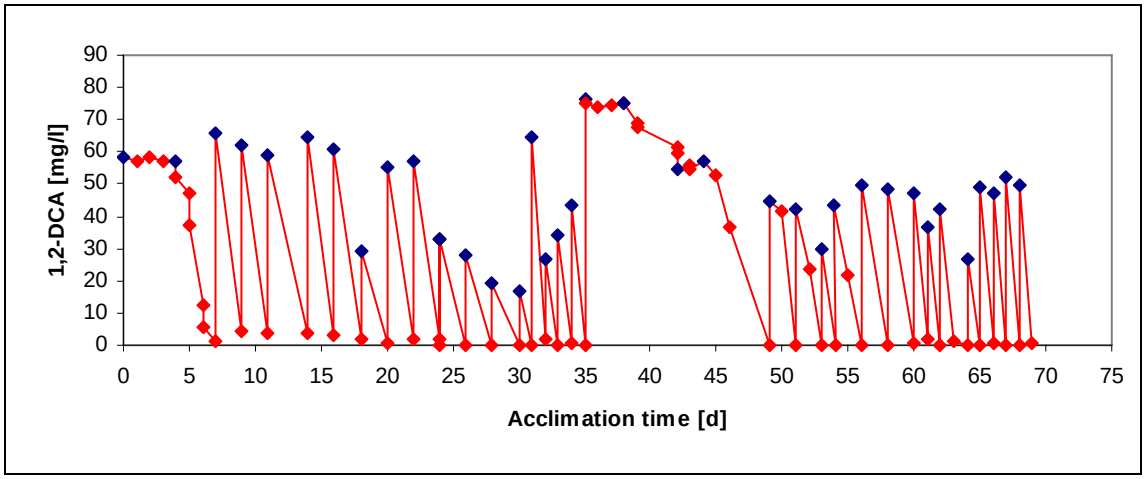

Figure 8.8. Trend of 1,2-DCA concentration in the SBR operated in fed batch modality. Blue points indicate the beginning of a new cycle.

8.2.1.2 Acclimation to 1,2-DCB

According to what observed in the batch tests on 1,2-DCB, which was degraded only by the sludge acclimated to 1,2-DCA, degradation of 1,2-DCB was performed in the SBR with the 1,2-DCA acclimated sludge.

Since the 98th day, four cycles, each of them lasting 6 to 8 days, were performed; 1,2-DCB was fed together with 1,2-DCA, which was the growth substrate of the acclimated sludge, and which could act as co-substrate in the metabolic process of 1,2-DCB degradation.

Results of the cycles are shown in Table 8.12 and Figure 8.9.

Table 8.12. Concentration of 1,2-DCA and 1,2-DCB in the SBR operated in fed-batch modality.

Time [d]	1,2-DCA [mg/l]	1,2-DCB [mg/l]
CYCLE N. 1		
0	45.7	14.3
1	22.1	12.5
2	10.9	11.9
6	0	10.4
Mean specific removal rate [$g/(d \cdot g_{VSS})$]	0.107	0.003
CYCLE N. 2		
0	53.6	16.3
1	28.1	14.2
2	12.1	11.3
5	0	9.8
Mean specific removal rate [$g/(d \cdot g_{VSS})$]	0.116	0.006
CYCLE N. 3		
0	49.4	13.2
1	24.7	10
3	13.7	6.9
6	0	3.4
Mean specific removal rate [$g/(d \cdot g_{VSS})$]	0.112	0.009
CYCLE N. 4		
0	43.5	14.8
1	21.1	12.9
2	11.7	7.8
4	0	2.1
Mean specific removal rate [$g/(d \cdot g_{VSS})$]	0.102	0.011

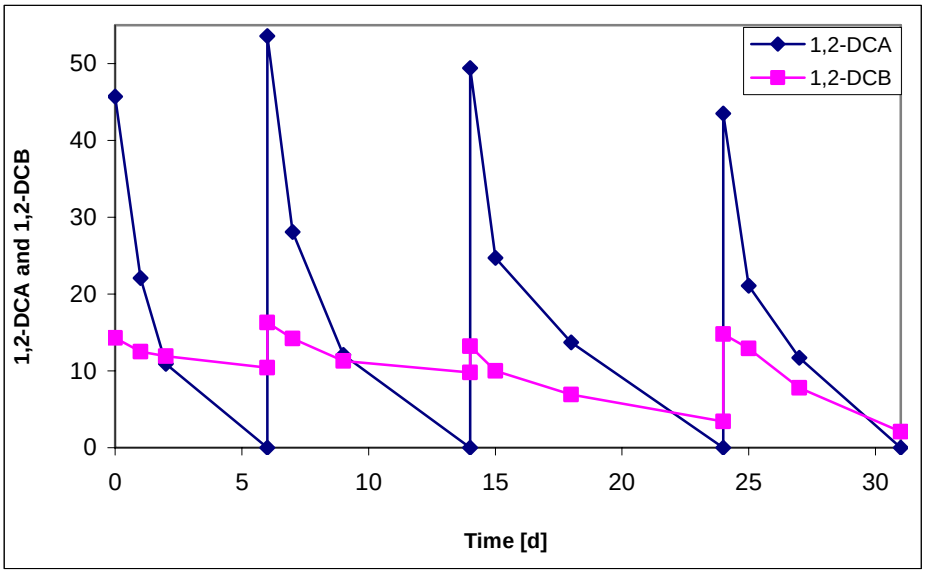

Figure 8.9. Concentration profiles of 1,2-DCA and 1,2-DCB in the SBR operated in fed-batch modality.

8.2.2 SBR operated in daily cycles modality

8.2.2.1 Degradation of 1,2-DCA

From the 70th to the 98th day of acclimation, degradation of 1,2-DCA took place in the SBR operated in daily cycles modality.
Degradation trend is shown in Table 8.13 and in Figure 8.10.

Table 8.13. 1,2-DCA concentration in the influent and effluent of the SBR operated in daily cycles modality.

Time [d]	1,2-DCA [mg/l]	Chlorides [mg/l]	mmol 1,2-DCA	mmol Cl	$mmol_{Cl}$ /$mmol_{1,2\text{-}DCA}$	$N\text{-}NO_3$ [mg/l]
70	45.1	49.7	0.95	2.46	2.59	18.4
71	0.4	91.2				17.6
71	48.6	40.6	1.02	2.87	2.82	18.4
72	0.7	89				18.9
72	42.9	42.3	0.89	2.57	2.87	19.2
73	0.8	85.6				16.8
73	39.7	43.8	0.82	1.42	1.72	17.4
74	0.9	67.7				17.2
74	44.8	44.7	0.92	1.71	1.85	19.0
75	1.3	73.5				18.4
75	52.8	42.3	1.08	2.22	2.06	18.9
76	1.9	79.8				16.3
76	49.6	45.6	1.04	2.11	2.04	17.9
77	0.8	81.3				19.4
77	49.3	50.8	1.02	2.32	2.27	18.0
78	1.3	89.9				17.9
78	44.3	51.3	0.92	2.15	2.34	18.3
79	0.9	87.6				18.9
79	46.8	53.2	0.98	1.52	1.55	18.8

to be continued

follows from the previous page

80	0.5	78.9				17.3
80	43.2	51.2	0.90	1.69	1.88	17.9
81	0.8	79.8				16.4
81	42.9	49.8	0.89	1.71	1.92	18.4
82	1.1	78.6				16.8
82	39.7	48.7	0.82	1.46	1.78	19.3
83	0.9	73.4				19.9
83	47.4	49.6	0.97	1.79	1.84	18.0
84	1.6	79.8				17.5
84	58.9	52.1	1.20	2.05	1.71	17.7
85	2.3	86.7				18.7
85	55.7	54.6	1.15	2.42	2.10	18.1
86	1.5	95.4				17.1
86	52.9	52.8	1.11	2.14	1.93	17.5
87	0.6	88.9				17.9
87	49.4	56.7	1.03	2.43	2.36	19.5
88	0.7	97.8				19.1
88	36.13	56.3	0.76	2.66	3.49	18.9
89	0.2	101.2				18.2
89	45.9	45.6	0.97	2.40	2.48	17.1
90	0.3	86.1				19.5
Mean specific degradation rate [$g_{DCA}/(d \cdot g_{VSS})$]	0.19					

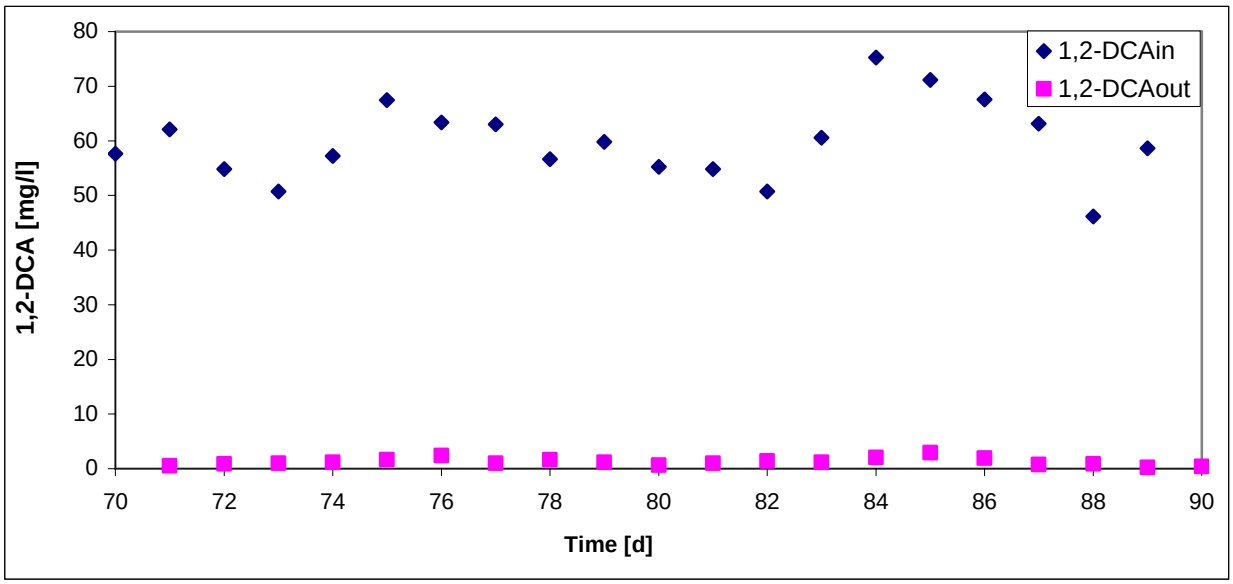

Figure 8.10. Trend of 1,2-DCA degradation in SBR operated in daily cycles modality. DCA_{in}: 1,2-DCA in the influent; DCA_{out}: 1,2-DCA in the effluent.

On the 90th day, a kinetic test was performed, obtaining a mean specific removal rate of 0.19 $g_{DCA}/d \cdot g_{VSS}$ and a maximum specific removal rate of 0.48 $g_{DCA}/d \cdot g_{VSS}$.

8.2.2.2 Degradation of 1,2-DCA and 2-CP in presence of phenol

Since the 500th day of reactor operation during which 1,2-DCA, 1,2-DCB and 2-CP were fed, a series of tests were performed in the SBR, in order to investigate the effects of the presence of **phenol** on degradation of **1,2-DCA** and **2-CP**. The tests started with the measurement of the maximum specific removal rates for 1,2-DCA and 2-CP; before feeding the two substrates, for one month 1,2-DCA was fed daily to the reactor as the only substrate. Then, phenol was added at different concentrations, and specific removal rates for 1,2-DCA and 2-CP were measured and compared to the ones observed when the two substances were fed without phenol.

Five different phases can be defined, each of them differing from the others for the typology and concentration of substrates fed to the SBR; the phases are detailed in Table 8.14.

Table 8.14. Phases of the experimentation on the effects of phenol at different concentrations on the degradation of 1,2-DCA and 2-CP (*: previously the system was fed with only 1,2-DCA for around one month.

Phase n.	1,2-DCA [mg/l]	2-CP [mg/l]	Phenol [mg/l]	Length [d]
1*	50	25	0	16
2	50	50	0	12
3	50	50	25	4
4	50	50	50	11
5	50	50	25	13

At the end and during any phase (with the exception of Phase n.3), kinetic tests were performed, in order to calculate the maximum specific removal rates of 1,2-DCA and 2-CP reached in the phase. Results of the tests are shown in Tables from 8.15 to 8.24 and in Figures from 8.11 to 8.15

Table 8.15. Results of Phase n.1; feeding of 1,2-DCA (50 mg/l) and 2-CP (25 mg/l). Previously the system was fed with only 1,2-DCA for around one month.

Time [d]	1,2-DCA [mg/l]	2-CP [mg/l]	Chlorides [mg/l]	Nitrates [mg/l]	Mean specific removal rate 1,2-DCA [$g_{DCA}/d \cdot g_{VSS}$]	Mean specific removal rate 2-CP [$g_{CP}/d \cdot g_{VSS}$]	VSS [mg/l]
0	14.05	20.32	54.87	34.16	0.05	0.08	256
1	1.565	0.00	57.37	27.53	-		
1	61.8	18.39	18.89	11.35	0.23	0.07	
2	4.07	0.00	108.56	38.29	-		
2	72.54	15.95	52.70	33.06	0.28	0.06	
3	0.49	0.00	104.83	30.87	-	-	

Table 8.16. Kinetic test on 1,2-DCA and 2-CP at the end of Phase n.1.

Time [h]	1,2-DCA [mg/l]	2-CP [mg/l]	Chlorides [mg/l]	Nitrates [mg/l]
0	72.54	15.95	52.70	33.06
1	55.91	15.60	57.19	30.89
2	46.97	13.43	60.42	29.68
3	38.87	12.09	67.98	30.44
4	43.66	12.05	70.66	29.63
24	0.49	0.00	104.83	30.87
Maximum specific removal rate [$g/(d \cdot g_{VSS})$]	1.05	0.16		

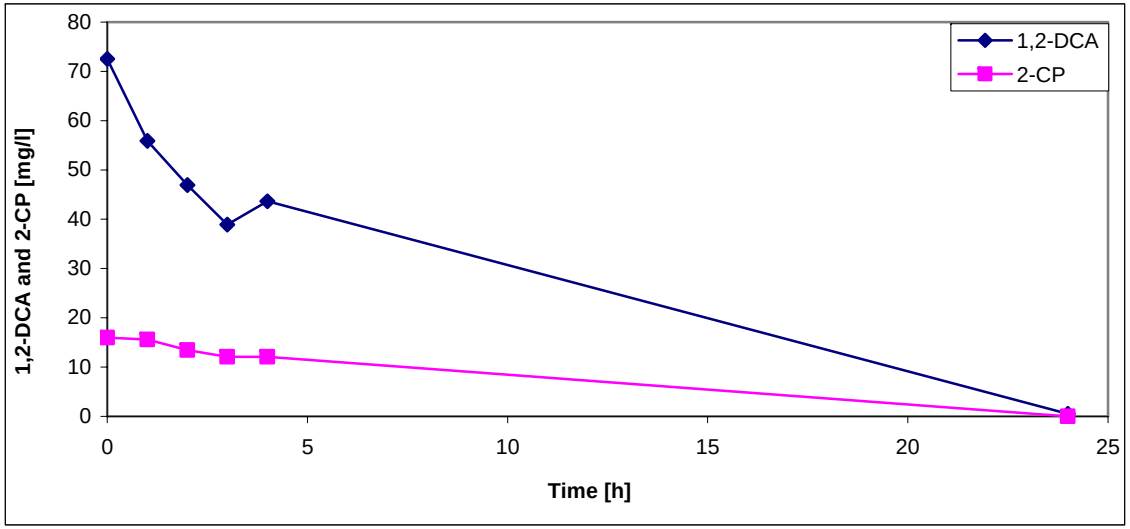

Figure 8.11. Kinetic test on 1,2-DCA and 2-CP at the end of Phase n. 1.

Table 8.17. Results of Phase 2; feeding of 1,2-DCA (50 mg/l) and 2-CP (50 mg/l).

Time [d]	1,2-DCA [mg/l]	2-CP [mg/l]	Chlorides [mg/l]	Nitrates [mg/l]	Mean specific removal rate 1,2-DCA [$g_{DCA}/d \cdot g_{VSS}$]	Mean specific removal rate 2-CP [$g_{CP}/d \cdot g_{VSS}$]	VSS [mg/l]
0	n.a.	47.56	42.75	41.82	n.a.	0.04	
1	n.a.	36.82	52.53	45.10	n.a.	-	294
1	63.29	49.96	38.13	43.79	0.09	0.16	
2	38.04	1.48	64.93	44.05	-	-	
6	-	-	83.18	43.38	-	-	
6	30.94	50.44	42.42	38.94	0.10	0.17	
7	0.72	0.00	79.64	40.10	-	-	
7	93.42	47.97	42.03	34.49	0.30	0.15	
8	0.00	0.00	130.40	32.70	-	-	
8	75.60	52.72	49.72	31.81	0.24	0.17	
9	0.03	0.00	129.38	27.20	-	-	312
9	63.38	48.87	53.33	31.06			
12	0.00	0.00			-	-	

Table 8.18. Kinetic test on 1,2-DCA and 2-CP at the end of Phase n.2.

Time [h]	1,2-DCA [mg/l]	2-CP [mg/l]	Chlorides [mg/l]	Nitrates [mg/l]
0	63.38	48.87	53.33	31.06
1	50.17	46.26	61.21	30.60
2	43.05	43.17	67.15	29.57
3	35.79	38.44	72.59	29.96
4	31.79	37.47	85.11	29.23
24	0.00	0.00		
Maximum specific removal rate [$g./(d \cdot g_{VSS})$]	0.61	0.27		

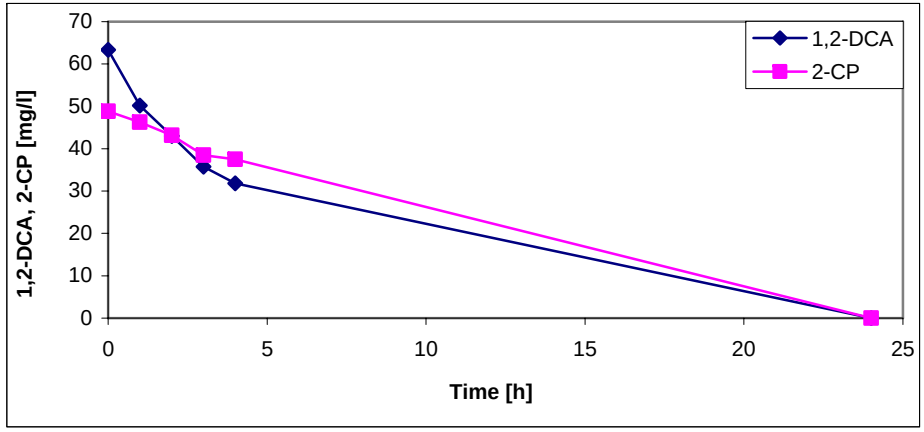

Figure 8.12. Kinetic test on 1,2-DCA and 2-CP at the end of Phase n. 2.

Table 8.19. Results of Phase 3; feeding of 1,2-DCA (50 mg/l), 2-CP (50 mg/l) and phenol (25 mg/l).

Time [d]	1,2-DCA [mg/l]	2-CP [mg/l]	Phenol [mg/l]	Chlorides [mg/l]	Nitrates [mg/l]	Mean specific removal rate 1,2-DCA [$g_{DCA}/d \cdot g_{VSS}$]	Mean specific removal rate 2-CP [$g_{CP}/d \cdot g_{VSS}$]	VSS [mg/l]
0	87.23	44.30	20.51	31.44	22.49	0.13	0.11	
1	38.36	1.33	0.00	42.76	20.19	-	-	
1	83.33	57.21	20.77	34.19	33.23	0.17	0.15	
2	18.56	0	0.05	91.60	31.36	-	-	
2	64.13	55.14	19.18	44.75	32.34	-	0.14	382
3	n.a.	0	0	105.21	19.29		-	
3	n.a.	49.39	20.96	46.36	28.08		0.13	
4	n.a.	0	0.09	91.92	14.71		-	

Table 8.20. Results of Phase 4; feeding of 1,2-DCA (50 mg/l), 2-CP (50 mg/l) and phenol (50 mg/l).

Time [d]	1,2-DCA [mg/l]	2-CP [mg/l]	Phenol [mg/l]	Chlorides [mg/l]	Nitrates [mg/l]	Mean specific removal rate 1,2-DCA [$g_{DCA}/d \cdot g_{VSS}$]	Mean specific removal rate 2-CP [$g_{CP}/d \cdot g_{VSS}$]	Mean specific removal rate Phenol [$g_{phenol}/d \cdot g_{VSS}$]	VSS [mg/l]
0	55.63	49.4	47.27	35.82	17.78	-	0.13	0.11	
3	0.03	0	0.06	98.3	3.87	-	-	-	
3	76.13	50.74	42.9	45.82	30.26	0.12	0.09	0.10	
4	31.45	17.94	0.05	69.52	22.97	-	-	-	
4	65.66	54.3	39.56	37.42	30.24	0.12	0.14	0.09	
5	18.79	0	0	71.72	15.56	-	-	-	
5	61.16	45.72	40.04	37.38	32.65	0.14	0.11	0.10	
6	1.48	0.27	0.24	86.5	19.15	-	-	-	
6	29.7	48.81	43.06	40.32	29.43	0.07	0.11	0.10	
7	0.00	3.58	0	67.65	14.7	-	-	-	418
7	37.33	45.87	43.08	38.12	26.83	0.09	0.11	0.10	
10	0.16	0	0.2	80.65	1.86	-	-	-	
10	46.1	51	43.45	39.71	33.04	0.11	0.09	0.10	
11	0.18	14.42	0	92.29	21.52		-		

Table 8.21. Results of the first kinetic test performed on the fourth day of Phase n. 4. Feeding of 1,2-DCA (50 mg/l), 2-CP (50 mg/l) and phenol (50 mg/l).

Time [h]	1,2-DCA [mg/l]	2-CP [mg/l]	Phenol [mg/l]	Chlorides [mg/l]	Nitrates [mg/l]
0	65.66	54.3	39.56	37.42	30.24
1	60.2	52.26	32.76	38.79	29.97
2	54.2	51.33	25.41	39.52	30.09
3	53.56	47.6	12	42.76	31.55
4	50.92	46.97	7.09	45.24	32.03
24	18.79	0	0	71.72	15.56
Maximum specific removal rate [g/(d·g_{VSS})]	0.23	0.08	0.51		

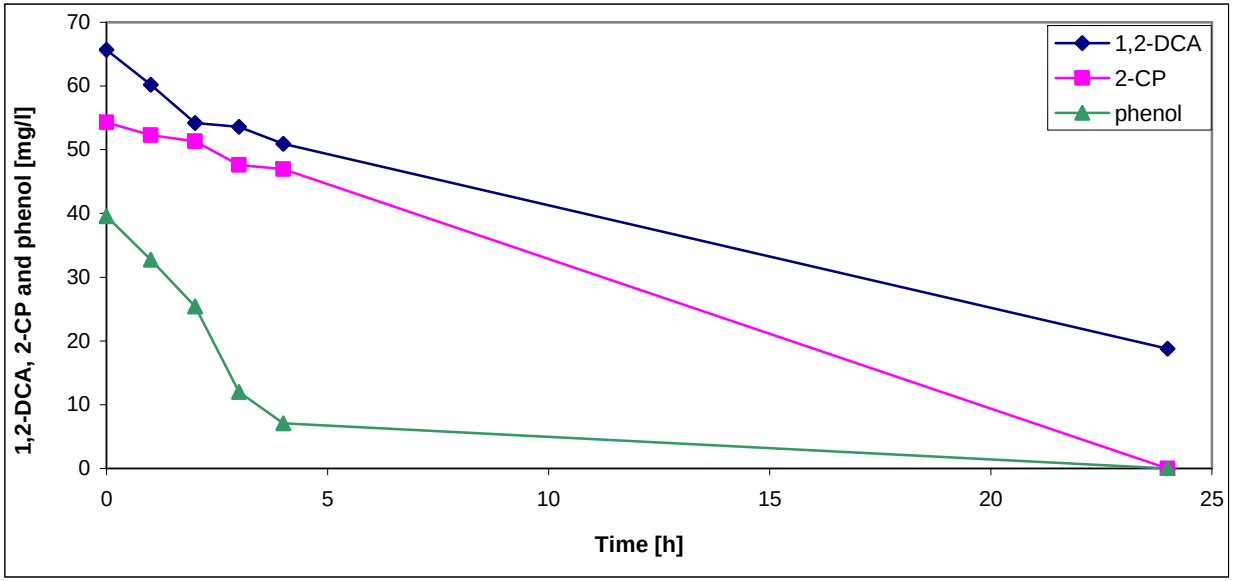

Figure 8.13. Kinetic test performed on the fourth day of Phase n. 4.

Table 8.22. Results of the second kinetic test performed at the end of Phase n. 4. Feeding of 1,2-DCA (50 mg/l), 2-CP (50 mg/l) and phenol (50 mg/l).

Time [h]	1,2-DCA [mg/l]	2-CP [mg/l]	Phenol [mg/l]	Chlorides [mg/l]	Nitrates [mg/l]
0	46.1	51	43.45	39.71	33.04
1	35.48	50.44	40.51	42.79	33.8
2	31.02	48.3	36.8	45.97	33.73
3	26.3	48.3	32.36	48.79	33.49
4	24.23	46.3	30.17	51.57	32.49
24	0.18	14.42	0.00	92.29	21.52
Maximum specific removal rate [g/(d·g_{VSS})]	0.31	0.05	0.15		

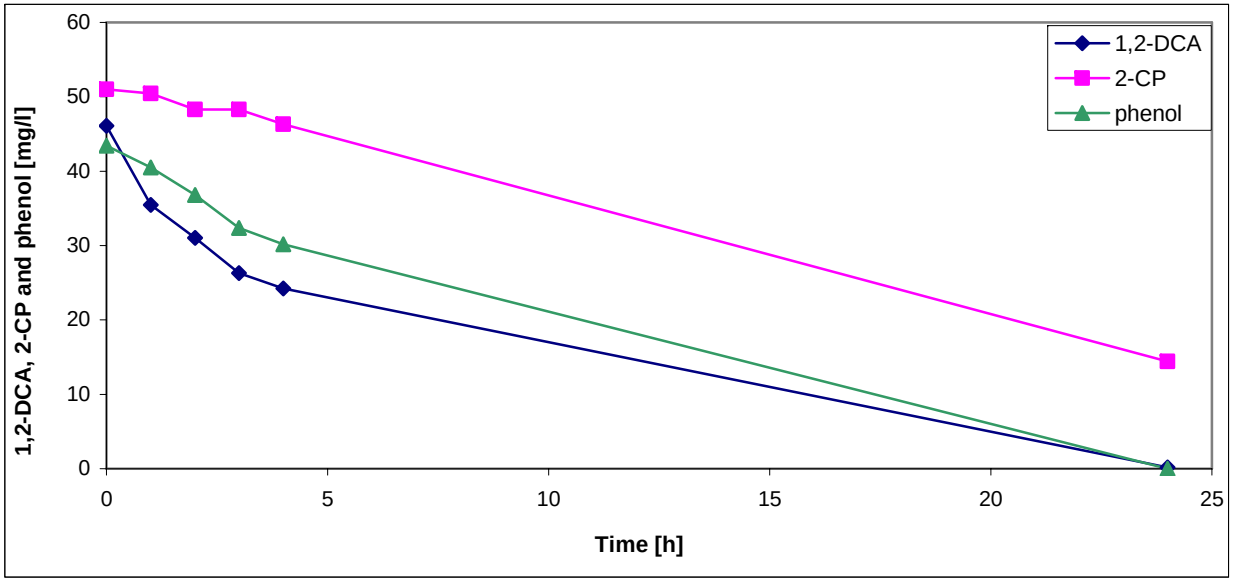

Figure 8.14. Kinetic test performed at the end of Phase n.4.

Table 8.23. Results of Phase 5; feeding of 1,2-DCA (50 mg/l), 2-CP (50 mg/l) and phenol (25 mg/l).

Time [d]	1,2-DCA [mg/l]	2-CP [mg/l]	Phenol [mg/l]	Chlorides [mg/l]	Nitrates [mg/l]	Mean specific removal rate 1,2-DCA [$g_{DCA}/d \cdot g_{VSS}$]	Mean specific removal rate 2-CP [$g_{CP}/d \cdot g_{VSS}$]	Mean specific removal rate Phenol [$g_{phenol}/d \cdot g_{VSS}$]	VSS [mg/l]
0	36.37	55.36	20.56	48.13	31.89	0.08	0.12	0.04	
1	0.8	0.00	0.00	93.48	20.12	-	-	-	
1	37.56	44.39	18.36	47.94	31.21	0.08	0.10	0.04	
2	0.42	0.00	0.00	94.77	16.71	-	-	-	458
2	34.36	45.68	20.72	48.53	30.96	0.08	0.10	0.05	
3	0.00	0.00	0.00	91.48	22.74	-	-	-	
3	30.73	49.94	20.08	47.41	32.57	0.07	0.11	0.04	
6	n.a.	0.00	0.00	84.43	24.25	-	-	-	
6	n.a.	51.23	24.64	43.91	41.28	-	0.11	0.05	
7	1.79	0.18	0.24	81.27	38.52	-	-	-	
7	28.50	48.39	21.16	44.54	44.37	0.06	0.11	0.05	
8	0.18	0.11	0.20	82.73	34.84	-	-	-	
8	26.96	50.64	20.30	44.83	42.88	0.06	0.11	0.04	
9	0.00	0.00	0.23	79.45	34.76	-	-	-	
9	20.39	49.08	20.07	44.14	44.47	0.04	0.11	0.04	
10	-0.13	0.00	0.13	77.11	33.63	-	-	-	
10	61.81	53.65	22.87	48.56	48.17	0.13	0.11	0.05	486
13	0.03	0	1.5	99.34	27.16				

Table 8.24. Results of the kinetic test performed at the end of Phase n.5. Feeding of 1,2-DCA (50 mg/l), 2-CP (50 mg/l) and phenol (25 mg/l).

Time [h]	1,2-DCA [mg/l]	2-CP [mg/l]	Phenol [mg]	Chlorides [mg/l]	Nitrates [mg/l]
0	61.81	53.65	22.87	48.56	48.17
1	55.16	52.50	20.64	57.49	47.02
2	48.19	52.00	17.06	51.89	46.96
3	44.77	50.97	15.02	52.10	45.89
4	39.61	49.44	12.26	55.70	44.67
24	0.03	0	1.5	99.34	27.16
Maximum specific removal rate [$g_{/(d \cdot g_{VSS})}$]	0.27	0.05	0.13		

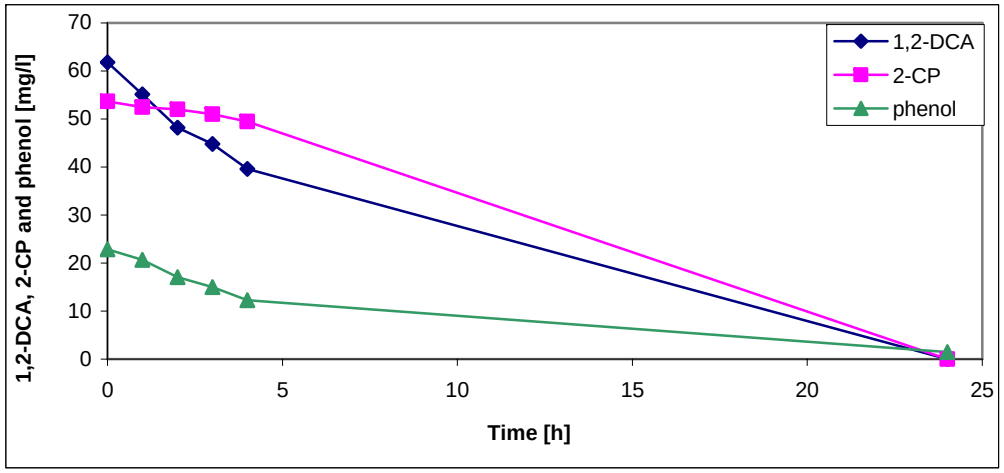

Figure 8.15. Kinetic test performed at the end of Phase n.5.

8.2.2.3 Effects of operating parameters variation on 1,2-DCA degradation

After 547 days of reactor operation, a series of tests were performed in the SBR, in order to investigate how the variation of operating parameters could affect 1,2-DCA removal.
Therefore, this part of experimentation was developed in four phases, that are detailed in Table 8.25.

Table 8.25. Variation of operating parameters in the SBR; substrate fed was 1,2-DCA in all the phases.

Phase n.	Temperature [°C]	Mineral medium aeration	Mineral medium composition
1	30	Yes	Minerals aqueous solution
2	20	Yes	Minerals aqueous solution
3	20	No	Minerals aqueous solution
4	20	No	Tap water

During and at the end of any phase, kinetic tests were performed in order to check biomass activity in the different operating conditions.

Phase n.1: degradation of 1,2-DCA at 30°C with aerated mineral medium

Before the beginning of this phase, the SBR was fed with 1,2-DCA (the biomass growth substrate) for one month. In this phase, the system was fed with 1,2-DCA and aerated mineral medium, and was kept at 30°C.
During this phase, two kinetic tests were performed: one on the 14th day of the phase and the other one at the end of the phase, which lasted 18 days. Degradation of 1,2-DCA observed in the first phase and kinetic results are shown in Tables from 8.26 to 8.28 and in Figures 8.16 and 8.17.

Table 8.26. Results of phase n.1.

Time [d]	1,2-DCA [mg/l]	Chlorides [mg/l]	N-NO_3 [mg/l]	Mean specific removal rate 1,2-DCA [$g_{DCA}/d \cdot g_{VSS}$]	mmol 1,2-DCA oxidized	mmol chlorides produced	mmol 1,2-DCA/ mmol Cl
0	17.5	45.38	49.83	0.04	0.53	0.96	1.81
1	0.56	56.38	44.76				
1	59.25	33.18	17.94	0.19	1.86	3.32	1.79
2	0	71.12	20.67				
2	38.27	56.07	29.77	0.12	1.19	2.98	2.51
3	0.28	90.18	45.29				
3	122.19	40.65	45.03	0.36	3.4	8.2	2.41
4	13.6	134.44	11.86				
4	131.91	43.29	19.97	0.11	3.09	7.57	2.45
7	33.13	129.82	35.77				
7	46.37	51.78	34.29	-	-	2.52	-
8	n.a.	80.65	30.14				
11	n.a.	9.14	13.31	-	-	4.9	-
14	1.30	65.13	65.9				
14	48.75	28.96	51.49	0.16	1.53	2.98	1.95
15	0.02	63.04	60.61				
15	47.90	34.27	38.27	0.16	1.5	1.98	1.32
16	0.04	56.9	51.76				
16	42.92	35.44	45.53	0.14	1.34	3.11	2.32

to be continued

follows from the previous page

17	0.21	70.95	44				
17	34.92	52.12	47.73	0.12	1.09	2.1	0.11
18	0	76.15	35.19				

Table 8.27. Kinetic test performed on the 14th day of the Phase n.1.

Time [h]	1,2-DCA [mg/l]	Chlorides [mg/l]	$N\text{-}NO_3$ [mg/l]
0	55.2	28.96	51.49
1	45.6	n.a.	n.a.
2	28.7	37.75	44.99
3	18.7	n.a.	n.a.
4	13.8	48.96	1.68
24	0.02	63.04	60.61
Maximum specific removal rate [$g_{/}(d \cdot g_{VSS})$]	1.13		

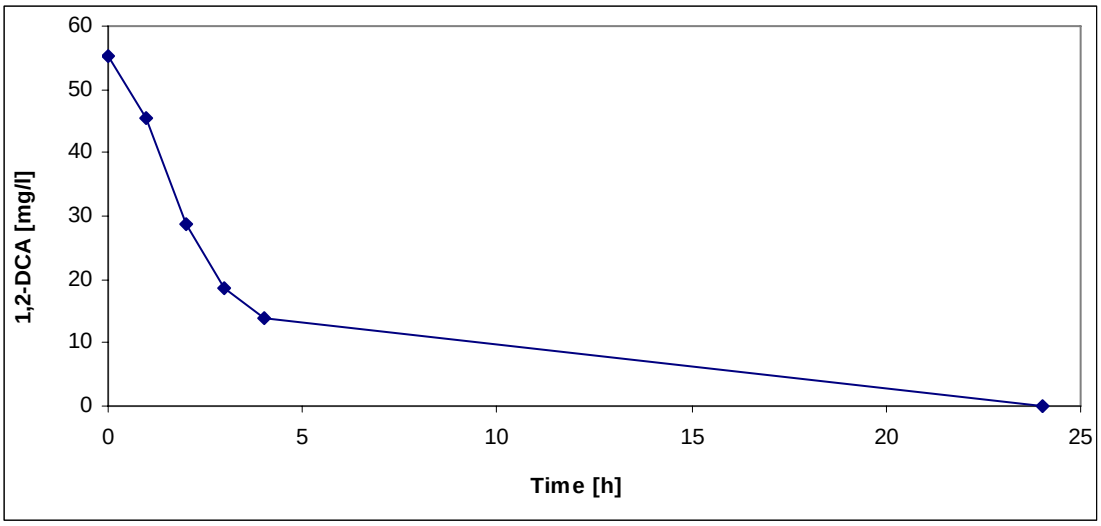

Figure 8.16. Trend of 1,2-DCA during the kinetic test performed on the 14th day of the Phase n.1.

Table 8.28. Kinetic test performed at the end of the Phase n.1.

Time [h]	1,2-DCA [mg/l]	Chlorides [mg/l]	$N\text{-}NO_3$ [mg/l]
0	54.5	52.12	47.73
1	32.04	n.a	n.a
2	26.62	61.3	41.59
3	17.4	n.a	n.a
4	12	74.38	36.64
24	0.21	100.95	43.99
Maximum specific removal rate [$g_{/}(d \cdot g_{VSS})$]	1.16		

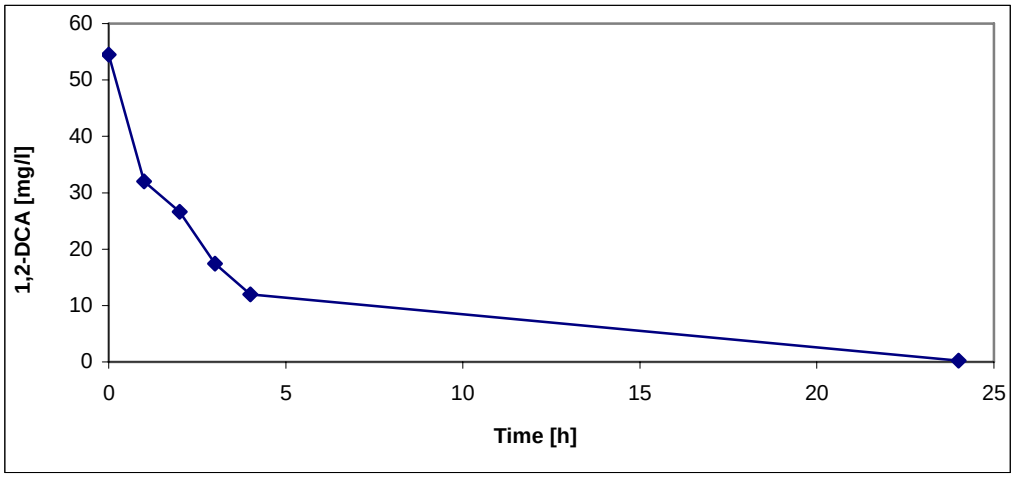

Figure 8.17. Trend of 1,2-DCA during the kinetic test performed at the end of the Phase n. 1.

Phase n.2: degradation of 1,2-DCA at 20°C with aerated mineral medium

In this phase, the system was fed with 1,2-DCA and aerated mineral medium, and was kept at 20°C.

During this phase, two kinetic tests were performed: one on the 6th day of the phase and the other one at the end of the phase, which lasted 18 days. Degradation of 1,2-DCA observed in the second phase and the kinetic results are shown in Tables from 8.29 to 8.31 and in Figures 8.18 and 8.19.

Table 8.29. Results of phase n. 2.

Time [d]	1,2-DCA [mg/l]	Chlorides [mg/l]	N-NO_3 [mg/l]	Mean specific removal rate 1,2-DCA [$g_{DCA}/d \cdot g_{VSS}$]	mmol 1,2-DCA oxidized	mmol chlorides produced	mmol 1,2-DCA/ mmol Cl
0	52.96	43.37	30.96	0.06	1.62	3.36	2.08
1	1.28	81.85	40.19				
1	43.24	47.92	35.67	0.14	1.35	3.62	2.67
2	0.07	89.26	43.05				
2	43.79	48.51	37.7	0.15	1.37	2.59	1.89
3	0.03	78.12	38.71				
3	40.51	45.02	39.2	0.12	1.27	2.86	2.26
4	0.02	77.72	49.99				
4	33.73	49.91	40.58	0.1	1.06	2.89	2.74
7	0.03	82.98	45.87				
7	46.21	44.73	37.55	0.04	1.45	4.06	2.81
8	0	91.2	55.44				
11	26.59	45.49	53.8	0.08	0.83	2.08	2.49
14	0	69.24	51.29				
14	49.62	46.5	52.3	0.14	1.48	3.9	2.64
15	2.43	91.1	44.39				
15	56.57	43.18	44.66	0.16	1.77	4.55	2.57
16	0.07	95.24	45.55				
16	50.92	51.96	21.06	0.14	1.59	3.14	1.97
17	0	87.92	23.42				
17	32.71	47.03	18.87	0.02	1.02	2.61	2.55
18	0	76.85	27.28				

Table 8.30. Kinetic test performed on the 6th day of the Phase n. 2.

Time [h]	1,2-DCA [mg/l]	Chlorides [mg/l]	$N\text{-}NO_3$ [mg/l]
0	46.21	44.73	37.55
1	35.2	n.a	n.a
2	30.6	58.84	31.76
3	27.4	n.a	n.a
4	20.7	70.99	26.86
24	0.7	91.20	55.44
Maximum specific removal rate [$g_{/(d \cdot g_{VSS})}$]	0.69		

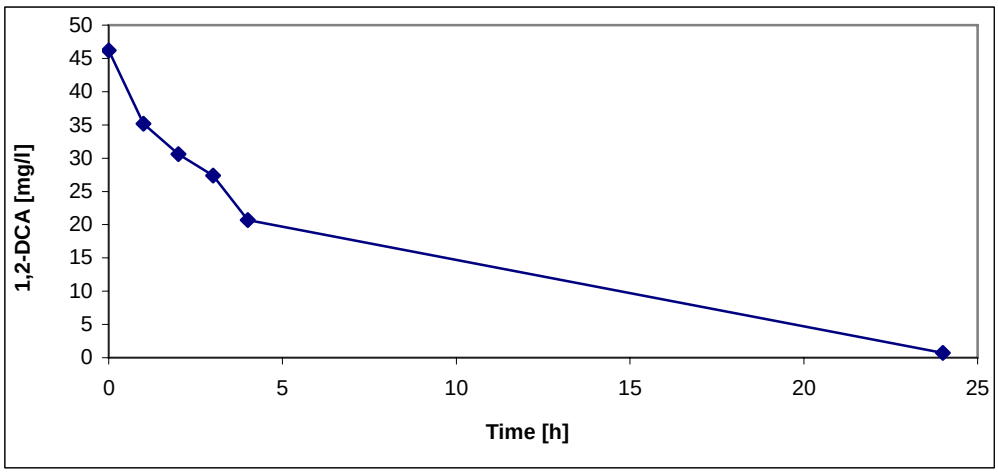

Figure 8.18. Trend of 1,2-DCA during the kinetic test performed on the 6th day of the Phase n. 2.

Table 8.31. Kinetic test performed at the end of the Phase n. 2.

Time [h]	1,2-DCA [mg/l]	Chlorides [mg/l]	Nitrates [mg/l]
0	32.71	47.03	18.87
1	28.13	n.a	n.a
2	21.6	59.65	20.23
3	17.3	n.a	n.a
4	8.8	70.07	21.2
24	0	95.86	56.30
Maximum specific removal rate [$g_{/(d \cdot g_{VSS})}$]	0.65		

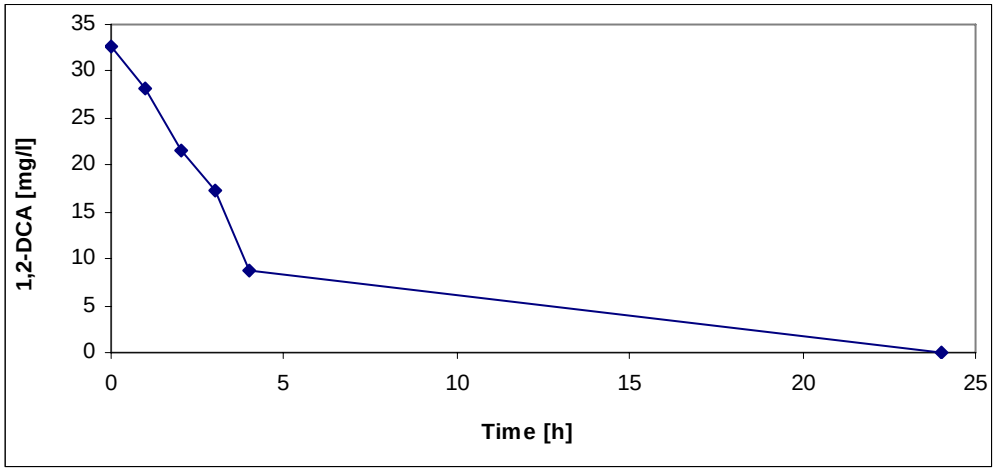

Figure 8.19. Trend of 1,2-DCA during the kinetic test performed at the end of the Phase n. 2.

Phase n. 3: degradation of 1,2-DCA at 20°C with non-aerated mineral medium

In this phase, the system was fed with 1,2-DCA and non-aerated mineral medium, and was kept at 20°C.

During this phase, two kinetic tests were performed: one on the 10th day of the phase and the other one at the end of the phase, which lasted 24 days. Degradation of 1,2-DCA observed in the third phase and the kinetic results are shown in Tables from 8.32 to 8.34 and in Figures 8.20 and 8.21.

Table 8.32. Results of phase n. 3.

Time [d]	1,2-DCA [mg/l]	Chlorides [mg/l]	N-NO_3 [mg/l]	Mean specific removal rate 1,2-DCA [$g_{DCA}/d \cdot g_{VSS}$]	mmol 1,2-DCA oxidized	mmol chlorides produced	mmol 1,2-DCA/ mmol Cl
0	43.37	38.92	14.76	0.12	3.89	2.87	1.36
1	0.07	83.46	36.89				
1	34.67	43.29	27.17	0.1	2.95	2.72	1.08
2	0.05	77.04	28.94				
2	62.54	36.6	18.42	0.18	4.2	2.15	1.95
3	0.13	84.59	24.37				
3	78.53	44.21	8.82	0.08	5.08	2.07	2.46
6	0	102.34	47.8				
6	59.78	48.39	13.94	-	3.66	-	-
7	n.a.	90.23	31.29				
7	n.a.	42.97	10.94	-	2.77	-	-
8	0.24	74.66	18.73				
8	42.54	48.23	9.34	0.11	2.49	1.87	1.33
9	0.1	76.66	23.13				
9	40.66	44.48	8.49	0.1	3.63	2.89	1.25
10	0.59	85.98	27.68				
10	56.53	43.27	11.88	*0.05*	3.01	1.7	1.77
13	0	77.66	62.22				
13	26.5	34.9	16.68	0.07	2.16	2.61	0.83
14	0.06	59.6	42.53				
14	35.66	42.81	11.5	*0.05*	2.62	2.39	1.1
16	0.63	72.8	52.18				

to be continued

follows from the previous page

16	63.51	26.09	14.98	0.16	2.75	1.43	1.92
17	2.22	57.53	27.41				
17	34.43	43.82	10.17	0.02	2.59	2.4	1.08
22	0	73.44	44.77				
22	53.25	35.5	15.8	0.14	2.7	1.62	1.66
23	0.18	66.34	28.69				
23	43.18	32.96	11.1	-	2.71	-	-
24	n.a.	63.98	34.74				

Table 8.33. Kinetic test performed on the 10th day of the Phase n.3.

Time [h]	**1,2-DCA [mg/l]**	**Chlorides [mg/l]**	**N-NO_3 [mg/l]**
0	56.53	43.27	11.88
1	51.68	n.a	n.a
2	45.55	54.26	23.54
3	42.76	n.a	n.a
4	36.4	66.75	36.98
24	0	77.66	62.22
Maximum specific removal rate [$g/(d \cdot g_{VSS})$]	0.66		

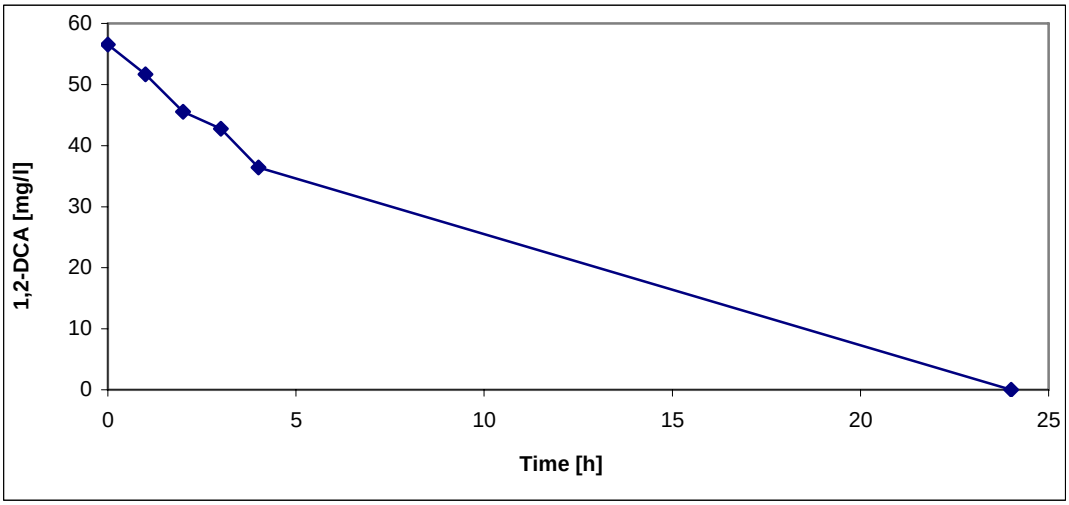

Figure 8.20. Trend of 1,2-DCA during the kinetic test performed on the 10th day of the Phase n. 3.

Table 8.34. Kinetic test performed at the end of the Phase n.3.

Time [h]	1,2-DCA [mg/l]	Chlorides [mg/l]	N-NO_3 [mg/l]
0	46.2	32.96	11.1
1	41.31	n.a	n.a
2	34.84	47.7	15.8
3	28.51	n.a	n.a
4	23	46.15	12.29
24	0	65.46	22.61
Maximum specific removal rate [$g_{/(d \cdot g_{VSS})}$]	0.63		

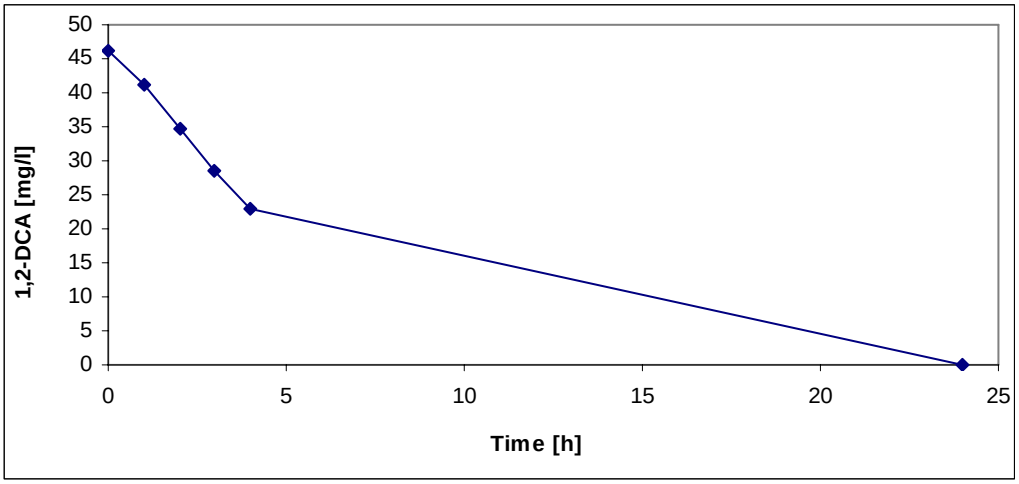

Figure 8.21. Trend of 1,2-DCA during the kinetic test performed at the end of the Phase n. 3.

Phase n. 4: degradation of 1,2-DCA at 20°C with non-aerated tap water

In this phase, the system was fed with 1,2-DCA and non-aerated tap water, and was kept at 20°C.During this phase, two kinetic tests were performed: one on the 7th day and the other one at the end of the phase, which lasted 26 days. Degradation of 1,2-DCA observed in the third phase is shown in Tables from 8.35 to 8.37 and in Figures 8.22 and 8.23.

Table 8.35. Results of phase n. 4.

Time [d]	1,2-DCA [mg/l]	Chlorides [mg/l]	N-NO_3 [mg/l]	mmol 1,2-DCA/ mmol Cl	mmol 1,2-DCA oxidized	mmol chlorides produced	Mean specific removal rate 1,2-DCA [$g_{DCA}/d \cdot g_{VSS}$]
0	n.a.	42.86	13.87	-	2.86		-
3	n.a.	75.54	23.88			-	
4	n.a.	29.77	8.67	-	1.62	-	-
5	0	48.27	10.99				
5	40.47	33.92	5.42	1.21	2	1.66	0.1
6	1.98	56.77	6.6				

to be continued

follows from the previous page

6	64.7	35.87	5.88	1.73	1.64	0.95	0.15
7	9.35	54.66	7.38				
7	52.46	30.36	5.66	1.64	0.81	0.49	0.05
10	0	39.65	6.01				
10	40.59	31.86	8.55	0.85	0.5	0.59	0.07
11	13.52	37.54	11.94				
11	35.94	28.38	4.5	0.69	0.98	1.43	0.06
12	13.95	39.63	5.32				
12	66.63	33.99	3.91	0.42	0.36	0.85	0.06
13	53.13	38.09	6.04				
13	71.01	27.98	10.04	0.21	0.26	1.29	0.03
14	64.46	31	11.81				
14	67.16	27.03	4.07	1.06	0.58	0.55	0.05
17	33.34	33.65	5.76				
17	47.02	46.23	6.91	0.5	0.73	1.46	0.07
18	31.19	54.52	5.2				
18	29.51	25.64	5.07	0.14	0.32	2.25	0.02
19	24.96	29.31	5.6				
19	28.76	25.84	5.07	0.13	0.39	2.95	0.01
20	24.57	30.27	6.61				
20	25.52	26.49	5.03	0.08	0.15	1.94	0.01
21	23.12	28.16	4				
24	68.82	24.91	4.95	0.5	0.21	0.43	0.02
21	52.9	27.37	5.46				
24	56.29	25.5	4.98	0.08	0.09	1.21	0.01
25	53.87	26.54	4.95				
25	57.96	25.17	4.85	0.25	0.13	0.5	0.05
26	49.84	26.63	5.08				

Table 8.36. Kinetic test performed on the 7th day of the Phase n. 4.

Time [h]	**1,2-DCA [mg/l]**	**Chlorides [mg/l]**	**N-NO_3 [mg/l]**
0	52.46	30.36	5.66
1	50.65	n.a	n.a
2	49.76	32.64	10.88
3	46.39	n.a	n.a
4	45.74	35.99	8.45
24	9.35	63.07	6.65
Maximum specific removal rate [$g_{/}(d \cdot g_{VSS})$]	0.11		

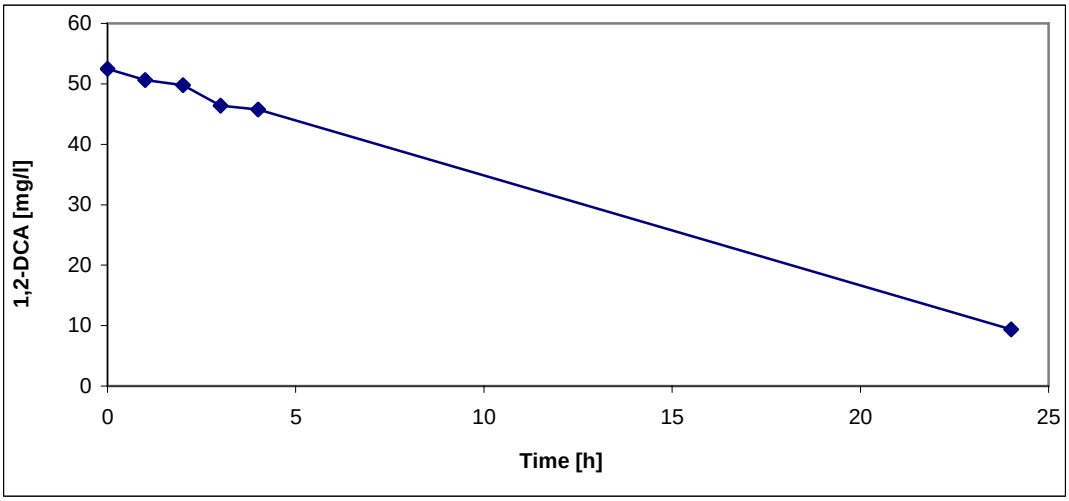

Figure 8.22. Trend of 1,2-DCA during the kinetic test performed on the 7th day of the Phase n. 4.

Table 8.37. Kinetic test performed at the end of the Phase n. 4.

Time [h]	1,2-DCA [mg/l]	Chlorides [mg/l]	$N\text{-}NO_3$ [mg/l]
0	50.58	21.65	5.42
1	53.09	n.a	n.a
2	54.72	23.88	4.72
3	52.15	n.a	n.a
4	52.97	24.78	4.89
24	49.6	25.40	4.66
Maximum specific removal rate [$g_{/}(d \cdot g_{VSS})$]	0.01		

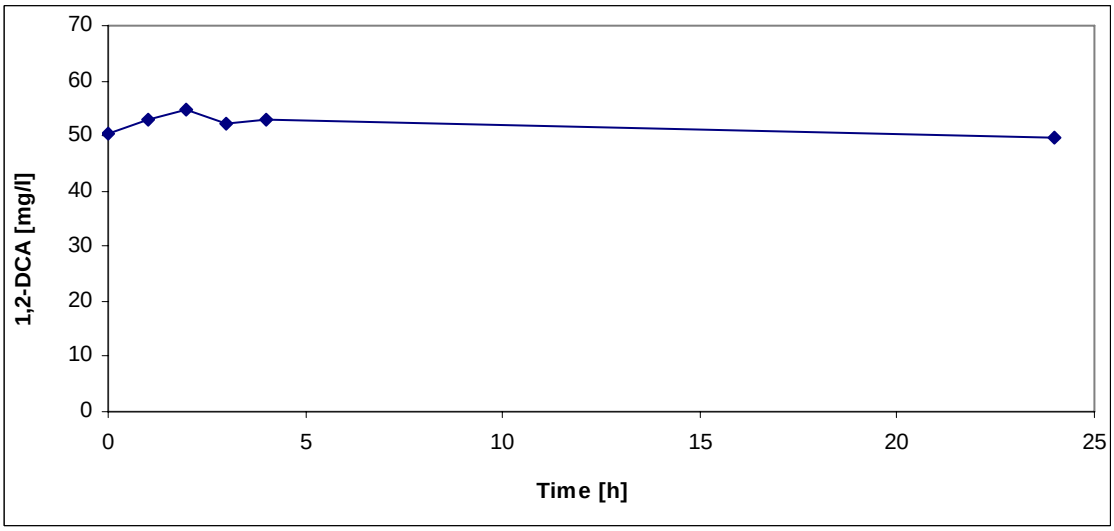

Figure 8.23. Trend of 1,2-DCA during the kinetic test performed at the end of the Phase n. 4.

Degradation of 1,2-DCA in the four phases

In Table 8.38 the average values of the maximum specific removal rates observed in the four phases are shown, while in Table 8.39 and in Figure 8.24 the 1,2-DCA concentrations in the influent and the effluent and removal efficiencies observed in all the four phases are shown.

Table 8.38. Maximum specific degradation rates achieved in the four phases.

Phase n.	Maximum specific degradation rate [$g_{DCAc}/(d \cdot g_{VSS})$]
1	0.38
2	0.21
3	0.31
4	0.06

Table 8.39. Trend of 1,2-DCA in the influent and in the effluent of the system and efficiency removal during the test on variation of operating parameters.

Time [d]	1,2-DCA$_{in}$ [mg/l]	1,2-DCA$_{out}$ [mg/l]	Removal efficiency [%]	Time [d]	1,2-DCA$_{in}$ [mg/l]	1,2-DCA$_{out}$ [mg/l]	Removal efficiency [%]
0	25.58	n.a.	-	**43**	n.a.	n.a.	-
1	77.02	0.56	98	**44**	52.74	0.24	100
2	49.2	0	100	**45**	50.42	0.01	100
3	154.07	0.28	99	**46**	70.1	0.59	99
4	163.57	13.6	91	**49**	32.86	0	100
7	57.5	33.13	80	**50**	44.22	0.06	100
11	n.a.	n.a.	-	**52**	78.75	0.63	99
14	60.45	1.3	98	**53**	42.69	2.22	97
15	59.4	0.02	100	**58**	66.03	0	100
16	53.22	0.04	100	**59**	53.54	0.18	100
17	43.3	0.21	100	**60**	n.a.	n.a.	-
18	65.67	0	100	**65**	50.18	0	100
21	53.62	1.28	98	**66**	80.23	1.98	96
22	54.3	0.07	100	**67**	65.05	9.35	88
23	50.23	0.03	100	**70**	50.33	0	100
24	41.83	0.02	100	**71**	44.57	13.52	73
25	57.3	0.03	100	**72**	82.62	13.95	69
28	32.97	0	100	**73**	88.05	53.13	36
29	61.53	0	100	**74**	83.28	64.46	27
30	70.15	2.43	96	**77**	58.3	33.34	60
31	63.14	0.07	100	**78**	36.59	31.19	47
32	40.56	0	100	**79**	35.66	24.96	32
36	53.78	0	100	**80**	31.64	24.57	31
37	42.99	0.07	100	**81**	85.34	23.12	27
38	77.55	0.05	100	**84**	69.8	52.9	38
39	97.37	0.13	100	**85**	71.87	53.87	23
42	74.13	0	100	**86**	62.72	49.84	31

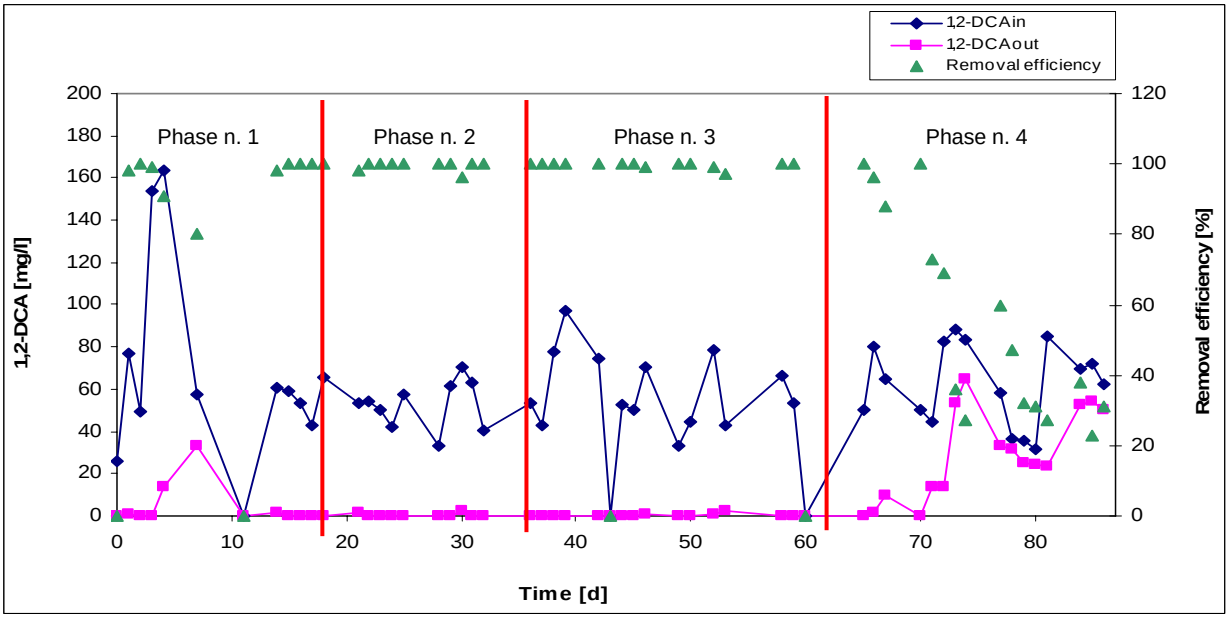

Figure 8.24. Trend of 1,2-DCA in the influent and in the effluent of the system and removal efficiency during the four parts of the test on variation on operating parameters.

Trend of nitrates in the four phases

Nitrates were monitored during the four phases. Their trend is shown in Figure 8.25.

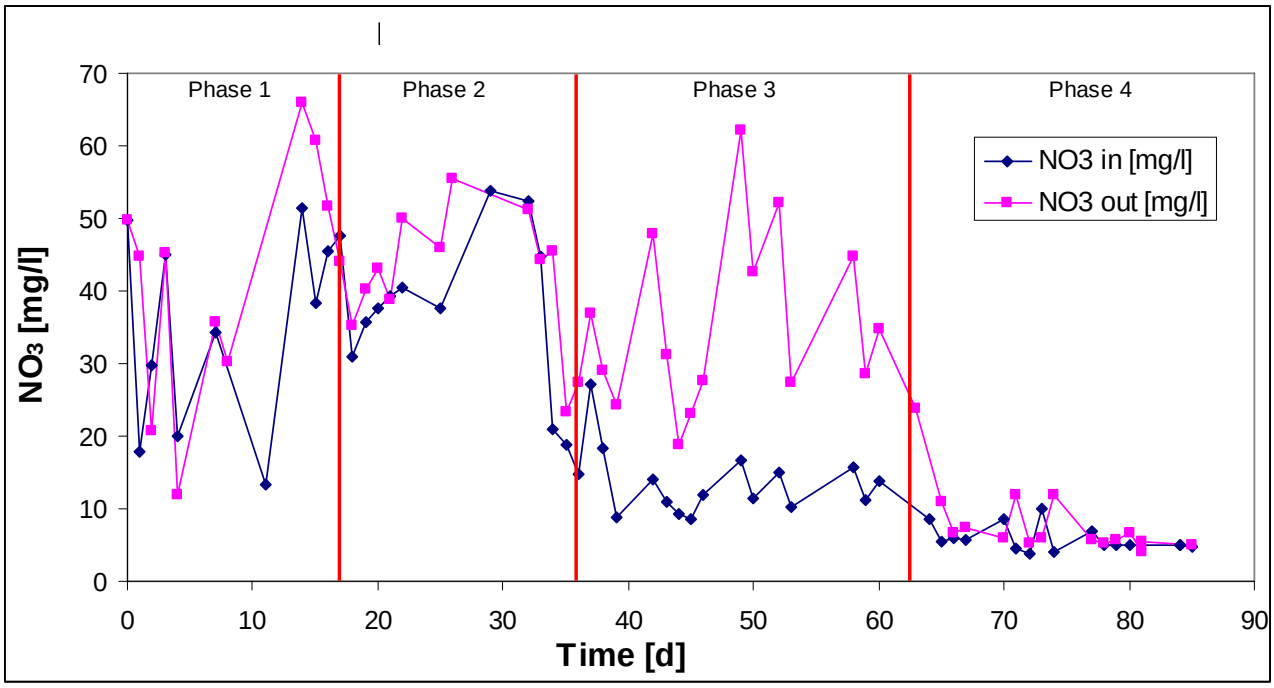

Figure 8.25. Trend of nitrates during the four phases of the test on the effects of variation of operating parameters.

8.3 MSBR

In the MSBR, degradation of the chlorinated compounds fed singularly or in combination has been performed. Degradation of the chlorinated compounds has been performed with cycles of variable length depending on their degradation rate; when the substances were degraded in one or two days, the MSBR was operated with daily cycles. MSBR was also operated with two cycles per day, in order to increase the daily chlorinated compounds load entering the system and to investigate degradation capability of the acclimated biomass.

8.3.1 Degradation of 1,2-DCA and 1,2-DCB

8.3.1.1 Degradation of 1,2-DCA

Degradation of 1,2-DCA was carried out in the MSBR with daily cycles in different moments of the whole experimentation, in order to investigate the variation of specific removal rates of 1,2-DCA with the increase of sludge acclimation. Specifically, degradation of 1,2-DCA in daily cycles was performed starting from the 180th day, from the 275th day and from the 438th day of sludge acclimation. 1,2-DCA degradation with two cycles per day took place since the 99th day and since the 498th day of reactor operation. In any phase, degradation was carried out for several days before performing the kinetic tests for the calculation of specific removal rates.

Degradation of 1,2-DCA with one cycle per day

Degradation of 1,2-DCA on the 180th day of acclimation

On the 180th day of acclimation to 1,2-DCA, the sludge was inoculated into the MSBR, and 1,2-DCA degradation took place with daily cycles, for 8 days.

Trend of 1,2-DCA degradation is shown in Figure 8.26 and Table 8.40. At the end of the 8 days, a kinetic test on 1,2-DCA was performed, in order to define the maximum specific removal rate achieved. The results of this kinetic tests are shown in Figure 8.27 and Table 8.41.

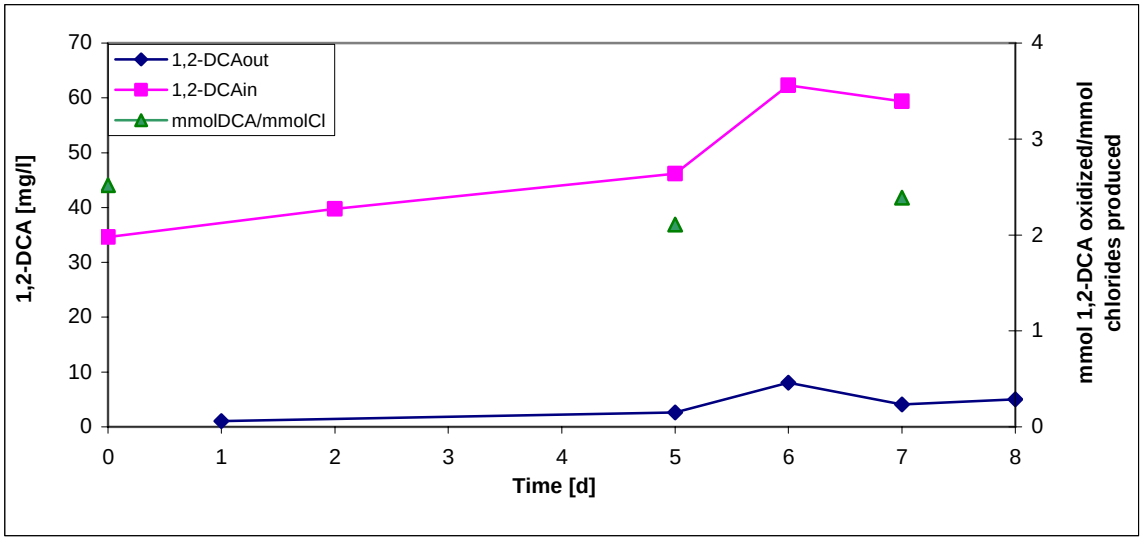

Figure 8.26. Trend of 1,2-DCA in the influent and effluent to the system, and ratio between mmoles of chlorides produced and mmoles 1,2-DCA oxidized.

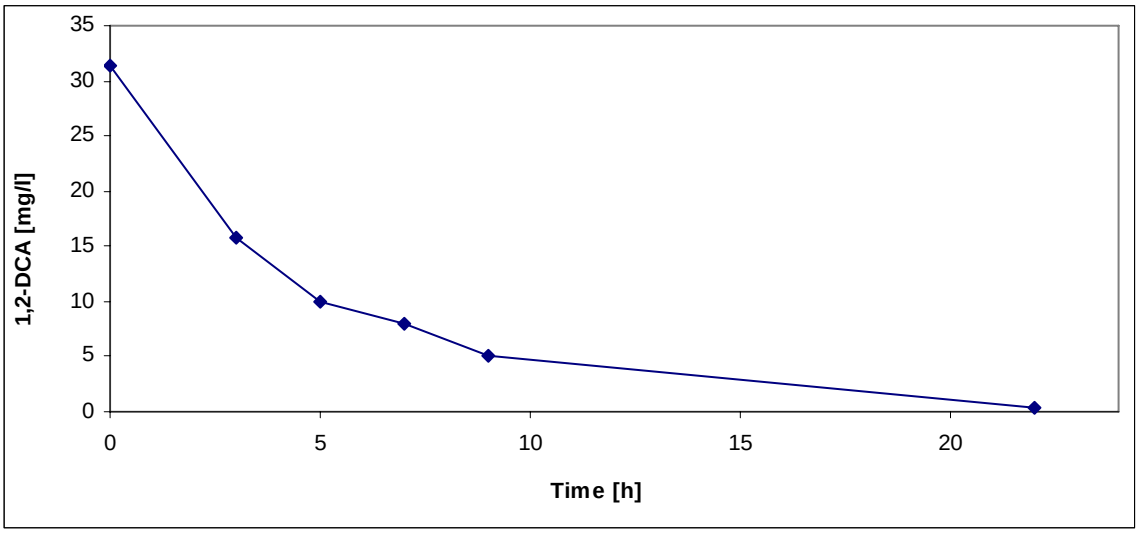

Figure 8.27. 1,2-DCA concentration profile in the kinetic test performed on the eighth day of the phase.

.

Table 8.40. Trend of 1,2-DCA when fed in the MSBR with daily cycles.

Time [d]	1,2-DCA [mg/l]	Chlorides [mg/l]	$N\text{-}NO_3$ [mg/l]	mmol 1,2-DCA oxidized	mmol Chlorides produced	mmol Chlorides/ mmol 1,2-DCA	Mean specific removal rate [$g_{1,2\text{-}DCA}/(g_{VSS}\cdot d)$]
0	27.09	81.70	30.10	0.56	1.40	2.52	0.12
1	0.8	105.40	29.80				
2	31.10	87.44	21.40	0.62	1.30	2.11	0.14
5	2.07	109.40	21.60				
5	36.14	81.30	20.30	0.63	1.51	2.39	0.14
6	6.30	106.80	21.50				
6	48.77	75.10	24.40	0.97	2.09	2.16	0.21
7	3.20	110.40	23.03				
7	46.48	69.60	0.10	0.90	1.70	1.88	0.20
8	3.93	98.30	20.76				
							Average mean specific removal rate 0.16

Table 8.41. Trend of 1,2-DCA in the kinetic test performed on the 8th day of the phase.

Time [h]	1,2-DCA [mg/l]	Chlorides [mg/l]	$N\text{-}NO_3$ [mg/l]	Mean specific removal rate $[g_{1,2\text{-}DCA}/g_{SSV}\cdot d]$	Maximum specific removal rate $[g_{1,2\text{-}DCA}/g_{SSV}\cdot d]$
0	31.31	52.91	42.87	0.19	0.70
3	15.69	54.42	43.52		
5	10.04	58.54	43.93		
7	8.01	63.19	44.46		
9	5.10	64.87	44.52		
22	0.28	67.29	45.49		

Degradation of 1,2-DCA on the 275th day of acclimation

Since the 275th day of sludge acclimation, the MSBR was fed with 1,2-DCA at initial concentration in the reactor at the beginning of the cycle of 50 mg/l. The substrate was fed for one month, and in Table 8.42 the last four days of degradation are reported. A kinetic test on the degradation of the substrate was performed on the last day, in order to calculate the maximum specific removal rate; results are reported in Table 8.43 and in Figure 8.28.

Table 8.42. Degradation of 1,2-DCA in the MSBR around the 300th day.

Time [d]	1.2-DCA [mg/l]	Chlorides [mg/l]	N-NO_3 [mg/l]	mmol 1,2-DCA oxidized	mmol chlorides produced	mmol Chlorides/ mmol 1,2-DCA	Mean specific degradation rate 1,2-DCA [$g_{1,2\text{-DCA}}/g_{VSS}$·d]
0	33.95	5.27	10.2	0.71	0.58	0.81	0.14
1	3.49	14.29	10.49				
1	51.24	8.54	8.09	0.92	1.63	1.77	0.18
2	11.47	33.71	12.39				
2	65.52	42.33	18.76	1.46	3.70	2.53	0.25
3	2.71	99.37	22.03				
						Average value mean specific removal rate	0.22

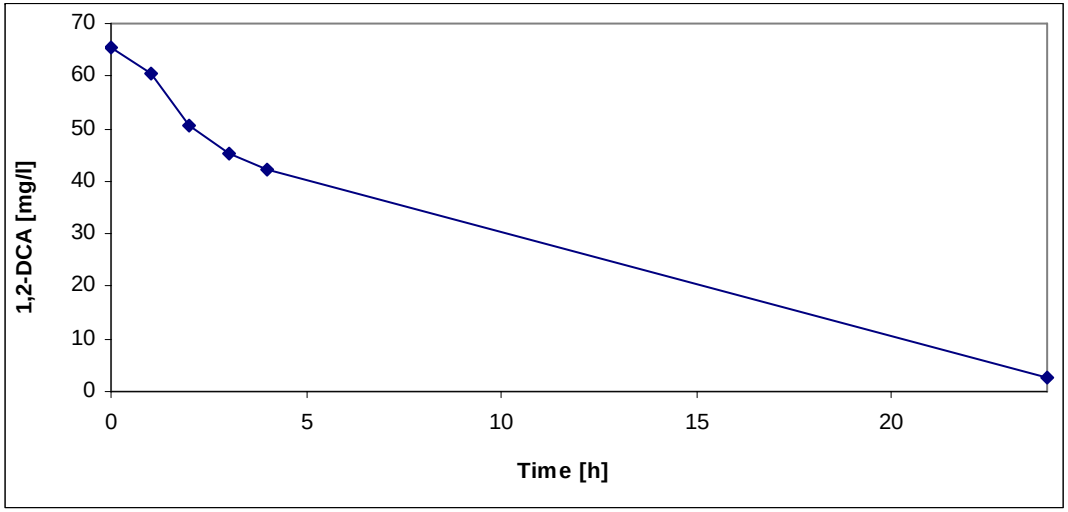

Figure 8.28. Kinetic test on degradation of 1,2-DCA on the 305th day.

Table 8.43. Kinetic test on 1,2-DCA on the 305th day.

Time [h]	1,2-DCA [mg/l]
0	65.52
1	60.32
2	50.52
3	45.38
4	42.24
24	2.71
Mean specific removal rates [$g_{c.c.}/g_{VSS}$·d]	0.24
Max specific removal rates [$g_{c.c.}/g_{VSS}$·d]	1.06

Degradation of 1,2-DCA on the 438th day

Since the 438th day, the MSBR was fed with 1,2-DCA at initial concentration in the reactor at the beginning of the cycle of 50 mg/l. The substrate was fed for two months, and some kinetic tests were periodically performed, in order to calculate the maximum specific removal rate; the results of the final kinetic test, performed on the last day of 1,2-DCA feeding, are reported in Table 8.44 and in Figure 8.29.

Table 8.44. Kinetic test for degradation of 1,2-DCA at the 498th day.

Time [h]	1,2-DCA [mg/l]	Chlorides [mg/l]	Nitrates [mg/l]	mmol 1,2-DCA oxidized	mmol Chlorides produced	mmol $Cl^-_{produced}$/ mmol $1,2\text{-}DCA_{oxid}$
0	52.02	55.1	28.17	1.45	2.88	1.99
1	31.2	66.2	28.9			
2	22.64	72.1	27.71			
3	14.68	78.5	28.2			
4	10.08	84.3	28.93			
5	5.78	88.02	29.46			
24	2.71					
Mean specific removal rates [$g_{c.c.}/g_{VSS}$·d]	0.24					
Max specific removal rates [$g_{c.c.}/g_{VSS}$·d]	1.36					

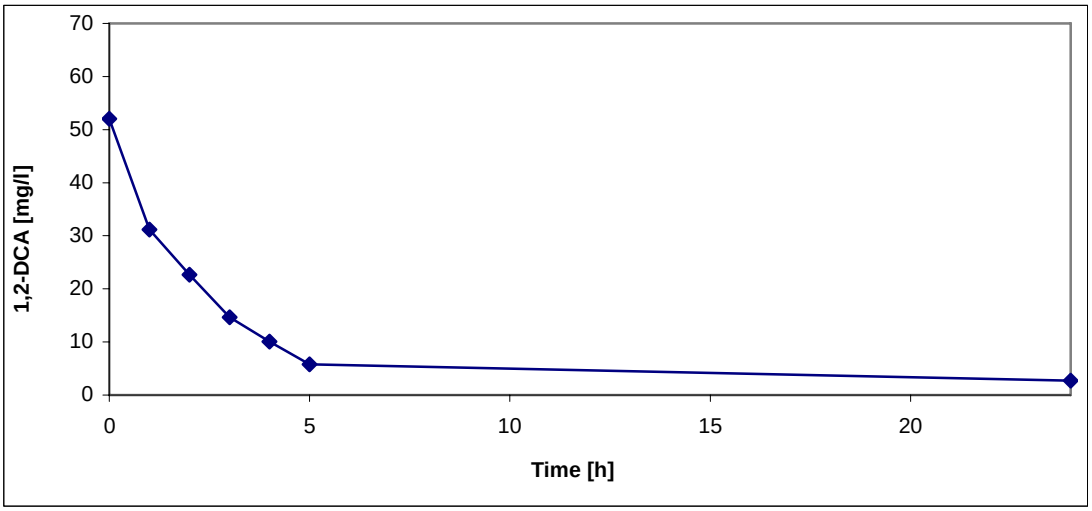

Figure 8.29. Kinetic test on degradation of 1,2-DCA on the 498th day.

Degradation of 1,2-DCA with two cycles per day

For some periods of the experimentation, degradation of 1,2-DCA took place with two cycles per day, in order to investigate the degradation capability of the biomass fed with a higher chlorinated compounds daily load.
Degradation with two cycles per day took place since the 99th day and since the 498th day of sludge acclimation.

Degradation of 1,2-DCA since the 99th day of acclimation

Degradation of 1,2-DCA with two cycles per day took place for the first time starting from the 99th day of sludge acclimation, and lasted around 18 days. At the end of the 18 days, a kinetic test was performed, in order to calculate the maximum specific removal rate of 1,2-DCA. Results are shown in Table 8.45 and in Figure 8.30.

Table 8.45. Kinetic test on degradation of 1,2-DCA fed with two cycles per day since the 99th day of acclimation.

Time [h]	**1,2-DCA [mg/l]**	**Chlorides [mg/l]**	**N-NO_3 [mg/l]**	**mmol 1,2-DCA oxidized**	**mmol Chlorides produced**	**mmol $Cl^-_{produced}$/ mmol 1,2-DCA_{oxid}**
0	56.4	18.64	61.41	1.19	2.97	2.49
1	44.3	11.30	77.34			
2	33.1	5.93	83.71			
3	24.0	8.81	92.19			
4	21.5	0.79	96.19			
5	16.5	1.56	103.64			
24	0.3	2.16	111.57			
Mean specific removal rates [$g_{c.c.}/g_{VSS}$·d]	0.51					
Max specific removal rates [$g_{c.c.}/g_{VSS}$·d]	1.11					

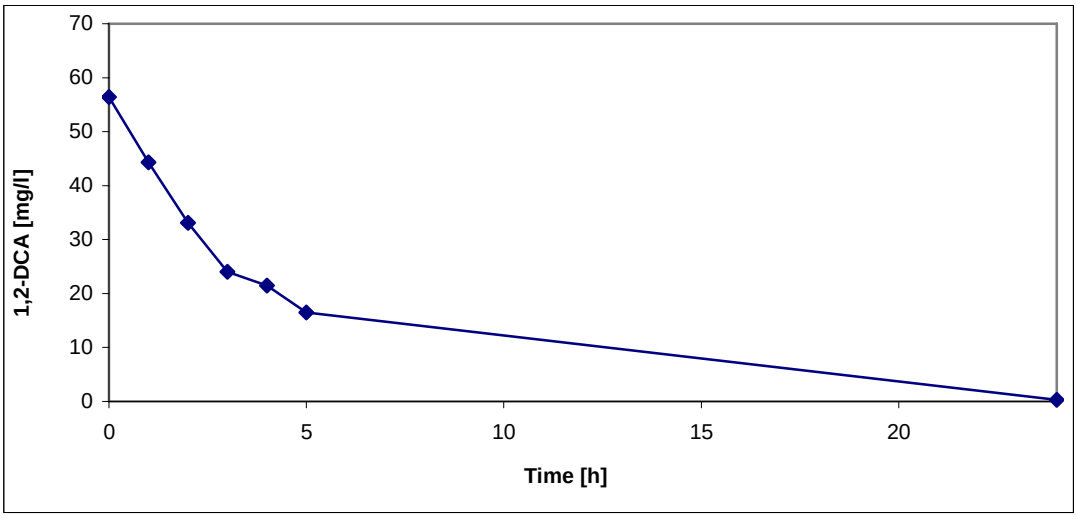

Figure 8.30. Kinetic test on degradation of 1,2-DCA fed with two cycles per day since the 99th day of acclimation

Degradation of 1,2-DCA since the 498th day

Starting from the 498th day, the MSBR was fed again with 1,2-DCA with two cycles per day for 40 days. At the end of the 40 days, two kinetic tests were performed in order to calculate the maximum specific removal rate of 1,2-DCA. Results of the kinetic tests are shown in Tables 8.46 and 8.47 and in Figures 8.31 and 8.32.

Table 8.46. First kinetic test on degradation of 1,2-DCA fed with two cycles per day since the 498th day.

Time [h]	1,2-DCA [mg/l]	Chlorides [mg/l]	N-NO_3 [mg/l]	mmol 1,2-DCA oxidized	mmol Chlorides produced	mmol $Cl^-_{produced}$/ mmol 1,2-DCA_{oxid}
0	58.63	60.49	13.31	1.77	4.11	2.32
1	40.28	75.61	9.16			
2	21.43	84.7	3.93			
3	14.66	95.05	1.81			
4	9.12	99.5	0.85			
5	5.04	108.83	1.17			
9	2.05	107.5	2.97			
Mean specific removal rates [$g_{c.c.}/g_{VSS}$·d]	0.79					
Max specific removal rates [$g_{c.c.}/g_{VSS}$·d]	1.83					

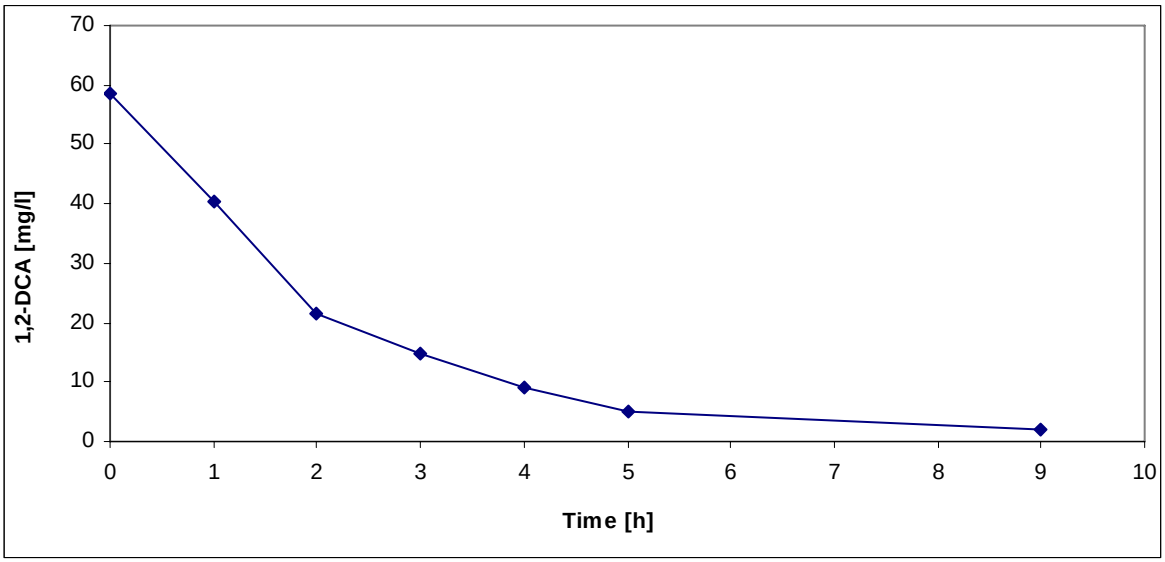

Figure 8.31. First kinetic test on degradation of 1,2-DCA fed with two cycles per day since the 498th day.

Table 8.47. Second kinetic test on degradation of 1,2-DCA fed with two cycles per day since the 498th day.

Time [h]	1,2-DCA [mg/l]	Chlorides [mg/l]	$N\text{-}NO_3$ [mg/l]	mmol 1,2-DCA oxidized	mmol Chlorides produced	mmol $Cl^-_{produced}$/ mmol 1,2-DCA$_{oxid}$
0	51.12	95.6	13.12	1.19	2.59	2.17
1	35.66	100.45	11.65			
2	24.85	106.77	8.16			
3	18.16	114.21	4.58			
4	15.85	116.92	1.52			
5	12.99	125.19	0.38			
9	4.37					
Mean specific removal rates [$g_{c.c.}/g_{VSS}$·d]	0.66					
Max specific removal rates [$g_{c.c.}/g_{VSS}$·d]	1.39					

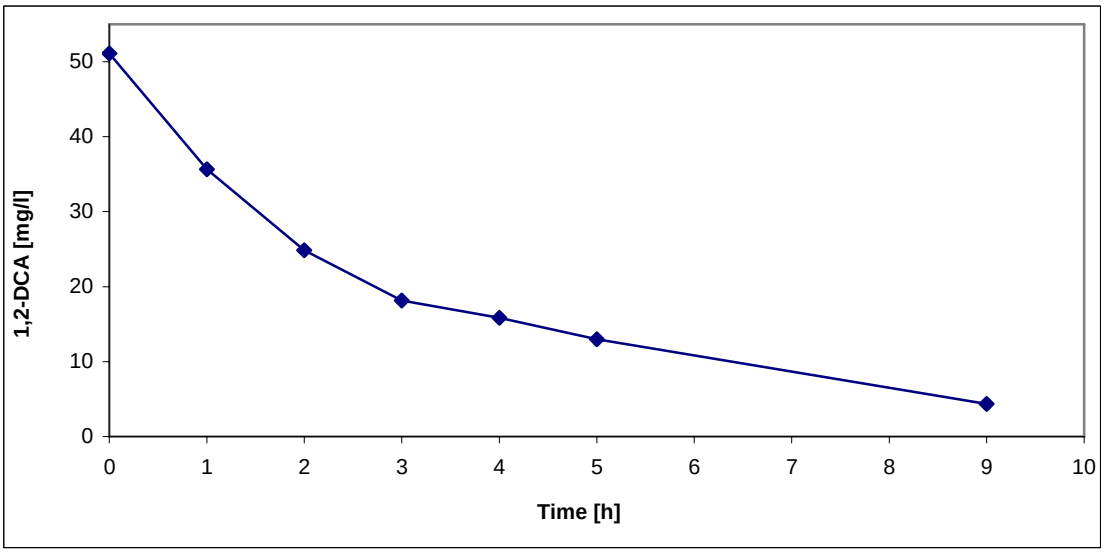

Figure 8.32. Second kinetic test on degradation of 1,2-DCA fed with two cycles per day since the 498th day.

8.3.1.2 Degradation of 1,2-DCB

On the 188th day of sludge acclimation, after feeding the MSBR with 1,2-DCA, it was fed with 1,2-DCB at a concentration of 15 mg/l at the beginning of the cycle.
Two cycles were performed, one lasting 6 days and the other 4 days. Maximum specific removal rates are calculated on a daily basis.
Results of degradation of 1,2-DCB in the MSBR are reported in Figure 8.33 and in Table 8.48.

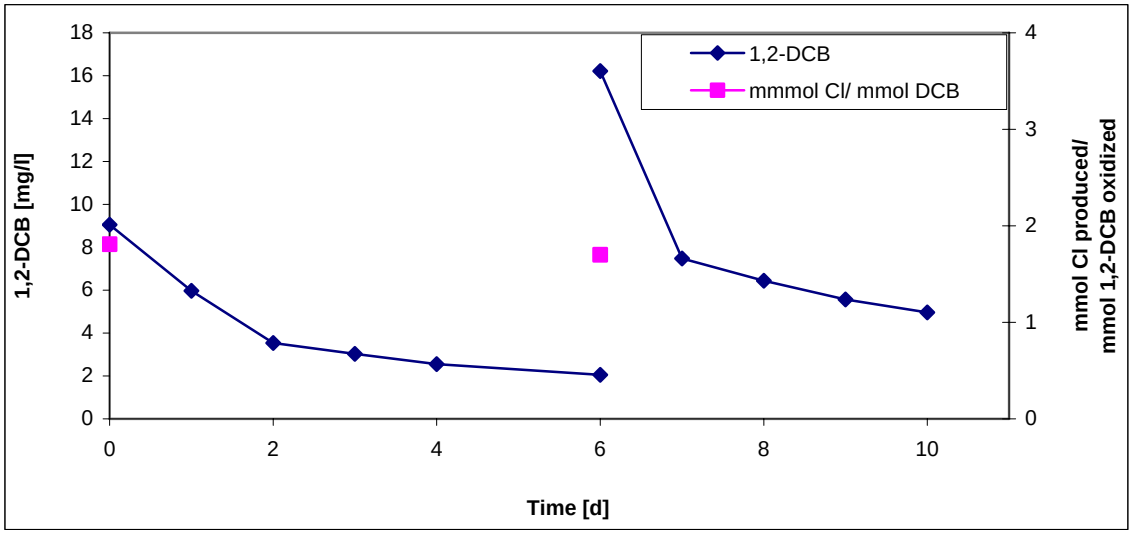

Figure 8.33. Degradation of 1,2-DCB in the MSBR.

Table 8.48. Degradation of 1,2-DCB in the MSBR.

Time [d]	1,2-DCB [mg/l]	Chlorides [mg/l]	Nitrates [mg/l]	mmol 1,2-DCB oxidized	mmol Chlorides produced	mmol Cl^-/ mmol 1,2-DCB	Mean specific removal rate [$g_{1,2\text{-}DCB}/g_{VSS}\cdot d$]	Maximum specific removal rate [$g_{1,2\text{-}DCB}/g_{VSS}\cdot d$]
0	9.05	50.18	22.53	0.10	0.18	1.81	0.01	0.027
1	5.97	49.18	24.00					
2	3.54	49.66	25.37					
3	3.03	48.92	26.78					
4	2.55	52.15	26.93					
6	2.05	53.24	27.52					
6	16.22	52.37	36.10	0.16	0.27	1.70	0.03	0.10
7	7.48	54.51	36.31					
8	6.44	55.39	34.93					
9	5.57	56.12	34.93					
10	4.96	56.98	36.26					
							Average value mean specific removal rate 0.02	**Average value max specific removal rate** 0.06

8.3.1.3 Degradation of 1,2-DCA and 1,2-DCB

Starting from the 198th day of sludge acclimation, after feeding the MSBR with 1,2-DCB, it was fed with 1,2-DCA and 1,2-DCB together. Initial concentrations of the two substrates at the beginning of the cycle in the reactor were 50 mg/l of 1,2-DCA and 15 mg/l of 1,2-DCB. Three cycles were performed: the first lasted 7 days, the second 8 days, and the last one lasted three days.

At the end of the third cycle, a kinetic test was performed in order to check the maximum specific removal rate of both substrates fed.

The trend of 1,2-DCA and 1,2-DCB in the cycles are reported in Figure 8.34 and Table 8.49; the results of the kinetic test are reported in Figure 8.35 and in Table 8.50.

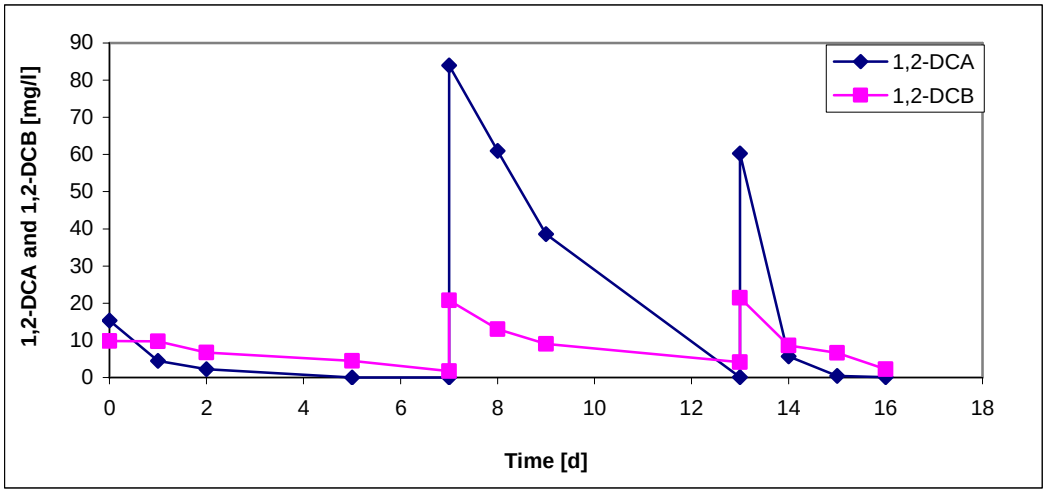

Figure 8.34. Trend of 1,2-DCA and 1,2-DCB when fed together.

Table 8.49. Trend of 1,2-DCA and 1,2-DCB when fed together in the MSBR in cycles of variable length. Maximum specific removal rates are calculated on a daily basis.

Cycle n.	Time [d]	1,2-DCA [mg/l]	1,2-DCB [mg/l]	Chlorides [mg/l]	$N\text{-}NO_3$ [mg/l]	1,2-DCA mean specific removal rate [$g_{1,2\text{-}DCA}/g_{VSS}\cdot d$]	1,2-DCA maximum specific removal rate [$g_{1,2\text{-}DCA}/g_{VSS}\cdot d$]	1,2-DCB mean specific removal rate [$g_{1,2\text{-}DCB}/g_{VSS}\cdot d$]	1,2-DCB maximum specific removal rate [$g_{1,2\text{-}DCB}/g_{VSS}\cdot d$]
1	0	15.30	9.80	53.00	14,90	0.031	0.03	0.005	
	1	4.50	9.70	63.30	14,90				-
	2	2.20	6.70	56.60	14,70				
	5	0.00	4.45	59.50	16,70				
	7	0.00	1.70	68.30	17,50				
2	7	84.00	20.78	45.80	23,70	0.082	0.13	0.016	0.04
	8	61.00	12.97	51.90	21,60				
	9	38.60	9.07	61.40	19,80				
	13	0.11	4.15	108.00	22,80				
3	13	60.27	21.41	50.80	19,00	0.140	0.38	0.045	0.097
	14	5.67	8.59	74.10	19,70				
	15	0.42	6.64	79.40	22,10				
	16	0.05	2.23	96.20	23,43				
					Average value	0.08	0.18	0.02	0.04

Table 8.50. Kinetic test performed at the end of the three cycles in which 1,2-DCA and 1,2-DCB were fed together in the MSBR.

Time [h]	1,2-DCA [mg/l]	1,2-DCB [mg/l]	1,2-DCA mean specific removal rate [$g_{1,2\text{-DCA}}/g_{VSS}\cdot d$]	1,2-DCA maximum specific removal rate [$g_{1,2\text{-DCA}}/g_{VSS}\cdot d$]	1,2-DCB mean specific removal rate [$g_{1,2\text{-DCB}}/g_{VSS}\cdot d$]	1,2-DCB maximum specific removal rate [$g_{1,2\text{-DCB}}/g_{VSS}\cdot d$]
1	42.23	14.40	0.19	0.43	0.041	0.044
2	39.30	14.27				
3	36.00	14.12				
4	32.00	13.77				
5	29.86	13.31				
6	25.80	12.94				
24	9.90	12.10				

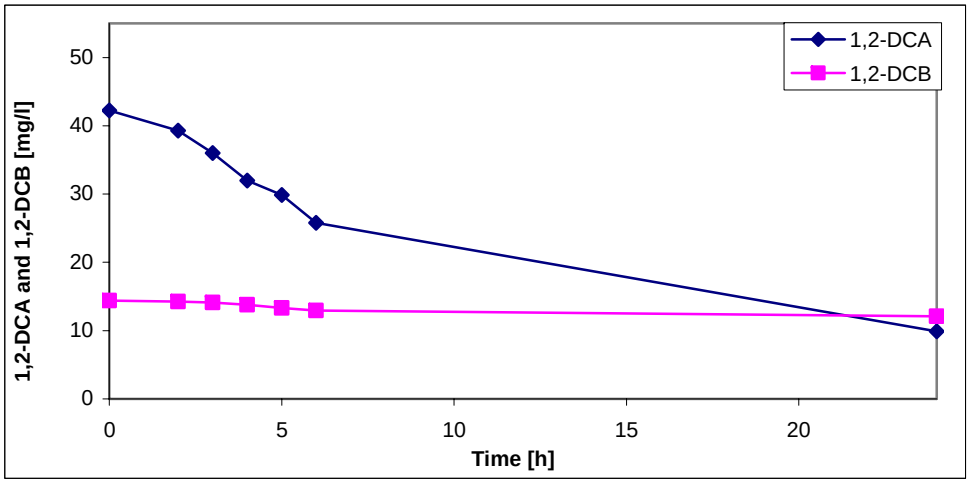

Figure 8.35. Kinetic test performed at the end of the three cycles in which 1,2-DCA and 1,2-DCB were fed together in the MSBR.

8.3.1.4. Specific removal rates for 1,2-DCA and 1,2-DCB

With reference to paragraphs n. 8.3.1, 8.3.2 and 8.3.3, specific removal rates of 1,2-DCA and 1,2-DCB observed when they were fed to the system singularly or in combination are summed up in the Table 8.51.

Table 8.51. Comparison of the specific removal rates for 1,2-DCA and 1,2-DCB when fed singularly or in combination.

Substances fed	Mean specific removal rate 1,2-DCA [$g_{1,2\text{-DCA}}/g_{VSS}\cdot d$]	Maximum specific removal rate 1,2-DCA [$g_{1,2\text{-DCA}}/g_{VSS}\cdot d$]	Mean specific removal rate 1,2-DCB [$g_{1,2\text{-DCB}}/g_{VSS}\cdot d$]	Maximum specific removal rate 1,2-DCB [$g_{1,2\text{-DCB}}/g_{VSS}\cdot d$]
1,2-DCA (180th day)	0.16	0.70	-	-
1,2-DCA (275th day)	0.24	1.06	-	-
1,2-DCA (498th day)	0.24	1.36	-	-
1,2-DCA 2cycles/d (99th day)	0.51	1.11	-	-
1,2-DCA 2cycles/d (498th day)	0.79	1.83	-	-
1,2-DCB (188th day)	-	-	0.02	0.06
1,2-DCA+1,2-DCB(198th day)	0.43	0.18	0.04	0.04

8.3.2 Degradation of 2-CP

After the tests with 1,2-DCA and 1,2-DCB, i.e. at the 200th day of sludge acclimation, degradation of 2-CP in the MSBR started.
2-CP was degraded in the MSBR in combination with other substances, used either as co-substrates or in order to check the effect on 2-CP degradation by acclimated biomass.
For degradation of 2-CP, initially cycles of variable length were operated, while, when a certain acclimation of the sludge to 2-CP was observed, daily cycles were operated.

8.3.2.1 Degradation of 2-CP with phenol

One cycle of 3 days length was performed; MSBR was fed with **phenol** at 35 mg/l and **2-CP** at 15 mg/l. Phenol was degraded, while no degradation of 2-CP was observed.
Results of the cycle are reported in Table 8.52 and Figure 8.36. Maximum specific removal rates are calculated on a daily basis.

Table 8.52. Degradation of phenol with 2-CP.

Time [d]	2-CP [mg/l]	Phenol [mg/l]	Max specific degradation rate 2-CP [$g_{2\text{-CP}}/g_{SSV}\cdot d$]	Max specific degradation rate phenol [$g_{fenolo}/g_{SSV}\cdot d$]
0	14.69	34.73	0	0.08
1	15.82	0.68		
2	14.94	2.15		
3	15.24	3.14		

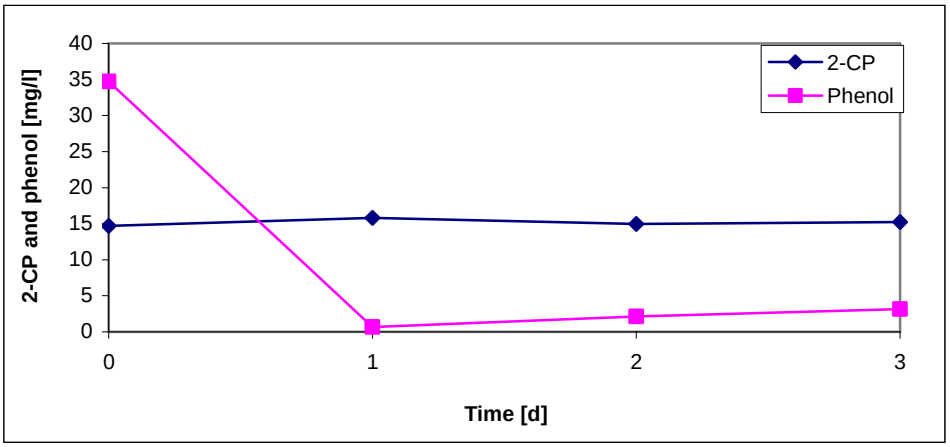

Figure 8.36. Degradation of 2-CP with phenol in the MSBR.

8.3.2.2 Degradation of 2-CP with phenol and 1,2-DCA

On the 203rd day of sludge acclimation two cycles were started, in which **2-CP** was fed together with **1,2-DCA** and **phenol**. Each cycle lasted 4 days; the results are reported in Table 8.53 and Figure 8.37. Consumption of nitrates together with the other substrates was observed during the cycles. Maximum specific removal rates (shown in Table 8.54) are calculated on a daily basis.

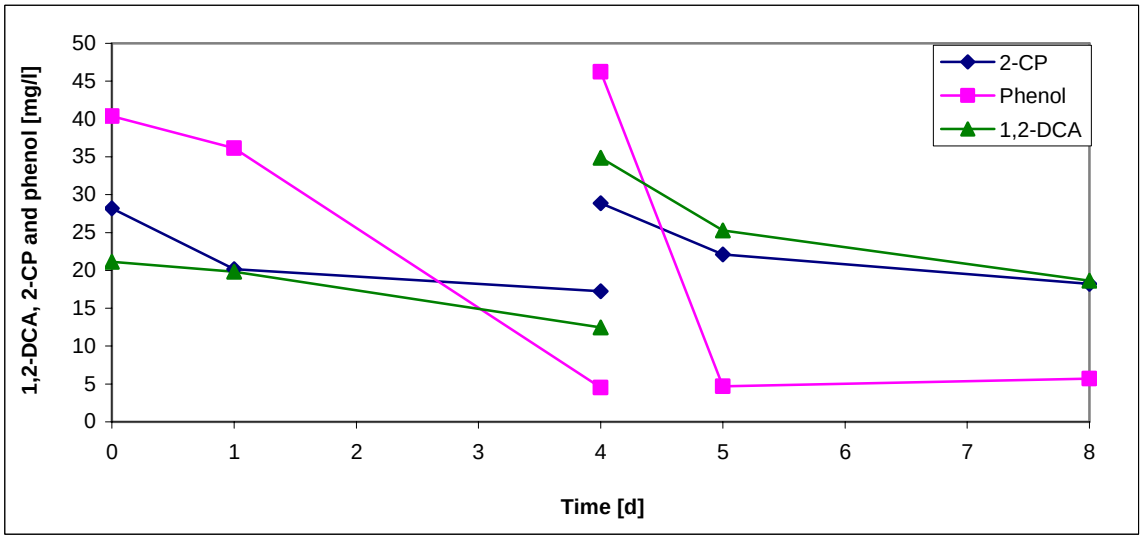

Figure 8.37. Degradation of 2-CP together with 1,2-DCA and phenol.

Table 8.53. Degradation of 2-CP together with 1,2-DCA and phenol.

Time [d]	1,2-DCA [mg/l]	2-CP [mg/l]	Phenol [mg/l]	Chlorides [mg/l]	$N\text{-}NO_3$ [mg/l]	mmol 1,2-DCA oxidized	mmol 2-CP oxidized	mmol chlorides produced	mmol Chlorides theoretically produced	$mmol\ chlorides_{theor}$/ $mmol\ chlorides_{produced}$
0	21.14	28.19	40.35	47.23	24.97	0.184	0.179	0.07	0.55	7.81
1	19.83	20.15	36.15	47.41	22.58					
4	12.48	17.23	4.52	48.06	18.43					
0	34.88	28.88	46.22	49.09	22.76	0.34	0.17	0.01	0.85	85
1	25.29	22.10	4.7	47.68	21.04					
4	18.66	18.22	5.7	49.26	19.02					

Table 8.54. Maximum specific removal rates (on a daily basis) observed during the two cycles in which 2-CP was fed with phenol and 1,2-DCA.

Maximum specific removal rate 1,2-DCA $[g_{1,2\text{-}DCA}/g_{VSS}\cdot d]$	Maximum specific removal rate 2-CP $[g_{2\text{-}CP}/ g_{VSS}\cdot d]$	Maximum specific removal rate phenol $[g_{phenol}/ g_{VSS}\cdot d]$
0.02	0.02	0.03
0.04	0.03	0.20

8.3.2.3 Degradation of 2-CP with 1,2-DCA in absence of phenol

Degradation of 2-CP and 1,2-DCA with one cycle per day

Degradation of 2-CP with 1,2-DCA on the 211th of acclimation

Since degradation of phenol and of 1,2-DCA was not improved by the presence of phenol, from the 211th day of sludge acclimation, degradation of **2-CP** was operated in presence of **1,2-DCA** but in absence of phenol. Besides, in comparison to the previous phase, in which 2-CP and 1,2-DCA were degraded with phenol at 50 mg/l, initial concentration of 2-CP in the reactor at the beginning of the cycle was lowered to 25 mg/l.

Results of the cycles and of the two kinetic tests performed are shown in Tables from 8.55 to 8.57, and in Figures 8.38 and 8.39.

Table 8.55. Degradation of 1,2-DCA and 2-CP in the MSBR with one cycle per day.

Time [d]	1,2-DCA [mg/l]	2-CP [mg/l]	Chlorides [mg/l]	$N\text{-}NO_3$ [mg/l]	mmol 1,2-DCA oxidized	mmol 2-CP oxidized	mmol Chlorides theoretically produced	mmol Chlorides produced	$mmol\ chlorides_{theor}$/ $mmol\ chlorides_{produced}$
0	53.12	24.46	33.74	33.37	0.89	0.05	0.94	1.83	2.4
1	11.06	21.29	46.66	33.37					
1	56.74	31.72	35.73	30.43	1.14	0.03	1.17	2.31	3.9
2	3.00	29.93	45.64	31.52					
2	42.19	31.91	34.08	28.43	0.76	0.02	0.79	1.54	1.1
3	6.29	30.48	57.92	31.98					
3	44.73	34.86	39.32	50.81	0.78	0.05	0.83	1.61	5.5
4	8.12	31.74	44.21	52.37					

Table 8.56. First kinetic test on degradation of 2-CP and 1,2-DCA.

Time [h]	1,2-DCA [mg/l]	2-CP [mg/l]	Chlorides [mg/l]
0	44.73	34.86	38.62
2	42.47	33.67	41.13
4	40.46	32.62	43.84
6	33.63	31.94	46.79
24	8.12	31.74	55.17
Mean specific removal rates $[g/g_{VSS}\cdot d]$	0.08	0.01	
Max specific removal rates $[g/g_{VSS}\cdot d]$	0.12	0.06	

Table 8.57. Second kinetic test on degradation of 2-CP and 1,2-DCA.

Time [h]	1,2-DCA [mg/l]	2-CP [mg/l]	Chlorides [mg/l]
0	23.7	40.4	39.32
1	20.3	40.2	
2	20.2	39.3	
3	18.2	39	
4	16.5	38.3	40.00
5	13.8	38.5	
6	9.6	38.1	
24	0.5	32.4	54.21
Mean specific removal rates $[g/g_{VSS}\cdot d]$	0.10	0.04	
Max specific removal rates $[g/g_{VSS}\cdot d]$	0.26	0.04	

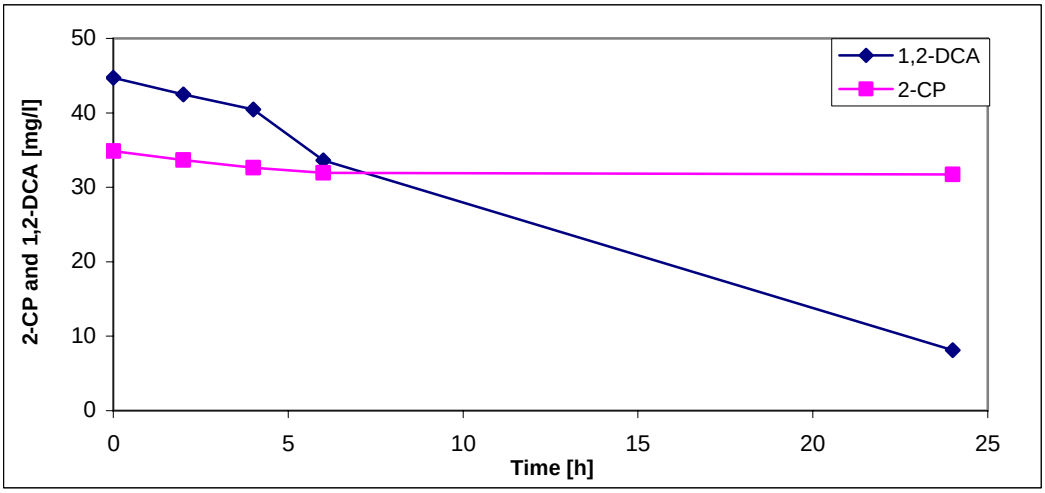

Figure 8.38. First kinetic test on the degradation of 2-CP (25 mg/l) together with 1,2-DCA, in absence of phenol.

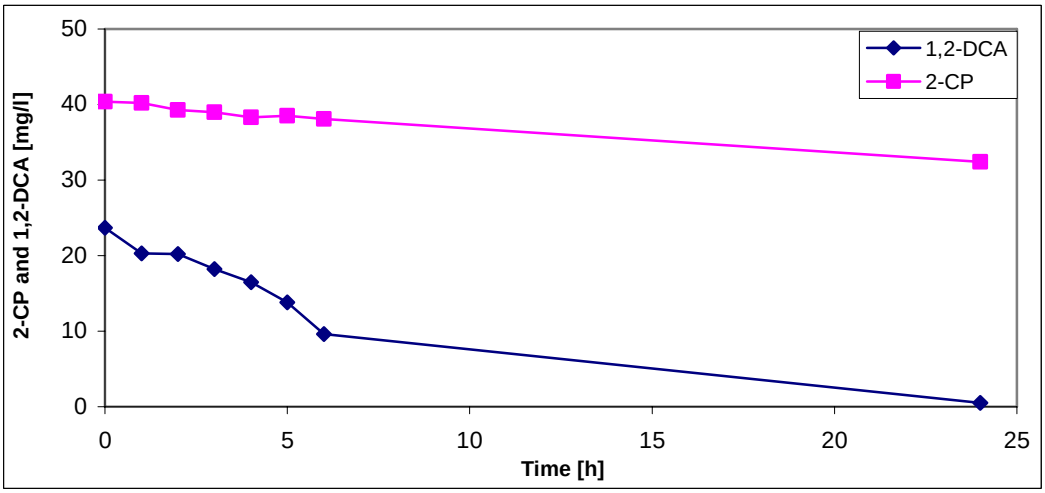

Figure 8.39. Second kinetic test on the degradation of 2-CP (25 mg/l) together with 1,2-DCA, in absence of phenol.

Degradation of 2-CP with 1,2-DCA on the 407th day

Degradation of 2-CP with only 1,2-DCA was also performed with daily cycles since the 407th day. Feeding of **2-CP** and **1,2-DCA** lasted 31 days and occurred after feeding the two substrates together with 1,2-DCB for around 37 days.

The concentration of the two substrates in the reactor at the beginning of the cycle was 50 $mg_{1,2\text{-}DCA}$/l and 25 $mg_{2\text{-}CP}$/l. Trend of 1,2-DCA and 2-CP concentrations are shown in Table 8.58 and in Figure 8.40.

At the end of the 31 days, a kinetic test was performed in order to calculate maximum specific removal rates of the two chlorinated compounds fed. Results of the kinetic test are reported in Table 8.59 and in Figure 8.41.

Table 8.58. Degradation of 1,2-DCA and 2-CP since the 407th day.

Time [d]	1,2-DCA [mg/l]	2-CP [mg/l]	Mean specific removal rate 1,2-DCA [$g_{1,2\text{-DCA}}/g_{VSS}\cdot d$]	Mean specific removal rate 2-CP [$g_{2\text{-CP}}/g_{VSS}\cdot d$]
0	55.90	23.95	0.22	0.07
1	1.44	5.75		
7	54.23	24.19	0.21	0.09
8	1.78	2.09		
8	58.52	29.13	0.24	0.04
9	0.29	0		
9	48.33	20.52	0.07	0.03
12	0.05	0		
12	42.67	21.98	0.17	0.09
13	1.5	0.45		
13	48.07	21.07	0.19	0.09
14	1.02	0.38		
14	55.80	25.62	0.21	0.10
15	3.43	0.96		
15	75.96	24.57	0.41	0.13
16	0.85	0.58		
16	62.42	22.46	0.11	0.04
19	0.72	0		
19	70.43	27.91	0.36	0.13
20	3.38	3.11		
20	51.70	21.10	0.28	0.11
21	0	0.53		
21	56.22	20.18	0.30	0.11
22	0.24	0.29		
22	64.63	18.31	0.35	0.10
23	0.27	0		
23	63.43	22.03	0.11	0.12
26	0.65	0		
26	48.36	12.10	0.26	0.07
27	1	0		
27	54.68	25.30	0.30	0.14
28	0.34	0.41		
28	57.45	17.07	0.31	0.09
29	0	0		
29	59.81	24.92	0.32	0.14
30	0.47	0		
30	68.44	29.96	0.36	0.14
31	2.27	3.12		
			0.25	**0.10**

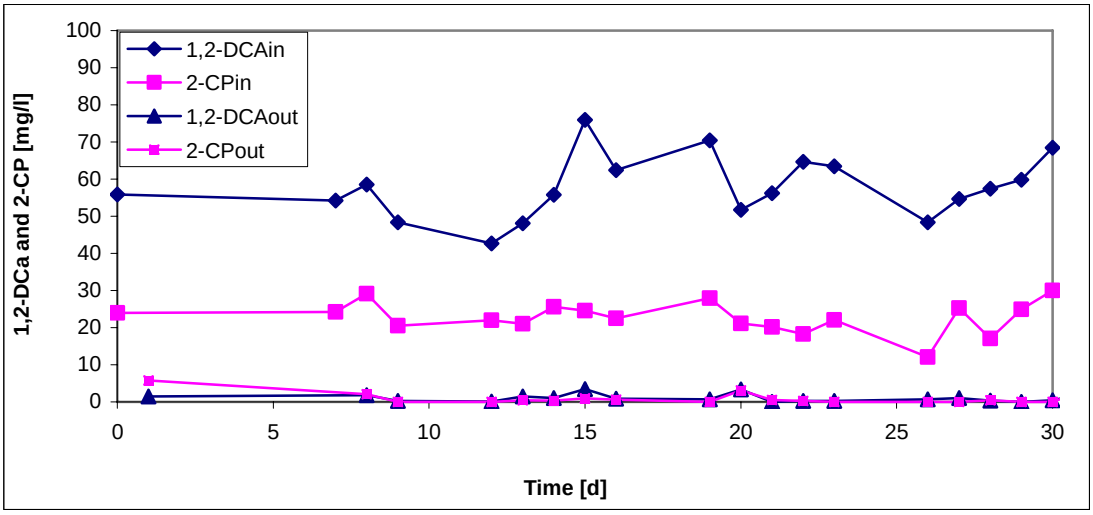

Figure 8.40. Degradation of 1,2-DCA and 2-CP since the 407th day.

Table 8.59. Kinetic test on degradation of 1,2-DCA and 2-CP on the 438th day.

Time [h]	1,2-DCA [mg/l]	2-CP [mg/l]
0	53.56	23.45
1	42.73	22.32
2	42.11	21.96
3	34.74	19.93
4	32.83	17.75
24	2.27	3.12
Mean specific removal rates [g/g$_{VSS}$·d]	0.37	0.14
Max specific removal rates [g/g$_{VSS}$·d]	1.05	0.20

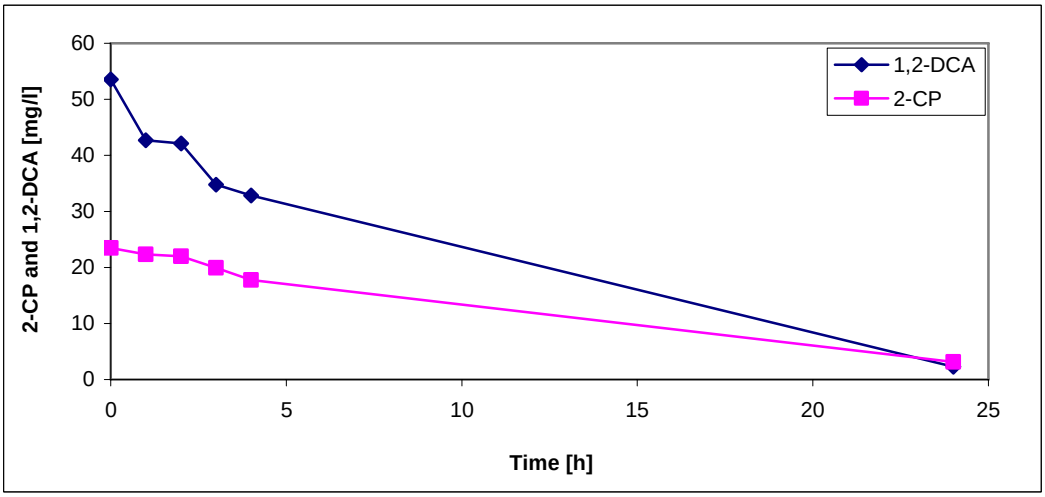

Figure 8.41. Kinetic test on degradation of 1,2-DCA and 2-CP on the 438th day.

Degradation of 2-CP and 1,2-DCA with two cycles per day

Degradation of 1,2-DCA at 50 mg/l and 2-CP at 15 mg/l

Since the 538th day of acclimation, degradation of **1,2-DCA** and **2-CP** was carried out with **two cycles per day**; 1,2-DCA concentration in the reactor at the beginning of the cycle was 50 mg/l, while 2-CP concentration was 15 mg/l. After 15 days of the beginning of this phase, a kinetic test was performed, in order to calculate the maximum specific removal rate. Results of the kinetics are shown in Table 8.60 and in Figure 8.42.

Table 8.60. Kinetic test on degradation of 1,2-DCA and 2-CP with two cycles per day, with initial 2-CP concentration of 15 mg/l.

Time [h]	1,2-DCA [mg/l]	2-CP [mg/l]	Chlorides [mg/l]	N-NO_3 [mg/l]	mmol 1,2-DCA oxidized	mmol 2-CP oxidized	mmol Chlorides produced	mmol Chlorides theoretically prodcuced	mmol Cl_{theor}/ mmol Cl_{prod}
0	46.11	15.12	80.49	33.42	1.15	0.00	1.11	2.3	2.1
1	32.5	17.01	90.57	14.43					
2	23.5	14.50	100.62	3.47					
3	17.13	17.23	110.41	2.54					
4	12.63	19.64	116.00	3.68					
5	9.5	19.60	120.05	3.83					
24	0.3	18.76							
Mean specific removal rates [g/g_{VSS}·d]	0.21	0							
Max specific removal rates [g/g_{VSS}·d]	1.03	0							

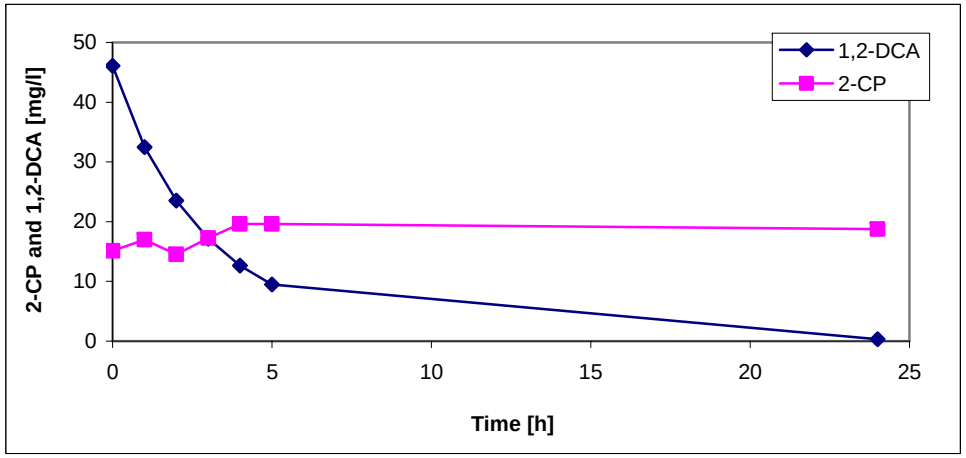

Figure 8.42. Kinetic test on degradation of 1,2-DCA and 2-CP with two cycles per day, with initial 2-CP concentration of 15 mg/l.

Degradation of 1,2-DCA at 50 mg/l and 2-CP at 10 mg/l

On the 568th day, for the degradation of **1,2-DCA** together with **2-CP** in **two cycles per day**, the initial concentration of 2-CP was **lowered** down to 10 mg/l. After 13 days, two kinetic tests were performed; the results are shown in Figure 8.61 and 8.62 and in Table 8.43 and 8.44.

Table 8.61. First kinetic test on degradation of 1,2-DCA and 2-CP with two cycles per day, with initial 2-CP concentration of 10 mg/l.

Time [h]	1,2-DCA [mg/l]	2-CP [mg/l]	Chlorides [mg/l]	Nitrates [mg/l]	mmol 1,2-DCA oxidized	mmol 2-CP oxidized	mmol Chlorides theoretically prodcuced	mmol Chlorides prodcuced	mmol Cl_{theor}/ mmol Cl_{prod}
0	46.53	12.68	92.19	22.71	1.30	0.10	1.02	2.7	2.64
1	24.5	11.11	99.37	15.30					
2	12.71	9.75	108.81	4.65					
3	9.61	7.67	115.56	0					
4	6.28	9.45	126.42	0					
5	4.88	9.55	128.36	0					
24	0.41	6.33							
Mean specific removal rates [g/g_{VSS}·d]	0.21	0.03							
Max specific removal rates [g/g_{VSS}·d]	1.34	0.09							

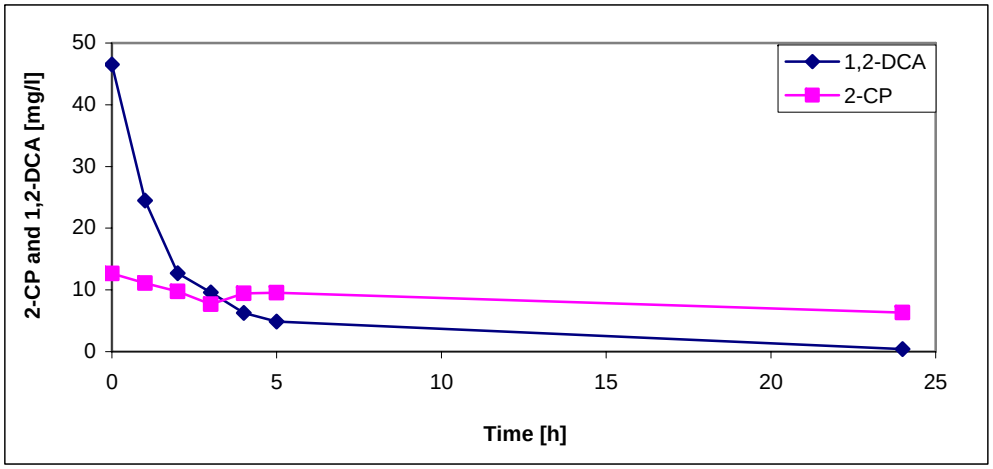

Figure 8.43. First kinetic test on degradation of 1,2-DCA and 2-CP with two cycles per day, with initial 2-CP concentration of 10 mg/l

Table 8.62. Second kinetic test on degradation of 1,2-DCA and 2-CP with two cycles per day, with initial 2-CP concentration of 10 mg/l.

Time [h]	1,2-DCA [mg/l]	2-CP [mg/l]	Chlorides [mg/l]	N-NO_3 [mg/l]	mmol 1,2-DCA oxidized	mmol 2-CP oxidized	mmol Chlorides produced	mmol Chlorides theoretically prodcuced	mmol Cl_{theor}/ mmol Cl_{prod}
0	41.13	13.40	93.15	25.50	1.11	0.09	2.62	2.30	0.88
1	23.80	12.54	98.4	14.65					
2	19.50	11.83	105.6	3.87					
3	8.69	11.61	110.4	0					
4	6.03	10.13	118.9	0					
5	5.77	10.54	123.15	0					
24	0.78	8.48							
Mean specific removal rates [g/g_{VSS}·d]	0.18	0.02							
Max specific removal rates [g/g_{VSS}·d]	1.18	0.07							

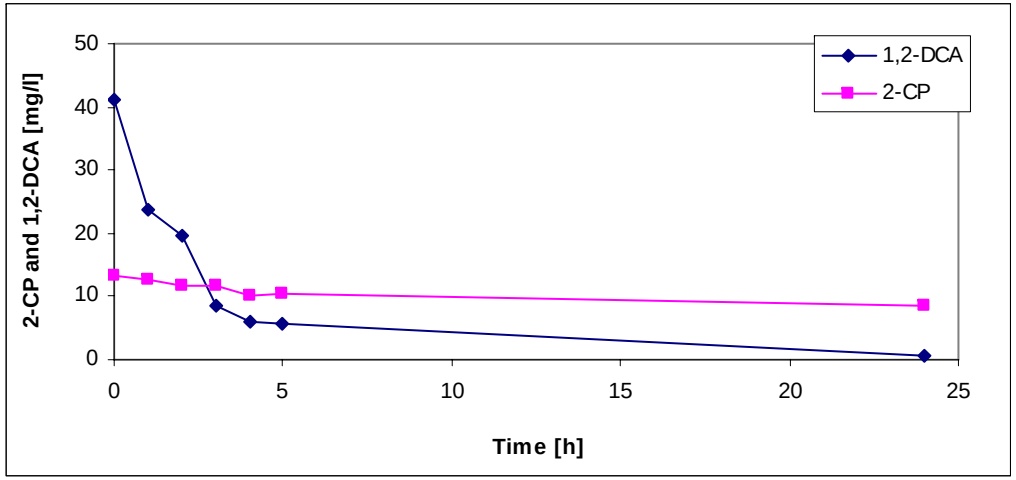

Figure 8.44. Second kinetic test on degradation of 1,2-DCA and 2-CP with two cycles per day, with initial 2-CP concentration of 10 mg/l.

8.3.2.4 Degradation of 2-CP with phenol and 1,2-DCB

Starting from the 216th day of acclimation, two cycles, each of them lasting one week, have been performed in which **2-CP** was fed in the reactor with **1,2-DCB** and **phenol**.
Results of chlorinated compounds trend and of the kinetic tests are shown in Figure 8.45 and in Table 8.63 and 8.64. Maximum specific removal rates are calculated on a daily basis.

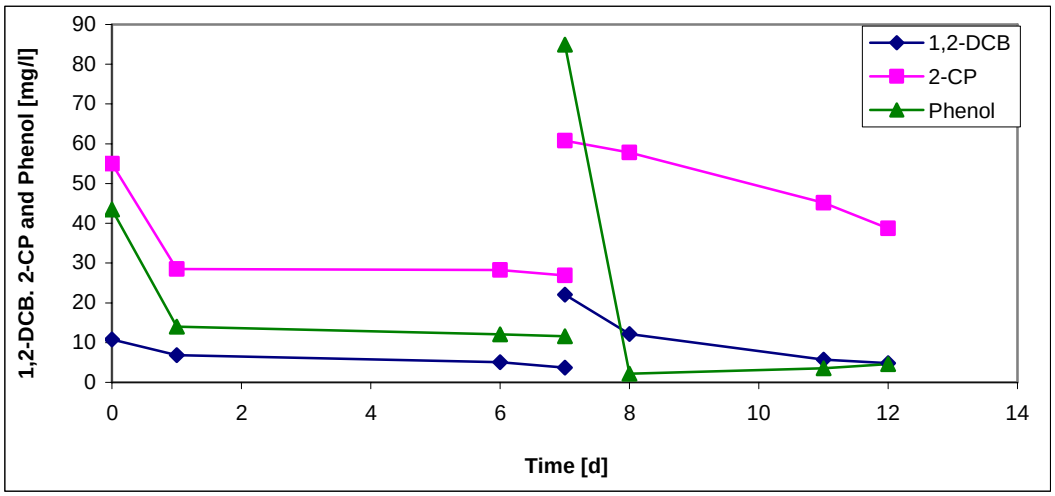

Figure 8.45. Trend of 2-CP, 1,2-DCB and phenol when fed together.

Table 8.63. Trend of degradation of 2-CP with 1,2-DCB and phenol in the first cycle performed.

Time [d]	**1,2-DCB [mg/l]**	**2-CP [mg/l]**	**Phenol [mg/l]**	**Chlorides [mg/l]**	**N-NO_3 [mg/l]**	**mmol 1,2-DCB oxidized**	**mmol 2-CP oxidized**	**mmol chlorides produced**	**mmol chlorides theoretically produced**	**mmol $Chlorides_{theor}$/ mmol $Chlorides_{produced}$**
0	10.81	55.02	43.49	37.78	20.46	0.101	0.460	0.690	0.662	0.96
1	6.84	28.46	13.98	45.01	12.75					
6	5.1	28.23	12.05	47.35	1.42					
7	3.71	26.86	11.61	49.42	0.5					
Mean specific removal rate **[g/g_{VSS}·d]**	0.006	0.03	0.03							
Maximum specific removal rate **[g/g_{VSS}·d]**	0.03	0.19	0.21							

Table 8.64. Trend of degradation of 2-CP with 1,2-DCB and phenol in the second cycle performed.

Time [d]	**1,2-DCB [mg/l]**	**2-CP [mg/l]**	**Phenol [mg/l]**	**Chlorides [mg/l]**	**N-NO_3 [mg/l]**	**mmol 1,2-DCB oxidized**	**mmol 2-CP oxidized**	**mmol chlorides produced**	**mmol chlorides theoretically produced**	**mmol $Chlorides_{theor}$/ mmol $Chlorides_{produced}$**
0	22.09	60.75	84.94	29.49	18.02	0.25	0.36	0.61	0.86	1.4
1	12.15	57.8	2.15	32.71	15.35					
6	5.7	45.18	3.55	32.62	0.27					
7	4.87	38.7	4.55	31.46	0.13					
Mean specific removal rate **[g/g_{VSS}·d]**	0.01	0.01	0.03							
Maximum specific removal rate **[$g/_{VSS}$·d]**	0.05	0.01	0.37							

8.3.2.5 Degradation of 2-CP with phenol, 1,2-DCA and 1,2-DCB

Starting from the 231st day of acclimation, degradation of **2-CP** with **phenol** and **1,2-DCA** and **1,2-DCB** took place in the MSBR both with 2 cycles lasting few days, and with daily cycles.
Then weekly cycles were performed, in which initial concentration of the substrates fed were 15 mg/l for 1,2-DCB and 50 mg/l for all the other substrates. In daily cycles, 2-CP concentration was lowered down to 25 mg/l, while the concentration of the other substrates was the same of the other cycles.
Trend of the substrates fed are shown in Figure 8.46 and in Tables from 8.65 to 8.67. Maximum specific removal rates are calculated on a daily basis.

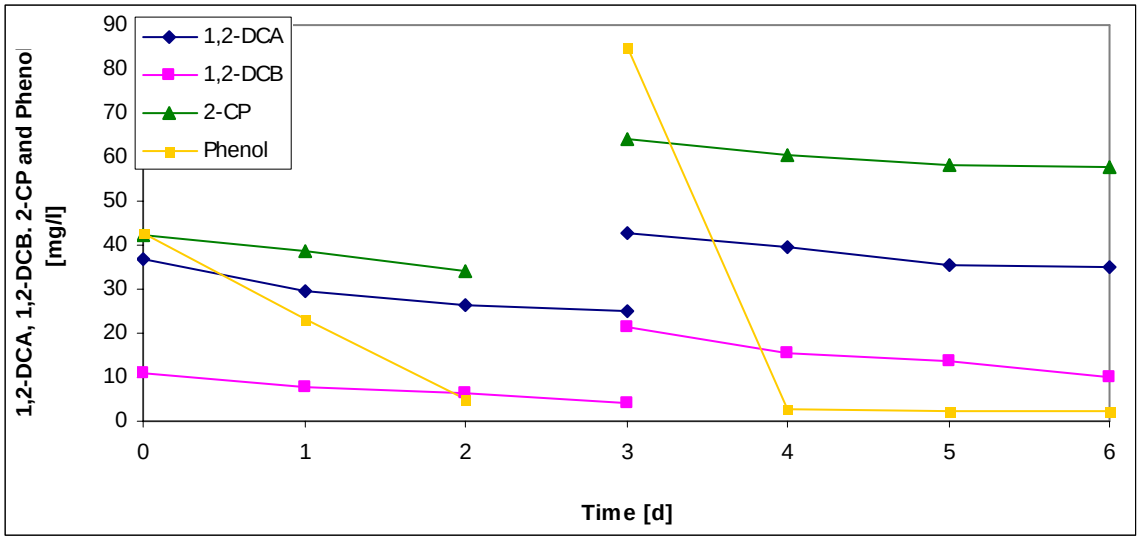

Figure 8.46. Degradation of 2-CP with phenol, 1,2-DCA and 1,2-DCB fed together in the MSBR in the two cycles lasting 3 days.

Table 8.65. Degradation of 2-CP, phenol, 1,2-DCA and 1,2-DCB fed together in the MSBR in the first cycle.

Time [d]	1,2-DCA [mg/l]	1,2-DCB [mg/l[	2-CP [mg/l]	Phenol [mg/l]	Chlorides [mg/l]	$N\text{-}NO_3$ [mg/l]	mmol 1,2-DCA oxidized	mmol 1,2-DCB oxidized	mmol 2-CP oxidized	mmol Chlorides theoretically produced	mmol Chlorides produced	mmol $Chlorides_{theor}$/ mmol $Chlorides_{produced}$
0	36.6	10.82	42.40	42.58	17.98	8.58	0.65	0.09	0.69	1.48	0.97	2.23
1	29.50	7.91	38.69	23.18	28.39	4.06						
2	26.30	6.49	34.1	5.19	28.44	0.74						
3	25.00	4.06			34.38	0.07						
Maximum specific removal rate **[g/g_{VSS}·d]**	0.03	0.01	0.02									

Table 8.66. Degradation of 2-CP, phenol, 1,2-DCA and 1,2-DCB fed together in the MSBR in the second cycle.

Time [d]	1,2-DCA [mg/l]	1,2-DCB [mg/l[	2-CP [mg/l]	Phenol [mg/l]	Chlorides [mg/l]	$N\text{-}NO_3$ [mg/l]	mmol 1,2-DCA oxidized	mmol 1,2-DCB oxidized	mmol 2-CP oxidized	mmol Chlorides theoretically produced	mmol Chlorides produced	mmol $Chlorides_{theor}$/ mmol $Chlorides_{produced}$
0	42.9	21.15	63.94	85.09	13.53	8.59	0.16	0.19	0.10	0.8	0.28	2.85
1	39.7	15.65	60.4	2.65	13.39	0.13						
2	35.3	13.70	58.3	2.31	14.09	0.26						
3	35.1	9.99	57.63	2.12	14.05	0.14						
Maximum specific removal rate **[g/g_{VSS}·d]**	0.01	0.02	0.01									

Table 8.67. Degradation of 2-CP with phenol, 1,2-DCA and 1,2-DCB in the MSBR in daily cycles.

Time [d]	1.2-DCA [mg/l]	1.2-DCB [mg/l]	2-CP [mg/l]	Phenol [mg/l]	N-NO_3 [mg/l]	Mean specific removal rate 1,2-DCA [$g_{1.2\text{-DCA}}/g_{SSV}\cdot d$]	Mean specific removal rate 1,2-DCB [$g_{1.2\text{-DCB}}/g_{SSV}\cdot d$]	Mean specific removal rate 2-CP [$g_{2\text{-CP}}/g_{SSV}\cdot d$]
0	49.48	20.05	24.79	36.31	32.29	0.152	0.057	0.061
1	28.39	12.15	19.2	2.76	22.15			
1	36.71	17.83	26.07	35.46	19.1	0.112	0.094	0.078
2	21.09	4.81	18.89	1.67	6.74			
2	20.76	16.2	23.76	37.53	16.52	0.010	0.005	0.029
3	18.69	15.08	21.06	8.21	2.22			
3	26.44	27.88	21.74	32.17	5.63	0.055	0.032	0.014
4	15.10	21.16	20.48	0.63	2.86			
4	54.43	20.05	32.29	18.99	11.36	0.060	0.015	0.166
5	42.02	16.95	17.02	3.34	0.18			
5	56.27	27.27	15.86	29.36	5.78	0.045	0.064	0.020
6	47.01	14.02	13.99	10.45	0			
	Average value of mean specific removal rate [$g./(d\cdot g_{VSS}$]					0.07	0.05	0.05

8.3.2.6 Degradation of 2-CP with 1,2-DCA and 1,2-DCB in absence of phenol

Since there was evidence of the negative effects of phenol on degradation of 2-CP when fed with **1,2-DCA** and **1,2-DCB**, **2-CP** was fed with the other two substrates in absence of phenol. Starting from the 370th day, 37 daily cycles were operated, and finally a kinetic test was performed in order to calculate the maximum specific removal rates of the chlorinated compounds fed. Before being fed with 1,2-DCA, 1,2-DCB and 2-CP, the MSBR was fed for around 60 days with the three chlorinated compounds plus TCE.

Results are shown in Table 8.68; on the 18th and on the 37th days, two kinetic test were performed in order to calculate the maximum specific removal rates of the chlorinated compounds fed; the results are shown in Tables 8.69 and 8.70 and in Figures 8.47 and 8.48. The substrates were fed with initial concentration in the reactor of 50 $mg_{1,2\text{-}DCA}/l$, 15 $mg_{1,2\text{-}DCB}/l$ and 25 $mg_{2\text{-}CP}/l$.

Table 8.68. Degradation of 1,2-DCA, 1,2-DCB and 2-CP.

Time [d]	1,2-DCA [mg/l]	1,2-DCB [mg/l]	2-CP [mg/l]	Chlorides [mg/l]	$N\text{-}NO_3$ [mg/l]	Mean specific removal rate 1,2-DCA [$g_{1,2\text{-}DCA}/g_{VSS}\cdot d$]	Mean specific removal rate 1,2-DCB [$g_{1,2\text{-}DCB}/g_{VSS}\cdot d$]	Mean specific removal rate 2-CP [$g_{1,2\text{-}CP}/g_{VSS}\cdot d$]
0	62.99	22.90	21.38	68.73	21.21	0.36	0,07	0,02
1	7.47	11.64	18.84	103.64	13.66			
1	58.10	13.02	17.45	61.71	17.51	0.12	0,00	0,04
4	0.00	11.68	0.84	112.35	13.17			
4	67.59	35.23	14.57	76.10	18.97	0.21	0,10	0,03
5	29.67	17.46	9.76	95.16	16.87			
5	70.15	31.00	20.19	65.82	20.72	0.23	0,08	0,02
6	28.22	16.35	17.27	88.47	18.24			
6	64.79	23.61	26.80	65.67	21.86	0.24	0,00	0,02
7	21.77	22.93	22.45	78.07	17.30			
7	47.66	17.72	28.60	65.93	21.40	0.22	0,03	0,03
8	8.50	11.70	22.75	85.58	17.25			
8	42.13	12.70	31.26	61.09	20.40	0.08	0,01	0,05
11	0.00	8.41	4.32	99.19	13.93			
11	43.88	11.68	24.28	63.96	24.30	0.18	0,02	0,07
12	11.02	8.04	10.94	89.53	22.67			
12	45.94	12.24	22.90	62.75	27.88	0.18	0,02	0,08
13	13.31	8.85	8.82	79.87	20.30			
13	47.74	13.02	21.76	57.18	26.05	0.21	0,03	0,06
14	7.39	7.41	9.88	96.26	21.20			
14	49.72	12.89	20.11	60.72	14.02	0.22	0,02	0,05
15	7.73	9.45	10.62	85.32	5.46			
15	46.12	14.17	18.23	62.86	9.09	0.06	0,01	0,02
19	0.00	9.41	0.00	93.41	4.95			
19	53.11	19.01	19.91	67.04	16.32	0.18	0,04	0,05
20	18.75	10.47	11.12	80.74	17.83			
20	38.81	8.34	21.80	59.29	20.71	0.16	0,01	0,11
21	8.65	5.55	0.66	68.49	13.30			
21	41.27	6.21	20.92	62.38	28.76	0.20	0,01	0,11
22	3.24	4.12	0.34	66.70	16.88			
22	40.41	8.59	19.54	51.45	28.19	0.20	0,01	0,10
23	2.52	6.75	0.67	88.29	26.89			
27	62.14	16.26	28.42	53.32	30.48	0.31	0,04	0,10
28	3.05	9.39	9.20	85.44	19.13			
28	42.55	12.50	25.48	61.27	27.88	0.27	0,03	0,11
29	0.72	7.43	8.01	90.17	15.96			
29	40.37	12.07	24.76	42.23	25.77	0.09	0,01	0,05
32	0.00	7.27	0.17	78.57	25.10			
32	30.20	7.99	19.55	56.22	25.98	0.09	0,00	0,10
33	15.94	7.94	4.21	52.65	26.22			
33	40.63	11.63	23.21	3.57	26.82	0.13	0,01	0,07
35	1.32	7.02	0.15	87.36	23.05			
35	43.86	14.71	21.91	37.29	28.92	0.27	0,05	0,10
36	2.22	7.16	6.72	83.18	21.38			
36	42.41	7.69	25.45	54.58	27.70	0.21	0,02	0,09
37	1.87	4.27	7.64	96.86	30.92			
				Average removal rates		**0.19**	**0.03**	**0.06**

Table 8.69. Kinetic test performed on the 18th day of degradation of 2-CP with 1,2-DCA and 1,2-DCB.

Tempo [h]	**1,2-DCA [mg/l]**	**1,2-DCB [mg/l]**	**2-CP [mg/l]**
0	40.41	8.59	19.54
1	34.88	8.12	18.87
2	27.49	7.65	17.83
3	26.15	7.49	15.68
4	24.88	6.89	12.14
24	2.52	6.75	0.67
Mean specific removal rate [$g_{c.c.}/g_{VSS}$·d]	0.21	0.01	0.03
Max specific removal rate [$g_{c.c.}/g_{VSS}$·d]	0.60	0.05	0.16

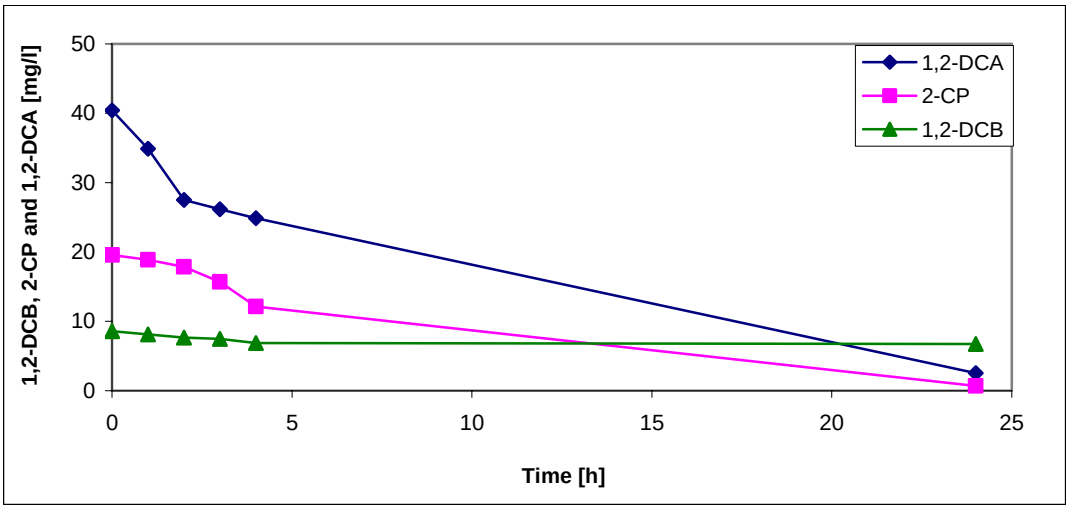

Figure 8.47. Kinetic test performed on the 18th day of degradation of 2-CP with 1,2-DCA and 1,2-DCB.

Table 8.70. Kinetic test performed on the 37th day of degradation of 2-CP with 1,2-DCA and 1,2-DCB.

Tempo [h]	1,2-DCA [mg/l]	1,2-DCB [mg/l]	2-CP [mg/l]
0	42.41	7.69	25.45
1	37.45	7.08	24.70
2	36.31	6.52	24.48
3	30.33	5.72	20.26
4	26.34	5.43	19.44
24	1.87	4.27	7.64
Mean specific removal rate [g/g_{VSS}·d]	0.26	0.02	0.11
Max specific removal rate [g/g_{VSS}·d]	0.61	0.10	0.26

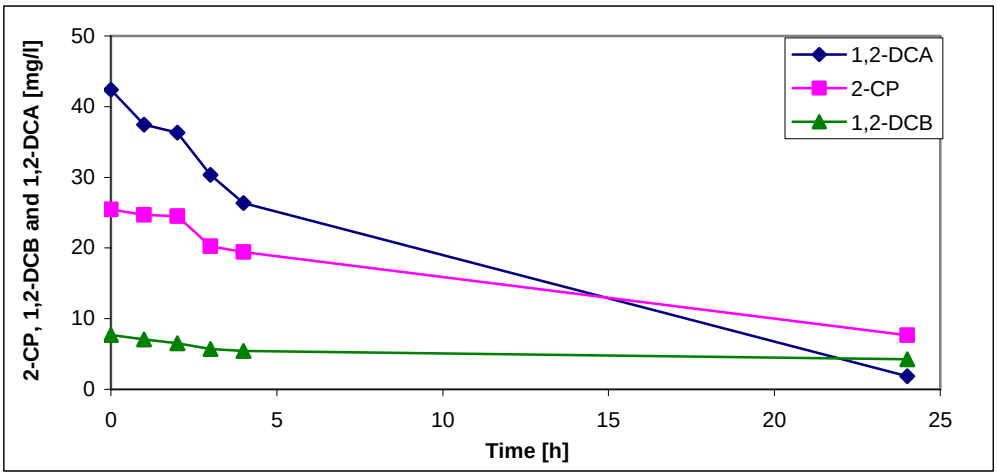

Figure 8.48. Kinetic test performed on the 18th day of degradation of 2-CP with 1,2-DCA and 1,2-DCB.

8.3.2.7 Summary of the specific removal rates observed when feeding 2-CP

Based on the tests performed on the degradation of 2-CP together with different substrates, a summary of the specific removal rates of all the substrates is reported in Table 8.71.

Table 8.71. Specific removal rates of the different chlorinated compounds fed together with 2-CP in the MSBR. *Maximum specific removal rates calculated on a daily basis. ** Specific removal rates achieved after acclimation with several daily cycles.

Substances fed	Mean specific removal rate 1,2-DCA [$g_{1.2\text{-}DCA}/g_{SSV}\cdot d$]	Max specific removal rate 1,2-DCA [$g_{1.2\text{-}DCA}/g_{SSV}\cdot d$	Mean specific removal rate 1,2-DCB [$g_{1.2\text{-}DCB}/g_{SSV}\cdot d$]	Max specific removal rate 1,2-DCB [$g_{1.2\text{-}DCB}/g_{SSV}\cdot d$]	Mean specific removal rate 2-CP [$g_{2\text{-}CP}/g_{SSV}\cdot d$]	Max specific removal rate 2-CP [$g_{2\text{-}CP}/g_{SSV}\cdot d$]
2-CP+phenol (200th day)	-	-	-	-	-	0*
2-CP + 1,2-DCA + phenol (203th day)	-	0.03*	-	-	-	0.03*
2-CP + 1,2-DCA (211th day)	0.10	0.26	-	-	0.04	0.04
2-CP + 1,2-DCA (407th day)	0.37	1.05	-	-	0.14	0.20
2-CP (15 mg/l) + 1,2-DCA 2 cycles/d (538th day)	0.21	1.03			0	0
2-CP (10 mg/l) + 1,2-DCA 2 cycles/d (568th day)	0.21	1.34			0.03	0.09
2-CP + 1,2-DCB + phenol (216th day)	-	-	0.01	0.02*	0.02	0.03*
2-CP + 1,2-DCA + 1,2-DCB + phenol (231st day)	0.02**	0.07*	0.05**	0.02*	0.05**	0.02*
2-CP + 1,2-DCA + 1,2-DCB (370th day)	0.26	0.61	0.02	0.10	0.11	0.26

8.3.3 Degradation of TCE

Since the 305th day, degradation of TCE took place in the MSBR. It was fed first in association with 1,2-DCA, then with 1,2-DCA and 1,2-DCB, and finally with 1,2-DCA, 1,2-DCB and 2-CP.
For all the different feeding modalities, several daily cycles were performed, in order to check if TCE degradation took place and how it influenced degradation rates of the other compounds; at the end of any phase a kinetic test was performed in order to calculate the maximum specific removal rates.

8.3.3.1 Degradation of TCE together with 1,2-DCA

On the 305th day of sludge acclimation, degradation of **TCE** with **1,2-DCA** started and was carried out with daily cycles. It was carried out for 13 days, and at the end of the phase, a kinetic test was performed. Concentration of 1,2-DCA at the beginning of the cycle in the reactor was 50 mg/l, while TCE was fed with a concentration of 10 mg/l.
Trend of 1,2-DCA and TCE are reported in Table 8.72; results of the kinetic test are reported in Table 8.73 and in Figure 8.49.

Table 8.72. Degradation of 1,2-DCA and of TCE.

Time [d]	1,2-DCA [mg/l]	TCE [mg/l]	Chlorides [mg/l]	N-NO_3 [mg/l]	mmol 1,2-DCA oxidized	mmol TCE oxidized	mmol Chlorides produced	mmol Chlorides theoretically produced	mmol $Cl^-_{produced}$/ mmol Cl^-_{theor}	Mean specific removal rate 1,2-DCA [$g_{1,2\text{-}DCA}/g_{SSV}\cdot d$]	Mean specific removal rate TCE [$g_{TCE}/g_{SSV}\cdot d$]
0	46.03	n.a.	54.84	45.99	1.02	-	1.77	2.04	0.87	0.17	-
1	2.17	n.a.	82.11	47.18							
5	28.28	6.02	45.50	40.78	0.62	0.11	1.38	1.55	0.89	0.10	0.02
6	1.65	0.00	66.81	49.12							
6	56.97	9.40	43.48	38.66	1.18	0.02	3.04	2.43	1.25	0.15	0.01
7	19.30	8.03	90.46	36.69							
7	57.40	7.97	51.49	33.96	1.41	0.13	2.16	3.22	0.67	0.14	0.03
8	20.80	0.30	84.80	14.71							
11	48.46	4.40	26.07	12.61	1.51	0.03	4.14	3.13	1.32	0.16	0.01
12	6.36	2.48	90.00	34.02							
12	59.16	4.62	62.06	35.62	1.20	0.04	2.46	2.51	0.98	0.16	0.01
13	17,65	2,40	100,01	36,41							
								Average value		**0.15**	**0.02**

Table 8.73. Kinetic test performed on the 13th day of degradation of 1,2-DCA and TCE.

Time [h]	1,2-DCA [mg/l]	TCE [mg/l]
0	59.16	4.62
1	49.12	4.44
2	46.36	4.36
3	40.01	4.25
4	31.82	4.18
24	17.65	2.4
Mean specific removal rate [g/g_{VSS}·d]	0.16	0.01
Max specific removal rate [g/g_{VSS}·d]	0.48	0.02

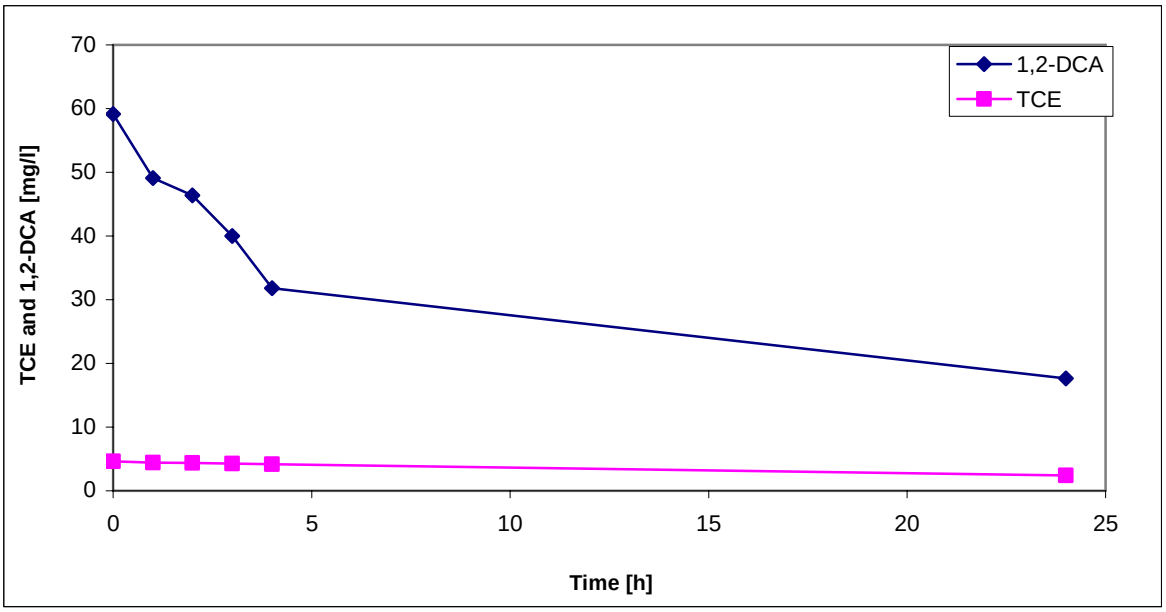

Figure 8.49. Kinetic test performed on the 13th day of degradation of 1,2-DCA and TCE.

8.3.3.2 Degradation of TCE together with 1,2-DCA and 1,2-DCB

Since the 318th day, **TCE** was fed into the MSBR together with **1,2-DCA** and **1,2-DCB** for 16 days. Degradation took place with daily cycles, and on the 16th day a kinetic test was performed in order to calculate the maximum specific removal rate of the substrates fed.
Trend of the chlorinated compounds fed are shown in Tables 8.74 and 8.75; results of the kinetic test are shown in Table 8.76 and in Figure 8.50.

Table 8.74. Trend of 1,2-DCA, 1,2-DCB and TCE when fed together in the MSBR.

Time [d]	1,2-DCA [mg/l]	TCE [mg/l]	1,2-DCB [mg/l]	Chlorides [mg/l]	$N\text{-}NO_3$ [mg/l]	Mean specific removal rate 1,2-DCA [$g_{1,2\text{-}DCA}/g_{SSV}\cdot d$]	Mean specific removal rate TCE [$g_{TCE}/g_{SSV}\cdot d$	Mean specific removal rate 1,2-DCB [$g_{1,2\text{-}DCB}/g_{SSV}\cdot d$
0	29.42	0.90	2.35	35.75	26.62	0.17	0.002	0
1	0.00	0.51	3.70	55.10	30.74			
1	20.01	0.35	1.43	35.56	26.75	0.03	0.000	0.00
5	0.00	0.27	3.52	62.10	39.80			
5	19.80	0.16	1.98	34.94	34.90	0.09	0.001	0
6	3.32	0.07	3.12	48.85	34.78			
6	33.49	0.99	4.37	32.07	32.12	0.18	0.002	0.02
7	1.49	0.63	1.79	52.16	32.12			
7	22.21	0.74	1.47	39.87	31.12	0.16	0.017	0
8	1.49	0.10	3.57	53.81	30.46			
8	22.21	1.30	2.94	37.16	31.36	0.06	0	0.00
11	0.03	0.35	1.53	53.47	33.09			
11	44.05	4.49	11.78	25.39	19.91	0.26	0.024	0.05
12	10.57	1.45	5.68	53.31	17.51			
12	43.30	5.11	11.26	21.20	17.50	0.29	0.008	0.03
13	5.90	4.09	6.97	50.66	14.33			
13	45.43	6.58	13.47	30.22	13.42	0.30	0.038	0.07
14	7.34	1.76	4.22	55.31	10.49			
14	35.01	3.57	8.52	40.78	8.35	0.24	0.011	0.01
15	4.09	2.18	7.84	70.40	13.49			
15	42.94	4.26	13.08	45.34	10.22	0.29	0.019	0.06
16	5.45	1.87	5.67	77.09	32.93			
				Average value		**0.17**	**0.01**	**0.02**

Table 8.75. Balance between chlorinated compounds oxidized and chlorides produced during degradation of 1,2-DCA, 1,2-DCB and TCE.

Time [d]	mmol 1,2-DCA oxidized	mmol TCE oxidized	mmol 1,2-DCB oxidized	mmol Chlorides produced	mmol Chlorides theoretically produced	mmol $Cl^-_{produced}$/ mmol Cl^-_{theor}
0 1	0.68	0.007	0	1.25	1.35	0.93
1 5	0.47	0.002	0	1.72	0.87	1.98
5 6	0.38	0.002	0	0.90	0.73	1.23
6 7	0.74	0.006	0.04	1.30	1.59	0.82
7 8	0.48	0.011	0	0.90	0.93	0.97
8 11	0.52	0.017	0.02	1.06	1.12	0.94
11 12	0.85	0.053	0.10	1.81	2.05	0.88
12 13	0.92	0.018	0.07	1.91	2.02	0.95
13 14	1.02	0.084	0.14	1.63	2.59	0.63
14 15	0.79	0.024	0.01	1.92	1.67	1.15
15 16	0.96	0.042	0.12	2.06	2.29	0.90

Table 8.76. Kinetic test for degradation of 1,2-DCA, 1,2-DCB and TCE.

Tempo [h]	1,2-DCA [mg/l]	TCE [mg/l]	1,2-DCB [mg/l]
0	42.94	4.26	13.08
1	39.91	4.15	12.98
2	37.75	4.06	12.72
3	35.16	3.62	12.48
4	29.98	3.46	12.34
24	5.45	1.87	5.67
Mean specific removal rate [$g_{c.c.}/g_{VSS}$·d]	0.17	0.02	0.03
Max specific removal rate [$g_{c.c.}/g_{VSS}$·d]	0.49	0.04	0.04

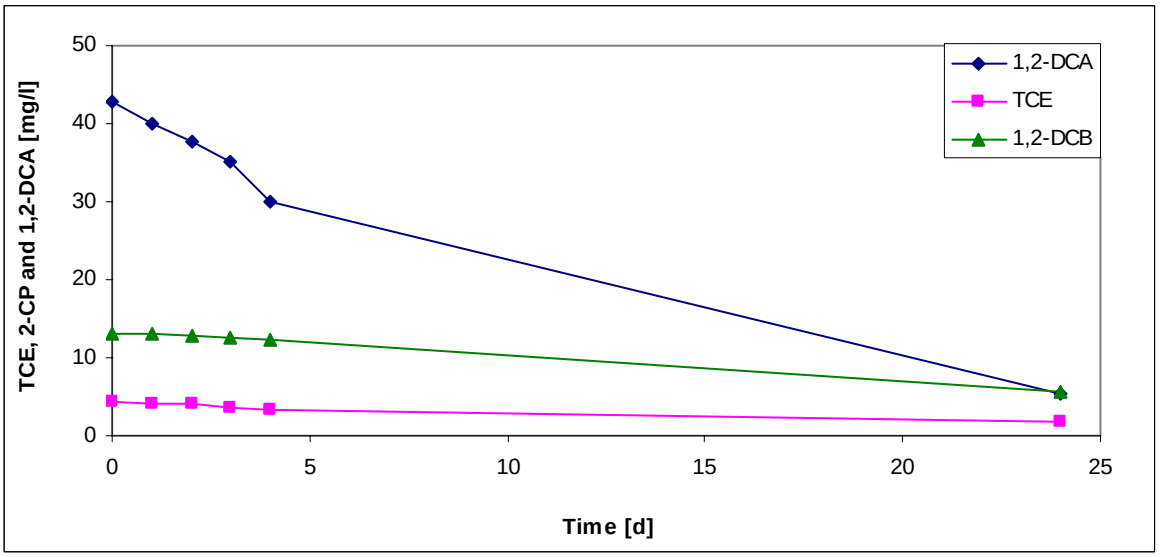

Figure 8.50. Kinetic test on degradation of 1,2-DCA, 1,2-DCB and TCE.

8.3.3.3 Degradation of TCE together with 1,2-DCA, 1,2-DCB and 2-CP

Since the 334th day, the MSBR was fed with **1,2-DCA**, **1,2-DCB**, **TCE** and **2-CP** for 36 days. Degradation took place with daily cycles, and on the 15th and on the 36th days two kinetic tests were performed in order to calculate the maximum specific removal rates of the substrates fed.

Trend of the chlorinated compounds fed are shown in Table 8.77; results of the kinetic tests are shown in Tables 8.78 and 8.79 and in Figures 8.51 and 8.52.

Table 8.77. Degradation of 1,2-DCA, 1,2-DCB, TCE and 2-CP.

Time [d]	1,2-DCA [mg/l]	TCE [mg/l]	1,2-DCB [mg/l]	2-CP [mg/l]	Chlorides [mg/l]	$N\text{-}NO_3$ [mg/l]	Mean specific removal rate 1,2-DCA [$g_{1.2\text{-}DCA}/g_{SSV}\cdot d$]	Mean specific removal rate TCE [$g_{TCE}/g_{SSV}\cdot d$]	Mean specific removal rate 1,2-DCB [$g_{1,2\text{-}DCB}/g_{SSV}\cdot d$]	Mean specific removal rate 2-CP [$g_{CP}/g_{SSV}\cdot d$]
0	27.69	3.72	14.07	35.94	43.79	0.00	0.22	0.01	0.05	0
1	0.00	3.10	8.35	36.93	68.70	10.22				
1	20.34	2.85	6.77	40.32	48.78	13.49	0.16	0.00	0.00	0.04
2	0.00	2.28	6.55	35.76	65.98	15.34				
2	36.65	2.72	7.61	12.09	48.20	18.70	0.10	0.00	0.01	0.00
5	0.00	1.40	5.82	11.27	76.95	5.60				
5	53.97	7.40	10.49	19.69	52.64	19.20	0.14	0.02	0.01	0
6	35.93	5.08	9.55	20.64	65.59	27.07				
14	52.78	10.13	21.26	18.02	69.40	28.64	0.25	0.06	0.09	0.07
15	13.17	2.02	9.17	9.31	92.99	20.08				
15	60.74	15.03	17.56	21.51	58.85	26.08	0.23	0.04	0.05	0.06
16	15.02	10.51	11.63	14.52	93.56	18.74				
16	56.93	8.06	12.64	20.22	62.80	28.69	0.15	0.01	0.02	0.05
19	0.00	6.21	6.15	2.49	110.20	31.15				
19	57.76	17.50	17.34	19.97	62.07	24.28	0.22	0.06	0.05	0.04
20	18.69	9.90	10.70	15.30	85.10	18.66				
20	54.82	7.68	10.37	26.87	59.32	23.53	0.27	0.02	0.02	0.12
21	9.92	5.50	8.25	11.06	80.54	22.17				
21	41.66	15.40	14.20	21.77	73.65	32.19	0.25	0.07	0.05	0.06
22	9.74	5.94	7.65	13.52	94.87	69.49				
22	47.64	9.11	11.26	23.74	67.28	37.73	0.25	0.03	0.03	0.11
23	8.21	3.83	6.61	6.91	86.37	19.43				
33	49.49	12.20	12.85	13.02	68.81	28.75	0.19	0.01	0.02	0.00
34	10.64	10.53	9.92	13.57	92.87	28.82				
34	51.86	15.77	14.60	18.18	64.73	26.86	0.29	0.05	0.06	0.04
35	6.02	8.30	5.87	11.63	94.62	21.50				
35	49.89	12.26	8.95	20.11	70.02	25.24	0.31	0.03	0.00	0.05
36	2.24	7.36	9.63	12.06	108.30	23.10				
					Average value		**0.20**	**0.03**	**0.03**	**0.04**

Table 8.78. First kinetic test for degradation of 1,2-DCA, 1,2-DCB, TCE and 2-CP, performed on the 15th day of the phase.

Tempo [h]	1,2-DCA [mg/l]	TCE [mg/l]	1,2-DCB [mg/l]	2-CP [mg/l]
0	60.74	15.03	17.56	21.51
1	58.18	15.39	16.98	21.22
2	55.11	14.28	16.84	19.74
3	53.30	12.65	16.67	17.12
4	50.56	14.11	16.31	17.86
24	25.02	10.51	11.63	14.52
Mean specific removal rate [$g_{c.c.}/g_{VSS}$·d]	0.20	0.04	0.05	0.05
Max specific removal rate [$g_{c.c.}/g_{VSS}$·d]	0.49	0.04	0.06	0.18

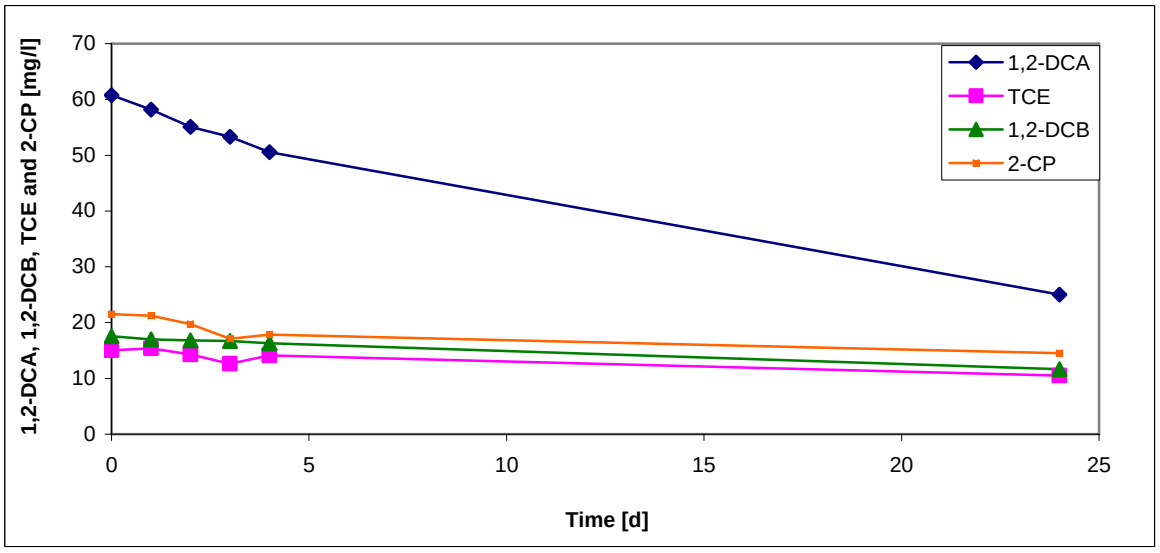

Figure 8.51. First kinetic test on the degradation of 1,2-DCA, 1,2-DCB, TCE and 2-CP, performed on the 15th day of the phase.

Table 8.79. Second kinetic test for degradation of 1,2-DCA, 1,2-DCB, TCE and 2-CP, performed on the 36th day of the phase.

Tempo [h]	1,2-DCA [mg/l]	TCE [mg/l]	1,2-DCB [mg/l]	2-CP [mg/l]
0	49.89	12.26	8.95	20.11
1	40.18	10.62	8.04	19.66
2	39.57	10.83	7.49	18.45
3	39.4	10.44	7.2	16.21
4	36.02	10.44	7.2	15.76
24	2.24	7.36	5.4	12.06
Mean specific removal rate [$g_{c.c.}/g_{VSS}$·d]	0.24	0.03	0.01	0.05
Max specific removal rate [$g_{c.c.}/g_{VSS}$·d]	0.52	0.09	0.09	0.19

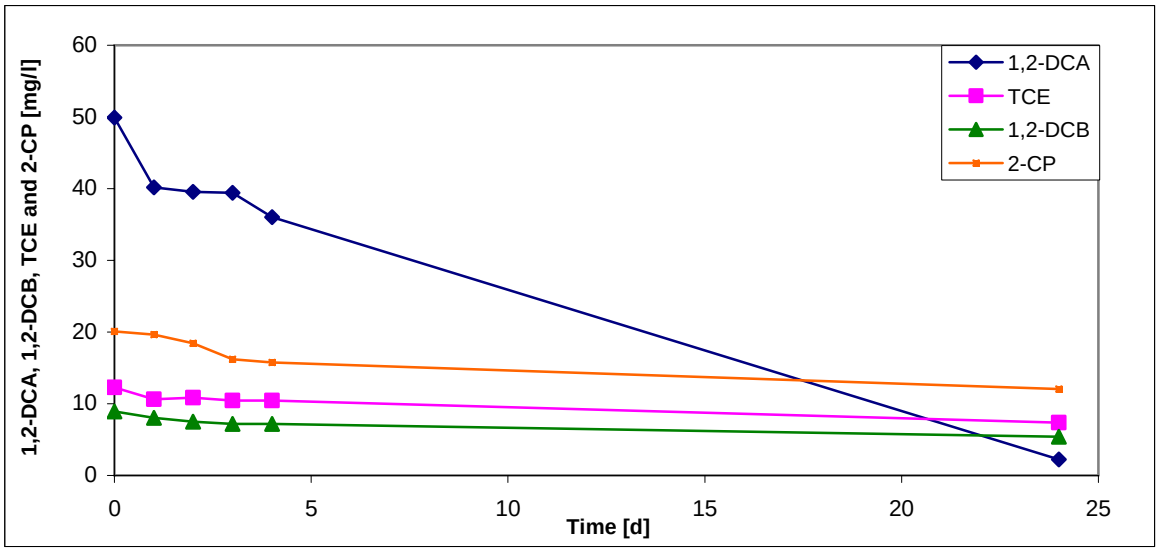

Figure 8.52. Second kinetic test on the degradation of 1,2-DCA, 1,2-DCB, TCE and 2-CP, performed on the 36th day of the phase.

8.4 Experimentation development

In order to clarify the development of the experimentation in time, the lengths of the different phases carried on either in batches, in the SBR and in the MSBR are explained in Figure 8.53 and in Table 8.80.

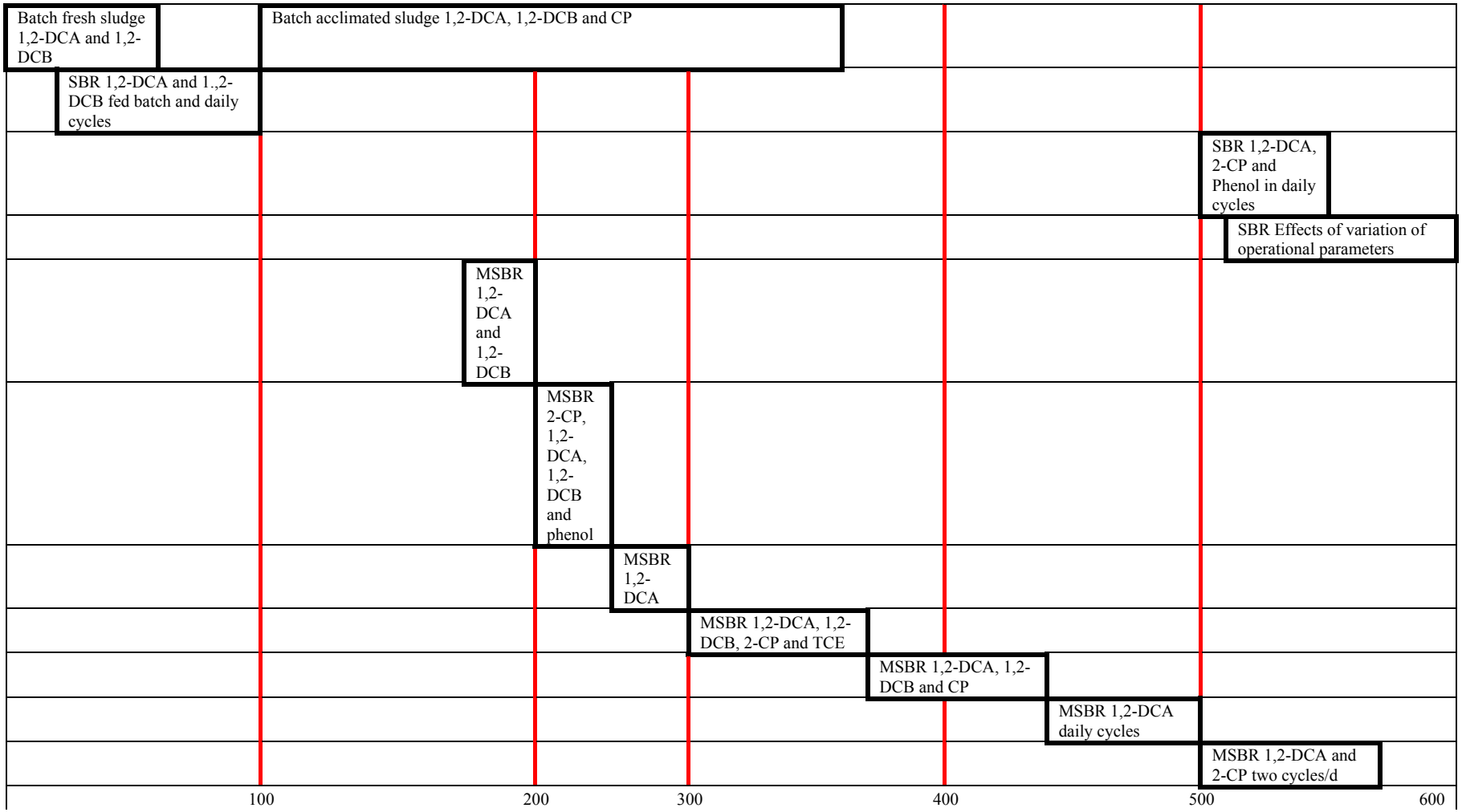

Figure 8.53. Development of the different phases carried out in the experimentation.

Table 8.80. Development of the different phases carried out in the experimentation.

Reactor	Feeding	Start [day n.]	Length [d]
Batch	Anaerobic batch test with 1,2-DCA; fresh sludge	0	21
Batch	Aerobic batch tests with 1,2-DCA; fresh sludge	0	22
Batch	Aerobic batch test 1,2-DCA; acclimated sludge	240	1
Batch	Aerobic batch test 1,2-DCA+methanol; acclimated sludge	330	1
Batch	Anaerobic batch tests 1,2-DCB; fresh sludge	23	21
Batch	Anaerobic batch test 1,2-DCB+methanol; fresh sludge	44	7
Batch	Aerobic batch test 1,2-DCB; fresh sludge	23	28
Batch	Anaerobic batch test 1,2-DCA+ methanol; fresh sludge	51	7
Batch	Aerobic batch test 1,2-DCB; acclimated sludge	270	21
Batch	Aerobic batch test 1,2-DCB + methanol; acclimated sludge	291	7
Batch	Aerobic batch test interactions 1,2-DCB and 1,2-DCA	298	9
Batch	Aerobic batch test 1,2-DCA, 1,2-DCB and methanol	307	7
Batch	Anaerobic batch test 2-CP; fresh sludge	300	28
Batch	Aerobic batch test 2-CP+phenol; fresh sludge	328	35
Batch	Anaerobic batch test 2-CP; acclimated sludge	300	21
Batch	Anaerobic batch test 2-CP+methanol; acclimated sludge	321	7
Batch	Anaerobic batch test 2-CP and 1,2-DCA; acclimated sludge	328	21
Batch	Anaerobic batch test 2-CP and 1,2-DCA+methanol; acclimated sludge	349	7
Batch	Anaerobic batch test 2-CP and phenol; acclimated sludge	356	7
Batch	Aerobic batch 2-CP; acclimated sludge	300	21
Batch	Aerobic batch 2-CP and methanol; acclimated sludge	321	7
Batch	Aerobic batch 2-CP and 1,2-DCA; acclimated sludge	328	21
Batch	Aerobic batch 2-CP and 1,2-DCA+ methanol; acclimated sludge	349	7
Batch	Aerobic batch 2-CP and phenol; acclimated sludge	356	7
Batch	Aerobic batch 2-CP, 1,2-DCA and phenol; acclimated sludge	363	7
Batch	Aerobic batch 2-CP, 1,2-DCB and phenol; acclimated sludge	370	7
Batch	Aerobic batch 2-CP, 1,2-DCB, 1,2-DCA and phenol; acclimated sludge	377	7
SBR	Degradation of 1,2-DCA in fed batch modality	25	69
SBR	Degradation of 1,2-DCB and 1,2-DCA in fed batch modality	98	21
SBR	Degradation of 1,2-DCA in daily cycles modality	70	28
SBR	Degradation of 1,2-DCA and phenol in daily cycles; 5 phases	500	47
SBR	Variation of operational parameters for 1,2-DCA degradation; 4 phases	547	86
MSBR	1,2-DCA degradation with daily cycles	180	8
MSBR	1,2-DCB degradation with daily cycles	188	10
MSBR	1,2-DCA and 1,2-DCB degradation with fed-batch cycles	198	17
MSBR	2-CP and phenol degradation with daily cycles	200	3
MSBR	1,2-DCA, 2-CP and phenol degradation with daily cycles	203	8
MSBR	1,2-DCA and 2-CP degradation with daily cycles	211	5
MSBR	1,2-DCB, 2-CP and phenol degradation with daily cycles	216	15
MSBR	1,2-DCB, 1,2-DCA, 2-CP and phenol degradation with daily cycles	231	12
MSBR	1,2-DCA degradation with daily cycles	275	30
MSBR	1,2-DCA and TCE degradation with daily cycles	305	13
MSBR	1,2-DCA, 1,2-DCB and TCE degradation with daily cycles	318	16
MSBR	1,2-DCA, 1,2-DCB, TCE and 2-CP degradation with daily cycles	334	36
MSBR	1,2-DCA, 1,2-DCB and 2-CP degradation with daily cycles	370	37
MSBR	1,2-DCA and 2-CP degradation with daily cycles	407	31
MSBR	1,2-DCA degradation with daily cycles	438	60

to be continued

follows from the previous page

MSBR	1,2-DCA degradation with two cycles per day	498	40
MSBR	1,2-DCA and 2-CP degradation with two cycles per day	538	30
MSBR	1,2-DCA and 2-CP (halved conc.) degradation with two cycles per day	568	20

Chapter 9
Discussion on the Experimental Results

9.1 Degradation of 1,2-DCA

9.1.1 Degradation of 1,2-DCA fed as unique substrate

9.1.1.1 Batch tests

Batch tests performed on fresh sludge indicated that 1,2-DCA was degraded by fresh sludge only under aerobic conditions; in anaerobic conditions, no degradation of 1,2-DCA could be observed, even after two weeks.

Degradation of 1,2-DCA was achieved in batch tests performed in **aerobic** conditions, and it was observed that specific removal rate of 1,2-DCA, measured on a daily basis, increased from the zero value up to 0.04 $g_{1,2\text{-}DCA}/(d \cdot g_{VSS})$ after 22 days of 1,2-DCA feeding.

9.1.1.2 Degradation in SBR

When 1,2-DCA (initial concentration in the reactor 50 mg/l) was fed to the SBR operated in fed batch modality and inoculated with sludge coming from the aerobic batch tests, degradation of the chlorinated compound started after a lag-phase of 5 days, and was then almost completely degraded after three days; afterwards, almost complete removal of 1,2-DCA was achieved after two days, and after 30 days of acclimation, complete degradation of 1,2-DCA was observed within one day. Then, degradation of 1,2-DCA took place in daily cycles modality, in order to supply the biomass with cyclic and regular amounts of substrate and favour its acclimation to the compound.

With the acclimation in fed-batch modality, no kinetic tests were performed, and so only mean specific removal rates on daily basis are available; at the end of the fed-batch operation, the mean specific removal rate achieved was 0.11 $g_{1,2\text{-}DCA}/(d \cdot g_{VSS})$.

On the 35^{th} day of degradation in fed-batch modality, 90% of the total sludge of the system was substituted with fresh non-acclimated sludge in order to increase the concentration of biomass in the system; this caused a temporary interruption of 1,2-DCA degradation, which took 14 days for the biomass to recover and start again the degradation of 1,2-DCA. This was due to the fact that in fresh sludge, bacterial strains capable of degrading 1,2-DCA were not selected, and this inhibited temporarily the degradation of the chlorinated compound; after 14 days, the proper bacterial strains were selected, and degradation of 1,2-DCA took place again.

After 20 days of the SBR operation in daily cycles modality, on the 90^{th} day of acclimation, mean and maximum specific removal rates were respectively 0.19 $g_{1,2\text{-}DCA}/(d \cdot g_{VSS})$ and 0.48 $g_{1,2\text{-}DCA}/(d \cdot g_{VSS})$.

Thus, an increase in mean specific removal rate of 1,2-DCA was observed with progressive acclimation of the sludge to the chlorinated compound, increasing from the value of 0.11 $g_{1,2\text{-}DCA}/(d \cdot g_{VSS})$ obtained in fed-batch modality up to 0.19 $g_{1,2\text{-}DCA}/(d \cdot g_{VSS})$ observed with daily cycles; this confirmed the hypothesis that **the acclimation played an important role in 1,2-DCA degradation potential by the biomass selected in this study**.

Besides, the mean specific removal rates observed during degradation in SBR was 5 times higher than the one observed in batch tests, thus giving evidence to **the effectiveness of the SBR system for the acclimation of a bacterial population**.

9.1.1.3 Degradation in MSBR with daily cycles

Degradation of 1,2-DCA with daily cycles took place in the MSBR in three different periods of the experimentation: since the 180th day for 8 days, since the 275th day for one month, and since the 438th day for two months. As can be seen from Table 9.1, maximum specific removal rates of 1,2-DCA increased with the progressive acclimation of the sludge.

Table 9.1. Mean and maximum specific removal rates of 1,2-DCA obtained in the MSBR fed with daily cycles in different periods of the experimentation.

Substances fed	Mean specific removal rate 1,2-DCA [$g_{1,2\text{-DCA}}/g_{VSS}\cdot d$]	Maximum specific removal rate 1,2-DCA [$g_{1,2\text{-DCA}}/g_{VSS}\cdot d$]
1,2-DCA (180th day)	0.16	0.70
1,2-DCA (275th day)	0.24	1.06
1,2-DCA (498th day)	0.24	1.36

These results, together with the removal rates obtained in the SBR operated in daily cycles, underline the **key role of acclimation for the degradation of 1,2-DCA**: maximum specific removal rates increased progressively in time, from the value of 0.48 $g_{1,2\text{-DCA}}/(d\cdot g_{VSS})$ observed on the 90th day of acclimation in the SBR up to the value of 1.36 $g_{1,2\text{-DCA}}/(d\cdot g_{VSS})$ achieved on the 498th day in the MSBR operated with two cycles per day.

9.1.1.4 Degradation in MSBR with two cycles per day

Degradation of 1,2-DCA was operated in the MSBR with two cycles per day in two different periods of the experimentation: one started on the 99th day of acclimation, while the other started on the 498th day of the experimentation. Mean and specific removal rates observed at the end of both phases are reported in Table 9.2, and underlined three main points:

- degradation of 1,2-DCA with **two cycles per day causes an increase of both mean and maximum specific removal rates** in comparison to the ones observed with one cycle per day.
- removal rates observed after around 500 days of degradation are 1.6 times higher than the ones observed after about 100 days. This underlines again the **importance of acclimation** for the degradation of 1,2-DCA: a sludge acclimated for a longer time by means of sequential cycles degrades more effectively the chlorinated compound;
- acclimated sludge, even after around 100 days of acclimation, is **capable of completely degrading 1,2-DCA after 12 hours**, thus indicating an interesting potential of remediation of polluted water fed with high frequency to the biological system.

Table 9.2. Mean and maximum specific removal rates of 1,2-DCA obtained in the MSBR fed with two cycles per day in different periods of the experimentation.

Substances fed	Mean specific removal rate 1,2-DCA [$g_{1,2\text{-DCA}}/g_{VSS}\cdot d$]	Maximum specific removal rate 1,2-DCA [$g_{1,2\text{-DCA}}/g_{VSS}\cdot d$]
1,2-DCA 2cycles/d (99th day)	0.51	1.11
1,2-DCA 2cycles/d (498th day)	0.79	1.83

9.1.1.5 Effects of variation of operating parameters on 1,2-DCA degradation

In order to foresee possible implementations of the SBR system at real scale, the effects of variation of some operating parameters on 1,2-DCA degradation was studied. Actually, it is reasonable to think that in a real scale implementation of the system, aeration of the mineral medium or of the polluted aqueous stream should be avoided or reduced in order to reduce management costs. Besides, a great cost saving would be achieved with the elimination of the system heating. Finally, also the addition of mineral salts to the system for the cellular growth should be avoided or reduced in order to decrease total management costs. Therefore, 1,2-DCA specific removal rates at different operating parameters were compared; temperature of the system was lowered from 30°C to 20°C, and aeration and mineral medium composition were varied. Sludge used for these tests had been acclimated to 1,2-DCA for about 600 days.

As it could be observed from the results, 1,2-DCA maximum specific removal rates observed at 30°C and with aerated mineral medium were in line with the ones observed in the MSBR in the last period of the acclimation: a mean value of 1.15 $g_{1,2\text{-}DCA}/g_{VSS}\cdot d$ was observed, which is very near to the value of 1.36 $g_{1,2\text{-}DCA}/g_{VSS}\cdot d$ achieved around the 500th day in the MSBR operated with daily cycles. Switching the temperature down to 20°C lowered the maximum specific removal rate down to 0.67 $g_{1,2\text{-}DCA}/g_{VSS}\cdot d$, thus indicating that **the system reached a more effective removal when operated at a higher temperature**.

Eliminating the aeration of the mineral medium fed to the system did not cause any appreciable decrease of the maximum specific removal rate, which stabilised at a value of 0.64 $g_{1,2\text{-}DCA}/g_{VSS}\cdot d$; this confirmed the fact that **the oxygen supplied to the system with the headspace left in the reactor and with the oxygen present in linked form in nitrates, was sufficient for the removal of 1,2-DCA at a concentration in the reactor of 50 mg/l**. Anyway, it is very interesting to observe (see Figure 8.25) the high nitrates consumption in Phase n. 3, which shows that the system entered anoxic conditions because of the lack of oxygen in molecular form. After consuming all the oxygen present in the reactor headspace microorganisms reduced the nitrates present, which was sufficient for completing the metabolic processes linked to 1,2-DCA oxidation. Therefore, it could be stated that **the bacterial population selected was capable of degrading 1,2-DCA under both aerobic and anoxic conditions, but not when the prolonged absence of molecular oxygen (for more than few days) set anaerobic conditions in the reactor.**

An appreciable **inhibition of the system was instead caused by the substitution of the mineral medium with tap water**: after 10 days degradation of 1,2-DCA was significantly lowered, and finally stopped 4 days later. Maximum specific removal rates observed during the kinetic test performed on the 7th day was very low (0.11 $g_{1,2\text{-}DCA}/g_{VSS}\cdot d$), while the one observed on the 26th day of this phase was almost zero (0.01 $g_{1,2\text{-}DCA}/g_{VSS}\cdot d$). This suggested **that the selected biomass needed a proper supply of mineral salts and microelements for their growth, that were not present in tap water**.

At this point feeding of the reactor with non aerated mineral medium was stopped, in order to avoid possible irreversible damages to the biomass, and the reactor was fed again with aerated mineral medium; degradation of 1,2-DCA took place again with the usual efficiency.

9.1.2 Degradation of 1,2-DCA with other substrates

9.1.2.1 Degradation of 1,2-DCA with 1,2-DCB

Degradation in batch tests

When sludge acclimated to 1,2-DCA was fed with 1,2-DCB, its capability of degrading 1,2-DCA decreased, and 1,2-DCA maximum specific removal rate decreased from the value of 0.58 down to the value of 0.50 $g_{1,2\text{-}DCA}/g_{VSS}\cdot d$. The test was performed on the 200th day of acclimation, and sludge came from the SBR. This result suggested a **negative influence of 1,2-DCB on degradation of 1,2-DCA**.

Degradation in SBR

When 1,2-DCA was degraded together with 1,2-DCB in SBR, it did not show a strong decrease of its mean specific removal rate, which remained stable to a value of 0.11 $g_{1,2\text{-}DCA}/g_{VSS}\cdot d$, i.e. the same value obtained in daily cycles.

Degradation in MSBR

More interesting was the degradation of 1,2-DCA and 1,2-DCB fed together in the MSBR. With the sludge acclimated from around 200 days, and the two substrates fed in fed-batch modality, a **decrease of 1,2-DCA maximum specific removal rate in comparison to when it was fed alone was observed**. It passed from the value of 0.70 $g_{1,2\text{-}DCA}/g_{VSS}\cdot d$ achieved in the same period in the MSBR fed with daily cycles with only 1,2-DCA, to the value of 0.18 $g_{1,2\text{-}DCA}/g_{VSS}\cdot d$.

Therefore a **negative effect of 1,2-DCB on degradation of 1,2-DCA could be supposed**; this was confirmed by the other tests operated both in the MSBR when 1,2-DCA and 1,2-DCB were fed together with 2-CP: at the end of the phase started on the 370th day, in which 1,2-DCA, 1,2-DCB and 2-CP were fed together, maximum specific removal rate for 1,2-DCA was 0.61 $g_{1,2\text{-}DCA}/g_{VSS}\cdot d$, which was lower than the one observed in the same period for 1,2-DCA fed alone in daily cycles (1.36 $g_{1,2\text{-}DCA}/g_{VSS}\cdot d$ on the 468th day of sludge acclimation). Since 2-CP had no negative effects on 1,2-DCA degradation (see below), the decrease of 1,2-DCA removal rate was imputable to the presence of 1,2-DCB.

In another test, started on the 231st day of acclimation, in which 1,2-DCA, 1,2-DCB, 2-CP and phenol were fed together, maximum specific removal rate for 1,2-DCA was 0.03 $g_{1,2\text{-}DCA}/g_{VSS}\cdot d$, but this was probably due to the presence of **phenol**, whose negative effects on degradation of the chlorinated compounds were shown in many tests.

Negative effect of 1,2-DCB on 1,2-DCA was not observed when the two substrates were fed together, in addition to TCE. In this case, 1,2-DCA maximum specific removal rate was around 0.50 $g_{1,2\text{-}DCA}/g_{VSS}\cdot d$ both in presence and in absence of 1,2-DCB. This rate, which was lower than the one observed in the same period in absence of TCE (see Table 9.1) was probably due to the fact that **TCE negatively affects removal of 1,2-DCA**, as will be explained below.

9.1.2.2 Degradation of 1,2-DCA with 2-CP

Degradation in batch test

In the batch test performed on the 363rd day of sludge acclimation, in which 1,2-DCA was fed together with phenol and 2-CP, mean specific removal rate of 1,2-DCA was very low (0.06 $g_{1,2\text{-}DCA}/g_{VSS}\cdot d$) in comparison to the one observed in the MSBR in the same period, when 1,2-DCA was fed alone in daily cycles (see Table 9.1). However, this effect was probably more due to the presence of **phenol**, rather than to 2-CP; actually, as it will be explained better, phenol showed to have a very strong negative effect on 1,2-DCA removal.

Degradation in SBR

Starting from the 500th day, sludge was fed in the SBR with 1,2-DCA (50 mg/l) and 2-CP (25 mg/l), for around one month. On the 530th day, maximum specific removal rate for 1,2-DCA was 1.05 $g_{1,2\text{-}DCA}/g_{VSS}\cdot d$, which was in line with what observed on the 498th day, when 1,2-DCA had been fed alone in the system for one month (see Table 9.1). This suggested that **2-CP at a concentration of 25 mg/l had no particular negative effects on removal of 1,2-DCA**.

Since the 531st day, 2-CP concentration was doubled up to 50 mg/l, and degradation took place for 12 days; at the end of this phase, 1,2-DCA maximum specific removal rate decreased down to 0.61 $g_{1,2\text{-}DCA}/g_{VSS}\cdot d$, thus indicating that the **sludge is partially affected by the presence of high concentrations of 2-CP**, and that probably **it takes a long acclimation time for the sludge to be capable of degrading higher concentrations of 2-CP**.

Degradation in MSBR

When degradation of 1,2-DCA and 2-CP (40 mg/l) was carried out in the MSBR since the 211th day, maximum specific removal rate observed for 1,2-DCA was 0.26 $g_{1,2\text{-}DCA}/g_{VSS}\cdot d$, which was lower than the one observed in the period when 1,2-DCA was fed alone (see Table 9.1). This was probably due to the fact that previously, the system was fed, in addition to 1,2-DCA, 2-CP, also with **phenol,** which showed to have negative effects on 1,2-DCA removal.

On the 438th day of acclimation, when the MSBR had been fed with 1,2-DCA and 2-CP (25 mg/l) for one month, maximum specific removal rate for 1,2-DCA observed was 1.05 $g_{1,2\text{-}DCA}/g_{VSS}\cdot d$, which was in line with the value of 1,2-DCA maximum specific removal rate observed for 1,2-DCA when it was fed alone in the same period (see Table 9.1). The higher removal rate observed on the 438th day in comparison to the one observed around the 220th day can be explained again with the **importance of the acclimation** for the degradation of 1,2-DCA: a sludge exposed for a longer time to 1,2-DCA is capable of degrading it with increasingly higher rates. Besides, it could also be stated that **1,2-DCA removal is not affected by the contemporary presence of 2-CP**.

Since the 538th day, 1,2-DCA and 2-CP (15 mg/l) were degraded together in the MSBR with two cycles per day; after one month of this kind of feeding, maximum specific removal rate observed for 1.2-DCA was 1.03 $g_{1,2\text{-}DCA}/g_{VSS}\cdot d$, which is slightly lower than the value observed when 1,2-DCA was fed alone in the system with two cycles per day in the same period (see Table 9.2).

After this phase, 2-CP was fed at lower concentration (10 mg/l), while 1,2-DCA was fed with the same concentration of the previous period; after one month of this kind of feeding, 1,2-DCA specific removal rate observed was higher, stabilising at a value of 1.26 $g_{1,2\text{-}DCA}/g_{VSS}\cdot d$. This showed once more the **importance of acclimation** of the sludge, that with the progressive exposition to 1,2-DCA improved its capability of degrading it.

9.1.2.3 Degradation of 1,2-DCA with 2-CP and phenol

Degradation in batch test

In the batch test performed on the 363rd day, in which 1,2-DCA was fed together with phenol and 2-CP, mean specific removal rate of 1,2-DCA was very low (0.06 $g_{1,2\text{-DCA}}/g_{VSS}\cdot d$) in comparison to the one observed in the MSBR in the same period, when 1,2-DCA was fed alone in daily cycles (see Table 9.1). Since 2-CP showed to have no negative effects on 1,2-DCA degradation, the observed decrease in 1,2-DCA degradation was probably due to the presence of **phenol**; actually, as it was observed during degradation in the other reactors, **phenol showed to have a very strong negative effect on 1,2-DCA removal**.

This was confirmed by what was observed during the batch tests in which 1,2-DCA was degraded together with 1,2-DCB, 2-CP and phenol since the 377th day; with these compounds fed, 1,2-DCA was degraded with a maximum specific removal rate of 0.03 $g_{1,2\text{-DCA}}/g_{VSS}\cdot d$; in this case, **partial inhibition of 1,2-DCA degradation was due both to the presence of phenol and of 1,2-DCB**.

Degradation in SBR

Important indications on the effects of phenol on degradation of 1,2-DCA and 2-CP were obtained in a proper phase of the experimentation. In this part of the study, starting from the 500th day and was into 5 phases, 1,2-DCA was fed always with the same concentration at the beginning of the cycle (50 mg/l), while phenol and/or 2-CP were fed in different concentrations (see Table 8.14).

As it can be observed in Figures from 8.11 to 8.15 and in Tables from 8.15 to 8.24, **the presence of phenol** at concentrations of both 25 and 50 mg/l, lead to a consequent **reduction of 1,2-DCA maximum specific removal rate**, which was lowered from the value of 0.61 (measured in the same test when 1,2-DCA and 2-CP at 50 mg/l were fed together) down to the value of 0.27 $g_{1,2\text{-DCA}}/g_{VSS}\cdot d$.

The negative influence of phenol on degradation of 1,2-DCA and 2-CP was quite evident, and was probably due to the fact that this substrate (which was more easily biodegradable by the biomass) was oxidized very rapidly, thus **consuming the oxygen present** in the system which did not allow the oxidation of the other chlorinated compounds, less degradable than phenol. This was also confirmed by the **consumption of nitrates observed during the kinetic tests in which phenol was fed**; this consumption was not observed during the kinetic tests without phenol. The presence of phenol, causing the fast consumption of oxygen present in molecular form, leads to the utilization as electron acceptors.

Degradation in MSBR

On the 203rd day of acclimation, 1,2-DCA and 2-CP were fed to the MSBR together with phenol. After 8 days, maximum specific removal rate for 1,2.DCA (measured on a daily basis) was very low (0.03 $g_{1,2\text{-DCA}}/g_{VSS}\cdot d$) in comparison to the rate observed on the 211th day (0.26 $g_{1,2\text{-DCA}}/g_{VSS}\cdot d$) in which 1,2-DCA and 2-CP were fed in the MSBR without phenol.

This confirmed the negative effect of phenol on degradation of 1,2-DCA.

In another test, started on the 231st day of acclimation, in which 1,2-DCA, 1,2-DCB and 2-CP were fed together, maximum specific removal rate for 1,2-DCA was 0.03 $g_{1,2\text{-DCA}}/g_{VSS}\cdot d$; this very low rate, which is in line with the one observed on the 203rd day (in which the same compounds with the exception of 1,2-DCB were fed) was due to the presence of phenol, since neither 2-CP nor 1,2-DCB showed to have any such strong effect on 1,2-DCA removal.

9.1.2.4 Degradation of 1,2-DCA with TCE and other substrates

Degradation of 1,2-DCA with TCE

Since the 305th day, degradation of 1,2-DCA and TCE took place in the MSBR. After 13 daily cycles, 1,2-DCA maximum specific removal rate in presence of TCE was 0.48 $g_{1,2\text{-}DCA}/g_{VSS}\cdot d$. This was lower than the rates observed for 1,2-DCA when fed alone in the system (see Table 9.1). **Therefore, a negative effect of TCE on 1,2-DCA removal was supposed.**

Degradation of 1,2-DCA with TCE and 1,2-DCB

When degraded with TCE and 1,2-DCB, 1,2-DCA showed to have a maximum specific removal rate of 0.49 $g_{1,2\text{-}DCA}/g_{VSS}\cdot d$, which was very similar to the one observed when 1,2-DCA was fed only with TCE, and was lower than the one observed when the compound was fed alone in the system, therefore suggesting **possible negative effects of TCE on 1,2-DCA removal**.

Degradation of 1,2-DCA with TCE, 2-CP and 1,2-DCB

When degraded with TCE, 1,2-DCB and 2-CP, 1,2-DCA showed to have a maximum specific removal rate of 0.49 $g_{1,2\text{-}DCA}/g_{VSS}\cdot d$, which was very similar to the one observed when 1,2-DCA was fed only with TCE and together with 1,2-DCB and TCE, and was lower than the one observed when the compound was fed alone in the system, therefore confirming **possible negative effects of TCE on 1,2-DCA removal**.

9.1.3 Conclusions on 1,2-DCA degradation

One of the most important indications given by the experimentation on 1,2-DCA was the **importance of sludge acclimation on its degradative potential**: a sludge exposed for a longer time to a chlorinated compound was capable of degrading it with higher degradation rates.

Another important factor observed in the experimentation was the **importance of the sequential cycles (both in SBR and in MSBR) modality for the acclimation of the sludge and for the selection of the proper bacterial populations deputed to the degradation of the chlorinated compounds**. This is due to the fact that the sequential and regular repetition of operational conditions and organic loads entering the biological system is fundamental in the selection of a particular bacterial strain.

The bacterial population selected in this experimentation was capable of degrading 1,2-DCA at initial concentration of 50 mg/l **within 12 hours**; the **increase of the organic daily load deriving from switching the cycles frequency from one to two per day increased also the maximum specific removal rate of the chlorinated compound fed**.

Temperature of the system was important in the degradation of the chlorinated compounds; higher specific removal rates of 1,2-DCA were observed when degradation was carried out at 30°C rather than at 20°C.

The **system showed to ensure the sufficient amount of oxygen** for the degradation of 1,2-DCA at 50 mg/l, by means of the previous aeration of the mineral medium and by the presence of the headspace inside the reactor.

Mineral salts and microelements showed to be fundamental in bacterial activity for 1,2-DCA degradation; if not already present in the water to be treated, a proper amount of salts and elements must be supplied to the biological system.

Other chlorinated compounds fed to the system together with 1,2-DCA showed to have different influence on degradation of 1,2-DCA. In particular **2-CP showed to have no particular influence** on 1,2-DCA removal, while **1,2-DCB negatively affected 1,2-DCA removal**. A **slight inhibitive effect on 1,2-DCA removal was also caused by the presence of TCE** in the feeding, while **phenol showed to have a very strong inhibiting potential on 1,2-DCA degradation**.
In all cases, **negative effects of these substances were totally reversible**, and degradation of 1,2-DCA took place again with high removal rates when the inhibiting substances were not fed anymore to the system.

9.2 Degradation of 1,2-DCB

9.2.1 Degradation of 1,2-DCB fed as unique substrate

9.2.1.1 Batch tests

When 1,2-DCB was fed in the batch tests inoculated with fresh sludge, its degradation was not achieved neither in aerobic, nor in anaerobic conditions. The **scarce biodegradability** of 1,2-DCB made it **degradable only by a bacterial population which was already acclimated** to a chlorinated compound: actually when the compound was fed in batch inoculated with acclimated sludge, **it was degraded but only in aerobic conditions**; thus its degradation was carried on in this experimentation in aerobic conditions.
However, even though the degradation of 1,2-DCB was carried out by means of an acclimated bacterial population, degradation of the chlorinated compound was very slow: 1,2-DCB mean specific removal rate was just 0.002 $g_{1,2\text{-DCB}}/g_{VSS}\cdot d$, which means around twenty times lower than the specific removal rate measured in the first batch tests carried out with 1,2-DCA.

9.2.1.2 Degradation in MSBR

Starting from the 188th day of sludge acclimation to 1,2-DCA, the MSBR was inoculated with the acclimated sludge and fed with 1,2-DCB at 10 mg/l initial concentration. During the two cycles, lasting respectively 4 and 6 days, maximum specific removal rate for 1,2-DCB (calculated on a daily basis) increased first up to the value of 0.027 $g_{1,2\text{-DCB}}/g_{VSS}\cdot d$ and then up to 0.10 $g_{1,2\text{-DCB}}/g_{VSS}\cdot d$, with an average value of 0.06 $g_{1,2\text{-DCB}}/g_{VSS}\cdot d$. This increase in specific removal rate in comparison to the batch test gave evidence of **the importance of sludge acclimation** for the degradation of a chlorinated compound, and also of the **effectiveness of the SBR system for the acclimation of a bacterial population** to a particular substance, due to the cyclic and regular repetition of operating conditions and of organic load

9.2.2 Degradation of 1,2-DCB with other substrates

9.2.2.1 Degradation of 1,2-DCB with 1,2-DCA

Degradation in batch test

On the 200th day of acclimation, a batch test was performed, in which 1,2-DCB was fed together with 1,2-DCA. Like in the batch inoculated with acclimated sludge in which 1,2-DCB was fed alone, also in presence of 1,2-DCA the mean specific removal rate of 1,2-DCB observed was 0.002 $g_{1,2\text{-DCB}}/g_{VSS}\cdot d$.

Therefore it was hypothesized that **1,2-DCB removal was not affected by the presence of 1,2-DCA**. This hypothesis was confirmed by the following tests carried out in the other reactors in different periods of the experimentation.

Degradation in SBR

Starting from the 98th day of acclimation, SBR was fed with 1,2-DCA and 1,2-DCB in fed-batch modality, with 4 cycles each of them lasting 4-6 days. With this feeding, 1,2-DCB specific removal rate grew from the value of 0.003 $g_{1,2\text{-}DCB}/g_{VSS}\cdot d$ up to the value of 0.01 $g_{1,2\text{-}DCB}/g_{VSS}\cdot d$, which was observed after just four cycles. This evident improvement in 1,2-DCB removal in comparison to the batch tests gave confirmation of the **importance of the acclimation of sludge in degradation of a chlorinated compound**, and gave also evidence of the **effectiveness of the SBR system for the acclimation of a bacterial population** to a particular substance.

Degradation in MSBR

Starting from the 198th day of acclimation, the MSBR was fed with 1,2-DCA (50 mg/l) and 1,2-DCB (15 mg/l). Degradation was carried out in fed-batch modality, with three cycles lasting 3-8 days. Maximum specific removal rate for 1,2-DCB (measured on a daily basis) was 0.04 $g_{1,2\text{-}DCB}/g_{VSS}\cdot d$, which is in line with the values measured in the same period in the MSBR when the compound was fed alone in the system. Again this confirmed that **1,2-DCB removal is not affected by the contemporary presence of 1,2-DCA**.

9.2.2.2 Degradation of 1,2-DCB with other substrates

Degradation of 1,2-DCB with methanol in batch tests

In the initial batch tests performed both with fresh and with acclimated sludge, methanol was added in the batch in order to check if it could act as co-substrate in 1,2-DCB degradation and subsequently if it could improve its degradation. However, **neither in aerobic nor in anaerobic conditions methanol showed to have positive effects on 1,2-DCB removal**.

In another test performed on 1,2-DCA and 1,2-DCB fed together with methanol, it was observed that 1,2-DCB was degraded more slowly when methanol was present, and this effect was more evident as much as methanol concentration increased; finally degradation of 1,2-DCB stopped when methanol was added at 400 mg/l. Therefore it could be stated that methanol is not helpful in 1,2-DCB degradation, and can even be detrimental for its degradation if present at high concentration.

Degradation of 1,2-DCB with 1,2-DCA, 2-CP, TCE and phenol

When 1,2-DCB was fed in batch test with **2-CP and phenol**, it was degraded with a maximum specific removal rate (calculated on a daily basis) of 0.003 $g_{1,2\text{-}DCB}/g_{VSS}\cdot d$, which was in line with the values observed in batch tests when 1,2-DCB was fed alone. The same value was obtained when 1,2-DCB was fed in batch with **1,2-DCA, 2-CP and phenol**.

Therefore, it could be stated that the presence of 1,2-DCA, 2-CP and phenol did not affect removal of 1,2-DCB.

Starting from the 216th day of acclimation, 1,2-DCB was fed in the MSBR together with **2-CP and phenol**, with cycles lasting one week. Maximum specific removal rate for 1,2-DCB was 0.03 $g_{1,2\text{-}DCB}/g_{VSS}\cdot d$, which was in line with the values observed when 1,2-DCB was fed as unique substrate and degraded in the MSBR; therefore, it could be stated again that the contemporary presence of 2-CP and phenol did not affect negatively 1,2-DCB removal.

When a similar test was performed, in which **1,2-DCA** was fed together with **1,2-DCB, 2-CP and phenol**, maximum specific removal rate of 1,2-DCB was 0.05 $g_{1,2\text{-}DCB}/g_{VSS}\cdot d$, which was

in line with the value obtained when 1,2-DCB was fed alone in the MSBR; the same value was observed for 1,2-DCB when it was fed in the MSBR together with **1,2-DCA and 2-CP, in absence of phenol**. This result confirmed again that 1,2-DCB removal is not affected by the presence of 1,2-DCA, 2-CP and phenol.
When 1,2-DCB was fed in the MSBR together with **1,2-DCA and TCE**, it was degraded with a specific removal rate of 0.04 $g_{1,2\text{-}DCB}/g_{VSS}\cdot d$; a similar value (0.06 $g_{1,2\text{-}DCB}/g_{VSS}\cdot d$) was observed when 1,2-DCB was fed in the MSBR with **1,2-DCA, 2-CP and TCE**. These results were a further confirmation of the fact that **1,2-DCB removal is not affected by the presence of the other compounds studied in this experimentation**.

9.2.3 Conclusions on 1,2-DCB degradation

1,2-DCB was removed by the biomass selected in this experimentation **with lower specific removal rates in comparison to 1,2-DCA**, and showed to **have partially inhibiting effects on some other substrates fed to the system**.
Similarly to what observed for 1,2-DCA, one of the most important indications given by the experimentation on 1,2-DCB was the **importance of sludge acclimation on its degradative potential**: a sludge exposed for a longer time to 1,2-DCB was capable of degrading it with higher degradation rates.
Also for 1,2-DCB there was evidence of the **importance of the sequential cycles (both in SBR and in MSBR) modality for the acclimation of the sludge and for the selection of the proper bacterial populations deputed to the degradation of the chlorinated compounds**.
The other chlorinated compounds fed to the system together with 1,2-DCB showed to have no influence on degradation of 1,2-DCB: neither 1,2-DCA nor 2-CP, phenol or TCE caused a decrease of 1,2-DCB removal rate in comparison to when it was fed alone.

9.3 Degradation of 2-CP and of phenol in presence of other substrates

9.3.1 Degradation of 2-CP with other substrates

Batch tests
When 2-CP was fed in batch tests as unique source of carbon and energy, it was not degraded by fresh sludge neither in aerobic nor in anaerobic conditions; the same results were obtained with the acclimated sludge.
2-CP was not even degraded when 1,2-DCA and methanol were added as possible co-substrates.
However, the addition of phenol together with 2-CP stimulated the degradation of the chlorinated compound, which was degraded only when phenol was present; phenol was degraded much more rapidly than 2-CP, and when phenol was consumed, degradation of 2-CP stopped until phenol was fed again. With this kind of feeding, 2-CP was degraded in batch tests by fresh sludge with a maximum specific removal rate (calculated on a daily basis) of 0.001 $g_{2\text{-}CP}/g_{VSS}\cdot d$.
A similar trend was observed with the acclimated sludge: in this case maximum specific removal rate for 2-CP was 0.08 $g_{2\text{-}CP}/g_{VSS}\cdot d$.
These tests gave very important indications:

- again **the importance of sludge acclimation for degradation of chlorinated compounds**: when batch test was performed with the acclimated sludge, 2-CP maximum specific removal rate was much higher than when the test was performed with fresh sludge;
- **phenol was a fundamental co-metabolite for the activation of the enzyme deputed to the degradation of 2-CP**; neither methanol (an easily biodegradable substrate) nor 1,2-DCA (the primary growth substrate for the acclimated biomass) were effective at all in the activation of 2-CP degradation, while phenol allowed to achieve 2-CP degradation with a good removal rate.

The positive effect of phenol for degradation of 2-CP was not improved by the contemporary presence of 1,2-DCA: in a batch test performed right after the ones with 2-CP and phenol, the two substrates were fed with also 1,2-DCA. 2-CP maximum specific removal rate was 0.04 $g_{2\text{-}CP}/g_{VSS}\cdot d$, which was slightly below the value observed in absence of 1,2-DCA, but in line with it.
Similarly, in a batch test in which **2-CP** was fed with **1,2-DCB and phenol**, and in another in which 2-CP was fed with **1,2-DCA, 1,2-DCB and phenol**, 2-CP wad degraded with a maximum specific removal rate of 0.04 $g_{2\text{-}CP}/g_{VSS}\cdot d$, thus indicating that **the presence of 1,2-DCB did not influence 2-CP removal**.

Degradation in SBR
When degradation of 2-CP was carried out in the SBR together with 1,2-DCA and in absence of phenol, 2-CP (initial concentration 25 mg/l) was degraded with a maximum specific removal rate of 0.16 $g_{2\text{-}CP}/g_{VSS}\cdot d$. When 2-CP was fed with an initial concentration of 50 mg/l together with 1,2-DCA and in absence of phenol, its maximum specific removal rate was 0.27 $g_{2\text{-}CP}/g_{VSS}\cdot d$.
However, when phenol at 50 mg/l was added in the system together with 2-CP and 1,2-DCA, maximum specific removal rate of 2-CP was lowered down to 0.08 $g_{2\text{-}CP}/g_{VSS}\cdot d$. After this test, the initial concentration of phenol was lowered down to 25 mg/l, but the effect of such a long exposition to the compound caused the further decrease of 2-CP maximum specific removal rate, down to the value of 0.04 $g_{2\text{-}CP}/g_{VSS}\cdot d$.

Degradation in MSBR
Degradation of **2-CP with phenol** in the MSBR was carried out with cycles of 3 days; maximum specific removal rate observed for 2-CP was 0.08 $g_{2\text{-}CP}/g_{VSS}\cdot d$, which was in line to what observed in the SBR when 2-CP was fed with phenol, and lower than the one observed in the SBR when 2-CP was fed without phenol.

When **2-CP** was degraded in cycles lasting three days together with **phenol and 1,2-DCA**, its maximum specific removal rate decreased down to the value of 0.03 $g_{2\text{-}CP}/g_{VSS}\cdot d$, which was lower than the one observed in the SBR fed with 1,2-DCA and without phenol.

When **2-CP** was degraded in cycles lasting one week with **phenol and 1,2-DCB** or with **phenol, 1,2-DCB and 1,2-DCA**, its maximum specific removal rate was 0.01 $g_{2\text{-}CP}/g_{VSS}\cdot d$, which was lower that the value observed in the SBR when 2-CP was fed with and without phenol.

When **2-CP** was degraded in daily cycles with **1,2-DCA and 1,2-DCB in absence of phenol**, 2-CP maximum specific removal rate reached the value of 0.26 $g_{2\text{-}CP}/g_{VSS}\cdot d$, which was much

higher than the value observed in MSBR when the three chlorinated compounds were degraded with phenol.

2-CP was fed in the MSBR **with 1,2-DCA in absence of phenol** in two different periods of the experimentation: the first started on the 211th day, while the second started on the 407th. In both periods, 2-CP and 1,2-DCA were fed with daily cycles.
During the first period, 2-CP maximum specific removal rate achieved an average value of 0.05 $g_{2\text{-}CP}/g_{VSS}\cdot d$; this value, which is quite low, is probably due to the fact that previously the system was fed with phenol for around 2 months, and this could have caused a partial inhibition of the degradative capability of the sludge towards the other compounds fed.
At the end of the second period in which 1,2-DCA was fed with 2-CP, the maximum specific removal rate for 2-CP increased up to the value of 0.20 $g_{2\text{-}CP}/g_{VSS}\cdot d$, which was in line with the value observed when 2-CP was degraded together with 1,2-DCA and 1,2-DCB in absence of phenol. **This result confirmed again the importance of the acclimation role in the degradation of the chlorinated compounds studied in this experimentation**. Besides, it confirmed that **feeding with daily cycles leads to a more effective degradation of the compounds fed**, because it allows the daily repetition of the same operating conditions and organic loads, which is fundamental for the acclimation of a bacterial population to a certain compound.

Since the 538th day, degradation of **1,2-DCA and 2-CP** was carried out with **two cycles per day**; in a *first phase*, 2-CP was fed with an initial concentration of 15 mg/l, while in a *second phase* it was fed at an initial concentration of 10 mg/l.
At the end of the *first phase*, lasting around one month, there was no 2-CP degradation; **at such a concentration, and with such a high daily load to the system, 2-CP was not degraded and accumulated**. At the end of the *second phase*, due to the longer time of exposition to this kind of feeding, and to the lower initial concentration of 2-CP, its maximum specific removal rate was 0.09 $g_{2\text{-}CP}/g_{VSS}\cdot d$. This result showed again the **importance of acclimation** for degradation of the chlorinated compound, but revealed also a certain **difficulty of the acclimated biomass in the degradation of 2-CP when it was fed with a high daily organic load**.

When **2-CP** was fed with **1,2-DCA, 1,2-DCB and TCE** with daily cycles, around the 340th day, 2-CP maximum specific removal rate was 0.19 $g_{2\text{-}CP}/g_{VSS}\cdot d$, a value which is slightly lower than the 0.26 $g_{2\text{-}CP}/g_{VSS}\cdot d$ observed when 2-CP was fed in daily cycles with 1,2-DCA and 1,2-DCB, but is in line with the value observed when 2-CP was fed with only 1,2-DCA in the MSBR. Therefore, **an unimportant effect of TCE on 2-CP was noticed**.

9.3.1.4 Conclusions on the results obtained with 2-CP

The results obtained, together with the ones coming from the batch tests, gave an important indication on the role played by **phenol as co-metabolite of 2-CP**: **phenol was helpful in the enzymatic activation of the biomass which was not capable of degrading 2-CP**, but it had a **negative effect on the degradation of chlorinated compounds in a system like the one equipped in this experimentation**. Actually, the high biodegradability of phenol caused the **rapid consumption of all the oxygen present in the reactor**, thus **preventing the complete and effective oxidation of the other compounds present in the system**.
This effect was not observed in the batch tests, because there a large headspace was present in comparison to the bulk liquid, and this ensured the degradation of all the compounds present.
Therefore it can be concluded that **phenol is effective for the activation of 2-CP removal by**

a non-acclimated sludge, but if the sludge is already capable of degrading 2-CP, its removal can be achieved with high efficiency in a system similar to the one equipped in this experimentation, without the addition of phenol. This brings also the advantage of a higher simplicity of management, of reagents costs reduction, and also to the elimination of the introduction of a possible pollution source.
The other compounds fed to the system in this experimentation showed no important effects on 2-CP removal, with the exception of TCE which slightly lowered 2-CP maximum specific removal rate.
Besides, the **importance of sludge acclimation to the compounds for their degradation capability** was confirmed again, and finally, the importance of **feeding with daily cycles rather than cycles lasting some days**, because it leads to a more effective degradation of the compounds fed.
In general, 2-CP was removed with **lower specific removal rates in comparison to 1,2-DCA**.

9.4 Degradation of TCE

9.4.1 Degradation of TCE with other substrates

In this experimentation, TCE was degraded only in the MSBR operated with daily cycles.

9.4.1.1 Degradation in MSBR with 1,2-DCA, 1,2-DCB and 2-CP

Starting from the 305th day of acclimation, TCE was fed with 1,2-DCA, with 1,2-DCA and 1,2-DCB, and with 1,2-DCA, 1,2-DCB and 2-CP in the MSBR; at the end of each phase, TCE maximum specific removal rate was always in the range of 0.02-0.05 $g_{TCE}/g_{VSS}\cdot d$; this showed that **TCE removal was not affected by the presence of any other compound studied in this experimentation**.
TCE was studied only in these three tests, because due to the fact that its molecule has three chlorinated substitutes, it was supposed that it would be hardly degraded by the biomass selected during the experimentation under aerobic conditions. Besides, due to the high volatile nature of the compound, it was not sure that the **decrease of its concentration observed in the MSBR was due to its real degradation or more to its volatilization out of the reactor or to the adsorption on the sludge**.

9.5 Summary of the specific removal rates in the different phases

All the maximum and mean specific removal rates of the substances degraded in the different phases of the experimentation are summed up in Table 9.3.

Table 9.3. Maximum and mean specific removal rates of all the substances degraded during the experimentation.(*: maximum specific removal rates calculated on a daily basis).

Feeding	Paragraph	Mean specific removal rate 1,2-DCA [$g_{1,2\text{-}DCA}/g_{VSS}\cdot d$]	Max specific removal rate 1,2-DCA [$g_{1,2\text{-}DCA}/g_{VSS}\cdot d$]	Mean specific removal rate 1,2-DCB [$g_{1,2\text{-}DCB}/g_{VSS}\cdot d$]	Max specific removal rates 1,2-DCB [$g_{1,2\text{-}DCB}/g_{VSS}\cdot d$]	Mean specific removal rate 2-CP [$g_{2\text{-}CP}/g_{VSS}\cdot d$]	Max specific removal rate 2-CP [$g_{2\text{-}CP}/g_{VSS}\cdot d$]	Mean specific removal rate TCE [$g_{TCE}/g_{VSS}\cdot d$]	Max specific removal rate TCE [$g_{TCE}/g_{VSS}\cdot d$]
1,2-DCA anaerobic batch test fresh sludge	8.1.1.1	0							
1,2-DCA aerobic batch test fresh sludge	8.1.1.1	0.04							
1,2-DCB anaerobic batch test fresh sludge	8.1.2.1			0					
1,2-DCB aerobic batch test fresh sludge	8.1.2.1			0					
1,2-DCB batch test with acclimated sludge	8.1.2.2			0.02					
2-CP anaerobic batch test with fresh sludge	8.1.3.1					0			
2-CP aerobic batch test with fresh sludge	8.1.3.1						0.0013*		
2-CP anaerobic batch test with acclimated sludge	8.1.3.2					0			
2-CP and phenol aerobic batch test with acclimated sludge	8.1.3.2					0.02	0.08*		
2-CP, 1,2-DCA and phenol aerobic batch test with acclimated sludge	8.1.3.2		0.06*				0.04*		
2-CP, 1,2-DCB and phenol aerobic batch test with acclimated sludge	8.1.3.2				0.003*		0.04*		
2-CP, 1,2-DCB, 1,2-DCA and phenol aerobic batch test with acclimated sludge	8.1.3.2		0.03*		0.003*		0.04		
1,2-DCA in SBR; fed batch modality	8.2.1.1	0.11							
1,2-DCB in SBR; fed batch modality	8.2.1.2	0.11		0.01					
1,2-DCA in SBR; daily cycles	8.2.2.1	0.19	0.48						
1,2-DCA (50 mg/l) and 2-CP (25 mg/l) in SBR with daily cycles	8.2.2.2	0.28	1.05			0.07	0.16		

Feeding	**Paragraph**	**Mean specific removal rate 1,2-DCA [$g_{1,2\text{-}DCA}/g_{VSS}\cdot d$]**	**Max specific removal rate 1,2-DCA [$g_{1,2\text{-}DCA}/g_{VSS}\cdot d$]**	**Mean specific removal rate 1,2-DCB [$g_{1,2\text{-}DCB}/g_{VSS}\cdot d$]**	**Max specific removal rates 1,2-DCB [$g_{1,2\text{-}DCB}/g_{VSS}\cdot d$]**	**Mean specific removal rate 2-CP [$g_{2\text{-}CP}/g_{VSS}\cdot d$]**	**Max specific removal rate 2-CP [$g_{2\text{-}CP}/g_{VSS}\cdot d$]**	**Mean specific removal rate TCE [$g_{TCE}/g_{VSS}\cdot d$]**	**Max specific removal rate TCE [$g_{TCE}/g_{VSS}\cdot d$]**
1,2-DCA (50 mg/l) and 2-CP (50 mg/l) in SBR with daily cycles	8.2.2.2	0.24	0.61			0.16	0.27		
1,2-DCA (50 mg/l), phenol (50 mg/l) and 2-CP (50 mg/l) in SBR with daily cycles	8.2.2.2	0.12	0.27			0.12	0.08		
1,2-DCA (50 mg/l), phenol (25 mg/l) and 2-CP (50 mg/l) in SBR with daily cycles	8.2.2.2	0.07	0.27			0.11	0.05		
Degradation of 1,2-DCA in SBR at 30°C with mineral medium on the 606th day	8.2.2.3	0.14	0.40						
Degradation of 1,2-DCA in SBR at 20°C with aerated mineral medium on the 635th day	8.2.2.3	0.10	0.22						
Degradation of 1,2-DCA in SBR at 20°C with non aerated mineral medium on the 650th day	8.2.2.3	0.10	0.32						
Degradation of 1,2-DCA in SBR at 20°C with non aerated tap water on the 675th day	8.2.2.3	0.06	0.01						
Degradation of 1,2-DCA in MSBR with daily cycles on the 180th day	8.3.1.1	0.16	0.70						
Degradation of 1,2-DCA in MSBR with daily cycles on the 275th day	8.3.1.1	0.24	1.06						
Degradation of 1,2-DCA in MSBR with daily cycles on the 438th day	8.3.1.1	0.24	1.36						
Degradation of 1,2-DCA in MSBR with two cycles/d on the 99th day	8.3.1.1	0.51	1.11						
Degradation of 1,2-DCA in MSBR with two cycles/d on the 438th day	8.3.1.1	0.79	1.83						

Feeding	**Paragraph**	**Mean specific removal rate 1,2-DCA [$g_{1,2\text{-}DCA}/g_{VSS}\cdot d$]**	**Max specific removal rate 1,2-DCA [$g_{1,2\text{-}DCA}/g_{VSS}\cdot d$]**	**Mean specific removal rate 1,2-DCB [$g_{1,2\text{-}DCB}/g_{VSS}\cdot d$]**	**Max specific removal rates 1,2-DCB [$g_{1,2\text{-}DCB}/g_{VSS}\cdot d$]**	**Mean specific removal rate 2-CP [$g_{2\text{-}CP}/g_{VSS}\cdot d$]**	**Max specific removal rate 2-CP [$g_{2\text{-}CP}/g_{VSS}\cdot d$]**	**Mean specific removal rate TCE [$g_{TCE}/g_{VSS}\cdot d$]**	**Max specific removal rate TCE [$g_{TCE}/g_{VSS}\cdot d$]**
Degradation of 1,2-DCB in MSBR with daily cycles on the 188th day	8.3.1.2			0.02	0.06				
Degradation of 1,2-DCA and 1,2-DCB in MSBR with daily cycles on the 198th day	8.3.1.3	0.43	0.18	0.02	0.04				
Degradation of 2-CP with phenol on the 200th day	8.3.2.1						0*		
Degradation of 2-CP with 1,2-DCA and phenol with daily cycles on the 203th day	8.3.2.2		0.03*				0.03*		
Degradation of 2-CP with 1,2-DCA with daily cycles on the 211th day	8.3.2.3.	0.10	0.26			0.04	0.04		
Degradation of 2-CP with 1,2-DCA with daily cycles on the 407th day	8.3.2.3.	0.37	1.05			0.14	0.20		
Degradation of 2-CP (15 mg/l) with 1,2-DCA with 2 cycles/d on the 538th day	8.3.2.3.	0.21	1.03			0	0		
Degradation of 2-CP (10 mg/l) with 1,2-DCA with 2 cycles/d on the 568th day	8.3.2.3.	0.21	1.34			0.03	0.09		
Degradation of 2-CP with 1,2-DCB and phenol with daily cycles on the 216th day	8.3.2.4			0.01	0.02*	0.02	0.03*		
Degradation of 2-CP with 1,2-DCA, 1,2-DCB and phenol with daily cycles on the 231st day	8.3.2.5	0.07**	0.03*	0.05**	0.02*	0.05**	0.02		
Degradation of 2-CP with 1,2-DCA and 1,2-DCB with daily cycles on the 407st day	8.3.2.6	0.26	0.61	0.02	0.10	0.11	0.26		
Degradation of TCE with 1,2-DCA on the 305th day	8.3.3.1	0.16	0.48					0.01	0.02
Degradation of TCE with 1,2-DCA and 1,2-DCB on the 318th day	8.3.3.2	0.17	0.49	0.03	0.04			0.03	0.04

Feeding	**Paragraph**	**Mean specific removal rate 1,2-DCA [$g_{1,2\text{-}DCA}/g_{VSS}\cdot d$]**	**Max specific removal rate 1,2-DCA [$g_{1,2\text{-}DCA}/g_{VSS}\cdot d$]**	**Mean specific removal rate 1,2-DCB [$g_{1,2\text{-}DCB}/g_{VSS}\cdot d$]**	**Max specific removal rates 1,2-DCB [$g_{1,2\text{-}DCB}/g_{VSS}\cdot d$]**	**Mean specific removal rate 2-CP [$g_{2\text{-}CP}/g_{VSS}\cdot d$]**	**Max specific removal rate 2-CP [$g_{2\text{-}CP}/g_{VSS}\cdot d$]**	**Mean specific removal rate TCE [$g_{TCE}/g_{VSS}\cdot d$]**	**Max specific removal rate TCE [$g_{TCE}/g_{VSS}\cdot d$]**
Degradation of TCE with 1,2-DCA, 2-CP and 1,2-DCB on the 318th day	8.3.3.3	0.24	0.52	0.01	0.09	0.03	0.19	0.05	0.05

9.6 Comparison with the degradation rates obtained in other studies

A comparison with the chlorinated compounds degradation efficiencies observed in other experimentations can be done only in terms of removal rate expressed as amounts of chlorinated compounds degraded per litre of mixed liquor per day. Actually, the extrapolation of specific removal rates (similarly to what done in this experimentation) from the past studies reported in the literature is not possible due to the fact that often degradation of the chlorinated compounds has been achieved by means of fixed bacterial population growth on solid supports, that did not allow to calculate the concentration of volatile suspended solids and subsequently the specific removal rates. Besides, in many studies the amount of biomass present in the biological system was expressed as amount of proteins instead of suspended solids.

Therefore, the degradation rates obtained in this experimentation have been converted in terms of removal measured in $mg_{cc}/(l \cdot d)$ consumed, and these values will be compared with the ones achieved in past studies. The best removal rates calculated for the various substances in this experimentation as compared to those obtained in past studies are reported in Table 9.4.

Table 9.4. Maximum removal rates in the present and in other studies.

Study	**Max removal rate 1,2-DCA [$mg_{1,2\text{-}DCA}/l \cdot d$]**	**Max removal rate 1,2-DCB [$mg_{1,2\text{-}DCB}/l \cdot d$]**	**Max removal rate 2-CP [$mg_{2\text{-}CP}/l \cdot d$]**	**Max removal rate TCE [$mg_{TCE}/l \cdot d$]**
Present experimentation	618.5	29.6	82.3	8.5
Janssen et al. (1984)	2851	-	-	-
Hage and Hartmans (1999)	1200	-	-	-
Freitas Dos Santos and Livingston (1995)	10800	-	-	-
Herbst and Wiesmann (1996)	14400	-	-	-
Dyer et al. (2003)	12.2	-	-	-
De Wildeman et al. (2001)	5	-	-	-
Seignez et al. (2001)	-	17	-	-
Monferran et al. (2005)	-	2.7	-	-
Lu et al. (2002)	-	-	0.7	-
Farrel and Quilty (2002)	-	-	122.9	-
Basu et al. (1996)	-	-	1.8	-
Sun et al. (2000)	-	-	51.2	-
Bae et al. (2002)	-	-	1.6	-
Kuo et al. (1996)	-	-	1.6	-
Chang and Fane (2001)	-	-	30	
Chang et al. (2004)	-	-	2.5	-
Majumder and Gupta (2007)	-	-	12.5	-
Frascari et al. (2006)	-	-	-	0.3
Han et al. (2007)	-	-	-	1.9
Volcik et al. (2005)	-	-	-	0.6
Johnston et al. (1996)	-	-	-	0.1

As it can be observed from the table, **1,2-DCA** removal rate observed in this experimentation is quite low if compared to the studies performed by Freitas Dos Santos and Livingston (1995) and by Herbst and Wiesmann (1996); however, in these two experimentations degradation of 1,2-DCA was achieved by means of selection of a specific bacterial strain, while in this experimentation, a mixed bacterial population was acclimated. Moreover the operation of the system with two cycles per day demonstrated that the system could easily treat a higher 1,2-DCA load.
The selected bacterial population in this experimentation was capable of degrading **1,2-DCB** with a removal rate higher than the other studies found in literature.
Also for degradation of **2-CP** the bacterial population selected in this experimentation showed a capability of degrading the chlorinated compound which was higher than most of the previous studies; only the study performed by Farrel and Quilty (2002) showed a higher 2-CP removal rate, which was however in the same order of magnitude.
Similarly, removal rate for **TCE** observed in this experimentation was higher than the ones observed in previous studies; however, this result is not noteworthy because it was not sure if TCE was really degraded or adsorbed on the sludge.

9.7 Chlorides production during compounds degradation

Measurements of chlorides were done in order to check if complete *mineralization* of the chlorinated compounds was achieved. If the chlorinated compound is mineralized, only H_2O, CO_2 and chlorides should be found as final by-products. According to the stoichiometric calculations, complete degradation of one mole of 1,2-DCA (as the complete degradation of one mole of 1,2-DCB) causes the production of two moles of chlorides, while the complete degradation of 2-CP causes the production of one mole of chlorides.
In general it was observed that when 1,2-DCA and 1,2-DCB were fed separately as single substrates, a stoichometric amount of chlorides was produced, while the simultaneous feeding of 2-CP, phenol or TCE did not allow the production of the stoichiometric amount of chlorides.
When **1,2-DCA** was fed as only substrate, its mineralization was generally observed. In particular, when the variation of the operating parameters was investigated, **mineralization** of 1,2-DCA fed as only substrate was observed when the reactor was fed with **aerated mineral medium**: the ratio between moles of chlorides produced in the reaction and moles of 1,2-DCA oxidized was 2. When the reactor was fed with **non aerated mineral medium**, the ratio between chlorides and 1,2-DCA lowered down to the value of 1.4, thus indicating that, even if the chlorinated compound was totally degraded, it was not completely mineralized, but probably formed some other by-products. When the reactor was fed with **non aerated tap-water**, the degradation of 1,2-DCA stopped and subsequently the ratio between chlorides and 1,2-DCA was close to the zero value.
When degradation of 1,2-DCA was operated with **two cycles per day**, the ratio between moles of chlorides found as by-products and moles of chlorinated compounds degraded was around the value of 2, thus indicating the complete mineralization of the chlorinated compound also when fed with two cycles per day.
When the MSBR was fed with **1,2-DCB**, even if the chlorinated compound was not completely degraded, the ratio between moles of chlorides produced and the moles of 1,2-DCB degraded was around the value of 1.8, thus indicating the **almost complete mineralization** of the chlorinated substance.

When 2-CP and the other compounds were fed to the MSBR, it was considered that one mole of 2-CP, if totally degraded, produces one mole of chlorides among the final by-products. Thus, the theoretical number of chlorides moles that should be produced during the degradation is given by the number of 2-CP moles, plus the double amount of 1,2-DCA moles. Therefore, the ratio used to check the mineralization of the chlorinated compounds was calculated between the number of chlorides moles that should have been theoretically produced and the number of chlorides moles really found among the final by-products. In case of total mineralization of the chlorinated compound, this ratio has the value of 1.
When **2-CP and 1,2-DCA** (with or without 1,2-DCB) were fed to the MSBR, the ratio between theoretical chlorides moles and chlorides moles produced was around the value of 2 or slightly higher, indicating that complete mineralization of the compounds was not achieved, even if the substrates were almost always completely degraded.
When **1,2-DCA** was fed (with or without **1,2-DCB**) together with **TCE**, the ratio between the theoretical chlorides moles and chlorides moles really produced set around the value of slightly more than 1, which indicates that in presence of TCE, 1,2-DCA is mineralized in the same way as it is in absence of trichloroethylene.

Conclusions

The experimentation carried out in this study focused on the use of an innovative technology (the MSBR) for the bioremediation of a synthetic groundwater polluted by four chlorinated compounds (1,2-dichloroethane, 1,2-dichlorobenzene, 2-chlorophenol and trichloroethylene).
Starting from a non acclimated fresh sludge, the selection of specific bacterial strains capable of degrading the four chlorinated compounds was achieved, by operating the MSBR with sequential cycles.
When fed with the chlorinated compounds studied, the fresh sludge showed no degradative capability, with the only exception of 1,2-dichloroethane which was degraded with progressively increasing rates.
Therefore, the acclimation of the fresh sludge started with 1,2-dichloroethane, which became the **growth and preferential substrate** for the sludge acclimated in the experimentation.
Degradation of 1,2-dichloroethane improved and its specific removal rate increased during the whole experimentation: maximum specific removal rates increased progressively in time, from the value of 0.48 $g_{1,2\text{-}DCA}/(d \cdot g_{VSS})$ observed on the 90^{th} day of acclimation in the SBR up to the value of 1.36 $g_{1,2\text{-}DCA}/(d \cdot g_{VSS})$ achieved on the 498^{th} day in the MSBR operated in daily cycles.
This trend, which was observed also for 1,2-dichlorobenzene and for 2-chlorophenol, showed that the acclimation of the sludge improved with the development of the experimentation, thus underlining one of the most important points that were proved in this experimentation: the **key role of the acclimation for the degradation of the chlorinated compounds**.
When the system was fed with 1,2-dichloroethane with two cycles per day, the system showed to capable of **completely degrading the substance within 12 hours**, thus indicating an interesting potential of remediation of polluted water fed with high load to the biological system. Besides, **feeding the system with a higher daily load of the chlorinated compound improved the degradation capability of the system**, and an increase of both maximum and mean specific removal rates was observed.

The system showed to operate degradation with higher efficiency when it was hold at 30°C rather than at 20°C; however, degradation rates showed to be good also at 20°C. Degradation rates were good also when the system was fed with non aerated mineral medium, which showed that the system equipped in this experimentation was suitable to ensure the necessary amount of oxygen for the degradation of the substances fed at the fixed concentrations. According to these results it can be hypothesized that **the system might be cost-effective when implemented at a larger scale, because it would need neither aeration nor heating systems**.
Degradation of the chlorinated compounds was interrupted when the system was fed with tap water instead of mineral medium; however, this can be due to the scarceness of mineral salts in tap water, whereas groundwaters are often rich of these elements.

The acclimated biomass showed to undergo a **negative effect** on 1,2-dichloroethane degradation when the compound was fed together with **1,2-dichlorobenzene**, while effects of **2-chlorophenol** on 1,2-dichloroethane degradation were observed only **when 2-chlorophenol was fed at high daily loads**.
Phenol showed a **strongly negative** effect on 1,2-dichloroethane degradation, which was due to the fact that this substrate (which is more easily biodegradable by the biomass) is oxidized very rapidly, thus **consuming the oxygen present** in the system which did not allow the oxidation of the other chlorinated compounds, less degradable than phenol.

A negative effect on 1,2-dichloroethane degradation, even if less strong than that of phenol, was observed when trichloroethylene was fed to the system.
However, for all the substances causing inhibition of 1,2-dichloroethane degradation, when their feeding to the system was interrupted and only 1,2-dichloroethane was supplied again the **complete recover of the biomass was observed**, thus demonstrating the **completely reversible effects of the inhibiting substances**.

1,2-dichlorobenzene was degraded during all the experimentation with lower removal rates than 1,2-dichloroethane, and showed to have **negative effects on both 1,2dichloroethane and 2-chlorophenol degradation**. On the other side, 1,2-dichlorbenzene **was not affected by the presence of both substrates**, neither it was affected by the presence of **phenol** and of **trichloroethylene**. Also for 1,2-dichlorobenzene, the **importance of the acclimation was evident**, and its maximum specific removal rate increased during the experimentation, when a higher sludge acclimation level was achieved.

Degradation of **2-chlorophenol** by fresh or acclimated sludge was not observed when the substrate was fed alone; for achieving its degradation, initially the sludge (both the fresh and the acclimated one), had to be fed with **phenol** as co-substrate. It was then supposed that phenol acted as a sort of *activator* of the enzyme responsible for 2-chlorophenol degradation.
In the presence of phenol as co-substrate, a good 2-chlorophenol removal was achieved; however when phenol was not fed anymore, the sludge showed to be able to degrade 2-chlorophenol with progressively increasing removal rates, which became very high at the end of the experimentation. Again, the importance of the **sludge acclimation for the degradation of a chlorinated compound was evident**.
Phenol showed to be not only **unnecessary for 2-chlorophenol** degradation, but it also showed to be **detrimental for the degradation of the other compound**. Actually, when it was fed again in presence of 2-chlorophenol, it caused the **rapid consumption of the oxygen** present in the system, due to its higher biodegradability, and subsequently caused the initially partial and finally total **inhibition of 2-chlorophenol** degradation.
Therefore it can be concluded that **phenol is effective for the activation of 2-CP removal by a non-acclimated sludge, but if the sludge is already capable of degrading 2-CP, its removal can be achieved with high efficiency in a system similar to the one designed in this experimentation, without the addition of phenol**. This brings also the advantage of a higher simplicity of management, of reagents costs reduction, and also to the elimination of the introduction of a possible pollution source, which favour the possible implementation of the experimented system at a larger scale.
When it was fed with two cycles per day, 2-chlorophenol was not completely degraded: the system showed to suffer for a high daily load of the compound.

Trichloroethylene was fed to the system in the last period of the experimentation, with the main aim of **verifying if its presence could interfere** with the removal of the other compounds; actually it was not expected to be degraded by the selected biomass, which was acclimated to compounds with maximum two chlorinated substitutes. Trichloroethylene showed to **partially inhibit degradation of the three other compounds**, but with a completely reversible effect; when it was not fed anymore to the system, degradation of the other compounds took place again with the usual removal efficiency.

The removal rates observed in this experimentation for the four chlorinated compounds studied were comparable to the ones obtained in past studies with mixed bacterial population.

1,2-dichloroethane removal rate observed in this experimentation was lower than the ones observed in some past studies in which degradation of the compound was achieved by means of pure cultures, but it was higher than other studies in which mixed bacterial populations were used.
The bacterial population selected in this experimentation was capable of degrading 1,2-dichlorobenzene with a removal rate higher than the other studies found in the literature.
Also for degradation of 2-chlorophenol the bacterial population selected in this experimentation showed a degradative capability that was higher than most of the previous studies; only one past study showed a higher 2-CP removal rate, which was however at the same order of magnitude.
Similarly, removal rate for trichloroethylene observed in this experimentation was higher than the ones observed in previous studies; however, this result is not noteworthy because it was not sure if TCE was really degraded or adsorbed on the sludge.

In conclusion, the system equipped in this experimentation showed to be effective in the removal of the chlorinated compounds studied; in the outlook of its possible implementation at a larger scale, the factors that imply the highest energy consumption (aeration of the mineral medium and heating) showed to be not necessary for achieving good removal of the polluting compounds, thus suggesting the possibility of reasonable management costs, comparable to the ones of a traditional MSBR.
The advantages obtained with a MSBR in comparison to a traditional SBR are the highest quality of the effluent in terms of solids content, which are totally absent in an effluent coming from a MSBR; on the other side, the highest costs deriving from the suction charge applied for filtration in a MSBR must be taken into account when projecting a system for remediation of a polluted water.

The strength of the system designed in this experimentation can be summed up into two main points:

1) differing from all the other systems equipped in the literature, this is the only system in which the **complete sealing of the reactor is achieved**; this ensures the complete elimination of the possibility of loss of chlorinated compounds by volatilization. In all the other systems found in previous studies, a even minimum volatilization of the compounds was counted; anyway, in this experimentation it was assumed that due to the high volatilization of the chlorinated compounds, this effect should be avoided as much as possible, especially when more than one compound is degraded in the system;

2) subsequently, in order to keep aerobic conditions inside the reactor, a **new system for oxygen supply was designed**: the aeration of the mineral medium before its feeding into the reactor, coupled to the headspace which was always present inside the reactor. This ensured the necessary amount of oxygen in all the experimentation for the degradation of the chlorinated compounds at the given concentrations (which are quite higher than the ones usually found in real polluted groundwater). Some problems of lack of oxygen were only observed when phenol, a more easily biodegradable substrate, was fed to the system; in this case oxygen was rapidly degraded and was not sufficient anymore for the degradation of the other compounds. However, since phenol showed to

be essential only in a first and short phase of acclimation of the sludge to 2-chlorophenol, this point was not a strong problem for the management of the system.

Appendix
The Experimentation in Berlin

In order to improve the know-how concerning Membrane Bioreactors management, six months of this study were spent at the Department of Chemical Engineering of the Technical University of Berlin (Germany). In this study, a cascade MBR operating nutrients removal from a synthetic municipal wastewater was studied. Recirculation sludge flow rates and metabolic conditions alternations were varied, and the subsequent effects on nutrients removal efficiency, with particular focus on phosphorus removal, were studied.

1 Phosphorus removal in different sludge recirculation configurations of a membrane bioreactor

The use of membrane bioreactors for enhanced nutrient removal requires the implementation of specific recirculation schemes for MLSS and nutrients distribution. In this study, a cascaded membrane bioreactor where phosphorus and nitrogen removal were accomplished by enhanced biological phosphorus removal and post-denitrification, respectively, was operated. Starting from a patented flow-scheme of the plant, four different configurations were set up by changing the internal sludge recirculation flows. The effects of the recirculation flows changes on COD and nutrients removal were investigated for the different configurations, with particular focus on phosphorus removal rate in anaerobic conditions and phosphorus uptake rate in aerobic conditions. The improvements in aeration costs saving and in the design simplicity were also evaluated for each configuration. In all four configurations, COD and nutrient removal efficiencies were stable, and, in comparison with the original set-up, higher phosphorus uptake rates and phosphorus release rates were observed in each modified configuration. One particular configuration was singled out as the most suited in terms of biomass distribution homogeneity and of aeration costs saving. In Table 1 the nomenclature used in this chapter is explained.

Table 1. Nomenclature used in this chapter.

AE	Aerobic
AN	Anaerobic
AN_0	Mixing basin
ATP	Adenosintriphosphate
AX	Anoxic
COD	Chemical oxygen demand (mg_{O2}/l)
DNR	Denitrification rate ($mg_{NO3\text{-}N}/g_{VSS}\cdot h$)
HRT	Hydraulic retention time (h)
MF	Membrane filtration chamber
$NADH_2$	Nicotinamide adenine dinucleotide
NR	Nitrification rate ($mg_{NH4\text{-}N}/g_{VSS}\cdot h$)
Nt	Total nitrogen concentration (mg/l)
PHA	Polyhydroxyalcanoates
PHB	Polyhydroxybutirate
PHV	Polyhydroxyvalerate
PRR	Phosphorus release rate ($mg_{PO4\text{-}P}/g_{VSS}\cdot h$)
Pt	Total phosphorus concentration (mg/l)
PUR	Phosphorus uptake rate ($mg_{PO4\text{-}P}/g_{VSS}\cdot h$)
R1	Recirculation flow no. 1
R2	Recirculation flow no.2

to be continued

follows from the previous page

R3	Recirculation flow no. 3
REC1	Recirculation flows configuration no. 1
REC2	Recirculation flows configuration no. 2
REC3	Recirculation flows configuration no. 3
REC4	Recirculation flows configuration no. 4
VFA	Volatile fatty acids

2 The topic of the study: a cascaded MBR for nutrients removal

Phosphorus and nitrogen removal from municipal and industrial wastewaters is one of the most important points in the control of eutrophication of receiving water bodies. Nitrogen removal is achieved in wastewater treatment plants through aerobic nitrification coupled to pre- or post-denitrification. The main advantage given by the operation with pre-denitrification is the smaller amount of oxygen to be supplied in the aerobic zone of the plant, because part of the organic substrate is already consumed by denitrifying heterotrophic microorganisms in the anoxic phase. Effluent quality, however, is limited by the recirculation ratio. On the other hand, post-denitrification often requires an external dosage of a carbon source in the anoxic zone, because the carbon present in the wastewater is consumed in the aerobic zone and subsequently is not enough for the denitrifying biomass metabolism. High nitrogen removal efficiencies obtained with post-denitrification make this treatment an attractive and often used alternative when stringent consents of nitrogen in the effluent are to be met (Kraume et al., 2005).
Phosphorus removal is achieved through physical processes (phosphorus precipitation), or through the biological process known as enhanced biological phosphorus removal (EBPR). EBPR is accomplished by a specific group of organisms, the Phosphorus Accumulating Organisms (PAOs), which accumulate phosphorus in higher amounts than required for cellular growth. Alternation of anaerobic with aerobic (or anoxic) conditions is required for proper PAO metabolism. As described in many studies (Arun et al., 1988; Kuba et al., 1996; Mino et al., 1995; Pereira et al., 1996; Petersen et al., 1998; Pijuan et al., 2004; Pijuan et al., 2005; Smolders et al., 1994a; Smolders et al, 1994b), in anaerobic conditions PAO take up organic substrate (preferably VFA) and synthesize PHA, which are constituted mostly by PHB and PHV. The energy for this process is given mainly by the hydrolysis of intracellularly stored polyphosphate (Poly-P) and partially by the degradation of internally stored glycogen, whose degradation also supplies the reducing equivalents for the synthesis of PHA. The hydrolysis of Poly-P in the anaerobic phase results in an increase of the orthophosphate concentration in the solution. In the following aerobic (or anoxic) zone, PAOs use the internally stored PHA to obtain the energy necessary for replenishing the intracellular Poly-P, for recovering the glycogen pool and for growth. Therefore, in aerobic or anoxic conditions, orthophosphates are taken up by PAOs in higher amounts in comparison with their growth requirements, and by removing the excess sludge from the plant a good removal of phosphorus from influent wastewater is achieved. According to several studies (Ahn et al., 2002; Jorgensen et al., 1995; Kerrn-Jespersen et al., 1993; Sorm et al., 1996; Sorm et al., 1998; Tsuneda et al., 2006), two main groups of PAOs exist, classified by the electron acceptor that they are able to use after the anaerobic conditions: one group is able to use only oxygen, while the other is capable of using both oxygen and nitrate as electron acceptors. This last group constitutes the so called Denitrifying PAOs (DNPAO).
The object of the present study was the optimization of the design of a cascaded membrane bioreactor with nitrogen removal through post-denitrification and phosphorus removal

through EBPR. Starting from a patented configuration of the plant, three changes of internal sludge recirculation flows have been operated. By changing both the sludge flow rate and the origin and the destination of the sludge recirculation flows, four configurations of the plant were in this way set-up. For each of the four configurations, the effects on nutrients removal after these changes have been observed, and the advantages of each configuration in terms of operational scheme simplicity and of operating costs reduction have been evaluated. Particularly, this work focuses on phosphorus removal through the different configurations of the plant, while nitrogen removal has been described with higher detail by Bracklow et al. (2007b).

3 Materials and methods

3.1 Cascaded membrane bioreactor

The plant used for the experimentation was a cascaded membrane reactor of 50.3 l total volume, operated at a HRT between 12.2 and 13.7 h and an influent flow rate between 3.7 and 4.1 l/h; the variations on HRT and flow rate depended on the extent of membrane fouling. The mean values of SRT in the whole plant and of HRT in each chamber can be seen in Table 2.

Table 2. Contact times and hydraulic retention time (HRT).

	Anaerobic zone AN [min]	Aerobic zone AE [min]	Anoxic zone AX [min]	Membrane chamber MF [min]	HRT [h]
REC 1	64	18	30	33	13.2
REC 2	69	21	35	26	12.6
REC 3	74	48	40	29	13.3
REC 4	54	35	59	43	14.2

The reactor was divided into anaerobic, aerobic and anoxic zones, as shown in Figure 1.

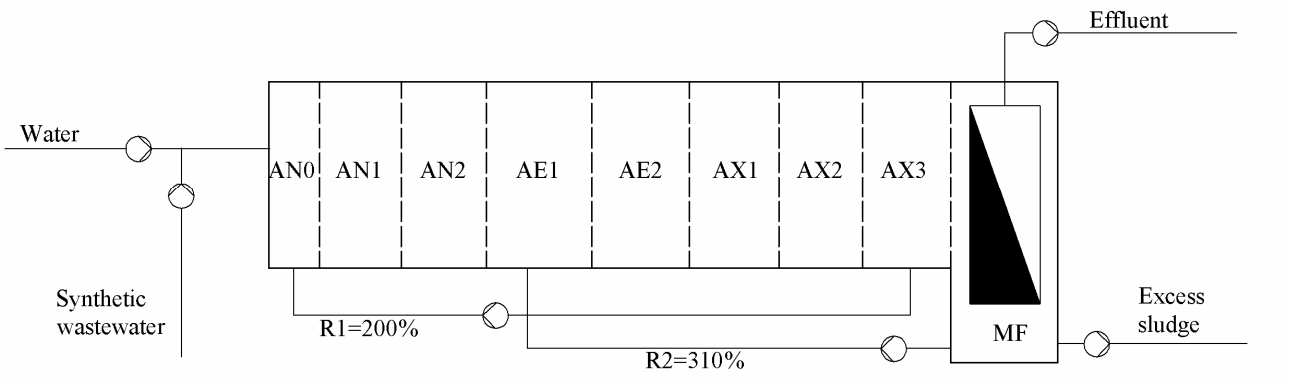

Figure 1. Basic flow scheme of the reactor (configuration 1).

Aerobic sections were not stirred, and were kept under continuous aeration to obtain oxygen concentrations higher than 2 mg/l. Anaerobic and anoxic sections were stirred continuously and not aerated.
In the filtration chamber, a plate and frame module (GKSS, Germany) made of polyacrylic nitril, with an effective area of 0.6 m^2 and a nominal cut-off of 37 nm, was implemented; this chamber was continuously aerated in order to reduce the fouling formation on the membrane surface and clogging of the channels in the membrane module. The effluent was extracted by means of a peristaltic pump which operated in cycles of 10 minutes followed by pauses of 3 minutes, to allow membrane relaxation.
In AN0, the influent was mixed with the recirculation sludge and with the NaOH necessary for pH control. The pH was set to a value of 7.4 in the first anaerobic chamber and was automatically corrected by dosing 4M NaOH in the AN1 section.
With this design, the reactor operated EBPR through the alternation anaerobic-aerobic zone, while the removal of nitrogen was accomplished through post-denitrification.

3.2 Feeding

The influent fed to the reactor was a synthetic wastewater, with its composition in terms of COD and nutrient concentration simulating municipal wastewater (Bracklow et al., 2007a). The wastewater concentrate was stored for one week in a tank, and was continuously pumped with water into the AN0 basin of the plant. The influent composition was set such that a mean COD value of 750 mg/L, and a C:N:P ratio of 100:10:1.7 were achieved. The mean values of COD, P_T and N_T measured in the synthetic influent in the different plant configurations are shown in Table 3.

Table 3 COD, P_T and N_T average values over each period obtained in the influent to the reactor in the four plant configurations, and phosphorus concentration in the sludge recirculated to the head of the plant.

	COD [mg/L]	**N_T [mg/L]**	**P_T [mg/L]**	**BIO-P [mg/L]**
REC1	908.6	68.9	14.4	398.9
REC2	764.3	64.8	14.8	373.4
REC3	711.2	66.0	12.8	394.4
REC4	786	81.7	18.6	417.0

3.3 Sludge recirculation flows

The reactor in its basic configuration (REC1) was built according to the ENREM (Enhanced Nutrient Removal in Membrane Bioreactor) patent (number WO03/057632, published on 17/07/03), a joint international patent by Berliner Wasserbetriebe and Veolia Water for the phosphorus and nitrogen removal in membrane bioreactors.
During the present study, three changes of the internal sludge recirculation flows have been operated, so to obtain four different configurations of the reactor design. For each of these configurations, the effects on nutrients removal and the metabolic dynamics in the different chambers of the reactor have been studied. The four configurations are shown in Figure 2.

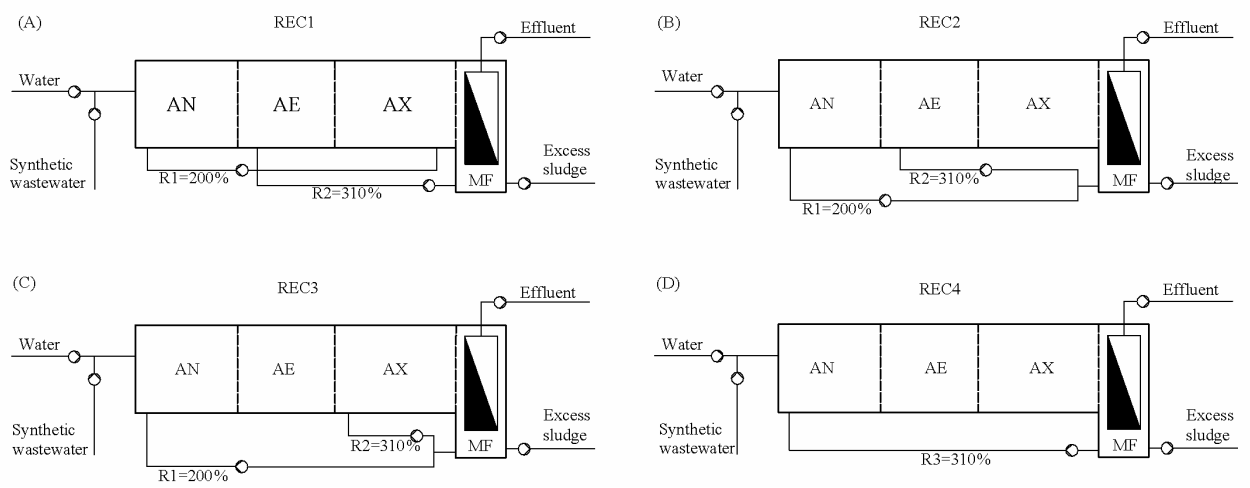

Figure 2. The four plant configurations studied during the experimentation. (A) Configuration no.1 (REC1); (B) Configuration no.2 (REC2); (C) Configuration no.3 (REC3); (D) Configuration no.4 (REC4).

In the first configuration (REC1), the flow scheme of the ENREM patent was reproduced. Two internal sludge recirculation flows were present: the first (R1), with a flow rate of 200% of the influent flow, recirculated sludge from AX3 to AN0, and the second (R2), with a flow rate of 310% of the influent flow, recirculated sludge from MF to AE1 (Figure 2(A)). This configuration was based on the assumption that for a better development of metabolic processes, no aerated sludge should be introduced into the anaerobic zone (R1), and on the consideration that the recirculation of aerated sludge into the aerobic zone could yield lower aeration costs (R2).

In the second configuration (REC2), two recirculation flows were present. The first recirculation (R1) had a flow rate of 200% of the influent flow, and brought sludge from MF to AN0; the second recirculation (R2), has not been changed from the REC1 configuration (Figure 2(B)). The change of recirculation flow operated in this configuration was made in order to check if it could improve the homogenization of the biomass distribution over the whole reactor, and to check if there would be a deterioration of the biological phosphorus removal process.

In the third configuration (REC3), two recirculation flows were present. The first (R1) was not changed in comparison with the REC2 configuration, while the second (R2), with a flow of 310% of the influent flow, recirculated sludge from MF to AX1. The change of the recirculation flow R2 was done in order to decrease the amount of sludge introduced into the aerobic zone, so to reduce the oxygen requirement, and, consequently, aeration costs. Besides, the new configuration should bring about adsorption processes of the carbon source on the biomass; a higher amount of adsorbed carbon available in the anoxic zone could improve the denitrification process.

In the fourth configuration (REC4) only one recirculation flow was present (R3); its flow rate was 310% of the influent flow, and it recirculated sludge from MF to AN0. The aim of this change of recirculation was an improved homogenization of the biomass distribution, and a much simpler plant design, which in case of implementation in a real plant is to be preferred.

3.4 Excess sludge

The excess sludge was extracted from the filtration chamber by means of a peristaltic pump, operating three times per day. The excess sludge pump flow rate was set in order to obtain an SRT in the plant of 25 days.

3.5 Analysis

Ions were measured by an Ion Chromatograph Dionex DX100; for the anions a IonPac AS4A-SC column was used. Phosphate was detected by a conductivity cell, while nitrate and nitrite were determined by a UV detector set to 214 nm. For the cations, a IonPac CS12A-SC column was used, and the detector was a conductivity cell.
Dr Lange cuvette tests were used for the determination of COD (kits LCK 114 and 314), total phosphorus (kits LCK 350 and 349) and total nitrogen (kits LCK 338 and 238).
For the volatile suspended solids measurement, the DIN 38414 Teil 3 method was used, while for the total suspended solids measurement, the DIN 38409 Teil 1 method was used.

4 Results

4.1 Mean removal efficiencies

The mean percentages of COD and nutrients removal in the four configurations are shown in Table 4.

Table 4. Percentage removal of COD and nutrients in the four configurations (mean values over each period after stable operation).

	COD removal [%]	**Pt removal [%]**	**Nt removal [%]**
REC1	96.9	97.4	90.6
REC2	96.6	99.0	89.7
REC3	97.6	99.3	90.8
REC4	97.1	99.3	92.1

As shown, COD and nutrients removal percentages were very similar in the four plant configurations. This shows that the changes of the recirculation flows did affect the metabolic processes neither negatively nor positively, in terms of nutrients and carbon removal. Subsequently, the analysis of the results of this study has been focused on the metabolic dynamics in the different chambers of the reactor, mainly in terms of phosphorus release and uptake rates, and, with less detail, in terms of nitrification and denitrification rates.

4.2 Change of the source of the sludge recirculated to the anaerobic zone

When the source of the sludge recirculated to the anaerobic zone of the reactor was switched from AX3 to MF, a deterioration of the phosphorus removal process was expected, because

an aerated sludge was introduced into a chamber in which strictly anaerobic conditions should have been kept. An estimation of oxygen consumed in the recirculation flow tube connecting MF with AN0 was made in order to check if oxygen was consumed by biomass before the mixed liquor could reach the anaerobic zone. OUR tests with the plant sludge were thus performed, and, by combining OUR values together with the HRT value of aerated sludge inside the tube, a consumption of 0.1 mgO_2/l in the recirculation tube was calculated. Since oxygen concentration in the membrane chamber sludge could even reach a value of 5 mgO_2/l, no relevant oxygen consumption in the recirculation tube could be assumed, and, subsequently, the sludge entering the anaerobic section from the membrane chamber was supposed to have a similar oxygen concentration of the one present in the source chamber. Unexpectedly, an increase in PRR in the first section of the anaerobic zone (AN0) was observed, while the mean PRR in the whole anaerobic zone and the PUR in the aerobic zone stayed at a constant value.
In Figure 3, the comparison between the typical phosphorus trends in the REC1 and the REC 2 is shown.

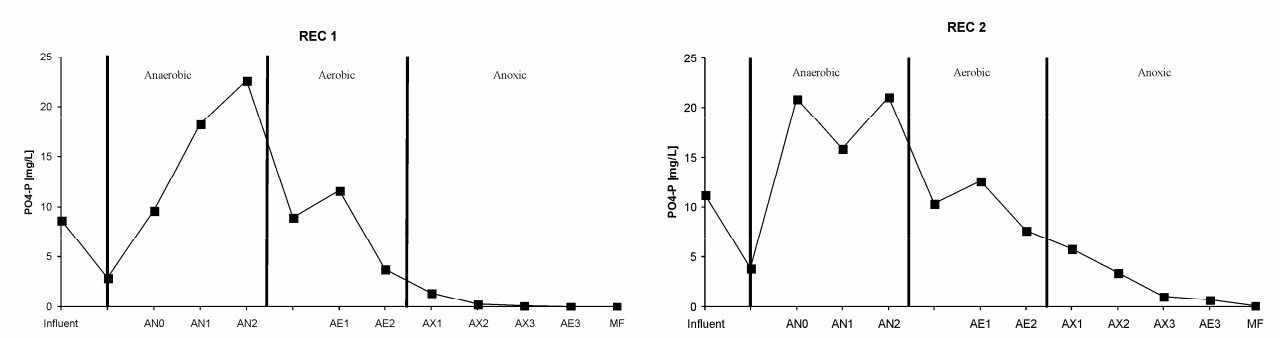

Figure 3. Comparison between the typical orthophosphate trends in REC1 and in REC2. Values shown at the beginning of the anaerobic zone were calculated by mass balance, taking into account the flow rate and the phosphorus concentration of both influent and recirculation flow.

As can be seen in Figure 3, in the second recirculation configuration the release of phosphorus in AN0 is much faster, while the concentration of phosphorus released at the end of the whole anaerobic zone is almost the same for both configurations. The decrease of PO_4-P in AN1 (REC2) is ascribed to the pH value which would lead to orthophosphate precipitation.
These results are shown in detail in Table 5, in which the mean values of PRR in AN0 and in the whole anaerobic zone, and the PUR in the whole aerobic and anoxic zones are shown for the four configurations. The mean values are calculated on the basis of four measurements per configuration, except for the REC3 configuration, for which ten measurements were performed; all measurements were performed when stable operating conditions were reached.

Table 5. Mean PRR values in AN0 and in the whole anaerobic zone, and PUR in aerobic and anoxic zones in the four configurations of the plant.

	PRR in AN_0 [mg PO_4-P/ (g VSS·h)]	**PRR in AN [mg PO_4-P/ (g VSS·h)]**	**PUR in AE [mg PO_4-P/ (g VSS·h)]**	**PUR in AX [mg PO_4-P/ (g VSS·h)]**
REC1	8.7	2.8	1.9	1.0
REC2	16.4	2.3	1.6	1.1
REC3	26.9	3.6	4.3	0.3
REC4	33.1	4.3	5.6	0.5

Despite the great difference in the PRR values in AN0, smaller differences in actual PRR values in the whole anaerobic zone can be observed in the different configurations. Concerning the PUR, very similar values can be observed in REC1 and in REC2, both for the aerobic and the anoxic zones.
The first change of recirculation did not affect the nitrogen removal. As shown in Table 6, nitrification and denitrification rate mean values were similar in REC1 and REC2 (Bracklow et al., 2007b).

Table 6. Mean nitrification and denitrification rates in the four configurations.

	NR [mgNH_4-N/gVSS·h]	**DNR [mgNO_3-N/gVSS·h]**
REC1	2.4	0.9
REC2	1.7	0.8
REC3	1.8	0.6
REC4	1.9	0.9

4.3 Change of the destination of the sludge recirculated from the filtration chamber

When the destination of the sludge recirculated with R1 from MF was switched from AE1 to AX1, a deterioration of the denitrification efficiency was expected, because a sludge rich in molecular oxygen was introduced into a chamber in which strictly anoxic conditions should be kept. Unexpectedly, denitrification rates were not affected by this change, as shown by Table 5.
More remarkable was the phosphorus release trend. As can be observed in Figure 4, where the comparison between the typical orthophospate trends in the REC2 and the REC3 is shown, the release of phosphorus in the anaerobic zone in REC3 was much higher than in REC2.

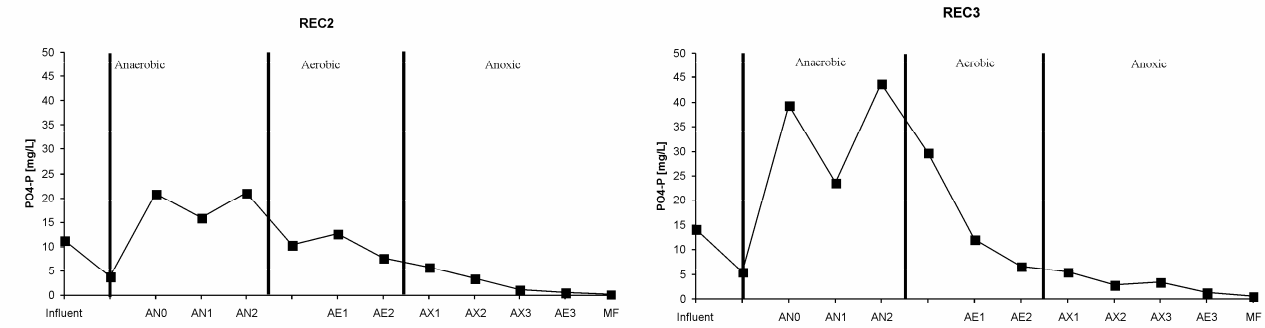

Figure 4. Comparison between the typical orthophosphate trends in REC2 and in REC3. Values shown at the beginning of the anaerobic zone were calculated by mass balance, taking into account the flow rate and the phosphorus concentration of both influent and recirculation flow.

As a consequence, the PRR in the anaerobic zone was higher in REC3 than in REC2, with a much higher PRR in AN0 observed in REC3 than in REC2. The PUR in the aerobic zone was higher in REC3, while the PUR in the anoxic zone had similar values in the two configurations (see Table 5). The low PUR values observed in the anoxic zone for both configurations was expected, due to the low concentration of PO_4-P in the mixed liquor of the anoxic chamber.

4.4 Switch to a single sludge recirculation flow

When only one recirculation flow was set up in the reactor, nitrification and denitrification rates were not affected, as shown in Table 6. Higher PRR in the anaerobic zone and PUR in the aerobic zone were observed, as can be seen from Table 3.

4.5 Solids distribution

One of the main aims of the changes of recirculation flows design was to achieve a more suited distribution of the biomass in the different sections of the plant, depending on the removal efficiencies and subsequently on the different biomass concentrations required in the different sections of the plant. In Table 7, the mean values of volatile suspended solids in anaerobic, aerobic and anoxic zones and in the whole reactor are reported (Bracklow et al., 2007b).

Table 7. Mean values of volatile suspended solids in anaerobic, aerobic and anoxic zones and in the whole reactor [g VSS/l].

	AN	**AE**	**AX**	**MF**	**Mean**
REC1	4.7	6.4	6.2	7.5	5.9
REC2	6.9	9.2	8.6	10.3	8.6
REC3	6.7	6.4	8.0	9.3	7.5
REC4	7.6	7.6	7.2	9.4	7.8

In Figure 5, the mean values of volatile suspended solids in anaerobic, aerobic and anoxic zones, in the filtration chamber, and in the whole reactor are shown. As expected, in REC2 the mean concentration in the whole reactor was higher than in REC1, due to the recirculation of a more concentrated sludge (the MF sludge) to the head of the plant. In REC3, the switch of the R1 destination from the aerobic to the anoxic zone led to a lower sludge concentration in the aerobic zone. In REC4 a more homogeneous distribution of the biomass in all the sections of the plant can be observed.

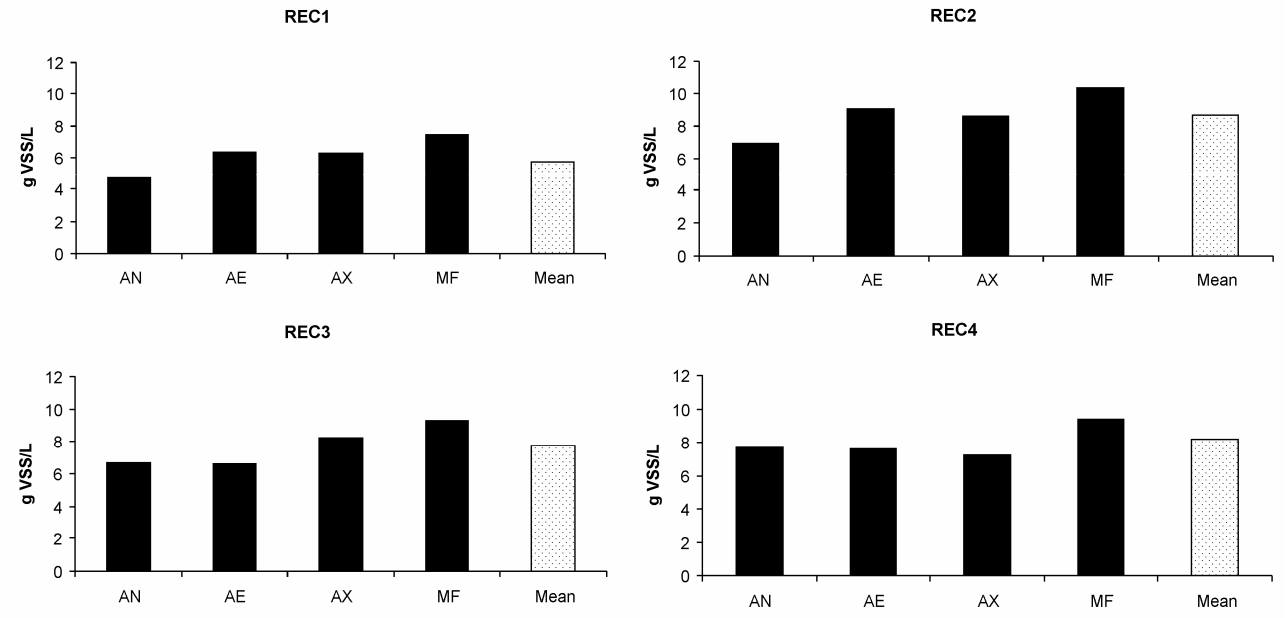

Figure 5. Mean volatile solids concentration in the anaerobic, aerobic and anoxic zones and in the filtration chamber, and mean value in the whole reactor for the different recirculation flows configurations.

5 Discussion

5.1 Carbon removal

The three changes of recirculation flows operated during the present study did not affect the carbon removal in the plant. As can be observed in Table 2, COD removal efficiency was similar in all of the four configurations, and stabilized at a value of around 97%; the COD value in the effluent was stable at a mean value always below 30 mg/l.

5.2 Nitrogen removal

The variations of the sludge recirculation flows did not affect, neither positively nor negatively, the nitrogen removal in the plant. The mean values of nitrogen removal in the four configurations of the plant are shown in Table 2. The mean removal percentage was stable at around 90% for the first three configurations, with a slight increase in the last configuration (mean nitrogen removal of 92.4%), while the total nitrogen concentration in the effluent of the plant was around 6.5 mgN_T/l for all the configurations. In the change from REC2 to REC3 - even if a deterioration of nitrogen removal was expected due to the introduction of an oxygen rich sludge into the anoxic zone, where strict absence of molecular oxygen should be kept - nitrogen removal in overall average was not affected, neither positively nor negatively, while some differences in nitrification and denitrification rates between different chambers in the various configurations were observed (Bracklow et al., 2007b).

5.3 Phosphorus removal

5.3.1 Change of the source of the sludge recirculated to the anaerobic zone

The difference in the PRR between REC1 and REC2 observed in the first chamber of the anaerobic zone has to be explained by the variations of alternation between anaerobic and aerobic or anoxic conditions, which was created with the change of the R2 recirculation. In REC1, the sludge recirculated to the anaerobic zone came from the anoxic zone, while in REC2 it came from the aerobic zone. As shown by past studies (Ahn et al., 2002; Kuba et al., 1996) in which the alternation of anaerobic-aerobic and anaerobic-anoxic conditions has been studied, the energy involved in the production of ATP in the alternation anaerobic-anoxic conditions is about 40% less than the one involved in the alternation anaerobic-aerobic conditions. In both cases (in the presence of an aerobic or anoxic zone subsequent to the anaerobic zone), the EBPR process in the anaerobic phase involves the same organic and inorganic compounds: in anaerobic conditions PAOs take up VFAs and store them as PHA, and the energy for this process is given by Poly-P hydrolysis and glycogen degradation, while the reduction equivalents ($NADH_2$) for PHA formation come from the degradation of glycogen (Arun et al, 1989; Kuba et al., 1996; Murnleitner et al.,1997; Smolders et al, 1994a). In aerobic conditions, $NADH_2$ reacts with the electron acceptor, which can be oxygen or nitrate, and the $P/NADH_2$ ratio (equal to the "ATP produced/$NADH_2$ utilized" ratio) is a measure of the energy production efficiency involved in this reaction (Kuba et al., 1996). In the same study, Kuba et al. (1996), showed that the energy involved in the phosphorylation, in terms of $P/NADH_2$ ratio, is 40% higher in case of alternation between anaerobic and aerobic conditions than in the alternation between anaerobic and anoxic conditions.
Since the PUR and PRR are a measure of the phosphorus uptake and release rate in a certain section of the plant, if the same PUR and PRR are observed in a section of the plant, and longer hydraulic retention times of the sludge are kept in that section, this implies that higher amounts of phosphorus are taken up or released. In the case of the present study, since the hydraulic retention times in anaerobic and aerobic zones in REC1 and in REC2 are the same, and the mean PRR and PUR for the two zones are similar in the two configurations, the production and consumption of the same amount of PHA, glycogen, VFA and orthophosphate in the EBPR process can be assumed in the two configurations. This fits with what was previously observed by Murnleitner et al. (1997), and Kuba et al. (1996), who states that the only difference between the alternation anaerobic-aerobic and anaerobic-anoxic is in the energy involved in the phosphorylation process, and not the compounds involved.
This difference in involved energy could explain the higher PRR in the first part of the anaerobic zone in REC2, which would bring to a faster release of phosphorus in the anaerobic zone. The fact that the amounts of organic compounds and of phosphorus involved did not increase, is shown by the fact that the mean PRR in the whole anaerobic zone and the PUR in the aerobic zone are the same in the two configurations. Since no higher phosphorus amounts were taken up in the aerobic phase or released in anaerobic phase, no improvement in general phosphorus removal can be stated with this change of recirculation flows.

5.3.2 Change of the destination of the sludge recirculated from the filtration chamber

The change of recirculation affected rather the PRR in the anaerobic zone and the PUR in the aerobic zone, leading to a large increase of both rates. This suggests that, differing from what was observed after the first recirculation flows change, there was a variation in the amounts of carbon and phosphate sources involved in the process. In particular, an increase in the amount of PHA, glycogen, VFA, orthophosphate and poly-P involved in the EBPR process can be assumed. This increase can be explained with the higher HRT in the aerobic zone obtained in

REC3, due to the switch of the recirculation R1 destination from the aerobic to the anoxic zone. As shown by the study of Petersen et al. (1998), a HRT increase in the aerobic zone leads to higher PHB utilization, and enlarging this observation to the case of the present study, an increase in the amount of glycogen stored in the PAO cell can be assumed. This assumption is supported by what was found by Kuba et al. (1996), who observed a linear relation for PHB and nitrates conversion rates as a function of growth, poly-P formation and glycogen synthesis, and by what was observed by Kerrn-Jespersen and Henze (1993), who found a linear relationship between the amount of acetate taken up in the anaerobic phase and the PUR in the aerobic phase. This suggests that the increase in the amount of one or more organic or inorganic compounds involved in the EBPR process can lead to an increase in the amount of the other released or taken up compounds. This was also observed in this study, because after the change of the recirculation R2, the HRT in the anaerobic zone did not change, while the phosphorus release rate in that zone increased. This must have had as a consequence that the net amount of orthophosphate released in the anaerobic zone increased, thus suggesting the presence of more glycogen available for this metabolic process.
The experimentation showed that the air flow rate for the aeration of the aerobic chambers was 30% lower in REC3 in comparison to the one employed in REC1, REC2 and REC4 to reach the same oxygen concentration in the aerobic section. In conclusion, the third recirculation configuration presents the advantage of a lower oxygen consumption, due to the lower biomass concentration in the aerobic zone, subsequent to the switch of the recirculation sludge destination from the aerobic to the anoxic zone. This change in the recirculation did not affect COD and nutrient removal, so the third recirculation flow configuration can be assumed more advantageous than the previous ones.

5.3.3 Switch to a single sludge recirculation flow

As shown by Table 3, COD and nutrients removal efficiencies were not affected by switching to a single recirculation flow. The increase of PRR in the anaerobic zone and of PUR in the aerobic zone can be attributed not to different metabolic processes, but rather to a higher P_T concentration in the feeding of the plant, observed during the fourth configuration as shown in Table 2.

In Table 2 also the values of the phosphorus stored in the sludge recirculated at the head of the plant are shown; this value gives an indication of the amount of phosphorus taken up in the aerobic zone, and as can be seen from the table, in the last configuration of the plant, when the phosphorus in the influent was higher than in the past configurations, a higher phosphorus concentration was also observed in the sludge recirculated to the head of the plant, thus indicating a higher amount of phosphorus available in the anaerobic section. This can explain the higher PRR in the anaerobic zone. The most homogeneous sludge concentration through the different sections of the plant was observed in REC4 in comparison with the other configurations. Anyway the lower sludge concentration obtained in the aerobic zone in REC3 in comparison with REC4, make configuration REC3 preferable in terms of aeration costs saving. An evaluation of the aeration cost saving achievable with configuration REC3 and of the advantages in the plant management due to the higher design simplicity achieved with configuration REC4 should be done in case of implementation of this plant scheme in a real wastewater treatment plant.

5.3.4 Solids distribution

From the analysis of the volatile suspended solids distribution through the sections of the plant, a more homogeneous distribution was observed in REC4, in comparison with the other configurations. The low sludge concentration in the aerobic zone in REC3 led to a lower oxygen requirement in comparison with REC2, and, consequently, to lower aeration costs. This, coupled to the fact that nitrification rate, as can be observed in Table 5, was not negatively affected by the lower amount of oxygen supplied (necessary to reach in REC3 the same oxygen concentration in the aerobic section that was obtained in the other configurations), makes configuration REC3 preferable in comparison with REC2.

5.3.5 Advantages achieved with the different changes of recirculation flows

Carbon and nutrients removal was not affected by the changes of recirculation flows operated during the experimentation. The advantages given by the different configurations are to be found in a lower oxygen consumption and in a simpler configuration, which is preferable if the plant is implemented at technical scale.

The advantage given by the configuration REC2 in comparison with the first one is a simpler operation due to the presence of only a single recirculation sludge origin.

The configuration REC3 presents the advantage of a lower oxygen consumption, due to the lower biomass concentration in the aerobic zone, subsequent to the switch of the recirculation sludge destination from the aerobic to the anoxic zone. This change in the recirculation did not affect COD and nutrient removal (nitrification was also not affected), so the configuration REC3 can be assumed more advantageous than the previous two.

The configuration REC4 showed the most homogeneous sludge concentration throughout the different sections of the plant; anyway the lower sludge concentration obtained in the aerobic zone in REC3 in comparison with REC4, makes configuration REC3 preferable in terms if aeration costs saving. An evaluation of the aeration cost saving achievable with REC3 configuration and of the advantages in the plant management due to the higher design simplicity achieved with REC4 configuration should be done in case of implementation of this plant scheme in a real wastewater treatment plant.

6 Conclusions

Starting from a patented configuration of a cascaded MBR, four different recirculation flows configurations have been set up in order to investigate their efficiency in nutrients removal and in operating costs saving.

- The change of the recirculation flows from the patented one not only did not affect the nutrients removal negatively, but also showed better metabolic kinetics in the phosphorus removal process.
- The average phosphorus and nitrogen removal efficiencies were similar, with a slight increase in the total phosphorus removal when the aerobic sludge, instead of the anoxic one, was recirculated into the anaerobic zone.
- Although nutrients removal was not improved, several advantages in terms of aeration costs saving, and in terms of management simplification can be achieved with the different recirculation flows configurations.
- All of the three configurations set up during this study showed improvements under different points of view in comparison with the patented flow scheme; the most advantageous one in terms of aeration proved to be REC3, in which 310% of the influent flow rate is recirculated from the filtration chamber to the anoxic zone, and

another flow (200% of the influent flow rate), is recirculated from the filtration chamber to the anaerobic zone.

An evaluation of the aeration cost saving achievable with configuration REC3 and of the advantages in the plant management due to the higher design simplicity achieved with configuration REC4, should be made in case of implementation of this plant scheme in a real wastewater treatment plant.

References

Adebusoye S.A., Picardal F.W., Ilori M.O., Amund O.O., Fuqua C., Grindle N. (2007). Aerobic degradation of di- and trichlorobenzenes by two bacteria isolated from polluted tropical soils. *Chemosph.*, 66, 1939–1946.

Ahn J., Daidou T., Tsuneda S., Hirata A. (2002). Characterization of denitrifying phosphate-accumulating organisms cultivated under different electron acceptor conditions using polymerase chain reaction-denaturing gradient gel electrophoresis assay. *Wat. Res.*, 36(2), 403-412.

Albrechtsen H.-J., Milton P., Nielsen P., Christensen T.H. (1996). Significance of biomass support particles in laboratory studies on microbial degradation of organic chemicals in aquifers. *Wat. Res.*, 30(12), 2977-2984.

Annachhatre A.P. and Gheewala (1996). Biodegradation of chlorinated phenolic compounds. *Biotech. Advant.*, 14 (1), 35-56.

Andreozzi R., Caprio V., Insola A., Marotta R. (1999). Advanced oxidation processes (AOP) for water purification and recovery. *Catal. Tod.*, 53, 51–59

Andreozzi R., Canterino M., Marotta R. (2006). Fe(III) homogeneous photocatalysis for the removal of 1,2-dichlorobenzene in aqueous solution by means UV lamp and solar light. *Wat. Res.*, 40, 3785 – 3792.

Andriukin A.A. (1979), Toxic effect of dichloroethane on the cardiovascular system. *Klin. Med. (Mosk.)*, 57, 43-47.

Antonelli M., Rozzi A., (2000). Conventional membrane processes. Proc. 52nd Sanitary engineering training course. Politecnico of Milan.

Aranzabal A., González-Marcos J.A., Ayastuy J.L., González-Velasco J.R. (2006). Kinetics of Pd/alumina catalysed 1,2-dichloroethane gas-phase oxidation. *Chemical Engineering Science*, 61, 3564 – 3576

Armenante P.M., Kafkewitz D., Lewandowski G.A., Jou C.-J. (1999). Anaerobic-aerobic treatment of halogenated phenolic compounds. *Wat. Res.* 33(3), 681-692.

Arun V., Mino T., Matsuo T. (1988). Biological mechanism of acetate uptake mediated by carbohydrate consumption in excess phosphorus removal systems. *Wat. Res.*, 22(5), 565-570.

Arun V., Mino T., Matsuo T. (1989). Metabolism of carboxylic acids located in and around the glycolytic pathway and the TCA cycle in the biological phosphorus removal process. *Wat. Sci. Tech.*, 21(4-5), 363-374.

Assessing the Human Health Risks of Trichloroethylene: Key Scientific Issues (2006). National Research Council, Board on Environmental Studies and Toxicology. Prepublication copy available at: http://newton.nap.edu/catalog/11707.html.

Bae H.-S., Yamagishi T., Suwa Y. (2002). Evidence for degradation of 2-chlorophenol by enrichment cultures under denitrifying conditions. *Microbiol.*, 148, 221–227.

Baker M. D., Mayfield C. I., Inniss W. E. (1980). Degradation of chlorophenols in soil, sediment and water at low temperature. *Wat. Res.*, 14(12), 1765-1771.

Barbeni M., Minero C. and Pelizzetti E. Borgarello E., Serpone N. (1987). Chemical degradation of chlorophenols with Fenton's reagent (Fe^{2+} + H_2O_2). *Chemosph.*, 16(10-12), 2225-2237.

Barreiro R., Pratt J.R. (1992). Toxic effects of chemicals on microorganisms, *Wat. Environ. Res*., 64(4), 632.

Basu S.K., Oleszkiewicz J.A., Sparling R. (1996). Dehalogenation of 2-chlorophenol in anaerobic batch cultures. *Wat. Res.*, 30(2), 315-322.

Baxendale J.H., Wilson J.A. (1957). The photolysis of hydrogen peroxide at high light intensities, Trans. Faraday Soc. 53, 344.

Bogoyavlenski V.F., Salikhova S.K.H., Karpova E.V. (1968). Clinical aspects and therapy for ethylene dichloride poisoning. *Sov. Med.,* 31, 107-109.

Bracklow U., Vocks M., Drews A., Kraume M. (2007a). Comparison of nutrients degradation in smallscale MBR fed with synthetic/domestic wastewater. *J. Haz. Mat.*, 144 (3), 620-626.

Bracklow U., Manigas L., Drews A., Vocks M., Kraume M. (2007b). Impact of different recirculation schemes on nutrients removal in a laboratory scale MBR. *Wat. Sci. Tech*., 56(6), 115-124.

Choi J., Shin C., Park T., Suh D. (2006). Characteristics of vanadia–titania aerogel catalysts for oxidative destruction of 1,2-dichlorobenzene. *Appl. Cat. A: General*, 311, 105–111.

Becker J.G., Stahl D.A., Rittmann B.E. (1999). Reductive Dehalogenation and Conversion of 2-Chlorophenol to 3-Chlorobenzoate in a Methanogenic Sediment Community: Implications for Predicting the Environmental Fate of Chlorinated Pollutants. *Appl. Environ. Microbiol.*, 65(11), 5169–5172.

Bosma T.N.P., van der Meer J.R., Schraa G., Tros M.E., Zehnder A.J.B. (1988). Reductive dechlorination of all trichloro- and dichlorobenzene isomers. *FEMS Microbiology Letters* 53(3-4), 223-229.

Bouhabila E.H., Aim R.B., Buisson H. (2001). Fouling characterisation in membrane bioreactors. *Sep.Pur. Tech.*., 22-23, 123–132.

Brigmon R. L., Bell N. C., Freedman D. L., Berry C. J. (1998). Natural Attenuation of Trichloroethylene in Rhizosphere Soils at the Savannah River Site. *J.Soil Cont.*, 7(4), 433-453.

DeWeerd K.A., Flanagan W.P., Brennan M.J., Principe J.M., Spivack J.L. (1998). Biodegradation of Trichloroethylene and Dichloromethane in Contaminated Soil and Groundwater. *Biorem. J.*, 2(1), 29-42.

Dilling W.L., Tefertiller N.B., Kallos G.J. (1975). Evaporation rates and reactivities of methylene chloride, chloroform, 1,1,1-trichloroethane, trichloroethylene, tetrachloroethylene, and other chlorinated compounds in dilute aqueous solutions. *Env.. Sci. Tech.*, 9, 833-838.

Cacciatore, D., Leigh, D. , Porter, B., Schaal, W. , Christensen, G., Brooks, P. (2005). Design and operations of an in situ anaerobic bioremediation system. *Proceedings of the 8th International In Situ and On-Site Bioremediation Symposium*, 4, 1770.

Carrol T., Booker N.A. (2000). Axial features in the fouling of hollow-fibre membranes. *J. Membrane Sci.*, 168, 203-212.

Case Studies in Environmental Medicine: Trichloroethylene (TCE) Toxicity. (2001). Agency for Toxic Substances and Disease Registry (ATSDR). Atlanta, GA.

Chang C.-C., Tseng S.-K., Chang C.-C., Ho C.-M. (2004). Degradation of 2-chlorophenol via a hydrogenotrophic biofilm under different reductive conditions. *Chemosph.*, 56, 989–997.

Chang C.-C., Tseng S.-K., Chang C.-C., Ho C.-M. (2003). Reductive dechlorination of 2-chlorophenol in a hydrogenotrophic, gas-permeable, silicone membrane bioreactor. *Biores. Tech.*, 90, 323–328.

Chang H.L., Alvarez-cohen L. (1995). Transformation capacities of chlorinated organics by mixed cultures enriched in methane, propane, toluene and phenol. *Biotech. Bioeng.*, 45, 440-449.

Chang Y.C., Hatsu M., Jung K., Yoo Y.S., Takamizawa K. (1998). Degradation of a Variety of Halogenated Aliphatic Compounds by an Anaerobic Mixed Culture. *J. Ferm. Bioeng.*, 86(4), 410-412.

Chang S., Fane A.G. (2001). The effect of fibre diameter on filtration and flux distribution – relevance to submerged hollow fibre modules. *J. Membrane Sci.*, 184, 221-231.

Chatterjee D., Dasgupta S., N Rao N. (2006). Visible light assisted photodegradation of halocarbons on the dye modified TiO_2 surface using visible light. *Solar Energy Mat. Solar Cells*,
90(7-8), 1013-1020.

Chen T.K., Ni C.H., Chen J.N., Lin J. (2003). High-strength nitrogen removal of opto-electronic industrial wastewater in membrane bioreactor – a pilot study. *Wat. Sci. Tech.*, 48(1), 191-198.

Cheryan M. (1998). Ultrafiltration and microfiltration Handbook. Technomic Publishing Company, Lancaster, Pennsylvania (USA).

Choo K.W., Lee C.H. (1996). Membrane fouling mechanism in the membrane-coupled anaerobic bioreactor. *Wat. Res.*, 30(8), 1771-1780.

Chua H.C., Arnot T.C., Howell J.A. (2002). Controlling fouling in membrane bioreactors a variable throughput. *Desalination*, 149, 225-229

Cicek N., Winnen H., Suidan M.T., Wrenn B.E., Urbain V., Manem J. (1998). Effectiveness of the membrane bioreactor in the biodegradation of high molecular weight compounds. *Wat. Res.*, 32(5), 1553-1563.

Cole J.R., Cascarelli A.L., Mohn W.W., Tiedje J.M. (1994). Isolation and Characterization of a Novel Bacterium Growing via Reductive Dehalogenation of 2-Chlorophenol. *Appl. Environ. Microbiol.*, 60(10), 3536-3542.

Coleman N.V., Mattes T.E., Gossett J.M., Spain J.C. (2002). Biodegradation of cis-dichloroethene as the sole carbon source by a alfa-Protobacterium. *Appl. Environ. Microbiol.*, 68, 2726-2730.

Cupitt L.T. (1980) Fate of toxic and hazardous materials in the air environment. Research Triangle Park, North Carolina, US Environmental Protection Agency, Environmental Sciences Laboratory, Office of Research and Development (EPA 600/3-80-084, PB 221948).

De Bont J.M.,. Vorage M.J.A.W., Hartmans S., Van Den Tweel W.J.J. (1986). Microbial Degradation of 1,3-Dichlorobenzene. *Appl. Environ. Microbiol.*, 52(4), 677-680.

De Wildeman S., Nollet H., Van Langenhove H., Verstraete W. (2001). Reductive biodegradation of 1,2-dichloroethane by methanogenic granular sludge in lab-scale UASB reactors. *Adv. Env. Res.*, 6, 17-27.

Defrance L., Jaffrin M.Y. (1999). Comparison between filtrations at fixed transmembrane pressure and fixed permeate flux: application to a membrane bioreactor used for wastewater treatment. *J. Membrane Sci.*, 152, 203-210.

Dilek S.F., Knappe D.R.U., Barlaz M.M. (2000). The fate of toluene, acetone and 1,2-Dichloroethane in a laboratory-scale simulated landfill. *Wat. Res.*, 34(12), 3063-3074.

Dinglasan-Panlilio M.J., Dworatzek S., Mabury S., Edwards E. (2006). Microbial oxidation of 1,2-dichloroethane under anoxic conditions with nitrate as electron acceptor in mixed and pure cultures. *FEMS Microbiol. Ecol*, 56(3), 355–364.

Dorndorf W., Kresse M., Christian W., Katritzki KG.(1975). Dichloroethane poisoning with myoclinic syndrome, epileptic attacks, and irreversible cerebral effects. *Arch. Psychiatr. Nervenkr.*, 220, 373-379.

Doyen W., Beeusaert L., Baèe B. (2000). Exploration of the possibilities of UF as an alternative pre-treatment step for drinking water production. *Proc. Journèes Internationales d'ètude du Cebedeau*, 4th -5th April 2000, Liège (B).

Drews A., Vocks M., Iversen V., Lesjen B., Kraume M. (2005). Influence of unsteady membrane bioreactor on EPS formation and filtration resistance. *Proc. ICOM 2005*, International Congress on Membrane Processes. Seoul, Korea.

Drury J.S., Hammons A.S. (1979). Investigations of selected environmental pollutants: 1,2-dichloroethane. Washington, DC, US Environmental Protection Agency, Office of Toxic Substances. (Report No. EPA-560/2-78-006, PB 295865).

Durnand-Borlier L., Baudin I., Glucina K. (2000). Treatment combinations of ultrafiltration and its applications to drinking water production. *Proc. Journèes Internationales d'ètude du Cebedeau*, 4th -5th April 2000, Liège (B).

Dyer M., van Heiningen E., Gerritse J. (2003). A field trial for in-situ bioremediation of 1,2-DCA *Eng. Geol.*, 70, 315–320.

Egli C., Scholtz R., Cook A.M., Leisinger T. (1987). Anaerobic dechlorination of tetrachloromethane and 1,2-dichloroethane to degradable products by pure cultures of Desulfobacterium sp. and Methanobacterium sp. *FEMS Microbiol. Let.*, 43(3), 257-261.

Fane A., Chang S. (2002). Membrane bioreactors: design and operational options. *Filtration+separation*. www.filtsep.com

Fane A.G., Fell C.J.D., Hodgson P.H., Leslie G., Marshall K.C. (1989) Microfiltration of biomass and biofluids:effects of membrane morphology and operating conditions. *Proc. of the 5th World Filtration Congress*, Nice (F), 320-329.

Farhataziz, A. Ross B. (1977) Selective specific rates of reactions of transients in water and aqueous solutions. Part III. Hydroxyl radical and perhydroxyl radical and their radical ions, *Natl. Stand. Ref. Data Ser.*, (USA Natl. Bur. Stand.), 59.

Farrell A., Quilty B. (2002). The enhancement of 2-chlorophenol degradation by a mixed microbial community when augmented with Pseudomonas putida CP1. *Wat. Res.*, 36, 2443–2450.

Fedorak P.M., Hrudey S.E. (1984). The effects of phenol and some alkyl phenolics on batch anaerobic methanogenesis, *Wat. Res.*, 18(3) 361.

Feitz A.J., Guan J., Chattopadhyay G., Waite T.D. (2002). Photo-Fenton degradation of dichloromethane for gas phase treatment. *Chemosph.*, 48, 401–406

Frascari D., Pinelli D., Nocentini M., Zannoni A., Fedi S., Baleani E., Zannoni D., Farneti A., Battistelli A. (2006). Long-term aerobic cometabolism of a chlorinated solvent mixture by vinyl chloride-, methane- and propane-utilizing biomasses. *J. Haz. Mat.*, B138, 29–39.

Freitas Dos Santos L.M., Livingston A.G. (1995). Novel membrane bioreactor for detoxification of VOC wastewaters: biodegradation of 1,2-dichloroethane. *Wat. Res.*, 29(1), 179-194.

Freitas dos Santos L. M., Pavasant P., Strachan L.F., Pistikopoulos E. N., Livingston A. G. (1997). Membrane attached biofilms for waste treatment fundamentals and applications. *Pure & Appl. Chern.*, 69(11), 2459-2469.

Gallego A., Fortunato M.S., Foglia J., Rossi S., Gemini V., Gomez L., Gomez C.E., Higa L.E., Korol S.E. (2003). Biodegradation and detoxification of phenolic compounds by pure and mixed indigenous cultures in aerobic reactors. *Int. Biodet. Biodeg.*, 52, 261 – 267.

Genevini P.L. (1996). The toxicity of depuration sludge. Presence of organic xenobiotics. Fondazione Lombardia per l'ambiente, 21.

Girard R., Martin P., Bourret J. (1969). Hémopathies graves et exposition à des dérivés chlorés du Benzene (à propos de 7 cas). *J. Med. Lyon.*, 50(1164), 771-773.

Goldman, P., Milne G. W. A., Keister D. B. (1968). Carbonhalogen bond cleavage. III. Studies on bacterial halidohydrolases. *J. Biol. Chem.*, 243, 428-434.

Gonzalez R.D., Lopez T., Gomez R. (1997). High specific surface area, well developed mesoporous structures, and highly dispersed active sites. *Catal. Today*, 35, 293.

González-Velasco, J.R., Aranzabal, A., López-Fonseca, R., Ferret, R., González Marcos, J.A., (2000a). Enhancement of the catalytic oxidation of hydrogen-lean chlorinated VOCs in the presence of hydrogen-supplying compounds. *Appl. Catal. B: Env.*, 24, 33–43.

González-Velasco, J.R., López-Fonseca, R., Aranzabal, A., Gutiérrez-Ortiz, J.I., Steltenpohl, P., (2000b). Evaluation of H-type zeolites in the destructive oxidation of chlorinated volatile organic compounds. *Appl. Catal. B: Env.*, 24, 233–242.

Gui P., Huang X., Chen Y., Qian Y. (2002). Effect of operational parameters on sludge accumulation on membrane surfaces in a submerged membrane bioreactor. *Desalination*, 15(1) 185-l 94.

Haber F., Weiss J. (1934). The catalytic decomposition of hydrogen peroxide by iron salts, *Proc. R. Soc. Series A*, 147, 332.

Hadengue M.A., Martin R. (1953). Un cas d'intoxication mortelle par le dichloréthane. *Soc. Med. Leg.*, 33, 247-249.

Hage J.C., Hartmans S. (1999). Monooxygenase-Mediated 1,2-Dichloroethane Degradation by Pseudomonas sp. Strain DCA1. *Appl. Environ. Microbiol.*, 65(6) 2466–2470.

Haggblom M.M., Young L. Y. (1995). Anaerobic Degradation of Halogenated Phenols by Sulfate-Reducing Consortia. *Appl. Environ. Microbiol.*, 61(4) 1546–1550.

Haigler B.E., Nishino S.F., Spain J.C. (1988). Degradation of 1,2-Dichlorobenzene by a Pseudomonas sp. *Appl. Environ. Microbiol.*, 54(2) 294-301.

Han Y.L., Tom Kuo M.C., Tseng I.C., Lu C.J. (2007). Semicontinuous microcosm study of aerobic cometabolism of trichloroethylene using toluene. *J. Haz. Mat*, 148, 583–591.

Hao O.J., Kim M.H., Seagren E.A., Kim H. (2002). Kinetics of phenol and chlorophenol utilization
by Acinetobacter species. *Chemosph.*, 46, 797–807.

Hardman, D. J., Slater J. H. (1981). Dehalogenases in soil bacteria. *J. Gen. Microbiol.*, 123, 117-128.

Hartmans S., DeBont J.A.M. (1992). Aerobic vinyl chloride metabolism in mycobacterium aurum L1. *Appl. Environ. Microbiol.*, 58, 1220-1226.

Herbst B., Wiesmann U. (1996). Kinetics and reaction engineering aspects of the biodegradation of dichloromethane and dichloroethane. *Wat. Res.*, 30(5), 1069-1076.
Hincapiè M., Maldonado M.I., Oller I., Gernjak W., Sànchez-Pèrez J.A., Ballesteros M.M., Malato S. (2005). Solar photocatalytic degradation and detoxification of EU priority substances. *Catalysis Today*, 101, 203–210.

Hinkel G.K. (1965). Oral dichloroethane intoxication of children. *Dtsch Gesudheitswes*., 20, 1327-1331.

Hoigné J., Bader H. (1983). Rate constants of reaction of ozone with organic and inorganic compounds in water. Part II. Dissociating organic compounds, *Wat. Res*., 17, 185.

Holler S., Trosch W. (2001). Treatment of urban wastewater in a membrane bioreactor at high organic loading rates. *Journal of Biotechnology*, 92, 95–101.

Holliger C., Schraa G., Stams A.J.M., Zehnder A.J.B. (1990). Reductive dechlorination of 1,2-dichloroethane and chloroethane by cell suspensions of methanogenic bacteria. *Biodegradation* 1(4), 253-261.

Hollingsworth R.L., Rowe V.K., Oyen F., Torkelson T.R., Adam E.M. (1958). Toxicity of o-dichlorobenzene; studies on animals and industrial experience. *Arch. Ind. Health*, 17, 180-187.

Howard C.J., Evenson K.M. (1976). Rate constants for the reactions of OH with ethane and some halogen substituted ethanes at 296 K. *J. Chem. Phys*., 64, 4303-4306.

Howard P.H. (1989). Handbook on environmental fate and exposure data for organic chemicals. Vol. II, Lewis Pub., Michigan, USA.

Huang H., Lu M., Chen J. (2001). Catalytic decomposition of hydrogen peroxide and 2-chlorophenol with iron oxides. *Wat. Res*., 35(9), 2291–2299.

Inguva S., Shreve G.S. (1999). Biodegradation kinetics of trichloroethylene and 1,2-dichloroethane by Burkholderia (Pseudomonas) cepacia PR020 and Xanthobacter autotrophicus GJ10. *Int. Biodet. Biodeg.*, 43, 57-61.

Janssen, D. B., Scheper A., Witholt B. (1984). Biodegradation of 2-chloroethanol and 1,2-dichloroethane by bacterial cultures. *Prog. Ind. Microbiol*., 20, 169-178.

Janssen, D. B., Scheper A., Dijkhuizen L., Witholt B. (1985). Degradation of halogenated aliphatic compounds by Xanthobacter autotrophicus GJ10. *Appl. Environ. Microbiol*. 49, 673-677.

Jechorek M., Wendlandt K.-D., Beck M. (2003). Cometabolic degradation of chlorinated aromatic compounds. *Journal of Biotechnology*, 102, 93-98.

Johnston J.J., Borden R.C.,. Barlaz M.A (1996). Anaerobic biodegradation of alkylbenzenes and trichloroethylene in aquifer sediment down gradient of a sanitary landfill. *Journal of Contaminant Hydrology*, 23, 263-283.

Jorgensen K.S., Pauli A.S.-L. (1995). Polyphosphate Accumulation among Denitrifying Bacteria in Activated sludge. *Anaerobe*, 1(3), 161-168.
Judd S. (2002). Submerged membrane bioreactors: flat plate or hollow fibre? *Filtration+Separation*. www.filtsep.com

Kang I.J., Lee C.H., Kim K.J. (2003). Characteristics of microfiltration membranes in a membrane
coupled sequencing batch reactor system. *Wat. Res.*, 37, 1192–1197.

Kao C.M., Prosser J. (1999). Intrinsic bioremediation of trichloroethylene and chlorobenzene: field and laboratory studies. *J. Haz. Mat.*, B69, 67–79.

Kao C.M., Chen S.C., Su M.C. (2001). Laboratory column study for evaluating a barrier system for providing oxygen and substrate for TCE biodegradation. *Chemosph.*, 44 925-934.

Kerrn-Jespersen J.P., Henze M. (1993). Biological phosphorus uptake under anoxic and aerobic conditions. *Wat. Res.*, 27(4), 617-624.

Kim J.-H., Suh D.J.,. Park T.-J,. Kim K.-L. (2000). Effect of metal particle size on coking during CO_2 reforming of CH_4 over Ni–alumina aerogel catalysts. *Appl. Catal. A*, 197, 191-200.

Kim J.S., Lee C.H., Chang I.S. (2001). Effect of pump shear on the performance of a crossflow membrane bioreactor. *Wat. Res.*, 35(9), 2137–2144.

Kim M.H., Hao O.J. (1999). Cometabolic degradation of chlorophenols by acinetobacter species. *Wat. Res.*, 33(2), 562-574.

Kiwi J., Pulgarin C., Peringer P., Gratzel M. (1993). Beneficial effect of homogeneous photo-Fenton pretreatment upon the biodegradation of anthraquinone sulfonate in wastewater treatment. *Appl. Catal. B: Environ.*, 3, 85.

Klecka G.M., Carpenter C.L., Gonsior S.J. (1998). Biological transformations of 1,2-dichloroethane in subsurface soils and groundwater. *J. Cont. Hydr.*, 34, 139–154.

Kong I.-C. (1998). Metal toxicity on the dechlorination of monochlorophenols in fresh and acclimated anaerobic sediment slurries. *Wat. Sci. Tech.*, 38(7), 143-150.

Krishnamoorthy S., Baker J.P., Amiridis M.D. (1998). Catalytic oxidation of 1,2-dichlorobenzene
over V 2 O 5 /TiO 2 -based catalysts. *Catal. Today*, 40, 39.

Krishnamoorthy S., Amiridis M.D. (1999). Kinetic and in situ FTIR studies of the catalytic oxidation of 1,2-dichlorobenzene over V2O5/Al2O3 catalysts. *Catal. Today*, 51, 203.

Krishnamoorthy S., Rivas J.A., Amiridis M.D. (2000). Catalytic Oxidation of 1,2-Dichlorobenzene over Supported Transition Metal Oxides. J. Catal., 193, 264.

Kuba T., Murnleitner E., Van Loosdrecht M.C.M., Heijnen J.J. (1996). A Metabolic Model for Biological Phosphorus Removal by Denitrifying Organisms. *Biotech. Bioeng.*, 52(6), 685-695.

Kraume M., Bracklow U., Vocks M., Drews A. (2005). Nutrients Removal In MBRs For Municipal Wastewater Treatment. *Wat. Sci. Tech.*, 51(6-7), 391-402.

Kuo C.-W.,. Sharak Genthner B.R. (1996). Effect of Added Heavy Metal Ions on Biotransformation and Biodegradation of 2-Chlorophenol and 3-Chlorobenzoate in Anaerobic Bacterial Consortia. *Appl. Environ. Microbiol.*, 62(7) 2317–2323.

Le-Clech P., Jefferson B., Judd S.J. (2003) Impact of aeration, solids concentration and membrane characteristics on the hydraulic performance of a membrane bioreactor. *J. Membrane Sci.*, 218, 117–129.

Lenczewski M., Jardine P., McKay L., Layton A. (2003). Natural attenuation of trichloroethylene in fractured shale bedrock. *J. Cont. Hydr*, 64, 151– 168.

Leisinger, T. (1983). Microorganisms and xenobiotic compounds. *Experientia*, 39, 1183-1191.

Li N., Descorme C., Besson M. (2007a). Ru/$Ce_xZr_{1\_x}O_2$, a novel and effective catalyst for the catalytic wet air oxidation of 2-chlorophenol. *Catal. Commun.*, 8, 1815–1819.

Li N., Descorme C., Besson M. (2007b). Catalytic wet air oxidation of 2-chlorophenol over Ru loaded $Ce_xZr_{1\_x}O_2$ solid solutions. *Appl. Catal. B: Env.*, 71(3-4), 262–270.

Liu R., Huang X., Feng S.Y., Qian Y. (2003). Hydrodynamic effect on sludge accumulation over membrane surfaces in a submerged membrane bioreactor. *Proc. Biochem.*, 39, 157-163.

López-Fonseca R., Aranzabal A., Steltenpohl P., Gutiérrez-Ortiz J.I., González-Velasco J.R. (2000).
Performance of zeolites and product selectivity in the gas-phase oxidation of 1,2-dichloroethane. *Catal. Tod.*, 62, 367–377.

Lorah M.M., Olsen L.D., Capone D.G., Baker J.E. (2001). Biodegradation of Trichloroethylene and Its Anaerobic Daughter Products in Freshwater Wetland Sediments. *Biorem. J.*, 5(2), 101-118.

Lu M., Chen J., Huang H. (2002). Role of goethite dissolution on the oxidation of 2-chlorophenol with hydrogen peroxide. *Chemosph.*, 46, 131-136.

Lu C., Lee C., Huang C. (1996). Biodegradation of chlorophenols by immobilized pure-culture microorganisms. *Wat. Sci. Tech.*, 34(10), 67-72.

Magara Y., Itoh M. (1991). The effect of operational factor on solid/liquid separation by iltra-membrane filtration in a biological denitrification system for collected human excreta treatment plants. *Wat. Sci. Tech.*, 23, 1583-1590.

Majumder P.S., Gupta S.K. (2007). Effect of carbon sources and shock loading on the removal of chlorophenols in sequential anaerobic–aerobic reactors. *Biores. Tech.* In press.

Madsen T., Licht D. (1992). Isolation and Characterization of an Anaerobic Chlorophenol-Transforming Bacterium. *Appl. Environ. Microbiol.*, 58(9), 2874-2878.

Martin G., Knorpp K., Huth K., Heinricht F., Mittermayer C. (1968) Clinical features, pathogenesis and therapy of dichloroethane poisoning. *Dtsch med. Wschr*., 93, 2002-2010.

Menschick H. (1957) Acute inhalation intoxications by symmetric dichloroethane. *Arch. Gewerbepathol. Gewerbehyg*., 15, 241-252.

Mercier-Bonin M., Daubert I., Leonard D., Maranges C., Fonade C., Lafforgue C. (2001) How unsteady filtration conditions can improve the process efficiency during cell cultures in membrane bioreactors. *Separation and Purification Technology*, 22-23, 601–615.

Mino T., Liu W., Kurisu F., Matsuo T. (1995) Modelling glycogen storage and denitrification capability of microorganisms in enhanced biological phosphate removal processes. *Wat. Sci. Tech.*, 31(2), 25-34.
Misra C., Gupta S. K. (2001). Hybrid reactor for priority pollutant-trichloroethylene removal. *Wat. Res*., 35(1), 160-166.

Mikkelson K.A. (1995). AquaSBR Design Manual. Aqua-Aerobic Systems.

Monferran M.V.,. Echenique J.R, Wunderlin D.A. (2005) Degradation of chlorobenzenes by a strain of Acidovorax avenae isolated from a polluted aquifer. Chemosphere 61, 98–106.

Morozov G.N. (1958). On acute dichloroethane poisoning. *Farmakol. Toksicol*., 21(1), 76-78.

Morgan J.W., Forster C.F., Evison L.M. (1990). A comparative study of the nature of biopolymers extracted from anaerobic and activated sludges. *Wat. Res.*, 6, 743-750.

Murnleitner E., Kuba T., Van Loosdrecht M.C.M., Heijnen J.J. (1997). An Integrated Metabolic Model for the Aerobic and Denitrifying Biological Phosphorus Removal. *Biotech. Bioeng.*, 54(5), 434-450.

Motosugi, K., Soda K. (1983) Microbial degradation of synthetic organochlorine compounds. *Experientia*, 39, 1214-1220.

Munoz I., Rieradevall J., Torrades F., Peral J., Domènech X. (2006) Environmental assessment of different advanced oxidation processes applied to a bleaching Kraft mill effluent. *Chemosph.*, 62, 9–16.

Musialik-Piotrowska A., Mendyka B. (2004). Catalytic oxidation of chlorinated hydrocarbons in two-component mixtures with selected VOCs. *Catal. Tod.*, 90, 139-144.

National Water Research institute and Orange County Water District (1994). Microfiltration for water treatment. August 25 & 26, Irvine, California.

Nocentini M., Frascari D., Zannoni A. (2005). Processi cometabolici in falda (Cometabolic processes in groundwater), in Bonifica dei siti contaminati, ed. McGraw-Hill, Milan):

Nobre Rosane C.M., Nobre Manoel M.M. (2004). Natural attenuation of chlorinated organics in a shallow sand aquifer. *J. Haz. Mat.*, 110, 129–137.

Ognier S., Wisniewski C., Grasmick A. (2002a) Membrane fouling during constant flux filtration in membrane bioreactors. *Membr. Tech.*, 7, 6-10.
Ognier S., Wisniewski C., Grasmick A. (2002b). Influence of macromolecule adsorption during filtration of a membrane bioreactor mixed liquor suspension. *J. Membrane Sci.*, 209. 27–37.

Ognier S., Wisniewski C., Grasmick A. (2004). Membrane bioreactor fouling in sub-critical filtration conditions: a local critical flux concept. *J. Membrane Sci*, 229, 171–177.

Ollis D., Al-Ekabi H. (1993). Photocatalytic Purification of Water and Air, Elsevier, New York.

Papenmeier, D.M., Rossin, J.A. (1994). Catalytic oxidation of dichloromethane, chloroform and their binary mixtures over a platinum alumina catalyst. *Industrial and Engineering Chemistry Research*, 33, 3094–3103.

Pearson C.R. and McConnell G. (l975). Chlorinated C1 and C2 hydrocarbons in the marine environment. *Proc. R. Soc. Lond. B*, 189, 305-332.

Pereira H., Lemos P.C., Reis M.A.M., Crespo J.P.S.G., Carrondo M.J.T., Santos H. (1996). Model for carbon metabolism in biological phosphorus removal processes based on *in vivo*^{13}C-NMR labelling experiments. *Wat. Res.*, 30(9), 2128-2138.

Pèrez-Moya M., Graells M., del Valle L.J., Centelles E., Mansilla H.D. (2007). Fenton and photo-Fenton degradation of 2-chlorophenol: Multivariate analysis and toxicity monitoring. *Catal. Tod.*, 124, 163–171.

Petersen B., Temminik H., Henze M., Isaacs S. (1998). Phosphate uptake kinetics in relation to PHB under aerobic conditions. *Wat. Res.*, 32(1), 91-100.

Peyton G.R., Glaze W.H. (1988). Destruction of pollutants in water with ozone in combination with ultraviolet radiation. Part 3. Photolysis of aqueous ozone. *Environ. Sci. Technol*., 22, 761-767.

Pijuan M., Saunders A.M., Guisasola A., Baeza J.A., Casas C., Blackall L.L. (2004). Enhanced Biological Phosphorus Removal in a Sequencing Batch Reactor Using Propionate as the Sole Carbon Source. *Biotech. Bioeng.*, 85(1), 56-67.

Pijuan M., Guisasola A., Baeza J.A., Carrera J., Casas C., Lafuente J. (2005). Aerobic phosphorus release linked to acetate uptake: Influence of PAO intracellular storage compounds. *Biochem. Eng. J.*, 26 (2-3), 184-190.

Poplawski K., Lichtenberger J., Keil F.J., Schnitzlein K.,. Amiridis M. D. (2000). Catalytic oxidation of 1,2-dichlorobenzene over ABO3-type perovskites. *Catal. Tod.*, 62, 329–336.

Pries F., van der Ploeg J.R., Dolfing J., Janssen D.B. (1994). Degradation of halogenated aliphatic compounds: the role of adaptation. *FEMS Microbiol. rev.*, 15, 279-295.

Pulgarin C., Kiwi J. (1996). Overview on photocatalytic and electrocatalytic pretreatment of industrial non-biodegradable pollutants and pesticides, *Chimia*, 50, 50.

Radding S.B., Lui D.H., Johnson H.L., Mill, T. (1977). Review of the environmental fate of selected chemicals. Washington DC, US Environmental Protection Agency, Office of Toxic Substances (EPA report no. 560/5-77-003, PB 267121).

Rajeshwar K. (1995). Photoelectrochemistry and the environment. *J. Appl. Electrochem.*, 25, 1067-1082.

Rapp P., Timmis K.N. (1999). Degradation of Chlorobenzenes at Nanomolar Concentrations by Burkholderia sp. Strain PS14 in Liquid Cultures and in Soil. *Appl. Environ. Microbiol.*, 65(6) 2547–2552.

Reemtsma T., Jekel M. (1997) Dissolved organics in tannery wastewaters and their alteration by a combined anaerobic and aerobic treatment, *Wat. Res.*, 31(5), 1035.

Rehfuss M., Urban J. (2005). Rhodococcus phenolicus sp. nov., a novel bioprocessor isolated actinomycete with the ability to degrade chlorobenzene, dichlorobenzene and phenol as sole carbon sources. *Syst. Appl. Microb.*, 28, 695–701.

Reineke W., Knackmuss H.J. (1984). Microbial Metabolism of Haloaromatics: Isolation and Properties of a Chlorobenzene-Degrading Bacterium. *Appl. Environ. Microbiol.*, 395-402.

Ridgway H.F., Flemming H.C. (1996). Biofouling of membranes. In Mallevialle J., Odendaal P.E., Wiesner M.R., Water Treatment membrane processes, Mc Graw Hill, New York, 6.1-6.62.

Field R.W., Wu D., Howell J.A., Gupta B.B. (1995). Critical flux concept for microfiltration fouling. *J. Membrane Sci*., 100, 259–272.

Rodrìguez S., Gàlvez J., Manuel I., Rubio M., Ibànez P., Gernjak W., Alberola I. (2005). Treatment of chlorinated solvents by TiO2 photocatalysis and photo-Fenton: influence of operating conditions
in a solar pilot plant. *Chemosph.*, 58, 391–398.

Rossin, J.A., Farris, M.M. (1993). Catalytic oxidation of chloroform over a 2% platinum alumina catalyst. *Ind. Eng. Chem. Res.*, 32, 1024–1029.

Safarzadehet-Amiri A., Bolton J.R., Cater S.R. (1997). Ferrioxalatemediated photodegradation of organic pollutants in contaminated water. *Wat. Res.*, 31(4) 787.

Sander P., Wittich R.-M., Fortnagel P., Wilkes H., Francke W. (1991). Degradation of 1,2,4-Trichloro- and 1,2,4,5-Tetrachlorobenzene by Pseudomonas Strains. *Appl. Environ. Microbiol.*, 57(5) 1430-1440.

Scherb, K. (1978). Investigations on the evaporation of low molecular weight chlorinated hydrocarbons from flow-gutter. Munch. *Beitr. Abwasser-Fisch. Flussbiol.*, 30, 235-248.

Schmidt E., Hellwig M., Knackmuss H.-J. (1983). Degradation of Chlorophenols by a Defined Mixed Microbial Community. *Appl. Environ. Microbiol.*, 46(5) 1038-1044.

Scholz W. and Fuchs W. (2000). Treatment of oil contaminated wastewater in a membrane bioreactor. *Wat. Res*., 34(1414), 3621-3629.
Schonborn H., Prellwitz W., Baum P. (1970). Consumption coagulation pathology of 1,2-dichloroethane poisoning. *Klin. Wochenschr*., 48, 822-824.

Schraa G., Boone M.L., Jetten M.S.M.,. Van Neerven A.R.W., Colberg P.J., Zehnder A.J.B. (1986). Degradation of 1,4-Dichlorobenzene by Alcaligenes sp. Strain A175. *Appl. Environ. Microbiol.*, 52(6), 1374-1381.

Scott J.A., Neilson D.J., Liu W., Boon P.N. (1998). A dual function membrane bioreactor system for enhanced aerobic remediation of high-strength industrial waste. *Wat. Sci. Tech.*, 38(4-5), 413-420.

Seignez C., Vuillemin A., Adler N., Peringer P. (2001). A procedure for production of adapted bacteria to degrade chlorinated aromatics. *J. Haz Mat.*, B84, 265–277.

Semprini L. (1997). In situ transformation of halogenated aliphatic compounds under anaerobic conditions. In: Ward C.H., Cherry J.A., Scalf M.R. (Eds.) Subsurface Restoration, AnnArbor Press Inc., Chelsea, MI, pp 429-450.

Seo G.T., Lee T.S., Moon B.H., Choi K.S., Lee H.D. (1997). Membrane separation activated sludge for residual organic removal in oil wastewater. *Wat. Sci. Tech.*, 36(12), 275-282.

Shim H., Ryoo D., Barbieri P., Wood T.K. (2001). Aerobic degradation of mixtures of tetrachloroethylene, trichloroethylene,dichloroethylenes and vinyl chloride by toluene-o xylene monoxygenase of Pseudomonas stutzeri OX1. *Appl. Microbiol. Biotechnol.*, 56, 265-269

Shimizu Y., Okuno Y.I., Uryu K., Ohtsubo S., Watanabe A. (1996). Filtration characteristics of hollow fibres microfiltration membranes used in membrane bioreactor for domestic wastewater treatment. *Wat. Res.*, 30(10), 2385-2392.

Smolders G.J.F., Van der Meij J., Van Loosdrecht M.C.M., Heijnen J.J. (1994a). Model of the Anaerobic Metabolism of the Biological Phosphorus Removal Process: Stoichometry and pH Influence. *Biotech. Bioeng.*, 43(6), 461-470.

Smolders G.J.F., Van der Meij J., Van Loosdrecht M.C.M., Heijnen J.J. (1994b). Stoichometric Model of the Aerobic Metabolism of the Biological Phosphorus Removal Process. *Biotech. Bioeng.*, 44(7), 837-848.

Sorm R., Bortone G., Saltarelli R., Jenicek P., Wanner J., Tilche A. (1996). Phosphate uptake under anoxic conditions and fixed-film nitrification in nutrient removal activated sludge system. *Wat. Res.* 30(7), 1573-1584.

Sorm, R.; Bortone, G.; Wanner, J.; Tilche, A. (1998). Behaviour of activated sludge from a system with anoxic phosphate uptake. *Wat. Sci. Tech.*, 37(4-5), 563-566.

Spain J.C., Nishino S.F. (1987). Degradation of 1,4-Dichlorobenzene by a Pseudomonas sp. *Appl. Environ. Microbiol.*, 53(5) 1010-1019.

Spence J.W. and Hanst P.L. (1978). Oxidation of chlorinated ethanes. *J. Air Pollut. Control Assoc.*, 28, 250-253.

Spiess E., Sommer C., Gorisch H. (1995). Degradation of 1,4-Dichlorobenzene by Xanthobacter Flavus 14p1. *Appl. Environ. Microbiol.*, 61(11), 3884–3888.

Stepnowski P., Siedlecka E.M., Behrend P., Jastorff B. (2002). Enhanced photo-degradation of contaminants in petroleum refinery wastewater. *Wat. Res.*, 36. 2167–2172.

Stucki, G., Leisinger T. (1983). Bacterial degradation of 2-chloroethanol proceeds via 2-chloroacetic acid. *FEMS Microbiol. Lett*., 16, 123-126.

Suh D.J., Park T.-J., Lee S.-H.,. Kim K.-L. (2001). Nickel–alumina composite aerogels as liquid-phase hydrogenation catalysts. *J. Non-Cryst. Solids*, 285, 309-316.

Sun B., Cole J.R., Sanford R.A., Tiedje J.M. (2000). Isolation and Characterization of Desulfovibrio dechloracetivorans sp. nov., a Marine Dechlorinating Bacterium Growing by Coupling the Oxidation of Acetate to the Reductive Dechlorination of 2-Chlorophenol. *Appl. Environ. Microbiol.*, 66(6) 2408–2413.

Suveev I.M., and Babichenko M.E. (1969). On the clinic and cure of acute intoxication with dichloroethane vapours. *Gig. Tr. Prof. Zabol.*, 13: 50-51.

Symons J.M., Prengle J.W., Belhateche D. (2007). Use of ultraviolet irradiation and hydrogen peroxide for the control of solvent contamination in small water utilities. *Journal of Hazardous Materials*, 22(2), 265-266**.**

Tang W.Z., Huang C. P. (1995). The effect of chlorine position of chlorinated phenols on their dechlorination kinetics by Fenton's reagent. *Waste Man.*, 15(8), 615-622.

Tang W.Z., Huang C. P. (1996). Effect of Chlorine Content of Chlorinated Phenols on Their Oxidation Kinetics by Fenton's Reagent. *Chemosph.*, 33(8), 1621-1635.

Taralunga M., Mijoin J., Magnoux P. (2006). Catalytic destruction of 1,2-dichlorobenzene over zeolites. *Catal. Commun.*, 7, 115–121.

Tartakovsky B., Manuel M.-F., Guiot S.R. (2005). Degradation of trichloroethylene in a coupled anaerobic–aerobic bioreactor: Modeling and experiment. *Biochem. Eng. J.*, 26, 72–81.

Tolot F., Soubrier B., Bresson J.-R., Martin P. (1969). Myélose proliférative d'évolution rapide. Rôle étiologigue possible des dérivés chlorés du benzène. *J. Med. Lyon 1164(3F)*, 761-768.

Tom Kuo M.C., Liang K.F., Han Y.L., Fan K.C. (2004). Pilot studies for in-situ aerobic cometabolism of trichloroethylene using toluene-vapor as the primary substrate. *Wat. Res.*, 38, 4125–4134.

Toxicological Profile for Trichloroethylene (Update) (1997). Agency for Toxic Substances and Disease Registry (ATSDR). Atlanta, GA.

Tovanabootr A., Semprini L. (1998). Comparison of TCE Transformation Abilities of Methane- and Propane-Utilizing Microorganisms. *Biorem. J.*, 2(2), 105-124.

Troisi F.M., Cavallazzi D. (1961). A fatal case of poisoning from inhalation of dichloroethane vapours. *Med. Lav.*, 52, 612-618.

Tsuneda S., Ohno T., Soejima K., Hirata A. (2006). Simultaneous nitrogen and phosphorus removal using denitrifying phosphate accumulative organisms in a sequencing batch reactor. *Biochem. Eng. J.*, 27(3), 191-196.

Ueda T., Hata K., Kikuoka Y. (1996). Treatment of domestic sewage biodegradation of alcohol ethoxylate nonionic surfactants by Pseudomoas sp. strain SC25A. *Microbiol.*, 142, 1123-1131.

Ueda T., Hata K., Kikuoka Y., Seino O. (1997). Effects of aeration on suction pressure in a submerged membrane bioreactor. *Wat. Res.*, 31(3), 489-494.

Ueda T., Hata K. (1999). Domestic wastewater treatment by a submerged membrane bioreactor with gravitational filtration. *Wat. Res.*, 33(12), 2888-2892.

Van Bentem A., Lawrence D., Horjus F., De Boer R., Koorneef E., Van Efferen B. (2001). MBR pilot research in Beverwijk: side studies. *H_2O*, October 2001.

Van den wijngaard A.J., Koen w. H., Van der kamp J., van der ploeg J., Pries F., Kazemier B, Janssen D.B. (1992). Degradation of 1,2-Dichloroethane by Ancylobacter aquaticus and Other Facultative Methylotrophs. *Appl. Environ. Microbiol.*, 58(3), 976-983

van Dijk L., Roncken G.C.G. (1997). Membrane bioreactors for wastewater treatment: the state of the art and new developments. Wat. Sci. Tech., 35(10), 35-41.

Verce M.F., Gunsch C.K.,, Danko A.S., Freedman D.L. (2002). Cometabolism of cis-1,2-dichloproethylene by aerobic cultures grown on vinyl chloride as the primary substrate. *Env. Sci. Tech.*, 36, 2171-2177.

Volcik Viktor, Hoffmann J., Ruzicka J., Sergejevova M. (2005). Trichloroethylene (TCE) removal in a single pulse suspension bioreactor. *J. Env. Manag.*, 74, 293–304.

Wang, Y., Shaw, H., Farrauto, R.J. (1992). Catalytic oxidation of trace concentrations of trichloroethylene over 1, 5% platinum on g-Alumina. Catalytic Control of Air Pollution. Mobile and Stationary Sources, Washington, pp. 125–140.

Yeh C., Wu H., Chenb T. (2003). Chemical oxidation of chlorinated non-aqueous phase liquid by hydrogen peroxide in natural sand systems. *J. Haz. Mat.,* B96, 29–51.

Yodaiken R.E., Babcock J.R. (1973). 1,2-Dichloroethane poisoning. *Arch. environ. Health*, 26, 281-284.

Yoon S.H., Kim H.S., Yeom I.T. (2004). The optimum operational condition of membrane bioreactor (MBR): cost estimation of aeration and sludge treatment. *Wat. Res.* 38 37–46.

Yu T.-Ch., Shaw, H., Farrauto, R.J. (1992). Catalytic oxidation of trichloroethylene over PdO catalyst on g-Al2O3. Catalytic Control of Air Pollution. *Mobile and Stationary Sources, Washington*, 141–152.

Yu S., Semprini L.(2002). Comparison of trichloroethylene reductive dehalogenation by microbial communities stimulated on silicon-based organic compounds as slow-release anaerobic substrates. *Wat. Res.*, 36, 4985–4996.

Weiss F. (1957). Lethal oral intoxications by dichloroethane. *Gewerbehygiene,* 15, 253-264.

Wilkes S., Brindle K., Passe D., Rosenberger S. (1999). The preliminary evaluation of a submerged membrane bioreactor based on a large diameter hollow fibre membranes for treatment of raw sewage. *Proc 2nd International Meeting on Membrane Bioreactors for Wastewater Treatment*, Cranfield University, Cranfield, UK.

Winnen H., Suidan M.T., Scarpino P.V., Wrenn B., Cicek N., Urbain V., Manem J. (1996). Effectiveness of the membrane bioreactor in the biodegradation of high molecular-weight compounds. *Wat. Sci. Tech.*, 34(9), 197-203.

Wirtschafter Z.T., Schwartz W.D. (1939). Acute ethylene dichloride poisoning. *J. Ind. Hyg. Toxicol.*, 21, 126-131.

Wisniewski C., Grasmick A. (1998). Floc size distribution in a membrane bioreactor and consequences for membrane fouling. *Coll. Surf. A*, 138 403–411

Wu W.-M., Nye J., Jain M.K., Hickey R.F. (1998). Anaerobic dechlorination of trichloroethylene (TCE) to ethylene using complex organic materials. *Wat. Res.*, 32(5), 1445-1454.

www.aicr.org.uk

www.atsdr.cdc.gov

www.epa.gov

www.frtr.gov

www.hc-sc.gc.ca

www.mallchem.com

www.speclab.com

www.titaniumart.com

Zannoni A., Frascari D., Pinelli D., Nocentini M., Fedi S., Zannoni D. (2004). Cometabolic treatment of chlorinated solvents: influence of operational conditions on the adaptation of indigenous biomass. In: Magar V.S., Kelley M.E. (Eds.) In situ and on-site bioremediation – 2003. Proceedings of the seventh international In Situ and On-site bioremediation Symposium (Orlando FL; June 2003), published by Battelle Press, Columbus, OH, Paper A-39 (on CD).

Zapata-Gayon C., Zapata-Gayon N., Gonzalez-Angulo A. (1982). Clastogenic chromosomal aberrations in 26 individuals accidentally exposed to ortho dichlorobenzene vapours in the National Medical Centre in Mexico City. *Arch. Environ. Health*, 37(4), 231-235.

Zhang S., van Houten R., Eikelboom D.H., Doddema H., Jiang Z., Wang Y.F (2003). Sewage treatment by a low energy membrane bioreactor. *Biores. Tech.*, 90,185–192.

Zhang Y., Crittenden J.C., Hand D.W. (1994). The solar photocatalytic decontamination of water. *Chem. Ind*. 19, 714-717.