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Environmental life cycle assessment of polyhydroxyalkanoates production from cheese whey

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Abstract

Cheese whey (CW) is the main by-product of the dairy industry and is often considered one of the main agro-industrial biowaste streams to handle, especially within the European Union, where the diary activities play an essential role in the agrarian economy. In the paper, Life Cycle Assessment (LCA) is used to analyse the feasibility of producing polyhydroxyalkanoates (PHA) as the main output of an innovative CW valorisation route which is benchmarked against a conventional anaerobic digestion (AD) process. To this aim, the LCA inventory data are derived from lab-scale PHA accumulation tests performed on real CW, while data from the literature of concern are used for modelling both the PHA extraction from the accumulating biomass and for the alternative CW valorisation through AD. The comparison shows that AD would have better environmental performances than the baseline PHA production scenario. For example, the climate change indicator values result 44.8 and -35.7 kg CO_2 eq./t CW for the baseline PHA recovery and AD, respectively. LCA proved to be a useful tool to highlight the weak points of

innovative processes and suggest proper improvements. Once improved and again analysed through the LCA, the PHA production process from CW shows that environmental performance comparable to AD may be achieved. With reference, again, to the climate change indicator the value can be reduced to -50.3 kg CO_2 eq./t CW for the improved PHA production process.

Keywords: dairy residues; PHA; biopolymers; bioenergy; LCA; environmental footprint.

1. Introduction

Cheese whey (CW), namely the liquid effluent from the precipitation and removal of the milk casein during the cheese-making process, is the main by-product of the dairy industry (Zotta et al., 2020). The dairy activities play an essential role in the agrarian economy of the European Union (EU), and CW is considered one of the most problematic agro-industrial biowaste streams to deal with (Asunis et al., 2019a; Zotta et al., 2020). The reasons are to be ascribed to both the high specific production, around 9-10 L of CW per kg of cheese produced (Prazeres et al., 2012), which results in around $40 \bullet 10^6$ t per year only in the sole EU (Eurostat, 2018), and the high organic load of 50-100 g COD/L which represents a severe hazard for the environment (Asunis et al., 2020). Illegal practices such as direct discharge into surface water bodies cause eutrophication and acidification effects. However, negative effects can also arise from some of the most applied solutions, as direct land spreading could decrease crop yields due to oxygen depletion, while a diet characterised by excessive amounts of whey may promote several pathologies in piglets (Ahmad et al., 2019)..

The above mentioned issues commit dairy companies to treating most of the CW generated, with an obvious increase in cheese production costs and, additionally, the need to tackle various technical difficulties. Chemical-physical and aerobic biological treatments do not allow any CW valorisation and are characterized by high costs associated with reagents, energy consumption, and high sludge production (Ahmad et al., 2019; Dąbrowski et al., 2017); moreover, the

performance of aerobic treatments is heavily affected by CW rapid acidification and filamentous growth (Ahmad et al., 2019).

Anaerobic digestion (AD) represents a valid option to reduce the organic load of CW and convert it into a methane-rich biogas, thus promoting the use of renewable energy (De Gioannis et al., 2014). Many studies were conducted on AD of CW reporting organic matter removal up to 90% and specific methane production ranging from 0.3 to 0.6 Nm^3 CH_4/kg TVS (Chatzipaschali and Stamatis, 2012; Erguder et al., 2001; Escalante et al., 2017; Labatut et al., 2011; Vivekanand et al., 2018). However, significant difficulties in maintaining stable performances were also experienced (Prazeres et al., 2012). Instability is mostly due to the high organic load and low alkalinity of CW, which may result in volatile fatty acids (VFA) accumulation and inhibition of the methanogenic activity (Escalante et al., 2017). All such constraints have somehow contributed to limit the implementation of full-scale AD plants within the dairy industries as compared to other sectors. Nevertheless, the number of full-scale AD plants (0.3-3.5 MW plant size) is increasing in the European dairy industry, favoured by the growing interest in bioenergy (Asunis et al., 2020).

An alternative to overcoming the technical issues affecting the commonly practiced CW treatment options could be found in a context of biowaste management that is deeply and rapidly changing.

As a result of the EU Action plan for the Green Deal implementation, intense research has started in the field of bio-based industries, where bio-based materials and fuels are expected to replace fossil-based ones (European Commission, 2020). This perspective is of particular interest for agro-industrial residues, as they are characterized by adequate qualitative homogeneity, an important prerequisite for the application of more sophisticated processes than those intended for the demolition/stabilization of qualitatively more heterogeneous organic substrates.

The characteristics of CW may support the development of waste biorefinery schemes aimed

at producing valuable bioproducts such as, among others, hydrogen (Asunis et al., 2019b), ethanol (Pasotti et al., 2020), lactic acid (Luongo et al., 2019), succinic acid (Longanesi et al., 2018), biosurfactant (Patowary et al., 2016) and biopolymers (Colombo et al., 2016).

Currently, the increasing demand for biodegradable plastics, fuelled by stringent EU regulation and policies against fossil-based plastics along with the need for a more sustainable development and circular bioeconomy, are driving the biopolymers industry.

Among biopolymers, polyhydroxyalkanoates (PHA) are gaining attention as a sustainable alternative to petroleum-based plastics due to several properties, relating to the different possible chemical compositions, which make them suited for a wide range of applications, from packaging to pharmaceutical (Ganesh Saratale et al., 2021, Tan et al., 2021).

PHA are produced by specific bacteria which under stress conditions accumulate them inside the cell for energy and carbon storage purposes (Amaro et al., 2019; Lee, 1996). Industrial PHA production still relies on the use of pure cultures and expensive substrates, but, interestingly, they can also be produced from low-cost organic substrates by selected mixed microbial cultures (MMCs). However, PHA production from organic waste by MMCs is still in the development stage (TRL 4-6) with limited pilot-scale experiences (Estévez-Alonso et al., 2021; Kourmentza et al., 2017).

Different authors have recently investigated PHA production from CW, with promising results in terms of productivity (Colombo et al., 2016; Oliveira et al., 2018, 2017), showcasing the possibility to recover clean energy in the form of hydrogen along with biopolymers (Asunis et al., 2019a; Colombo et al., 2019).

Although the implementation of the waste biorefinery concept can open a wide array of possibilities to shift waste management towards higher sustainability, several barriers including technical, environmental, economic, logistic aspects need to be overcome (Alibardi et al., 2020). In particular, the underlying question is: do relatively complex processes producing high added-value materials make sense in the field of agro-industrial waste management? Or is it (will it

105 be) preferable to rely on well-established processes such as anaerobic digestion (Technology
106 Readiness Level - TRL of 8-9)?

107 In such a dynamic context, a case study encompassing innovative solutions for CW
108 management and valorisation requires assessing not only technical aspects but also its
109 environmental performance (Roibás-Rozas et al., 2020), and to this aim, the Life Cycle
110 Assessment (LCA) methodology appears to be the appropriate tool (Christensen et al., 2020).

111 Quite a number of scientific manuscripts are available on the application of LCA to waste
112 biorefineries. From a broad LCA perspective, some authors have compared conventional
113 management methods, such as landfilling, composting, anaerobic digestion and incineration
114 against multi-feedstock and multi-product biorefinery systems (Khoshnevisan et al., 2020;
115 Papadaskalopoulou et al., 2019). Others have compared specific biorefinery pathways for the
116 valorisation of the same type of substrate (Gullón et al., 2018). Finally, starting from different
117 feedstocks, some authors have compared the environmental performance of biorefinery systems
118 suitable for the generation of the same product (Demichelis et al., 2020).

119 Focusing on the application of LCA to PHA production, the available studies are based on
120 data from laboratory or pilot facilities and most of them deal with feedstocks such as sugar cane
121 and corn starch (Cristóbal et al. 2016). Studies considering urban biowaste as feedstock recently
122 appeared (Andreasi Bassi et al., 2021), while there is a shortage of studies on CW. Zhong et al.
123 (2009) made a LCA comparing microbial PHA manufacturing using glucose, CW, and corn as
124 the carbon substrate. They concluded that PHA manufacturing from corn leads to lower CO_2
125 emissions and lower energy consumption than PHA manufacturing from glucose or CW. Koller
126 et al. (2013) analysed a PHA production system composed of ultrafiltration, hydrolysation and
127 fermentation of whey, identifying the environmental hot spots in terms of associated impacts.
128 Considering the whole process at the laboratory scale, the electricity needs for the fermentation
129 process were identified as the largest contributor to the overall impact.

130 To the best of the authors' knowledge, only a few LCA studies are available about PHA

production using dark fermentation (DF) as a preliminary step and, in general, they refer to substrates other than CW, such as wastewater from the food industry (Gurieff and Lant, 2007), mixed wastewater from the paper and food industries (Fernández-Dacosta et al., 2015a), anaerobically digested sewage sludge from wastewater of the agri-food industries (Vogli et al., 2020), and wastewater from the fish-canning industry (Roibás-Rozas et al., 2020). All these studies are based on results from laboratory and pilot-scale experiments, complemented by literature sources when needed.

In this study, a three-stage PHA production process from CW, based on: i) preliminary DF producing H_2-rich gas, ii) selection and enrichment of PHA-accumulating biomass in an aerobic sequencing batch reactor (SBR) operated under a feast-and-famine regime, iii) PHA accumulation in a batch reactor, was evaluated using LCA.

As the first approach, the PHA production system was compared with conventional AD of CW to be considered as a sort of benchmark in terms of environmental performances.

The LCA procedure was also applied in order to highlight the aspects of the PHA production process which could be improved in terms of environmental impact, also in light of the low TRL that characterizes this process (this issue is addressed by trying to model a full industrial process and introducing the sensitivity analysis). The novelty of this paper applying LCA in the design phase, can provide an important tool to contain environmental impacts during an early-stage development of innovative technologies, speeding up the transition towards higher TRLs. This kind of study can address research investment to improve the environmental sustainability of those are identified as the weakest points and to guide policy- and decision-makers towards more circular economy paths based on effective environmental sustainability (Albizzati et al., 2021; Christensen et al., 2020).

2. Materials and methods

The LCA is carried out according to the phases defined by ISO 14040:2006 (ISO, 2006) and

ISO 14044:2006 (ISO, 2006): goal and scope definition, inventory analysis (included in the present section), impact assessment and interpretation (reported in the Results section).

For a detailed description of the laboratory-scale experimental campaign for biopolymer production from CW, the reader is referred to our previous publication (Asunis et al., 2019a).

2.1 Goal and scope definition

In the present study, the main goal is comparing, by attributional LCA, an innovative three-stage process aimed at H_2 and PHA recovery with a benchmark management approach aimed at converting the CW organic load into a methane-rich biogas through AD. In this respect, the two main scenarios considered by the analysis are:

***Baseline DF+PHA production Scenario*:** 1) DF of CW; 2) valorisation of the H_2-rich biogas in a molten carbonate fuel cell (MCFC) to produce electric energy (EE) and thermal energy (TE); 3) use of VFAs from CW fermentation as the substrate for both the selection/enrichment of a PHA-accumulating biomass and PHA accumulation by the selected biomass;

***Benchmark AD Scenario*:** 1) AD of CW; 2) use of the CH_4-rich biogas in an internal combustion engine (ICE) to produce EE and TE; 3) land spreading of the solid fraction of the digestate as a soil improver.

The secondary goal of the LCA is aimed at highlighting the role of the different phases of the PHA production process, in terms of environmental impacts, in order to identify potential weaknesses of the process layout and, in turn, propose improvements.

The functional unit adopted for the comparison is 1 t of CW (produced at approximately 40 °C), characterized by total solids (TS) and total volatile solids (TVS) contents equal to 7.6% wet weight and 92.5% of TS (dry weight), respectively (Asunis et al., 2019a).

The LCA boundaries include DF or AD, energy recovery from the H_2-rich or CH_4-rich biogas, PHA-accumulating biomass selection, PHA accumulation and extraction, centrifugation of the AD outflow, treatment of the AD effluent, treatment of the effluents from PHA

182 production/extraction (Fig. 1).

183 Inventory data for AD of CW are collected from the literature (details in the inventory), while
184 data for the DF+PHA production process derive from our previous studies (Asunis et al.,
185 2019a). The production processes for utilities, fuels, chemicals, and manufactured materials
186 entering the processes and the generated emissions are included within the system boundaries.
187 Carbon dioxide emissions from biogas combustion are of biogenic origin and are assumed to
188 be carbon neutral (Evangelisti et al., 2017; Saer et al., 2013). Impacts caused by the construction
189 of the plants are not included within the system boundaries (Evangelisti et al., 2015, 2017). The
190 multi-functional issues of the compared scenarios are addressed by system expansion (Clift et
191 al., 2000; Finnveden et al., 2009), which is the most common choice in LCA applied to waste
192 management (Laurent et al., 2014), considering the substitution of market mix products, i.e.
193 referring to average data. . In particular, the recovered EE and TE are considered to replace,
194 respectively, EE from the Italian national power grid and TE produced from the combustion of
195 natural gas. PHA and the solid fraction of digestate are assumed to replace, respectively,
196 polyethylene terephthalate (PET) and inorganic fertilizers in terms of nitrogen (N), phosphorus
197 (P), and potassium (K) to be used in agriculture.

198 Mass and energy balances are conducted considering a daily inflow of 43.78 t/day of CW,
199 namely equal to 15 980 t/y of CW[1]. Inventory data are reported per functional unit.

200 Inventories for the utilities, fuels, chemicals and manufactured materials entering the
201 processes, for substituted products (electricity, heat, etc.), and wastewater treatment are
202 retrieved from Ecoinvent 3.0 (Steubinget al., 2016; Wernet et al., 2016).

203 **2.2 Inventory analysis**

204 In this phase, the studied systems are quantitatively described according to the material and

[1] corresponding to the CW production expected from a cheese factory able to process 20 000 t/y of milk, with a specific CW yield of 0.85 L CW/L milk (17 000 t/y of CW), ready for further processing of ricotta production with a yield of around 6%.

205 energy flows across the system boundary. Inventory data are gained by modelling the processes
206 and by using literature and experimental laboratory data.

207 **2.2.1 DF+PHA production Scenario**

208 The CW is fed to the process outlined in Figure 1, and the main operating parameters are
209 summarized in Table 1. The raw CW is processed by DF in a continuous stirred-tank reactor
210 (CSTR), operated in the pH range 5.5-6.0 (Asunis et al., 2019b). The specific hydrogen
211 production (SHP) from CW fermentation is assumed to be 0.075 Nm^3 H_2/kg TVS (equivalent
212 to ~11 Nm^3 H_2-rich gas/t CW, based on an observed volumetric composition of 45% H_2 and
213 55% CO_2 (Asunis et al., 2019b)). The fermented CW (DF outflow), containing 6.5% TS and 52
214 g COD/L (Asunis et al., 2019b), is sent to a centrifuge to separate a solid rich stream (10% by
215 weight) which is sent to treatment (assumed to be at 100 km, travelled by a freight lorry with a
216 capacity of 16-32 t). The liquid fraction of the fermented CW, rich in organic acids (mostly
217 short-chain fatty acids), is divided into equal parts: 50% by weight is fed to a sequencing batch
218 reactor (SBR) for the selection/enrichment of the PHA-accumulating biomass, the remaining
219 50% to a fed-batch reactor for PHA accumulation. This distribution of the fermented CW flow
220 derives from our previous studies, and it is such as to guarantee the availability of adequate
221 PHA-accumulating biomass on the basis of the observed efficiencies of biomass selection and
222 VFA conversion into PHA (see Section 2.1 in Supplementary Material).

223 The selection of the PHA-accumulating biomass is achieved through a feast-and-famine
224 regime, i.e. alternating periods of supply (feast) and absence (famine) of the carbon source under
225 fully aerobic conditions. A continuous process requires two parallel SBRs so that when the first
226 one is in the filling phase, the second one is in the settling phase. Two full cycles per day and
227 per each reactor (12 h for each cycle with 10 h of aeration time) are considered. In order to
228 guarantee an organic loading rate (OLR) of 1.2 kg COD/m^3 d, adopted in our previous studies
229 on biomass selection, it is necessary to provide 35 m^3 of water per t of FCW (Asunis et al.,

2019a). A biomass-free effluent (about 75% of the mixture of fermented CW and water) is drawn off at the end of the SBRs settling phase and sent to subsequent conventional treatment.

PHA accumulation is attained in a fed-batch reactor by feeding the fermented CW to the enriched PHA-accumulating biomass, derived from the previous SBR accumulation stage, adopting a pulse-wise regime controlled on the basis of dissolved oxygen (DO) evolution over time (Colombo et al., 2016). The total dosed C is calculated considering that the Carbon to Microorganism ratio has to be the same as in the selection reactor (Colombo et al., 2016). The accumulation phase is stopped when no DO variation is observed after substrate feeding. Our studies highlighted the possibility of obtaining a PHA accumulation of 35-40% by dry cell weight (Asunis et al., 2019a).

After a settling phase, the biomass is sent to the PHA extraction process, which is inventoried using data from the literature and assuming the use of NaCl and NaOH for PHA recovery (Anis et al. 2013). As reported in Figure 1, the settled PHA-rich biomass is centrifuged until a final mass equal to about 10% of the starting one is obtained; the biomass is then fed to the subsequent chemical treatment (Righi et al., 2017) while the centrifuge liquid outflow, poor in biomass, is sent to treatment. The biomass concentrate rich in PHA is subjected to a series of extraction/purification cycles alternating the addition of 0.8 g NaCl/g biomass, 0.4 g NaOH/g biomass and 0.16 g C_2H_6O/g biomass in CSTR reactors at 30°C, and further centrifugation (Anis et al. 2013).

The expected PHA production is 7.6 kg PHA per t of initial CW. Further details are reported in Section 2.1 of the Supplementary Material.

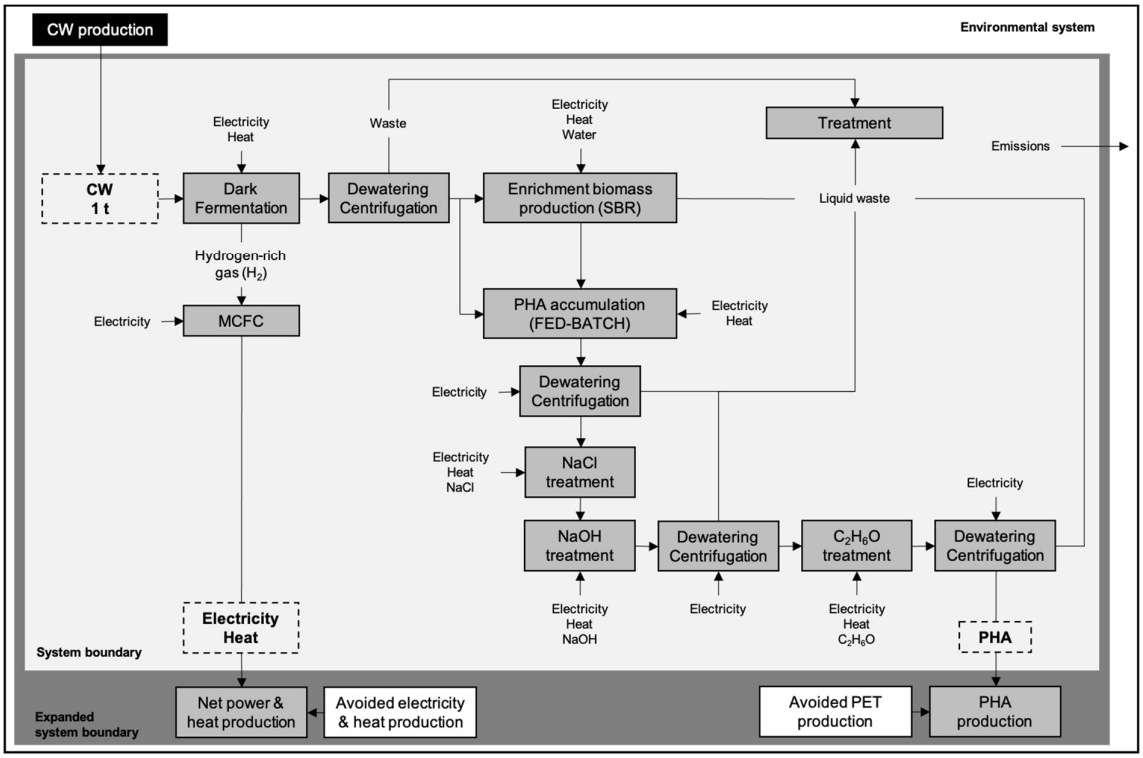


Figure 1. Processes included within the system boundaries of the baseline DF+PHA Scenario.

The analysis requires calculating the TE requirements for reactor heating, as well as the EE for stirring and pumping (Piccinno et al., 2016). The EE required for air insufflation into the SBR and the PHA accumulation reactors can be estimated in 0.5 kWh/kg O_2 (Metcalf & Eddy et al., 2013), while that of the different centrifugation stages in 5 kWh per t entering the centrifuge (Lombardi et al. al., 2017). Further details about the energy consumption are reported in Section 1 of the Supplementary Material.

The stoichiometric amount of O_2 required for aeration is calculated from the OLR (1.5 kg COD/m^3 d for the SBRs - Table 1), assuming an additional 25% excess.

The H_2-rich biogas is fed to a MCFC, with an EE efficiency of 0.5, a TE efficiency of 0.37 and an EE self-consumption of 0.02 (Carnevale and Lombardi, 2015). The energy produced is not enough to cover the energy demand of the whole process, in terms of both EE and TE (Table 2). The MCFC stack emissions are calculated by adopting an emission factor of 3.15E-06 kg

269 NO_x/Nm^3 H_2-rich gas (U.S. Environmental Protection Agency, 2015).

270 This scenario represents the baseline option regarding the production of PHA from CW, and
271 it will be identified with the abbreviation DF + PHA (standing for combination of dark
272 fermentation and PHA production); for the sake of simplicity, the associated H_2 production is
273 not always called up, unless required.

274

275 **Table 1**. Operating parameters for the baseline DF+PHA Scenario and for the benchmark AD Scenario

Operating parameters for the baseline DF+PHA Scenario			
Dark Fermentation reactor			
Parameter	Unit	Value	Reference
Flow rate	t/d	43.78	
Volume*	m^3	170	-
Hydraulic retention time (HRT)	d	3	(Valentino et al., 2017)
OLR	kg TVS/ m^3 d	23.43	
Operating temperature	°C	35	-
SHP	Nm^3 H_2/kg TVS	0.075	(Asunis et al., 2019b)
H_2-rich gas production	Nm^3/t CW	11	-
Volumetric composition of H_2-rich gas	%	45% H_2 - 55% CO_2	(Asunis et al., 2019b)
Lower heating value (LHV) of H_2-rich gas	kJ/Nm^3	11109	-
Selection/enrichment of PHA-accumulating biomass reactor			
Flow rate	t/d	709.25	
Number of reactors	-	2	-
Volume of each reactor	m^3	522	-
HRT	d	1	(Asunis et al., 2019a; Valentino et al., 2017)
OLR	kg COD/m^3 d	1.2	(Asunis et al., 2019a)
Operating temperature	°C	25	(Asunis et al., 2019a)
PHA accumulation reactor			
Flow rate	t/d	172.54	
Number of reactors	-	2	-
Volume	m^3	128	-
HRT	d	0.2	(Valentino et al., 2017)
OLR	kg COD/m^3 d	14	Valentino et al., 2017)
Operating temperature	°C	25	-
* calculated with a safety factor with respect to the volume calculated as V= flow rate•HRT (Piccinno et al., 2016)			

Operating parameters for the benchmark AD Scenario			
Anaerobic digestion reactor			
Parameter	Unit	Value	Reference
Flow rate	t/d	43.78	
Volume*	m^3	1045	-
HRT	d	20	(Asunis et al., 2020; Chatzipaschali and Stamatis, 2012; Erguder et al., 2001; Escalante et al., 2017; Labatut et al., 2011; Prazeres et al., 2012)
OLR	kg TVS/m^3 d	3.5	-
Operating temperature	°C	35	-
SMP	Nm^3 CH_4/kg TVS	0.3	(Asunis et al., 2020; Chatzipaschali and Stamatis, 2012; Erguder et al., 2001; Escalante et al., 2017; Labatut et al.,

			2011; Prazeres et al., 2012)
Biogas yield	Nm^3 biogas/t CW	32	-
Biogas composition (by vol.)	%	65% CH_4 - 35% CO_2	(Escalante et al., 2017)
LHV of methane	kJ/Nm^3	35220	-

* calculated with a safety factor with respect to the volume calculated as V= flow rate•HRT (Piccinno et al. 2016)

2.2.2 AD Scenario

AD is assumed to be carried out in single stage CSTR (Figure 2) operated as summarised in the second part of Table 1. The digestate outflow has a TS content of 4% wet weight. The specific methane production (SMP) of CW is assumed equal to 0.3 Nm^3 CH_4/kg TVS (or 32 Nm^3 biogas/t CW, considering a volumetric composition of biogas of 65% CH_4 and 35% CO_2), being in the lower end of the literature range (as detailed in Table 1). However, to take into account the already mentioned and possible unstable operation of the reactors (Prazeres et al., 2012), a decrease of the SMP will be considered in the sensitivity analysis, as well as its possible increase, to account for performances in the top end range.

According to most recent literature data (Bakkaloglu et al., 2021), a fugitive emission equal to 3.7% loss with respect to the estimated produced CH_4 is included. The digestate is then centrifuged for dewatering after adding a polyelectrolyte (18 kg per t of digestate dry matter) to obtain a TS content of around 20% (solid digestate) (Lombardi et al., 2017). The resulting liquid fraction is sent to a wastewater treatment plant (WWTP), while the solid fraction is supposed to be spread on agricultural soils according to the prescriptions of the current Italian legislation (Ministero delle politiche agricole, alimentari e forestali, 2016), assuming an average transportation distance of 100 km, travelled by a freight lorry with a capacity of 16-32 t.

Emissions from solid digestate spreading on land are accounted for according to the following emission factors: 4.6 mg CH_4/kg digestate, 6.2 mg N_2O/kg digestate and 505,9 mg NH_3/kg digestate (Boulamanti et al., 2013). Moreover, the consumption of diesel for digestate spreading is included, too, assuming to spread 170 kg of N per ha per year, as the maximum (corresponding to the most conservative case for the previously cited Italian legislation) and the specific consumption of 4.8 l of diesel per ha (Timonen et al., 2019).

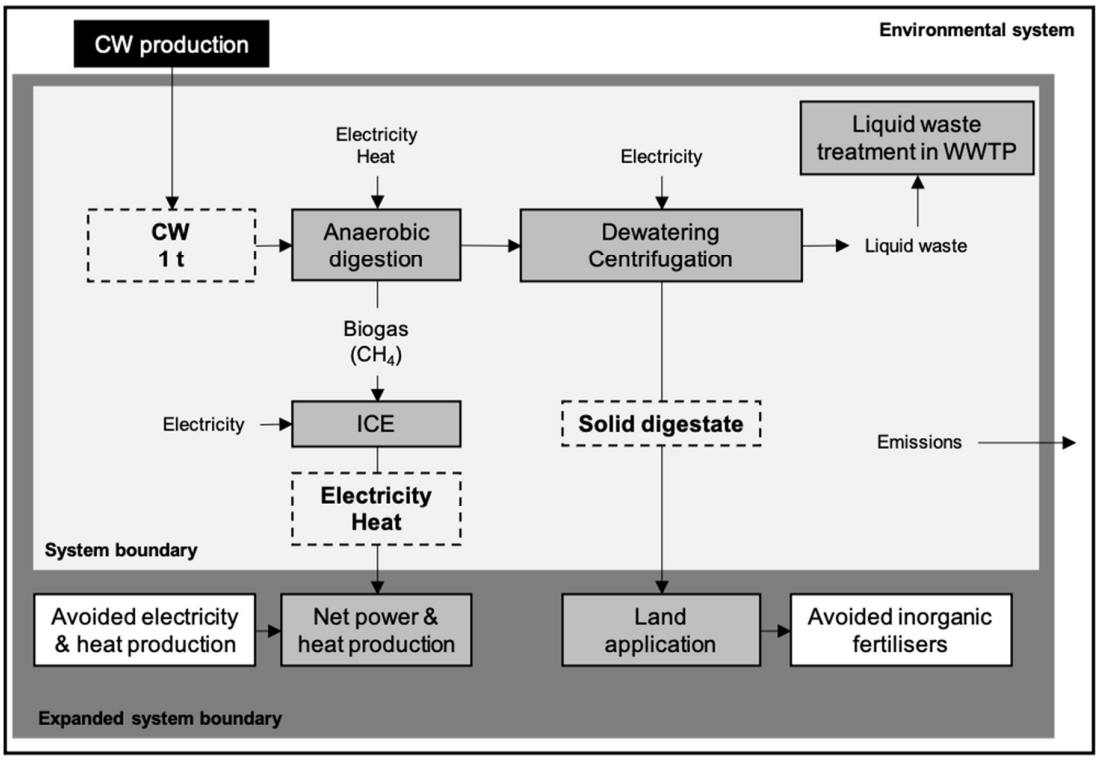


Figure 2. Processes included within the system boundaries of the benchmark AD Scenario.

The TE required by the scenario is calculated taking into account the heating requirements of the material entering the digester and the heat losses during AD. The EE consumption is associated with digestate stirring, pumping and centrifugation. Details on energy consumption calculations are given in Section 1 of the Supplementary Material.

The produced biogas is fed to an ICE with 0.42 EE efficiency and 0.43 TE efficiency (Jenbacher, 2019) and 0.04 EE self-consumption (Carnevale and Lombardi, 2015). In this scenario, the produced energy is enough to cover the needs of the plant in terms of both EE and TE (Table 2). The ICE stack emissions are calculated according to the following emission factors: 5E-04 kg NO_x/Nm^3 biogas (Jenbacher, 2019), 1.91E-05 kg PM/Nm^3, 8.29E-04 kg CO/Nm^3 biogas (Carnevale and Lombardi, 2015) and 4.34E+02 mg CH_4/MJ (Nielsen, 2018).

CO_2 emissions from biogas combustion are considered carbon-neutral because carbon is generated by a renewable carbon source.

This scenario represents the benchmark option, and it will be identified with the abbreviation

AD (standing for anaerobic digestion).

2.2.3 Avoided energy production and use of PHA and digestate

The produced electricity is supposed to be injected into the Italian national grid, while the recovered heat is supposed to be sold to a thermal user located nearby. The appropriate Ecoinvent records used for inventory of the avoided EE and TE are *Electricity, low voltage {IT}| market for* and *Heat, district or industrial, natural gas {Europe without Switzerland}, heat production, natural gas, at boiler condensing modulating > 100kW.*

The produced PHA is assumed to substitute PET according to a substitution rate PHA:PET equal to 1:0.72 (Roibás-Rozas et al., 2020). The assumed value integrated both the replacement ratio equal to 0.93 calculated by (Vega et al., 2019), basing on mechanical properties (yield strength and the density of the materials), and the efficiency of the extraction process (assumed equal to 77.17% according to average values from Fernández-Dacosta et al., (2015b)). Substitution ratio based on mechanical properties of the produced PHA, with respect to those of PET, strongly depends on the mixture and the purity of different obtained biopolymers (El-Hadi et al., 2002; Van de Velde and Kiekens, 2002), in our case mainly expected as a mixture of polyhydroxyvalerate and polyhydroxybutyrate. For this reason, due the uncertainty of the obtainaible mix of biopolymers, and hence of the overall mechanical properties, a sensitivity analysis with respect to the substitution ratio will be performed.

Consistently with the Italian legislation, 100% of the solid fraction of the digestate is reused onto agricultural soils with consequent benefits related to avoiding chemical fertilizers, estimated on the basis of the content of N, P, and K in the digestate (Rigamonti et al., 2013). Since for the present case the nutrient content (N, P and K) in the digestate is not available, the information was derived from literature data relating to the typical nutrient content in raw CW (N, P and K equal to 2.63%, 0.125% and 0.305% of the initial TS content, respectively (Aboukila et al., 2018)) and to the expected N and P removal during AD of 52% and 20%,

339 respectively (Escalante et al., 2017).

340 The summary of the inventory data for the DF+PHA and AD Scenarios is reported in Table

341 2.

342 **Table 2**. Inputs/outputs for the considered scenarios expressed per functional unit (1 t of CW).

Baseline DF+PHA production Scenario		
Parameter	Unit	Value
Dark fermentation reactor		
Electricity required	kWh/t CW	0.31
Heat required	kWh/t CW	1.31
Dewatering section		
Electricity required	kWh/t CW	4.94
Liquid waste to WWTP	m^3/t CW	0.10
Molten carbonate fuel cell		
Electricity required	kWh/t CW	0.15
Electricity produced	kWh/t CW	7.56
Heat produced	kWh/t CW	5.59
NO_x emission	kg/t CW	3.43E-05
Selection/enrichment reactors		
Electricity required	kWh/t CW	14.67
Heat required	kWh/t CW	1.46
Water required	kg/ t CW	15 750
Liquid waste to WWTP	m^3/t CW	11.81
PHA accumulation reactor		
Electricity required	kWh/t CW	40.99
Heat required	kWh/t CW	0.59
PHA extraction reactor		
Electricity required*	kWh/t CW	22.06
Heat required	kWh/t CW	4.05
NaCl required	kg/ t CW	17.52
NaOH required	kg/ t CW	8.76
C_2H_6O required	kg/ t CW	3.50
Liquid waste to WWTP	m^3/t CW	3.96
PHA produced	kg/ t CW	7.67
Benchmark AD Scenario		
Anaerobic digestion reactor		
Electricity required	kWh/t CW	1.00
Heat required	kWh/t CW	4.05
Internal combustion engine		
Electricity required	kWh/t CW	3.47
Electricity produced	kWh/t CW	86.66
Heat produced	kWh/t CW	88.72
CO_2 biogenic emission	kg/t CW	41.40
NO_x emission	kg/t CW	1.62E-02
PM emission	kg/t CW	6.20E-04
CO emission	kg/t CW	2.69E-02
Dewatering section		
Electricity required	kWh/t CW	4.81
Liquid waste to WWTP	m^3/t CW	0.77
Polyelectrolyte	kg/t CW	0.70
N distribution in the digestate	kg/t CW	1.04
P distribution in the digestate	kg/t CW	0.02
K distribution in the digestate	kg/t CW	0.05

*including the electricity required by centrifugation, reactor stirring and process units inlet/outlet pumping

2.3 Modified assumptions

The PHA production process from biodegradable residues is still under development, as confirmed by the rather low TRL. Thus, the possibility of implementing changes is well founded.

The LCA results obtained for the baseline DF+PHA Scenario pointed out the process phases requiring changes in order to improve the overall environmental performance. The changes are introduced below along with the related inventory. They can be classified as follows: improvements in the PHA production process; improvements in the PHA extraction process; implementation of a future electricity generation mix.

2.3.1 PHA production processes improvements

2.3.1.1 Modified performance parameters of PHA production

The baseline scenario for PHA production relates to our previous studies where process stability was attained, but the PHA production performance was in the medium-low range compared to literature about PHA production from whey-based substrate by MMCs. For instance, the PHA content obtained in our previous study (35-40%) is within the commonly reported range of 30-60%, although far from the value of 80%, which is currently the maximum PHA content reported in literature (Colombo et al., 2019). Therefore, it was decided to consider two additional improved PHA production scenarios, namely S-STOR1 and S-STOR2, characterized by better performance parameters regarding: i) the selection stage, in terms of growth yields ($Y_{X/OA}$), and ii) the accumulation stage, in terms of accumulation yields ($Y_{X/OA}$) and PHA content (kgPHA/kgVS). Further details about key assumptions and mass balance for such additional scenarios are reported in Section 2.2 of the Supplementary Material. The related required changes to the reactor volume and energy consumption are also considered.

According to these assumptions, a final PHA production of 9.9 and 17.5 kg PHA per t of CW fed to DF can be estimated for the S-STOR1 and S-STOR2 Scenarios, respectively.

2.3.1.2 Modified organic loading rate in the biomass selection phase

Another improvement concerns the need for dilution water in the selection/enrichment of the PHA-accumulating biomass, which is reduced from a value of 35 m^3/t CW for the corresponding baseline case to 5 m^3/t CW by increasing the OLR up to 8 kg_{COD}/m^3 d (vs. 1.2 kg_{COD}/m^3 d for the baseline scenario), in the scenario named S-DIL (standing for dilution). This assumption is supported by the experimental evidence that good storage responses have been reported for OLR up to around 12 kg_{COD}/m^3 d, whereas higher values have been shown to favour biomass growth rather than PHA accumulation (Dionisi et al., 2006; Valentino et al., 2017, 2014).

2.3.1.3 Valorisation of residues in the PHA production scenario

The baseline DF+PHA production Scenario is modified by introducing the AD-based valorisation of both the solid fraction of fermented CW and the residual biomass from PHA extraction obtained after the two centrifugation stages (see Figure 1). The related scenario is named S-ADrb (standing for anaerobic digestion of residual biomass).

The estimated biogas production of the two combined streams is equal to 13.4 Nm^3 biogas/t CW. The production of the methane-rich biogas would therefore add to the production of bio-H_2 achieved during the CW dark fermentation. The key assumptions and mass balance for this scenario are reported in Section 2.2 of the Supplementary Material. The actual methane yields and interferences of residual chemicals used for extraction will require properly designed experimental tests in the future, especially for the residual biomass stream.

The digestate produced by the AD section implemented into the PHA production process is further treated. The additional energy consumption for AD is calculated as reported in detail in Section 2.2 of the Supplementary Material.

2.3.2 Improvements in PHA extraction

The digestion of non-PHA material with acidic or alkaline reagents (such as NaOH in

393 DF+PHA Scenario) is seen as a cost-effective and green alternative to the more conventional
394 solvent extraction methods based on chlorinated organic solvents, such as chloroform, 1,2-
395 dichloromethane, and methyl chloride (Mannina et al., 2020). Green solvents such as dimethyl
396 carbonate (DMC), a versatile reagent with relatively low toxicity for human health and the
397 environment and a high recyclability rate, represent another feasible alternative (Samorì et al.,
398 2015). From a LCA perspective, Righi et al. (2017) evaluated different configurations for the
399 PHA extraction process with DMC, concluding that PHA extraction from dried biomass, with
400 PHA recovery after evaporation of the solvent (Section 3 of the Supplementary Material), leads
401 to better environmental performances. The possibility of recovering the used chemicals is highly
402 desirable at the industrial scale.

403 Since the extraction phase significantly contributes to the overall impact of the DF+PHA
404 production process, the alternative PHA extraction method using DMC, proposed by Righi et
405 al. (2017), is used in the updated inventory, resulting in an alternative scenario named S-DMC
406 (standing for use of DMC instead of NaOH and NaCl).

407 Details for the updated inventory are reported in Section 3 of the Supplementary Material.

408 **2.3.3 Future electricity mix**

409 The environmental performance of the benchmark AD and baseline DF+PHA Scenarios are
410 recalculated following the efficiency, renewable energy, and emissions reduction targets to be
411 achieved by 2030 (Terna and Snam, 2019) in the EU. The assumed share of electricity from
412 various sources (fossils, renewables, etc) in the current and future (2030) conditions is reported
413 in details in the Sectin 4 of the Supplementary Material.

414

415 An overview of the different scenarios assessed through LCA is shown in Table 3.

416 **Table 3**. Overview of the different scenarios investigated in this study.

Main scenarios		
Baseline DF+PHA	Coproduction of PHA and H_2 from CW (baseline	PHA yield: 7.6 [kg PHA/t CW]

	Benchmark AD	PHA production) Production of CH_4 from CW (benchmark AD)	Dilution factor: 35
Additional scenarios			
Modifications to the PHA production process	S-STOR1	Improvements in the PHA production performance parameters	PHA yield: 9.9 [kg PHA/t CW] Dilution factor: 35
	S-STOR2	Improvements in the PHA production performance parameters	PHA yield: 17.5 [kg PHA/t CW] Dilution factor: 35
	S-Dil	Reduction of water required for the PHA production	PHA yield: 7.6 [kg PHA/t CW] Dilution factor: 5
	S-ADrb	AD of the residues from the process and energy recovery	PHA yield: 7.6 [kg PHA/t CW] Dilution factor: 35
Modifications to the PHA extraction process	S-DMC	Improvements in the PHA extraction process	PHA yield: 7.6 [kg PHA/t CW] Dilution factor: 35
Modifications to the electricity mix	Future electricity mix	Evaluated for baseline DF+PHA and benchmark AD	PHA yield: 7.6 [kg PHA/t CW] Dilution factor: 35

2.4 Sensitivity analysis

As already anticipated, the sensitivity analysis is used to evaluate how some key values assumed in the inventory could affect the impact assessment results. Concerning the DF + PHA Scenario, PET substitution rate is rather uncertain and related to the purity of the obtained biopolymer mixture, and quite important for the results. While, regarding the AD Scenario, the SMP is the main parameter affecting the obtainable beneficial effects, with some uncertainties related to the stability of the process.

The sensitivity analysis is carried out through a Monte Carlo simulation generating random outcomes of the PET substitution and SMP (1000 samples each). In particular PET substitution rate is varied by ±30%, with respect to the original assumed vaule of 0.72, corresponding to the range 0.5-0.93, according to the values found in literature (Roibás-Rozas et al., 2020), and SMP is varied between 0.2 and 0.5 l CH_4/g TVS (originally equal to 0.3 l CH_4/g TVS), as reported in Escalante et al., 2017 and Labatut et al., 2011 (Escalante et al., 2017; Labatut et al., 2011).

The variation is made through a normal distribution, based on the mean of the expressed range values and a standard deviation equal to 4% and 7% respectively for PET substitution and SMP.

The sensitivity analysis is applied: i) considering the DF + PHA Scenario in the case

including all the possible improvements and their cumulative effects, called S-TOT+DMC Scenario, varying the PET substitution ratio and comparing it with the benchmark AD Scenario (base value of SMP 0.3 l CH_4/g TVS); ii) varying the SMP in the benchmark AD Scenario and comparing the results with the S-TOT+DMC Scenario (base value 0.72 PET substitution ratio).

2.5 Life cycle impact assessment method

The Environmental Footprint (EF) 3.0 method (European Commission, 2013; Zampori and Pant, 2019), developed by the Joint Research Centre of European Commission, is used to perform the Life Cycle Impact Assessment (LCIA), with its 16 impact categories: Climate change (CC) in kg CO_2 eq.; Ozone depletion (OD) in kg CFC-11 eq.; Ionising radiation (IR) in kBq U^{235} eq.; Photochemical ozone formation (POF) in kg NMVOC eq.; Particulate matter (PM) in disease incidence; Human toxicity, non-cancer (HTnc) in CTU_h (Comparative Toxic Unit for humans); Human toxicity, cancer (HTc) in CTU_h; Acidification (A) in mol H^+ eq.; Eutrophication, freshwater (Ef) in kg P eq.; Eutrophication, marine (Em) in kg N eq.; Eutrophication, terrestrial (Et) in mol N eq.; Ecotoxicity, freshwater (ETf) in CTU_e (Comparative Toxic Unit for ecosystems); Land use (LU) in pt (soil quality index); Water use (WU) in m^3 deprived; Resource use, fossils (RUf) in MJ; Resource use, minerals and metals (RUmm) in kg Sb eq. In the classification phase, all the material and energy flows of the inventory phase are identified according to their contribution to each impact category. The EF indicators calculation was performed with the aid of Simapro software, version 9.1, using an adapted method, which is not suitable for conducting the EF-compliant studies but can be used for other assessments. In this work, normalization and weighting steps are not performed.

3. Results and discussion

This section presents the environmental impacts as assessed according to the adapted EF 3.0 method.

The results expressed per functional unit (1 t of CW) are reported in Table 4 for the two main

scenarios (baseline DF+PHA production and benchmark AD). Table 4 shows that the benchmark AD Scenario has a better environmental profile than the baseline DF+PHA Scenario. In fact, while the AD Scenario has negative impact values for all impact categories (with the exception of Eutrophication marine indicator), the opposite occurs for the DF+PHA Scenario. This indicates that, based on the considered inventory, the production and exploitation of significant amounts of a methane-rich biogas results in larger benefits than the exploitation of relatively low amounts of a hydrogen-rich biogas and, most of all, despite the concomitant production of PHA. Similar findings were reported by Gurieff and Lant (2007), even if they calculated only the non-renewable CO_2 emission indicator. Therefore, the proposed process, although innovative, needs to be optimized to compete in terms of impacts with a more traditional management option.

More in detail, the higher environmental impact of the baseline DF+PHA Scenario is due to the higher consumption of energy and materials as compared to the benchmark AD Scenario, mainly due to the biomass selection and PHA extraction phases, as better explained in the contribution analysis.

Table 4. Results of the impact assessment performed according to the Environmental Footprint method.

		Baseline DF+PHA Scenario	**Benchmark AD Scenario**
Impact category	Unit	Value	Value
Climate change (CC)	kg CO_2 eq./t CW	4.48E+01	**-3.57E+01**
Ozone depletion (OD)	kg CFC-11 eq./t CW	1.28E-05	**-6.88E-06**
Ionising radiation (IR)	kBq U^{235} eq./t CW	1.02E+01	**-3.92E+00**
Photochemical ozone formation (POF)	kg NMVOC eq./t CW	1.32E-01	**-5.53E-02**
Particulate matter (PM)	disease incidence/t CW	1.64E-06	**-2.47E-07**
Human toxicity, non-cancer (HTnc)	CTU_h/t CW	2.41E-06	**-1.89E-07**
Human toxicity, cancer (HTc)	CTU_h/t CW	6.48E-08	**-1.03E-08**
Acidification (A)	mol H^+ eq./t CW	3.05E-01	**-1.39E-01**
Eutrophication, freshwater (Ef)	kg P eq./t CW	3.84E-02	**-9.60E-03**
Eutrophication, marine (Em)	kg N eq./t CW	3.61E-01	**1.13E-02**
Eutrophication, terrestrial (Et)	mol N eq./t CW	6.11E-01	**-1.78E-01**
Ecotoxicity, freshwater (ETf)	CTU_e/t CW	7.29E+03	**-3.22E+02**
Land use (LU)	pt/t CW	1.37E+03	**-9.42E+02**
Water use (WU)	m^3 deprived/t CW	6.95E+02	**-2.56E+01**
Resource use, fossils (RUf)	MJ/t CW	6.04E+02	**-7.13E+02**
Resource use, minerals and metals (RUmm)	kg Sb eq./t CW	9.70E-04	**-2.93E-04**

3.1 Contribution analysis

To investigate the main contributions that affect the overall environmental performance, the following key sub-processes and activities are identified: DF or AD, MCFC/ICE self-consumption, avoided electricity production, avoided heat production, MCFC/ICE air emissions, PHA-accumulating biomass selection, PHA accumulation, PHA extraction, avoided PET production, dewatering and land spreading of digestate (this lat one includes the avoided use of fertilisers and the impacts due to emissions and consumption from spreading). For each sub-process, the contributions of activities (consumption/production of energy and substances, emissions, treatment of effluents/waste, transport) are accounted for. Avoided impacts due to the substitution of conventional energy or fossil-based polymers or chemical fertilisers contribute with negative values.

Figure 3 summarises the percent contributions of the selected sub-processes to each impact category for the two main scenarios (larger and clearer figures for each impact indicator are reported in the Section 4 of the Suppelmentary Material). The weight of the sub-processes impacts is almost similar for all impact indicators.

Both the DF and AD processes result in limited impacts thanks to a low TE consumption made possible by the inflow temperature (40 °C) (the contribution is low compared to the others and it is not visible in Figure 3). Therefore, the difference between the two is due to PHA production, accumulation and extraction stages which strongly affect the consumption of electricity, chemicals, and water (see Section 5 of the Supplementary Material). In particular, the selection of the PHA-accumulating biomass involves considerable consumption of EE and water, and mainly affects the impact categories related to human toxicity (HTnc and HTc), as well as Em, ETf and WU.

The impact of the PHA extraction stage is mainly due to the use of chemicals; in particular, one of the most influenced impact categories is OD, as also pointed out by Vogli et al. (2020).

Details about the individual contributions are given in Section 5 of the Supplementary Material.

Although limited, in the AD Scenario the highest contributions to direct impacts are given by the EE required by dewatering (especially for the HTnc, HTc, Em and ETf indicators) and CH_4, NO_x and PM air emissions from biogas combustion (especially for the CC, POF, PM, Em and Et indicators).

The comparison in terms of energy recovery points out the role played by the amount of biogas produced by AD and DF. In fact, although hydrogen represents a potentially more attractive option than methane, the volume produced is much lower. This results in lower avoided impacts than the methane recovery in the AD Scenario. This also implies that, unlike the DF+PHA Scenario, the AD Scenario is energy self-sufficient.

PET substitution by PHA allows appreciable avoided impacts. As also mentioned in other studies (Roibás-Rozas et al., 2020), impact categories such as CC, POF, PM, A and RU (both indicators) are those most influenced by PHA recovery. However, such avoided effects are not high enough to balance the impacts related to resource consumption.

The evaluations carried out appear to suggest that the conventional AD process for CW valorization has a better environmental profile compared to the innovative scheme involving the integrated recovery of hydrogen and PHA. This result is obviously influenced by the adopted evaluation criteria. If economic considerations were included in the comparative assessment of the two Scenarios, one should also account for the notably higher – almost double – potential revenues obtainable by selling the produced PHA, compared to the revenues obtainable by selling the TE and EE produced in the AD Scenario[2].

However, a thorough assessment of the economic profile of the investigated process configurations would require a dedicated economic analysis that falls beyond the scopes of the present work.

[2] preliminary calculations are obtained considering the TE, EE and PHA productions calculated for the studied cases and assuming the following specific costs: 0.07 Euro/kWh for TE (Gruppo Hera, 2020); 0.16 Euro/kWh for EE (Ministero dello sviluppo economico, 2016); 3.4 Euro/kg for PHA (Colombo et al., 2019).

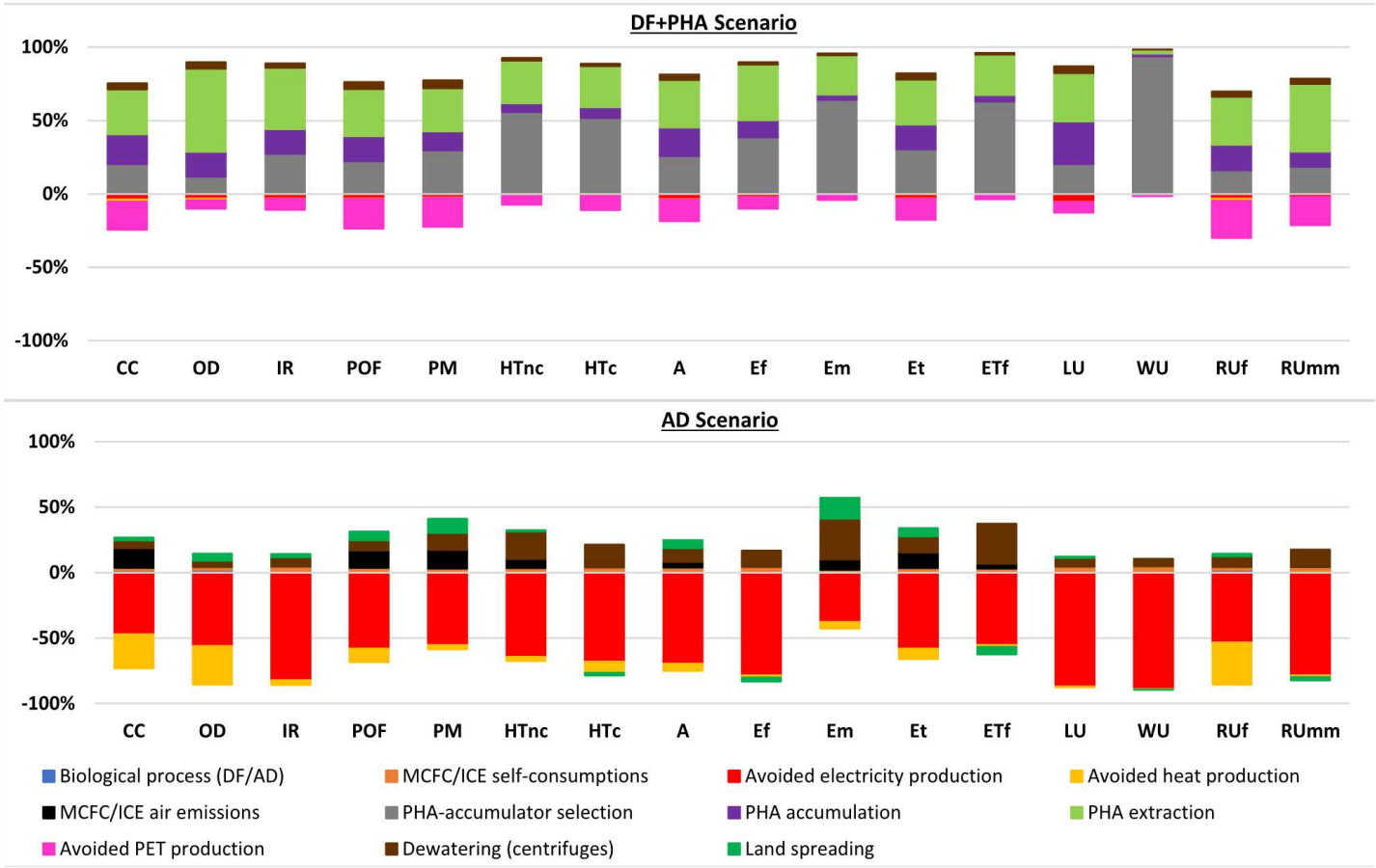


Figure 3. Sub-processes contributions to the considered impact categories for the baseline DF+AD Scenario and the benchmark AD Scenario.

528 Focusing on the CC impact category (see the devoted figure in the Section 6 of the
529 Supplementary Material), it is possible to highlight that the produced EE in the DF+PHA and
530 AD Scenarios replaces the supply from the Italian national grid, providing saving in CC effects
531 by, respectively, 4% and 47% (the specific CC coefficient for EE production in Italy is assumed
532 to be 0.43 kg CO_2 per kWh) of the overall impact. Considering that TE can also be recovered
533 by feeding the two types of biogas to MCFC (DF+PHA Scenario) and ICE (AD Scenario), the
534 savings from the avoided TE are 2% and 26% respectively (assumed specific coefficient for
535 thermal energy equal to 0.24 kg CO_2 per kWh). The DF+PHA Scenario takes also advantage
536 from PET substitution by PHA, which results in a CC contribution equal to -19% (3.05 kg CO_2
537 per kg PET).
538 PHA-accumulating biomass selection and PHA accumulation in the DF+PHA Scenario
539 generate large direct environmental burdens equal to 20% and 20% of the overall impact,
540 respectively, mainly due to water consumption (3.54E-04 kg CO_2 per kg H_2O) and wastewater
541 treatment (0.51 kg CO_2 per treated m^3) associated to the PHA-accumulating biomass selection,
542 as well as the electricity consumption for aeration during PHA accumulation. Finally, the main
543 direct CC impacts related to the PHA production process are associated to the PHA extraction
544 phase, accounting for 31% of the total CC impact, and the chemicals involved in the process
545 (0.17 kg CO_2 per kg NaCl; 0.84 kg CO_2 per kg NaOH; 1.23 kg CO_2 per kg C_2H_6O). It is worth
546 mentioning that PHA production displays significantly lower CC impacts compared to the PHA
547 extraction phase, as also discussed in Fernández-Dacosta et al. (2015a).

548 This shows that the environmental profile of PHA production may be improved by focusing
549 on the biochemical stages involved, i.e. biomass selection and PHA accumulation and
550 extraction. The results of the contribution analysis point out that the consumptions related to
551 the stages downstream of DF should be reduced and the associated impact savings enhanced.

552 Concerning the selection of the PHA-accumulating biomass, we highlighted how water

consumption/treatment accounts for 20% of the total CC. Adopting higher OLR values (as mentioned above) would allow for lower dilution of the fermented stream, and, in turn, a lower amount of water used, reducing the associated environmental impacts.

As for PHA extraction, the analysis shows that the indirect impacts from the used chemicals are the main contribution to CC, therefore adopting different and more effective reagents may help improve the environmental profile of the process with regard to global warming.

Concerning the avoided effects, improvements can also be gained by increasing the mass of PHA recovered and adopting AD for energy exploitation from the residual streams.

The effects resulting from the aforementioned changes are reported in the following section.

3.2 Results of improved PHA production

The results of the analysis for the improved Scenarios are presented in Table 5. For the sake of brevity, only the CC impact indicator is discussed, whilst the results for the other impact categories are reported in Section 7 of the Supplementary Material.

Table 5. CC for DF+PHA baseline and modified scenarios (AD as benchmark scenario) (first part of the table). CC for the overall proposed modifications to the baseline DF+PHA scenario (second part of the table).

Note that $\Delta\% = \frac{CC_{DF+PH\ \ modified} - C\ \ DF+PHA}{CC_{DF+PHA}} \cdot 100$

CC for DF+PHA baseline and modified scenarios (AD as benchmark scenario)				
	Scenario	**Unit**	**Climate Change**	**Δ%**
	Benchmark AD	kg CO_2 eq./t CW	-3.57E+01	
	Baseline DF+PHA	kg CO_2 eq./t CW	4.48E+01	
Modifications to the PHA production process	S-STOR1	kg CO_2 eq./t CW	2.97E+01	-34%
	S-STOR2	kg CO_2 eq./t CW	1.60E+01	-64%
	S-DIL	kg CO_2 eq./t CW	1.06E+01	-76%
	S-ADrb	kg CO_2 eq./t CW	2.58E+01	-42%
Modifications to the PHA extraction process	S-DMC	kg CO_2 eq./t CW	2.13E+01	-52%
Modifications to the electricity mix	Benchmark AD	kg CO_2 eq./t CW	-2.48E+01	
	Baseline DF+PHA	kg CO_2 eq./t CW	3.37E+01	-25%

Overall evaluation of the proposed modifications to the baseline DF+PHA scenario. CC results				
Scenario	**Modifications**	**Unit**	**Climate Change**	**Δ%**
Benchmark AD		kg CO_2 eq./t CW	-3.57E+01	
Baseline DF+PHA		kg CO_2 eq./t CW	4.48E+01	
S-TOT	S-STOR2+S-DIL+S-ADrb	kg CO_2 eq./t CW	-3.14E+01	-170%
S-TOT+DMC	S-STOR2+S-DIL+S-ADrb+S-DMC	kg CO_2 eq./t CW	-5.03E+01	-212%
TOT+DMC+Emix	The previus one changing the electricity mix	kg CO_2 eq./t CW	-4.65E+01	-204%

The S-STOR1 Scenario, characterised by better performance parameters (see Section 2.1 of the Supplementary Material), in particular by a higher PHA content of microbial cells (60% vs 40%), displays a reduction in the CC score by 34% compared to the DF+PHA baseline Scenario.

Further improvements in the environmental profile of the process are observed for the S-STOR2 Scenario. Attaining the maximum reported PHA content of ~80% implies a 64% reduction in the CC impact compared to the baseline case. Indeed, for the S-STOR1 Scenario, the contributions of the biomass selection and PET substitution are equal to, respectively, 15% (20% for the baseline case) and -26% (-19% for the baseline case) of the overall impact. For S-STOR2, the same contributions are 12% and -37%.

In S-DIL, the OLR increase in the selection reactor results in a CC score reduction 76% from the baseline Scenario. The reduction is more relevant than for the S-STOR1 and S-STOR2 Scenarios. In particular, the reduction in water supply leads to a lower impact, equal to 16% of the overall score (20% for the baseline DF+PHA Scenario). Moreover, the lower water requirement of biomass selection reduces energy consumptions, contributing to the overall impact by 5% (20% for the baseline case).

The application of AD to the DF+PHA process (S-ADrb Scenario) allows for a reduction in the CC impact by 42% from the corresponding baseline Scenario. In particular, energy recovery from biogas avoids conventional EE and TE production, providing savings in the CC impact of 15% (4% for the base case) and 8% (2% for the base case), respectively.

3.3 Results of improved PHA extraction

The use of DMC and its recyclability for PHA extraction has a beneficial effect on all the considered impact categories. In particular, the calculated CC score is 21.3 kg CO_2 eq. per t of CW, 52% lower than that of the corresponding baseline case. PHA extraction accounts for 9%

593 of the total CC impact (31% in the baseline scenario), mainly due to the treatment of the residual
594 biomass and to DMC supply (2.13 kg CO_2 per kg DMC). Detailed results are reported in Section
595 7 of the Supplementary Material.

596 **3.4 Effects of the future electricity mix**

597 The different boundary conditions related to the future electricity mix have effects on both
598 the main scenarios. For the DF+PHA baseline scenario, all the considered environmental
599 impact indicators improve, except for HTnc, HTc and Et. For the AD benchmark scenario, all
600 the considered environmental impact indicators worsen, except for HTnc, HTc, and Et. The
601 detailed results are reported in Section 7 of the Supplementary Material.

602 CC scores are 33.7 kg CO_2 eq. per t of CW for the DF+PHA Scenario (-25% compared to
603 the baseline case: 44.8 kg CO_2 eq. per t of CW) and -24.8 kg CO_2 eq. per t of CW for the AD
604 Scenario (+31% compared to the benchmark AD: -35.7 kg CO_2 eq. per t of CW). The use of
605 the projected electricity mix would reduce the impacts in terms of electricity consumption, but
606 at the same time the avoided effects in absolute terms. For this reason, the difference between
607 the two main scenarios shows a reduction.

608 **3.5 Discussion of the results**

609 According to the base assumptions, the benchmark AD Scenario displays a better
610 environmental profile than the baseline DF+PHA Scenario. However, the analysis of the
611 potential improvements in the DF+PHA Scenario, the choice of which is derived from previous
612 studies about PHA production from CW, proves that the gap between the two systems may be
613 reduced by optimizing (i) the selection stage with lower growth yields ($Y_{X/OA}$), (ii) the
614 accumulation stage with higher accumulation yields ($Y_{X/OA}$) and PHA contents (kgPHA/kgVS),
615 (iii) the selection stage with higher organic loading rate (OLR). It is necessary to underline that,
616 although theoretically and experimentally obtainable, the proposed improvements must be
617 verified on a case-by-case basis.

618 The potential of these possible improvements is remarkable considering their cumulative
619 effects (see Table 5, second part). The results for the other impact indicators are reported in the
620 Supplementary Material (Section 7).

621
622

623 The new scenario (S-TOT) including all the suggested modifications displays a CC score of
624 -31.4 kg CO_2 eq. per t of CW, with a reduction of 170% compared to the baseline DF+PHA
625 Scenario. The CC indicator, although of the same order of magnitude of the benchmark AD
626 Scenario, is still 12% higher.

627 Similar figures can be highlighted for the other impact indicators, with a substantial reduction
628 in the difference between the benchmark AD and S-TOT Scenarios, especially in the POF, A,
629 Et, and RUf impact categories. Moreover, S-TOT shows a better environmental profile for the
630 PM indicator compared to the benchmark AD (see Section 7 of the Supplementary Material).

631 If the DMC extraction process is also included (S-TOT+DMC Scenario), the CC score
632 changes to -50.3 kg CO_2 eq. per t of CW, with a reduction by 212% compared to the baseline
633 DF+PHA Scenario. In this case, the CC indicator is 41% lower than that of the benchmark AD
634 Scenario, and better environmental performances are also observed for POF, PM, HTnc, HTc,
635 A, Em, Et, RUf and RUmm (see Section 7 of the Supplementary Material).

636 Finally, if the S-TOT+DMC is evaluated with reference to the future electricity mix (S-
637 TOT+DMC+Emix), the CC indicator becomes -46.5 kg CO_2 eq. per t of CW, with a reduction
638 by 204% compared to the baseline DF+PHA scenario. In this case, the indicator value must be
639 compared to the one calculated for the AD Scenario considering the projected electricity mix,
640 equal to -24.8 kg CO_2 eq. per t of CW, indicating a 88% improvement. Better environmental
641 performances compared to the AD Scenario, considering the projected electricity mix, are also
642 recorded for POF, PM, A, Ef, Em, Et, ETf, LU, RUf and RUmm (see Section 7 of the
643 Supplementary Material).

644 **3.5.1 Results of the sensitivity analysis**

645 According to the assumtpions explained in paragraph 2.4, the impact assessment results are
646 recalculated, for S-TOT+DMC Scenario and the benchmark AD Scenario, obtaining a
647 probability distribution of the results per each impact category depending on the sample
648 outcomes (outcomes number considered equal to 1000). For conciseness reason, only the
649 probability distribution of the CC indicator is discussed here. The probability distribution of the
650 other indicators are reported in Figure 18 and 19 of the Supplementary Material.

651 According to the PET substitution ratio variation by ±30%, the CC final score of S-
652 TOT+DMC Scenario ranges from -6.17E+01 and -3.89E+01 kg CO_2 eq. per t of CW, showing
653 a 100% of convenience compared to the benchmark AD Scenario (-3.57E+01 kg CO_2 eq./t
654 CW). This result is quite encouraging for the innovative process of PHA production, providing
655 that efforts are made for its optimization.

656 Concurrently, varying the SMP between 0.2 and 0.5 l CH_4/g TVS fo the AD Scenario, its
657 final CC indicator ranges from -6.81E+01 and -1.94E+01 kg CO_2 eq. per t of CW, showing
658 about 17% of convenience compared to the S-TOT+DMC Scenario (-5.03E+01 kg CO_2 eq./t
659 CW).

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661

662 **4. Conclusions**

663 An innovative approach to CW valorization based on the combination of H_2-producing DF
664 and PHA production was evaluated by LCA and compared to AD as a benchmark option. LCA
665 gave an insight into the contributions to the overall impact related to the different steps of PHA
666 production, highlighting a number of weak points to be tackled. Consistently, improvements
667 were proposed and a new LCA-based comparison performed.

668 The proposed changes led to considerable improvements. The changes made to PHA
669 production – evaluated as a whole - allowed improving all the indicators, and, with particular

reference to the CC indicator, reaching the same order of magnitude of the AD benchmark scenario, while still remaining slightly less favorable. Further changes related to the PHA extraction process made it possible to calculate a CC value 41% indicator lower than the CC indicator related to AD. Finally, if also a future energy scenario based on renewable sources is included in the comparison, the CC indicator of the DF+PHA process was found to be 88% better than that of the AD benchmark scenario, and improved results were observed also for other indicators.

In conclusion, the PHA production process from an organic residue such as CW can be reasonably improved to achieve environmental performances close to those of a benchmark management system such as AD. Further efforts must be devoted to experimentally testing the proposed process improvements for any substrate under concern. The contribution to the development given by the application of the LCA procedure would find optimal fulfillment in the pilot scale experimentation so as to allow the system to be raised up to TRL 7.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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