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Effect of hydraulic retention time on the electro-bioremediation of nitrate in saline groundwater

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Abstract:	Bioelectrochemical systems (BES) have proven their capability to treat nitrate-contaminated saline groundwater and simultaneously recover value-added chemicals (such as disinfection products) within a circular economy-based approach. In this study, the effect of the hydraulic retention time (HRT) on nitrate and salinity removals, as well as on free chlorine production, was investigated in a 3-compartment BES working in galvanostatic mode, with the perspective of process intensification and future scale-up. Reducing the HRT from 30.1±2.3 to 2.4±0.2 hours led to a corresponding increase in nitrate removal rates (from 17±1 up to 131±1 mgNO3--N L-1d-1), although a progressive decrease in desalination efficiency (from 77±13 to 12±2%) was observed. Nitrate concentration and salinity close to threshold limits indicated by the World Health Organization for drinking water, as well as significant chlorine production, were achieved with an optimal HRT of 4.9±0.4 h. At the optimal HRT, specific energy consumption was low (6.8·10-2±0.3·10-2 kWh g-1NO3--Nremoved), considering that the supplied energy supports three processes simultaneously. A logarithmic equation correlated well with nitrate removal rates at the applied HRTs and may be used to predict BES behaviour with different HRTs. The galvanostatic mode exerted a selective pressure on the bacterial community of the cathode biofilm enriching a few dominant populations, including at genus level the taxa Rhizobium, Bosea, Fontibacter and Gordonia. The results provide useful information for the scale-up of BES treating multi-contaminated groundwater.
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Dear Editor,

With the approval of all the Authors, we are submitting the manuscript entitled "*Effect of hydraulic retention time on the electro-bioremediation of nitrate in saline groundwater*" by G. Puggioni, S. Milia, V. Unali, R. Ardu, E. Tamburini, M. Dolors Balaguer, N. Pous, A. Carucci and S. Puig, for its possible publication in Science of the Total Environment.

Nitrate and salinity simultaneously affect groundwater quality in many countries worldwide, hindering the exploitation of such an important water reservoir. Conventional remediation technologies used to remove nitrate and salinity from groundwater are characterized by many technical drawbacks and high operating costs. Our research group recently designed a proof-of-concept based on a 3-compartment bio-electrochemical system (BES) to simultaneously remove nitrates and salinity from groundwater and produce a value-added chemical. The possibility to maximise process performance and push the system toward its limits with the perspective of process scale-up represents a challenging opportunity. Though hydraulic retention time (HRT) is considered a key operating parameter for optimizing hydrodynamics and substrate distribution in conventional BES, its actual role is difficult to be predicted with multi-contaminated groundwater and more complex BES configurations like the one described in this study, where biotic and abiotic processes co-exist in the same reactor. In this study, we investigated how reducing the HRT (i.e., increasing the influent flowrates) may affect process performance in terms of, among the others, nitrate and desalination rates, chlorine production, and energy consumption. We think our results will positively contribute to developing novel, cost-effective, and efficient treatment alternatives based on BES for the remediation of multi-contaminated groundwater, and we consider Science of the Total Environment the ideal platform to share our findings.

We hereby declare that this manuscript represents the original work of the Authors, it has not been published previously, and it won't be submitted for publication elsewhere whilst under consideration by this Journal.

Kind regards,

The Authors.

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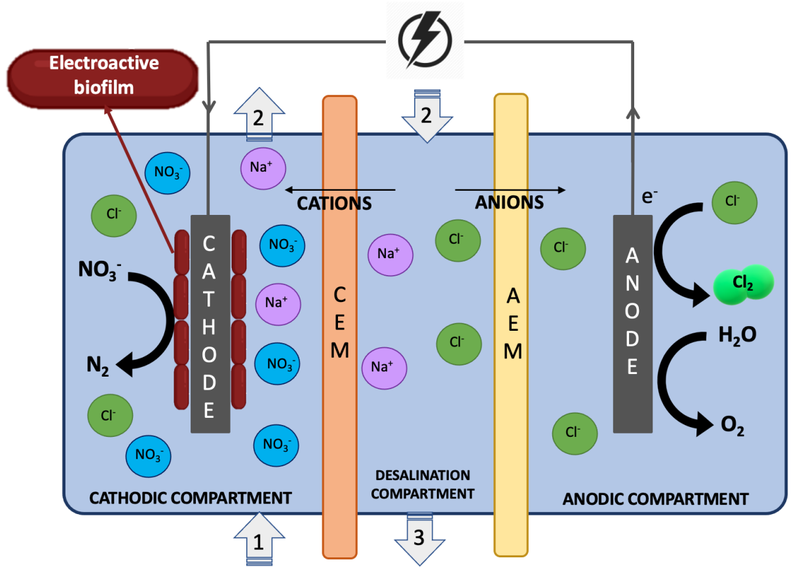
Graphical Abstract

Agro-industrial activities

Groundwater multi-contamination (nitrate, salinity)

Advanced bioelectrochemical systems



1 influent saline groundwater

2 denitrified saline groundwater

3 desalinized effluent groundwater

Decreasing hydraulic retention time (HRT)

- Increasing NO_3^- removal rates and efficiency
- Stable desalination rates
- Decreasing specific energy consumption
- Stable Cl_2 production

Highlights:

- Low HRT improves the nitrate removal performance
- Desalination performance is limited at low HRT
- 3 simultaneous processes are promoted with low energy consumption
- The relationship between nitrate removal rate and HRT follows a logarithmic trend

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Abstract:

Bioelectrochemical systems (BES) have proven their capability to treat nitrate-contaminated saline groundwater and simultaneously recover value-added chemicals (such as disinfection products) within a circular economy-based approach. In this study, the effect of the hydraulic retention time (HRT) on nitrate and salinity removals, as well as on free chlorine production, was investigated in a 3-compartment BES working in galvanostatic mode, with the perspective of process intensification and future scale-up. Reducing the HRT from 30.1±2.3 to 2.4±0.2 hours led to a corresponding increase in nitrate removal rates (from 17±1 up to 131±1 mgNO_3^--N $L^{-1}d^{-1}$), although a progressive decrease in desalination efficiency (from

77±13 to 12±2%) was observed. Nitrate concentration and salinity close to threshold limits indicated by the World Health Organization for drinking water, as well as significant chlorine production, were achieved with an optimal HRT of 4.9±0.4 h. At the optimal HRT, specific energy consumption was low ($6.8 \cdot 10^{-2} \pm 0.3 \cdot 10^{-2}$ kWh $g^{-1}NO_3^-$-$N_{removed}$), considering that the supplied energy supports three processes simultaneously. A logarithmic equation correlated well with nitrate removal rates at the applied HRTs and may be used to predict BES behaviour with different HRTs. The galvanostatic mode exerted a selective pressure on the bacterial community of the cathode biofilm enriching a few dominant populations, including at genus level the taxa *Rhizobium, Bosea, Fontibacter* and *Gordonia*. The results provide useful information for the scale-up of BES treating multi-contaminated groundwater.

Keywords: circular economy; denitrification; microbial electrochemical technology; saline groundwater; value-added products; water recovery.

1. INTRODUCTION

Groundwater is a critical freshwater reservoir fundamental for global water and food security. The spread of contaminants in groundwater can limit its use as drinking water, so actions must be taken to ensure a safe drinking water supply (Janža, 2022). Bioelectrochemical systems are emerging as sustainable alternatives for the treatment of contaminated groundwater. Such systems are based on the ability of electroactive microorganisms to perform oxidation and reduction reactions by exchanging electrons with an electrode (Pous et al., 2018; Wang et al., 2020). Therefore, they are particularly suitable for groundwater treatment, as they promote bioremediation without the supply of chemicals as electron acceptors/donors.

Most of the studies focus on the removal of one type of contaminant at a time (e.g., nitrate, organics, heavy metals, calcium, etc.), which is useful for a deep understanding and optimisation of the processes involved (Beretta et al., 2020; Ceballos-Escalera et al., 2022, 2021; Palma et al., 2018; Sevda et al., 2018; Verdini et al., 2015). However, groundwater matrices are highly complex and heterogeneous, influencing the behaviour of BES and representing a key aspect of process development and scale-up. One of the most intriguing challenges that researchers are currently facing is thus the application of BES to the bioremediation of multi-contaminated groundwater.

Among contaminants, nitrate is often found in groundwater at high concentrations co-existing with other pollutants. Nitrate contamination in groundwater is frequently due to inefficient farming practices and careless management of livestock activities (Kwon et al., 2021; Serio et al., 2018). The Nitrates Directive (91/767/EU) sets a nitrate concentration limit of 50 mgNO_3^- L^{-1} (11.3 mgNO_3^--N L^{-1}) in drinking water for human health, safety, and environmental protection. In this framework, the possibility of simultaneously removing nitrates and other contaminants from groundwater is of particular interest.

The presence of co-contaminants associated with nitrate can result from natural sources (e.g., arsenic derived from the reductive dissolution of arsenic-rich minerals) and anthropogenic activities (e.g., perchlorate derived from the production of car airbags, fireworks and fertilisers, Lian et al., 2016). Ceballos-Escalera et al. (2021) successfully removed nitrate and arsenic from groundwater using a tubular BES. The treatment combined nitrate reduction to dinitrogen gas and arsenite oxidation to arsenate (which shows less toxicity, solubility and mobility) within the same reactor. In this way, the ability of BES to denitrify without being affected by arsenite and under low electrical conductivity conditions (about 1 mS cm^{-1}) was demonstrated.

Wang et al., 2021 investigated the simultaneous removal of nitrate and perchlorate from groundwater with cathodic potential regulation. Results demonstrated that the mechanism of nitrate and perchlorate reduction in the BES was the direct electron transfer from the cathode to the bacteria, and the dominant bacterial community on the cathode was proven to have the ability to reduce nitrate and perchlorate. However, regardless of the potential applied to the cathode or not, nitrate inhibited the reduction of perchlorate.

The occurrence of high nitrate (30.0 $mgNO_3^-$-N L^{-1}) and salinity levels (3.3±0.3 mS cm^{-1}) in groundwater was recently dealt with by Puggioni et al. (2021). In this study, in contrast to the previous studies where co-contaminants required removal by oxidation/reduction, the treatment coupled reduction with a separation of co-contaminants. A proof-of-concept based on a 3-compartment BES allowed the simultaneous removal of nitrate (39±1 $mgNO_3^-$-N L^{-1} d^{-1}) and salinity (chloride removal rate of 13±2 gCl^- L^{-1} d^{-1}) from groundwater but also the production of value-added chemicals (i.e., free chlorine). The electroactive biomass attached to the cathode carried out the denitrification in the bio-cathodic compartment, while desalination took place in the central compartment thanks to electrochemically driven migration of ions across the two ion exchange membranes. In the anodic compartment, anions, mainly chloride, accumulated. Part of the accumulated chloride was converted into chlorine, which represents a value-added product that could also be used for disinfection in water treatment plants. The galvanostatic operation (applied current: 0.16 mA $cm^{-2}_{membrane}$) with pH control (< 9) in the bio-cathodic compartment resulted in high nitrogen and salinity removal efficiencies (69±2% and 63±5%, respectively) and significant recovery of free chlorine (26.8±3.4 $mgCl_2$ L^{-1}). Standard quality requirements for drinking water in terms of nitrate concentration (91/767/EU) and electrical conductivity (98/83/CE) were successfully met with this cell configuration. However, considering the high capital costs required to implement BES-based technologies (Zhang and Angelidaki, 2013) and the need to promote

the scale-up of these systems, the process needs to be further optimised by increasing nitrate removal rates, reducing energy consumption. The performance of such systems could be limited by the hydrodynamics and the corresponding substrate distribution (Vilà-Rovira et al., 2015). Hydrodynamics are reinforced at higher flowrates (lower HRTs). This strategy was confirmed by Ceballos-Escalera et al., 2021 and Pous et al., 2017, reaching higher denitrifying capacities in denitrifying BES. However, the role of the HRT may be different in more complex groundwater and BES configurations like the one described by Puggioni et al. (2021), where biotic (e.g., nitrate removal) and abiotic (i.e., desalination, chloride removal and chlorine production) processes co-exist in the same reactor. In the present work, the effects of increasing influent flowrates on simultaneous denitrification and desalination of groundwater in a 3-compartment cell were investigated, providing helpful information for its optimisation with a view to a future application at pilot scale. Moreover, the bacterial communities of biomass established under the galvanostatic mode used in the present study, and the potentiostatic mode previously tested by Puggioni et al. (2021), were characterised and compared.

2. MATERIALS AND METHODS

2.1 Reactor set-up

Two identical 3-compartment cells made of polycarbonate were used (Puggioni et al., 2021). The bio-cathode compartment contained the graphite felt cathode electrode (64 cm^2, degree of purity 99.9%, AlfaAesar, Germany), and it was physically separated from the central compartment by a cation exchange membrane (CEM 7000, Membrane International Inc., USA). The anode compartment, containing the anode electrode consisting of a titanium mesh coated with mixed metals oxide (Ti-MMO, 15 cm^2, NMT-Electrodes, South Africa), was physically separated from the central compartment by an anion exchange membrane (AEM 7001, Membranes International Inc., USA). A reference electrode (Ag/AgCl, +0.197 V vs SHE, mod. MF2052, BioAnalytical Systems, USA) was placed in the bio-cathode

compartment. Cathode, anode, and reference electrodes were connected to a multichannel potentiostat (Ivium technologies, IviumNstat, NL). The system was thermostatically controlled at 25±1 °C.

2.2 Groundwater characteristics

A synthetic medium mimicking nitrate concentration and salinity of groundwater from the nitrate vulnerable zone of Arborea (Sardinia, Italy) was fed to the bio-cathode compartment. This medium contained 216.6 mg L^{-1} KNO_3 (corresponding to 30.0 mgNO_3^--N L^{-1}); 10 mg L^{-1} NH_4Cl (corresponding to 2.6 mgNH_4^+-N L^{-1}), 4.64 mg L^{-1} KH_2PO_4; 11.52 mg L^{-1} K_2HPO_4; 350 mg L^{-1} $NaHCO_3$; 2000 mg L^{-1} NaCl and 100 µL L^{-1} of trace elements solution (Patil et al., 2010). The media was prepared using distilled water and pre-flushed with N_2 gas for 15 minutes to avoid any presence of oxygen. The medium's electrical conductivity and pH were 3.06±0.5 mS cm^{-1} and 8.2±0.3, respectively.

2.3 Experimental procedure

The cells were started-up and tested as described by Puggioni et al. (2021). The bio-cathode compartment was continuously fed with groundwater, and the effluent was sent into the central compartment to achieve desalination. Tap water was recirculated in the anode compartment and replaced periodically (about every 10 days). The potentiostat was set in galvanostatic mode at current of 10 mA (0.16 mA $cm^{-2}_{membrane}$). A pH control (< 9) was implemented to avoid excessive pH increases in the bio-cathode compartment by dosing HCl (1 M) in the bio-cathode recirculation line. The probe for continuous pH measurement (Mettler Toledo, mod. InPro 3253i/SG/225, USA) was connected to a transmitter (Mettler Toledo, mod. M300, USA), which recorded data every 10 minutes.

The enhancement of electro-bioremediation systems must be linked to the treatment capacity. In this sense, hydraulic retention time (HRT) was used as the operational parameter, as presented in Table 1.

Different HRTs were tested, from the previous proof-of-concept (Puggioni et al. 2021) value 30.1±2.3 (Test 1) to 2.4±0.2 h (Test 6), by increasing the influent flowrate. During Test 7, the same HRT of Test 5 (4.9±0.4 h) was applied to confirm the stability of the system. Each HRT was maintained for about one month. The nitrate concentration in the influent was also maintained at 29.3±3.5 mgNO_3^--N L^{-1}.

Table 1. Experimental procedure.

Tests	**Influent flowrate [L d^{-1}]**	**HRT Bio-cathode + central compartment [h]**	**HRT' central compartment [h]**	**NO_3^--N loading rate [mg L^{-1} d^{-1}]**
1	0.11	30.1±2.3	6.7±0.3	23.57±1.84
2	0.17	20.3±1.5	4.5±0.2	35.14±2.39
3	0.31	10.9±0.8	2.4±0.1	62.61±3.90
4	0.46	7.3±0.6	1.6±0.1	82.21±3.07
5	0.68	4.9±0.4	1.1±0.05	125.48±2.98
6	1.42	2.4±0.2	0.5±0.02	261.05±16.07
7	0.68	4.9±0.4	1.1±0.05	130.92±11.27

2.4 Analytical methods

Samples were periodically drawn from influent (once per week), effluent (three times per week), bio-cathode and anode compartments (three times per week) in order to evaluate overall cell performances. The same samples from the duplicate cell were taken once a week to confirm the process progress of the main cell. Liquid samples were analysed for quantification of anions, i.e., chloride (Cl^-), nitrite (NO_2^--N), nitrate (NO_3^--N), phosphate (PO_4^{3-}), and sulphate (SO_4^{2-}), using an ion chromatograph (ICS-90, Dionex-Thermofisher, USA) equipped with an AS14A Ion-PAC 5 μm column. Samples were filtered (acetate membrane filter, 0.45 μm porosity) and properly diluted with grade II water. The concentrations of the main cations, i.e., potassium (K^+) and sodium (Na^+), were determined

using an ICP/OES (Varian 710-ES, Agilent Technologies, USA): samples were filtered (acetate membrane filter, 0.45 μm porosity), acidified (nitric acid, 1% v:v) and diluted with grade I water.

Electrical conductivity and pH were measured using a benchtop meter (HI5522, Hanna Instruments, Italy).

The concentration of free chlorine was analysed using spectrophotometric techniques (DR1900, Hach Lange, Germany) and the DPD (N,N-diethyl-p-phenylenediamine) free chlorine method (DPD free chlorine reagent powder pillows Cat. 2105569, Hach Lange, Germany).

Nitrous oxide (N_2O) was measured using an N_2O liquid-phase microsensor (Unisense, Denmark) located in the effluent line of the reactors, thanks to a dedicated glass measuring cell. The resulting bio-cathode potentials were recorded every five minutes through potentiostat (Ivium technologies, IviumNstat, NL). Cell potential was periodically checked using a multimeter (K2M, mod. KDM-600C, Italy).

2.5 Calculations

Nitrate Removal Efficiency (N-RE) and Nitrate Removal Rate (N-RR) were calculated according to equations 1 and 2, respectively:

$$N - RE\ [\%] = \frac{C_{NO_3^- - N(inf)} - C_{NO_3^- - N(eff)}}{C_{NO_3^- - N(inf)}} \times 100 \qquad \textbf{(1)}$$

$$N - RR\ [mg\ N\ L^{-1}\ d^{-1}] = \frac{C_{NO_3^- - N(inf)} - C_{NO_3^- - N(eff)}}{HRT} \qquad \textbf{(2)}$$

Where $C_{NO3^-\text{-}N(inf)}$ and $C_{NO3^-\text{-}N(eff)}$ [$mgNO_3^-$-N L^{-1}] are nitrate concentrations in the influent and the effluent, respectively, while HRT [d] is the hydraulic retention time considering the volumes of the cathodic and central compartments.

The desalination performance was evaluated by calculating the electrical conductivity removal efficiency (EC-RE, equation 3), the chloride removal efficiency (Cl^--RE, equation 4), and the chloride removal rate (Cl^--RR, equation 5).

$$EC - RE\ [\%] = \frac{EC_{(inf)} - EC_{(eff)}}{EC_{(inf)}} \times 100 \quad \textbf{(3)}$$

$$Cl^- - RE\ [\%] = \frac{C_{Cl^-(inf)} - C_{Cl^-(eff)}}{C_{Cl^-(inf)}} \times 100 \quad \textbf{(4)}$$

$$Cl^- - RR\ [mg\ L^{-1}\ d^{-1}] = \frac{C_{Cl^-(inf)} - C_{Cl^-(eff)}}{HRT'} \quad \textbf{(5)}$$

where $EC_{(eff)}$ [mS cm^{-1}] and $C_{Cl\text{-}(eff)}$ [mg L^{-1}] represent the effluent electrical conductivity and chloride concentration, respectively. $EC_{(inf)}$ and $C_{Cl\text{-}(inf)}$ correspond to the electrical conductivity and chloride concentration of the solution in the bio-cathodic compartment (i.e., the influent to the central compartment), respectively, to consider the chloride input due to the acid dosage in this compartment. HRT' [d] is the hydraulic retention time of the central compartment.

The coulombic efficiency for nitrate reduction (εNO_x) was calculated according to equation 6 (Virdis et al., 2008):

$$\varepsilon NO_x[\%] = \frac{I}{n\,\Delta C_{NO_x} Q_{in} F} \times 100 \quad \textbf{(6)}$$

where I is the fixed current [A]; n is the number of electrons that can be accepted by 1 mol of oxidised nitrogen compound present in the bio-cathodic compartment, assuming N_2 is the final product; ΔC_{NOx} is the difference between the nitrate concentration in the cathodic influent and effluent [molNO_3^--N L^{-1}]; Q_{in} is the influent flow rate [L s^{-1}]; F is Faraday's constant [96485 Ce^- mol^{-1}].

The current efficiency (CE) was expressed as the percentage of the charge associated with the chloride removed from the central compartment to the amount of electric charge transferred (ECT) across the membranes (Ramírez-Moreno et al., 2019). CE [%] and ECT [C m^{-3}] were calculated using equations 7 and 8, respectively:

$$CE\ [\%] = \frac{v\ z\ F\left(C_{Cl^-(inf)} - C_{Cl^-(eff)}\right)}{ECT} \times 100 \quad \textbf{(7)}$$

$$ECT\ [C\ m^{-3}] = \frac{\int I\ dt}{V} \quad \textbf{(8)}$$

where v and z represent the stoichiometric coefficient and the valence of the chloride ion, respectively; V [m^{-3}] is the volume of water treated; dt is the time [s].

The specific energy consumption (SEC) was calculated according to equation 9 (Jingyu et al., 2017):

$$SEC\ [kWh\ m^{-3}] = \frac{I \int E\, dt}{V} \qquad \textbf{(9)}$$

where E is the cell potential [V].

2.6 Analysis of bacterial communities by NGS of 16S rRNA gene

The composition of the bacterial communities in the cathodic biofilm was characterised. Samples of the biofilms formed on the bio-cathode were axenically collected at the end of Test 5 (Table 1). Five cathode points were sampled, and the biomass was pooled into a composite sample to mitigate the effects of microscale heterogeneity on the bio-cathode. Biomass samples were stored at -20°C before DNA extraction. Genomic DNA was extracted from biomass samples (250 mg wet weight) using the DNeasy PowerSoil Pro Kit (QIAGEN), and DNA was subsequently purified using the DNeasy PowerClean Cleanup Kit (QIAGEN). The DNA quality and concentration were determined on agarose gel using a DNA quantitation standard. DNA samples were submitted to Bio-Fab Research Srl (Rome, Italy) for sequencing of the V3-V4 region of the bacterial 16S rRNA gene on an Illumina Miseq platform (Illumina, San Diego, CA) using 2 × 300 bp paired-end reads.

For data processing, raw sequences were demultiplexed by the sequencing facility. Reads were trimmed to remove primer sequences using the CutAdapt version 3.5. Sequences were imported into Quantitative Insights into Microbial Ecology (QIIME 2) version 2020-11 (Bolyen et al., 2019). Using the DADA2 pipeline (Callahan et al., 2016), reads with ambiguous and poor-quality bases were discarded, good quality reads dereplicated and denoised, and the paired reads merged. Chimeras and singletons were identified and removed from the dataset. DADA2 was used to produce alternative sequence variants

(ASVs), thus obtaining a filtered ASV-abundance table. For each ASV, a representative sequence was used for taxonomy assignment against the Silva database release 138 (Quast et al., 2013). The indices of diversity (richness as the number of observed ASV, Shannon with an e log base) and evenness (Pielou's) were used to assess the alpha-diversity by using the vegan R package (Oksanen et al., 2019). Read count data were normalised by Cumulative Sum Scaling (CSS) transformation using the metagenomeSeq package (Paulson et al., 2013). The Bray-Curtis similarity index between samples was calculated.

RESULTS AND DISCUSSION

3.1 Effect of the HRTs on denitrification and desalination performances

The system's enhancement was tested by increasing the influent flowrate and, thus, reducing the HRT within the system. Figure 1 shows the average NO_3^--N loading and removal rates at different influent flowrates.

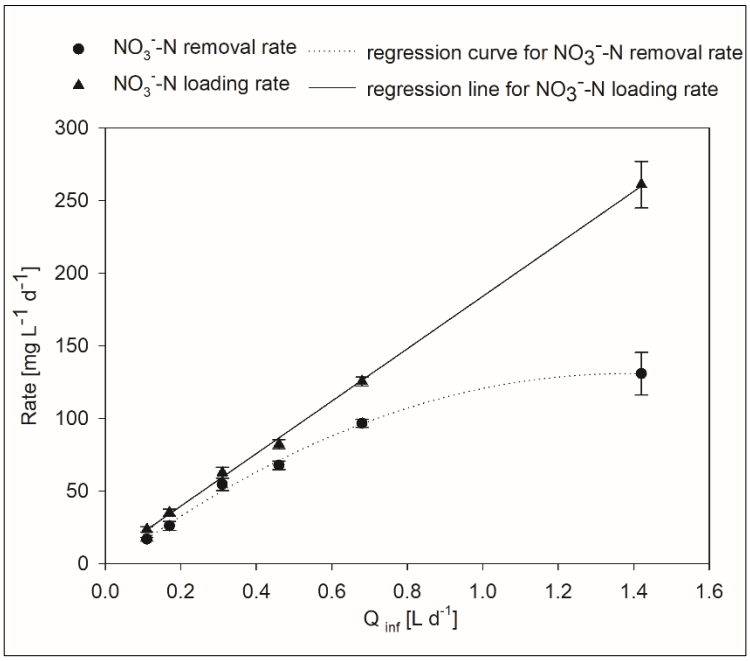


Figure 1. Average trend in nitrate-nitrogen loading into the system and nitrate-nitrogen removal rate as influent flowrate (Q_{inf}) increases.

The nitrate loading rate was increased from 23.6±1.8 mgNO_3^--N $L^{-1}d^{-1}$ in Test 1 to 261±16 mgNO_3^--N $L^{-1}d^{-1}$ in Test 6 by reducing the HRT. Nitrogen removal rate increased (from

16.9±1.3 mgNO_3^--N $L^{-1}d^{-1}$ in Test 1 to 130.8±14.7 mgNO_3^--N $L^{-1}d^{-1}$ in Test 6) but did not follow the same trend as the NLR, since a gradual deviation was observed.

The increase in the NRR with the influent flowrate could be explained by an increase in the denitrification activity of the autotrophic biomass due to the proper supply of nitrate and better hydrodynamic distribution (Pous et al., 2017; Vilà-Rovira et al., 2015).

Despite the increasing NRR, nitrate concentration in the effluent started to increase from Test 4 onward (Figure 2). The nitrate effluent concentration remained below the legal limits (<11.3 mgNO_3^--N L^{-1} from the Nitrate Directive 91/767/EU) throughout the experiment except during Test 6 (flowrate 1.42 L d^{-1} and HRT of 2.4±0.2 h), which exceeded this limit (with an average of 13.5±2.8 mgNO_3^--N L^{-1}, corresponding to a N-RE of 50±8 %).

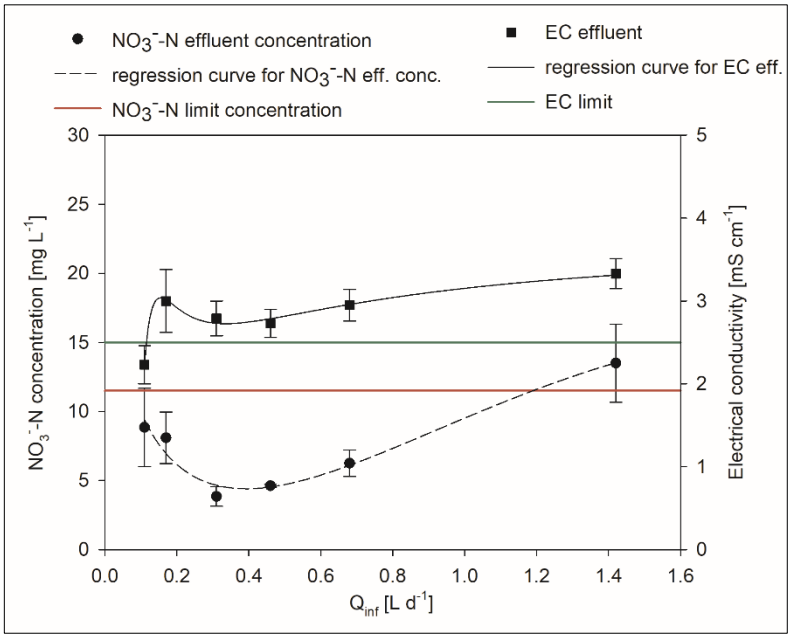


Figure 2. The average nitrate concentration and electrical conductivity (EC) trend in the effluent as influent flowrate increases.

During Test 6, low nitrite and nitrous oxide concentrations were detected in the effluent (0.22±0.08 mgNO_2^--N L^{-1} and up to 0.5 mgN_2O-N L^{-1}, respectively). Therefore, it is evident that during Test 6 (influent flowrate of 1.42 L d^{-1}), limiting operating conditions were reached for the system regarding nitrate removal.

Interestingly, the increase in nitrate removal rate as HRT decreased was also observed in previous studies. Figure 3 compares the trend in nitrate removal rate versus the HRT for the current study and those reported by Pous et al. (2017) and Ceballos-Escalera et al. (2021), exploiting tubular systems with hydraulically connected anode and cathode compartments. Although the systems were highly heterogeneous in terms of configuration (3-chamber plate cell vs tubular cells), materials (graphite felt vs granular graphite), and operating conditions (galvanostatic vs potentiostatic modes), the same mathematical model was able to fit the observed NRR vs HRT relationship.

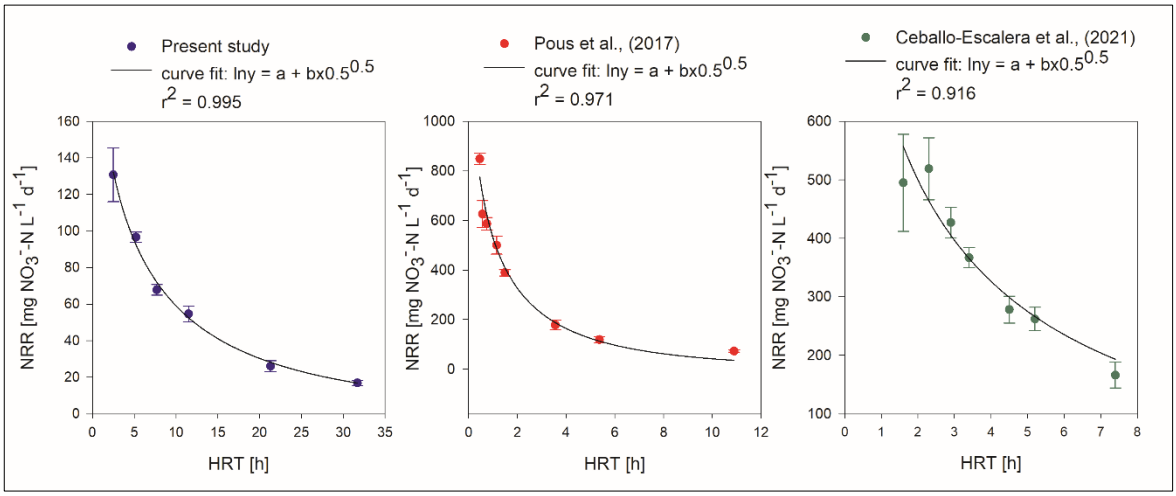


Figure 3. Comparison of nitrate removal rate (NRR) trends versus the HRT for the present study with that reported by Pous et al. (2017) and Ceballos-Escalera et al. (2021) and modelling of results.

This result is interesting as it confirms that increasing the influent flowrate (and thus reducing the HRT) can positively influence denitrification activity. Therefore, regardless of the type of configuration or operating conditions used, the process behaviour with different HRTs may be reasonably predicted, providing useful information in the perspective of reactor scale-up.

A final test (Test 7) was carried out by bringing the HRT value back to that corresponding to Test 5 (i.e., 4.9±0.4 h) to restore the denitrifying process and verify microbial activity. The performance in terms of nitrate removal observed during Test 5 (N-RE = 77±3%, and N-RR = 96.7±2.8 mgNO_3^--N $L^{-1}d^{-1}$) was immediately restored during Test 7, resulting in an

297 average N-RE of 89±4% and N-RR of 112±7.5 mgNO_3^--N $L^{-1}d^{-1}$. In addition, while in Test
298 6 (HRT of 1.4 h), the effluent concentration of nitrate exceeded the legal limits (13.5±3
299 mgNO_3^--N L^{-1}), the concentration was below the limits in Tests 5 and 7 (6±1 and 3±1
300 mgNO_3^--N L^{-1}, respectively). No nitrite or nitrous oxide were detected in the outlet during
301 Test 7. The slight increase in performance observed between Test 5 and Test 7 demonstrates
302 that biomass growth may have contributed in a small part to the increase in denitrifying
303 performance. This result confirms the limiting conditions for denitrification reached in Test
304 6, during which a general decline in terms of nitrate removal and production of intermediates
305 were observed. However, this condition turned out to be reversible according to Test 7,
306 demonstrating not a biomass inhibition condition but just an operational limit in Test 6. Since
307 the applied current was initially much higher than that theoretically required to remove the
308 nitrate input (10 mA applied vs approx. 1.4 mA theoretically required in Test 1), the
309 coulombic efficiency for nitrate removal was always above 100%, decreasing as the HRT
310 decreased, and reaching values close to 100% during Test 6.

311 An almost opposite trend to that of nitrate removal was observed for the desalination process.
312 The desalination process showed the best performance in Test 1 (with an effluent
313 conductivity of 2.2±0.2 mS cm^{-1}), which met the required limit of 2.5 mS cm^{-1} (98/83/CE
314 Directive) but exceeded this value in Test 2 and gradually worsened in subsequent tests
315 (Figure 2). Figure 4 shows the trend of electrical conductivity in the influent and effluent of
316 the central desalination compartment and the desalination efficiency. As expected, the
317 overall conductivity removal rate throughout the experiment was 23.4±7.3 mS cm^{-1} d^{-1}, so it
318 did not vary substantially as the HRT decreased. Thus, the desalination trend was limited
319 only by a physico-chemical effect due to insufficient charge replenishment as HRT
320 decreases. This effect could easily be overcome by increasing the applied current in
321 proportion to the increase in influent flowrate.

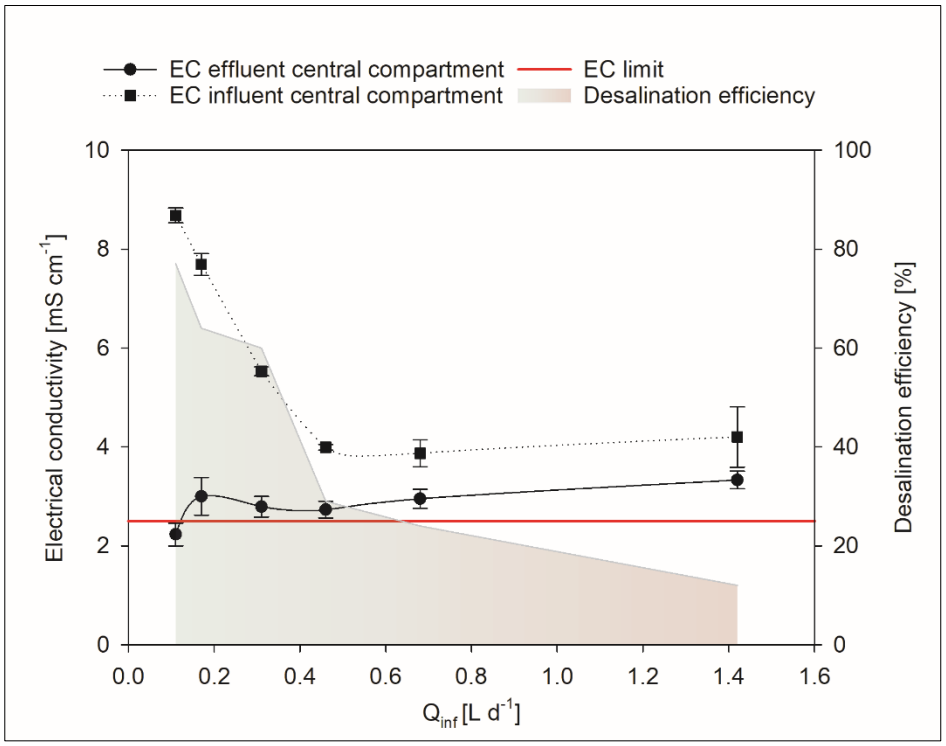


Figure 4. Average trend of central compartment influent and effluent electrical conductivity, and desalination efficiency versus the influent flowrate. The red line indicates the electrical conductivity limit for freshwater (2.5 mS cm^{-1}).

The influent conductivity of the central desalination compartment (corresponding to the effluent of the bio-cathode compartment) dropped from 8.7±0.2 mS cm^{-1} in Test 1 to 4.2±0.6 mS cm^{-1} in Test 6, likely as a result of the increased influent flowrate which probably led to a faster turnover of the solution in the bio-cathodic compartment, reducing the accumulation of both Cl^- ions due to the acid dosage, and cations migrating from the central compartment through the CEM. The average chloride concentration measured in the cathode chamber (and thus including the influent and acid dosage) decreased from 3622±443 in Test 1 to 1385±56 mgCl^- L^{-1} in Test 6, while the sodium concentration decreased from 2355±370 to 1040±182 mgNa^+ L^{-1}, respectively, for Test 1 and Test 6.

On the other hand, the effluent electrical conductivity increased slightly to 3.3 mS cm^{-1}. The electrical conductivity trend in the bio-cathode compartment and effluent trend resulted in a reduction of the overall desalination efficiency, which dropped to 12±2% in the last test (from 77±13% in Test 1). Coherently, the current efficiency related to the removal of chloride in the central compartment decreased during the experiment from 89±14% in Test

1 to 59±15% in Test 6. This performance could be related to the variation of the influent flowrate and may be explained by insufficient HRT (passing from 6.7±0.3 h in Test 1 to 0.5±0.02 h in Test 6) in the central desalination compartment. Calculating the theoretical quantity of chloride ions that can be transferred through the membranes by applying a current of 10 mA to the varying HRT gives 2.8 g L^{-1} for Test 1 and 0.22 g L^{-1} for Test 6. These values are very close to those actually obtained and correspond to 2.5±0.4 and 0.13±0.03 $gCl^-_{removed}$ L^{-1}, respectively, for Test 1 and Test 6, confirming the above results. Thus, the HRT decrease did not allow sufficient ions to migrate through the membranes to observe a significant reduction in effluent electrical conductivity. The adverse effect of low HRT on desalination performance was already demonstrated for the technology closest to the present study, i.e., MDCs (microbial desalination cells). Indeed, Jingyu et al. (2017) reported that HRT influences the removal of total dissolved solutes (TDS), increasing with the HRT, resulting in a higher current generation in MDC.

Chlorine production in the anodic compartment was monitored throughout the whole experimentation, and an average chlorine concentration of 14±3 $mgCl_2$ L^{-1} was observed. An essential aspect of monitoring is the durability of materials in contact with chlorine, as it is a powerful oxidant, and it tends to attack and damage them. For this reason, it was decided to replace the solution in the anodic chamber periodically (about every 10 days, producing an average concentration of 16±1 $mgCl_2$ L^{-1}) to avoid system damage. Higher values of chlorine concentration (approx. 30 $mgCl_2$ L^{-1}) were obtained by Puggioni et al. (2021) in the same system at the highest HRT tested but without the periodic replacement of the solution. In addition to being an effective disinfectant, chlorine has the additional advantage that its residue can protect the downstream flow from the point of disinfection (the WHO recommends a residual concentration of free chlorine greater than or equal to 0.5 mg L^{-1} after at least 30 minutes of contact with a pH below 8.0).

3.2 Considerations on pH development during the process

Increasing the influent flow rate also had an effect on pH trend in the different compartments. pH control plays a significant role in ensuring optimal denitrifying microbial activity, as a neutral pH is strictly necessary for this biological process (Clauwaert et al., 2009). Such control has become essential to optimise water desalination performance. Several studies demonstrated that the pH gradient between the anode and cathode compartments could lead to potential losses (of approximately 0.095 V) that adversely affect the desalination efficiencies of MDCs (Jingyu et al., 2017).

During the experiment, the periodic dosage of acid to control the pH in the bio-cathodic compartment remained constant over the entire experimental period. This occurrence resulted in a difference mainly in the effluent pH as a function of the influent flowrate. Figure 5 shows that while the influent pH mainly remained constant, the effluent pH increased from near-acidic (4.1±1.2) in Test 1 to slightly alkaline (7.8±0.3) in Test 6.

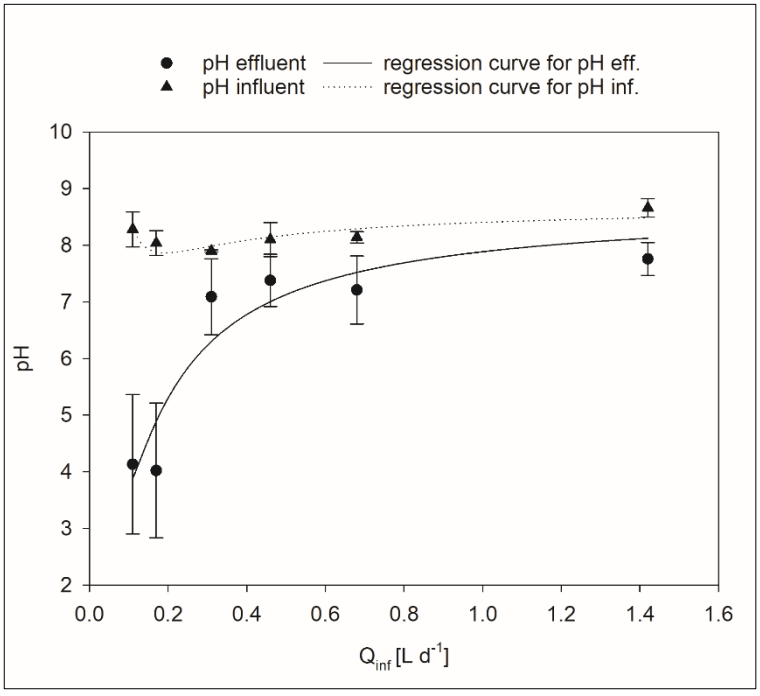


Figure 5. Average influent and effluent pH trend versus the influent flowrate.

Acidic pH values in the effluent corresponding to the first tests may be due to the higher HRT in the central desalination chamber (6.7±0.3 h in Test 1) that allowed protons produced at the anode (pH 2.0±0.7) to pass through the AEM, because of their small size. By reducing the HRT (to 0.5±0.02 h in Test 6) as the influent flowrate increased, the solution replacement led to a slowing down of the pH increase in the bio-cathode compartment and a lower passage of protons through the AEM into the effluent. In addition, the acid dosage per m^3 of treated water was reduced as the influent flowrate increased, which means lower operating costs for pH balancing.

3.3 Bacterial community diversity on the bio-cathode of the 3-compartment BES

Cathodic biomass was collected at the end of Test 5. The bacterial community composition of the biomass is shown in Figure 7A. The most abundant phyla of Bacteria in the biomass were Proteobacteria (44.0%) followed by Actinobacteriota (16.0%), Firmicutes (11.8%), Bacteroidota (10.8%), Planctomycetota (5.1%) and Chloroflexi (4.8%). The other less abundant phyla were all below the 3%, while the unassigned sequences accounted for 1.1% in the composition of bacterial community. At order level, the most abundant taxa were Rhizobiales (17.0%), Corynebacteriales (7.4%), and Burkholderiales (6.6%), followed by Xanthomonadales (4.5%), Alteromonadales (4.3%), and Thermomicrobiales (4.2%). At genus level, seven most abundant taxa accounted for more than 20% of the total community, including the genera *Rhizobium* (3.9%) and *Bosea* (3.1%) in Rhizobiales, *Mycobacterium* (3.2%) and *Gordonia* (2.4%) in Corynebacteriales, *Fontibacter* (2.6%) in Cytophagales, *Clostridium sensu strictu* (2.4%) in Firmicutes as well as the uncultured JG30-KF-CM45 in Thermomicrobiales (3.2%).

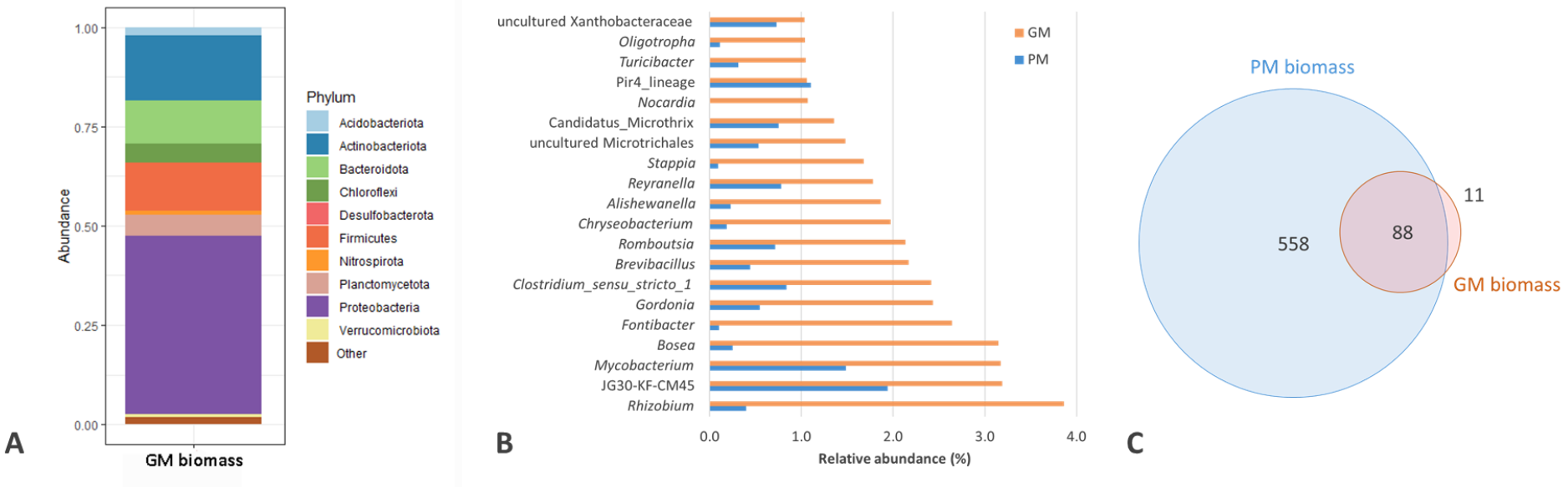
In order to compare the biomass established under the galvanostatic mode (GM), used in the present study, and potentiostatic mode, previously tested by Puggioni et al. (2021), a sample of the biofilm formed on the bio-cathode of the cell working in potentiostatic mode (PM)

was also analysed and the difference in the bacterial communities between the GM and PM biomass investigated in terms of alpha-diversity and community composition.

The community in GM biomass was characterised by a minor bacterial alpha-diversity as highlighted by a lower number of ASVs (i.e., reduced richness) and a higher community dominance (i.e., reduced evenness) in comparison to the PM biomass (Table S1). A marked difference in the bacterial community composition was also evident at the different taxonomic ranks. As compared to the PM biomass, the GM bio-cathodic community is characterised by the increase in the relative abundances (RAs) of Proteobacteria (+11.2%) and Firmicutes (+6.4%), and the decrease in RAs of Planctomycetota (-8.9%) and Chloroflexi (-7.3%). At order level, the more pronounced changes in RAs were the enrichment in Rhizobiales (+7.9%), Corynebacteriales (+5.3%), Alteromonadales (+3.5%), and Xanthomonadales (+3.4%). The comparison between PM and GM also showed the reduction in Pirellulales (-3.1%). Moreover, Caldilineales and Anaerolineales were not detected in the GM cathodic biofilm, while they accounted for 3.4% and 2.6% in the PM biomass, respectively. At genus level, the highest differences were found for the taxa *Rhizobium*, *Bosea*, *Fontibacter*, *Gordonia*, which were all below the 0.5% in PM biomass, while they predominated the composition of GM bacterial community (Figure 7B). Out of the 646 ASVs found in the PM biomass, 88 ASVs were shared between the two communities, while 11 and 558 ASVs were unique of GM and PM biomass, respectively (Figure 7C). Among the ASVs unique of the GM biomass, ASV01 affiliated to an uncultured lineage of Burkholderiales was also the most abundant ASV, accounting for 2.5% of the GM bacterial community (Table S2). Other ASVs exclusively found in the GM biomass were ASV026 (1.1%) and ASV027 (1.1%) affiliated to the genera *Fontibacter* and *Nocardia*, respectively.

Overall, biodiversity was severely restricted under galvanostatic mode. Presented results suggested that test conditions exerted a selective pressure on the bacterial community of the cathodic biofilm influencing its organisation and enriching few dominant populations. Moreover, an active role in denitrifying biomass has been previously proposed for several bacteria dominating the GM bio-cathodic biomass. More specifically, isolates affiliated to Rhizobiales have been proved to denitrify under autotrophic and heterotrophic conditions (Vilar-Sanz et al., 2108), and the genus *Rhizobium* has been implied in denitrification in MFC system for treating saline wastewater (Xu et al., 2019). *Clostridium sensu strictu* has been detected at a high amount in MEC biomass and suggested to be responsible for autotrophic denitrification in a bioelectrochemically-assisted constructed wetland system (Sotres et al., 2015; Xu et al., 2017). Recently, the genus *Fontibacter* has been found to be enriched after long term adaptation in a BES for nitrate removal from coke wastewater effluent (Tang et al., 2017) and a species of the genus, isolated from an MFC, has been proved to couple oxidation of organic matter to Fe(III) reduction (Zhang et al., 2013). On the contrary, other dominant populations in the GM biomass, such as Corynebacteriales, have been less extensively described, and their metabolic role in bioelectrochemical systems is far to be undiscovered.

Figure 7. Bacterial communities of the biofilms formed on the bio-cathode of the 3-compartment bio-electrochemical cells **A:** Bar plot showing the contribution at phylum level in cathodic biomass under galvanostatic mode (GM). **B:** twenty most abundant genera in GM biomass and comparison with biomass established under potentiostatic mode (PM). **C:** Venn chart showing the overlap of ASVs in bacterial communities of cathodic GM and PM biomasses.



3.4 Sustainability perspective on the application of BES for simultaneous denitrification and desalination

In order to move towards scaling-up of the technology for groundwater treatment, the system must be both technically and economically feasible. For this reason, a preliminary cost-benefit analysis was carried out comparing the main operational costs associated with the technology and the potential benefits obtained according to experimental data.

The operating costs of a technology depend significantly on the energy consumption of the process. Figure 6 presents the profiles of the specific energy consumption (SEC) per gram of NO_3^--N removed and SEC per volume of water treated as a function of influent flowrate and compared with the trend in nitrate removal rate.

During the experiment, an optimisation was observed not only in the removal of nitrate but also in energy consumption, which was significantly reduced to values of $5.1 \cdot 10^{-2} \pm 0.7 \cdot 10^{-2}$ kWh $g^{-1}NO_3^-N_{removed}$ and 0.5±0.03 kWh $m^{-3}_{water\ treated}$ (starting from $35.2 \cdot 10^{-2} \pm 3.6 \cdot 10^{-2}$ kWh $g^{-1}NO_3^-N_{removed}$ and 6.1±0.4 kWh $m^{-3}_{water\ treated}$, respectively).

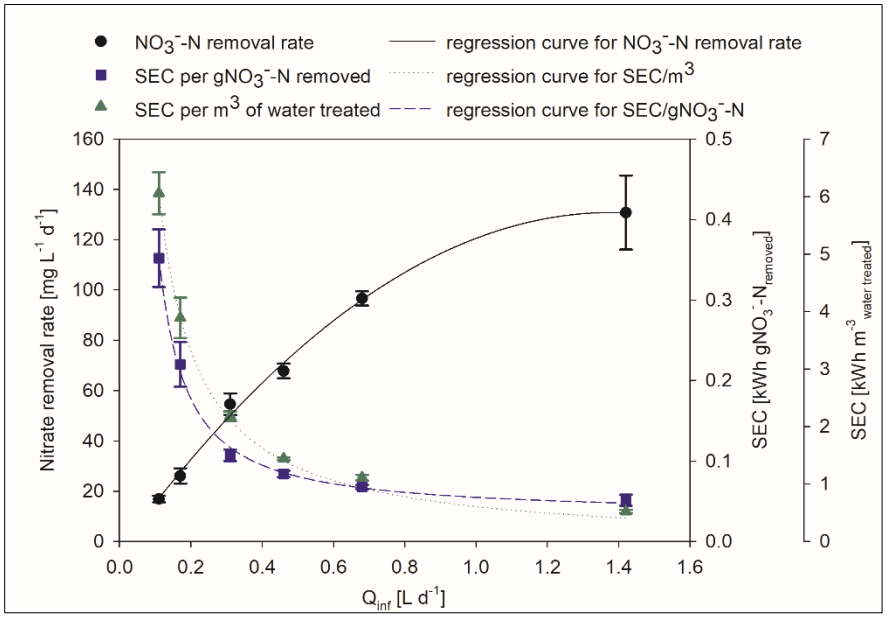


Figure 6. Average trends in specific energy consumed (SEC) per gram of nitrate-nitrogen removed and per volume of water treated, and nitrate removal rate as the inlet flow rate changes.

Pous et al. (2015) reported a list of specific energy consumptions for various technologies such as bioelectrochemical systems (BES), biofilm electrode reactor (BER), membrane bioreactor (MBR), electrodialysis (ED) and reverse osmosis (RO) (Zhao et al., 2011; Twomey et al., 2010; McAdam and Judd, 2008; Ortiz et al., 2008). Compared to the reported values, the energy consumption per m^3 of treated water was within the consumption range reported for desalination technologies, i.e., electrodialysis and reverse osmosis (between 0.04 and 2.09 kWh $m^{-3}_{treated\ water}$). The energy consumption per gram of nitrate removed obtained in the present study was in line with those of the technologies reported for nitrate removal only (BES and BER mainly), thus between $0.16 \cdot 10^{-2}$ and $7 \cdot 10^{-2}$ kWh $g^{-1}NO_3^-$-$N_{removed}$. Specifically, the values obtained in this study are closer to those of BER ($7 \cdot 10^{-2}$ kWh $g^{-1}NO_3^-$-$N_{removed}$), which applies a potential difference between the electrodes, in contrast to BES where the potential of the cathode electrode is fixed. This type of catalytical operation produces hydrogen in the cathode chamber, which is then used by bacteria to reduce nitrate. In the present study, the current was fixed, and the potential established at the cathode (approximately -1.3 V vs Ag/AgCl) was suitable for hydrogen production. According to Pous et al. (2015), fixing the cathode potential makes it possible to control the reduction of nitrate in the end products and implies less energy consumption. In the present study, however, the aim is not only to remove nitrate but also to reduce the electrical conductivity of water, as well as the production of value-added products (chlorine). In fact, during the process, part of the chloride accumulated in the solution of the anodic compartment is converted into free chlorine (Puggioni et al., 2021). Thus, the energy applied is used to carry out three processes simultaneously with consumption comparable to systems carrying out a single process (i.e., only denitrification or desalination). Under optimal operating conditions (HRT = 4.9±0.4 h), the total cost of energy consumption is 0.23 € m^{-3}, assuming an energy cost of 0.21 € kWh^{-1} (Eurostat, 2021). This value is competitive,

considering that Ceballos-Escalera et al. (2022) estimated an operating cost of 0.14 € m^{-3} only for the bio-electrochemical nitrate removal.

From an economic point of view, the production of chlorine also plays an important role. Chlorine is a disinfectant agent that is highly used in water treatment plants, and its market value is growing significantly due to the rising demand from the agrochemical and pharmaceutical industries. Moreover, the rising demand for water treatment applications combined with increased awareness of better hygiene practices resulting from the impact of the Sars-CoV-2 pandemic will drive the need for chlorine among industrialists. Greaves et al. (2022) demonstrated that Sars-CoV-2 is successfully eliminated by disinfection with free chlorine in both deionised water and wastewater. Web-based chlorine market data show a forecast growth of the chlorine value at a CAGR (compound annual growth rate) between 3.5 and 4.5% for the period 2021-2027.

In the present study, up to 0.17 gCl_2 per $gCl^-_{removed}$ was produced, and this production can easily be increased by switching to a continuous mode in the anodic chamber or by stripping the chlorine produced. In fact, Puggioni et al. (2021) showed higher production rates at the start of the batch that gradually decreased to a plateau over long periods of operation. Therefore, switching to continuous mode would increase production rates while avoiding chlorine accumulation and excessive concentrations in the anode chamber. Optimising the chlorine capture system seems essential to maximise its production and reduce the contact time with the materials in the bioelectrochemical cell.

4. CONCLUSIONS

At higher flowrates (and lower HRT, between 7.3±0.6 and 2.4±0.2 h), an increase in nitrate removal was found, reaching removal rates of 131 mgNO_3^--N $L^{-1}d^{-1}$. The operating limit for denitrification was reached at an HRT of 2.4±0.2 h, during which an effluent nitrate concentration above legal limits (91/767/EU) and the presence of intermediates were observed. Desalination performance was reduced (from 77±13% in Test 1 to 12±2% in Test 6), but the effluent electrical conductivity remained close to the legal limits (98/83/CE). In addition, biodiversity in the cathodic biomass was severely restricted under galvanostatic mode and populations previously identified under denitrifying conditions in BES were enriched.

The tests carried out in the present study demonstrate the economic potential of the proposed technology thanks to the possibility of considerably reducing energy consumption while simultaneously increasing denitrification performance. Such result was achieved simply by acting on the treated flowrate (by reducing hydraulic retention times) and not on the reactor volumes, which would imply additional costs in terms of materials and space. Finally, chlorine production represents an enormous potential for possible real application as it would reduce the costs of any on-site disinfection or, in general, an economic return if it were to be resold.

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Table 1. Experimental procedure.

Tests	Influent flowrate [L d^{-1}]	HRT Bio-cathode + central compartment [h]	HRT' central compartment [h]	NO_3^--N loading rate [mg L^{-1} d^{-1}]
1	0.11	30.1±2.3	6.7±0.3	23.57±1.84
2	0.17	20.3±1.5	4.5±0.2	35.14±2.39
3	0.31	10.9±0.8	2.4±0.1	62.61±3.90
4	0.46	7.3±0.6	1.6±0.1	82.21±3.07
5	0.68	4.9±0.4	1.1±0.05	125.48±2.98
6	1.42	2.4±0.2	0.5±0.02	261.05±16.07
7	0.68	4.9±0.4	1.1±0.05	130.92±11.27

Figure 01

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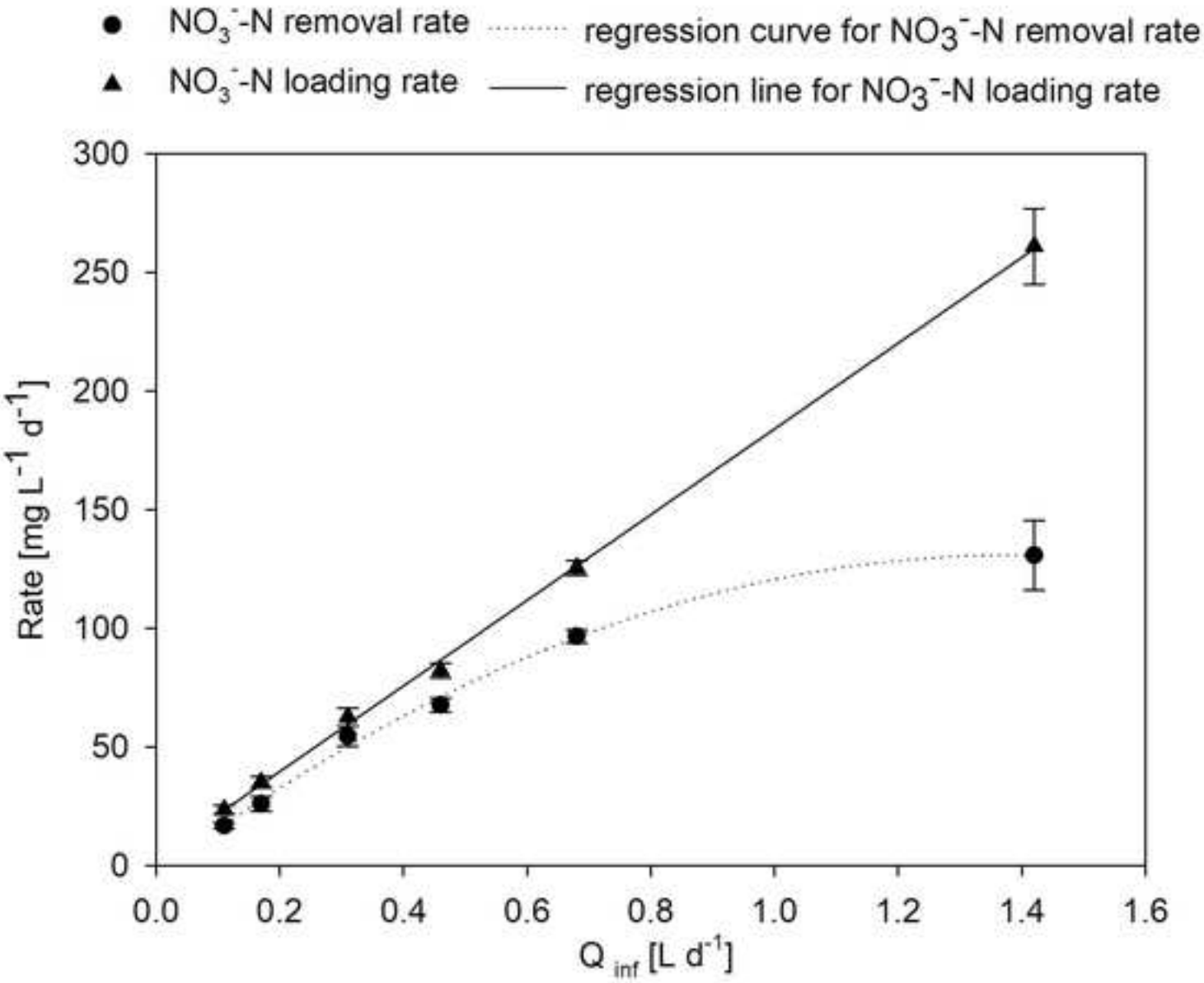

Figure 02

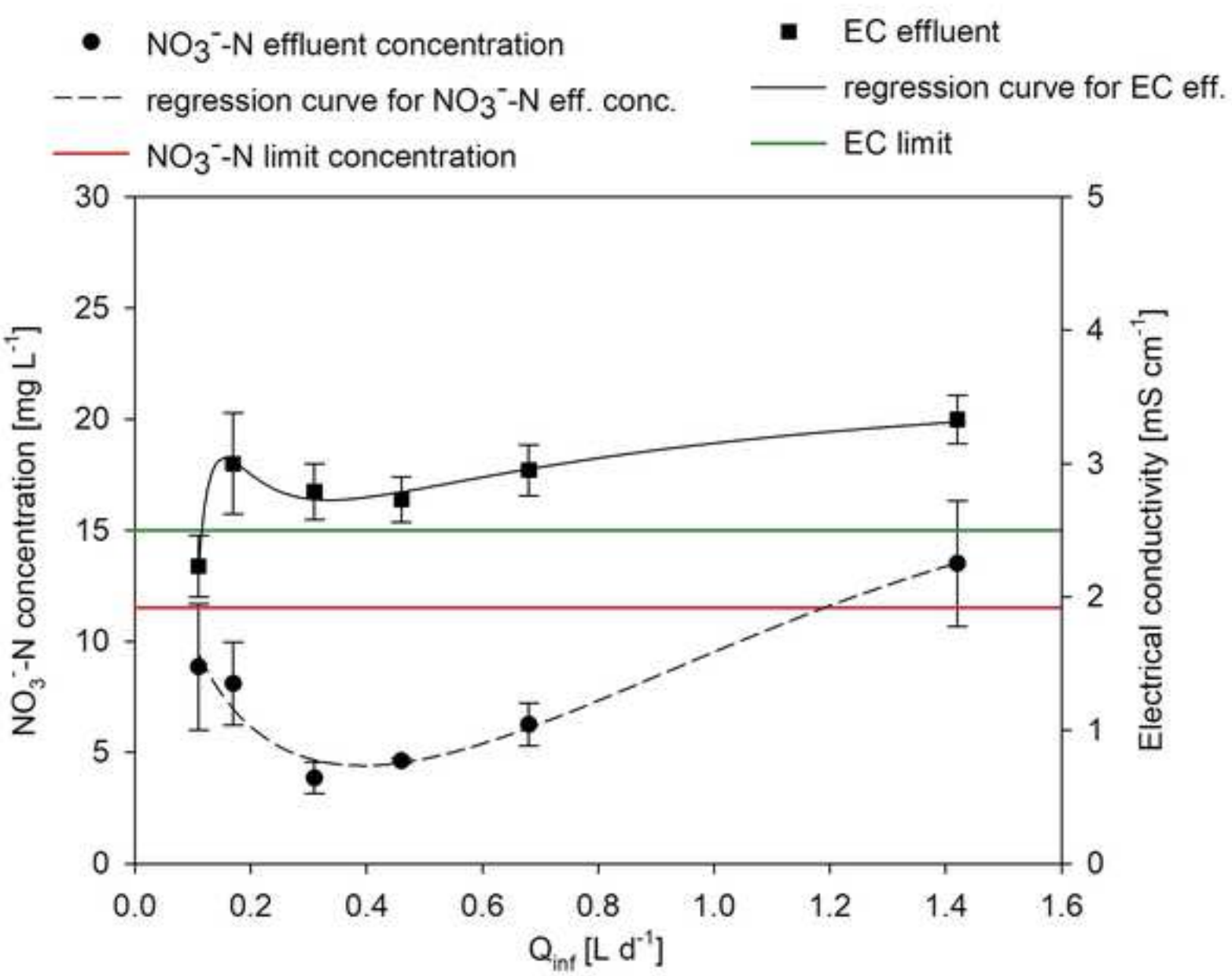

Figure 03

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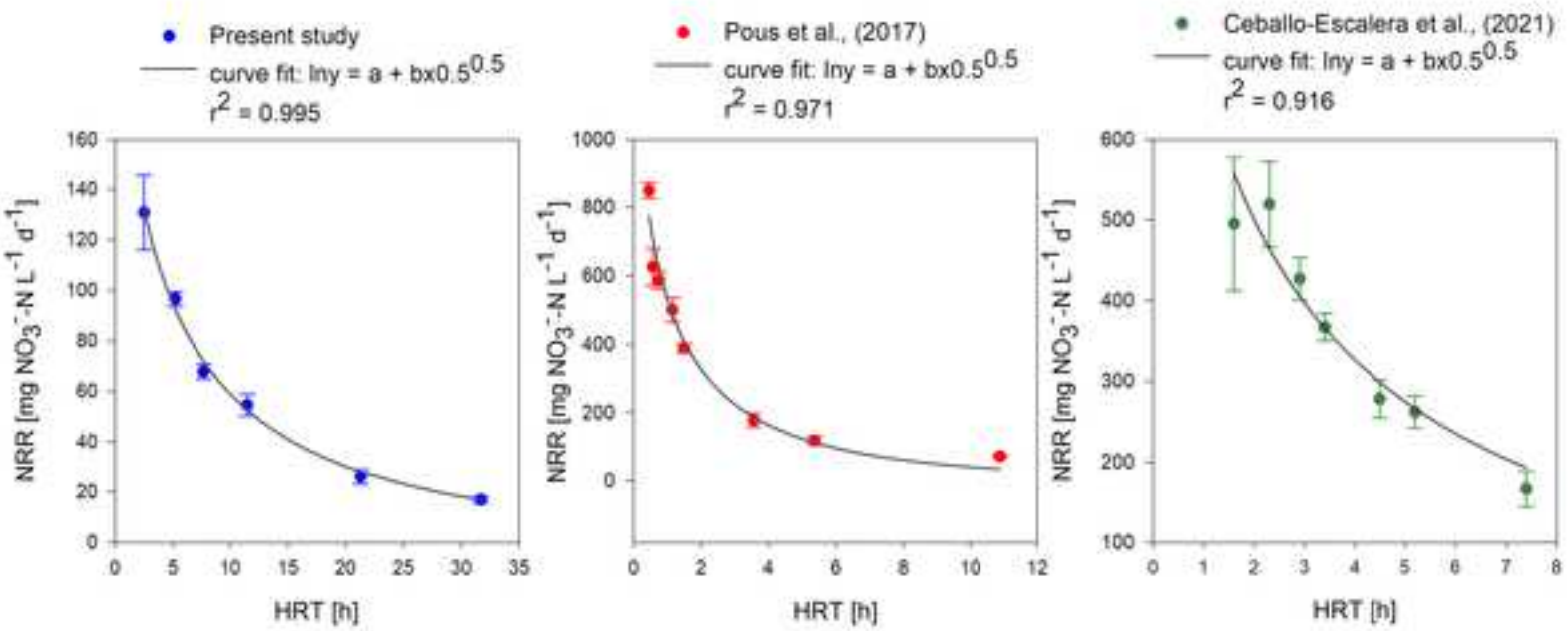

Figure 04

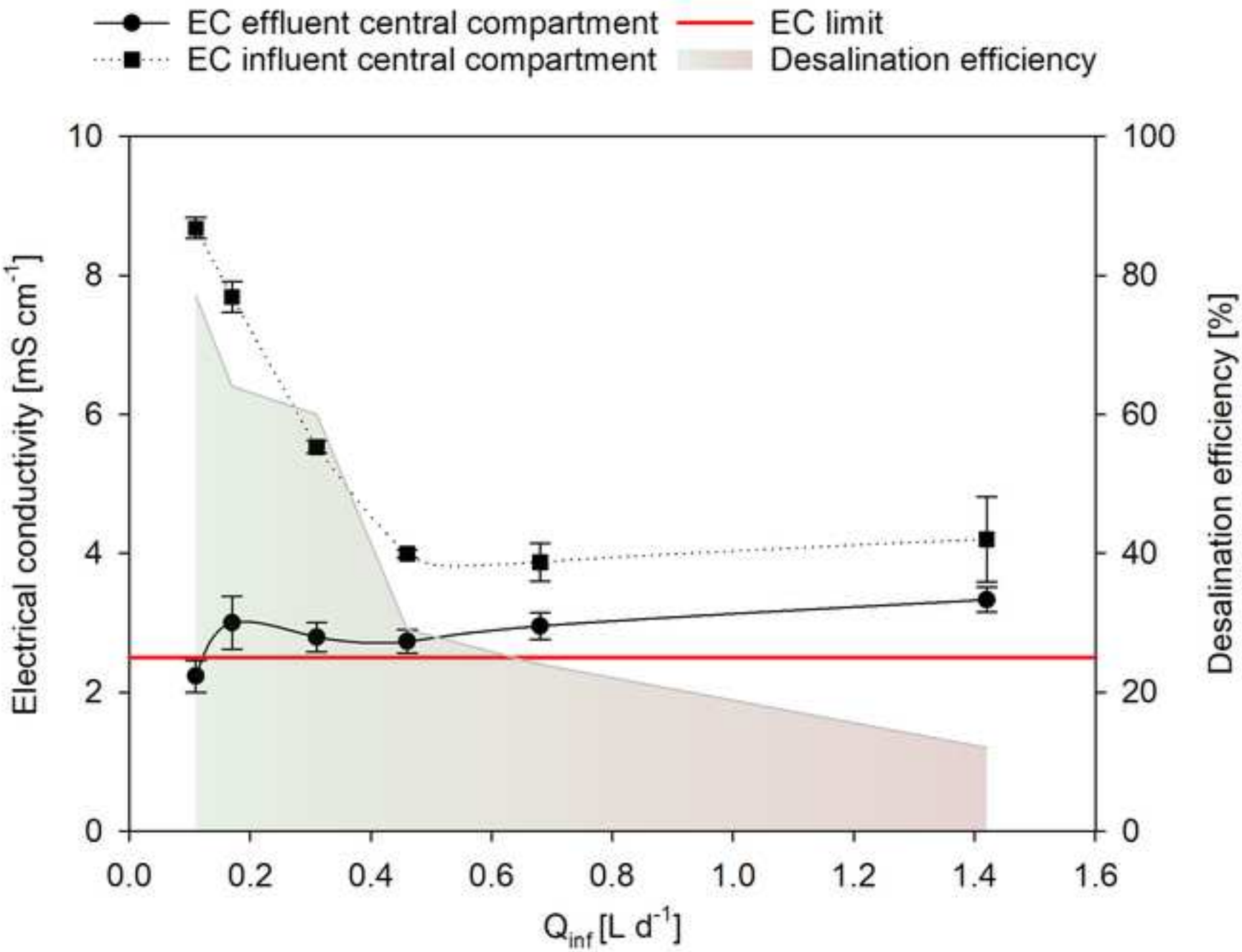

Figure 05

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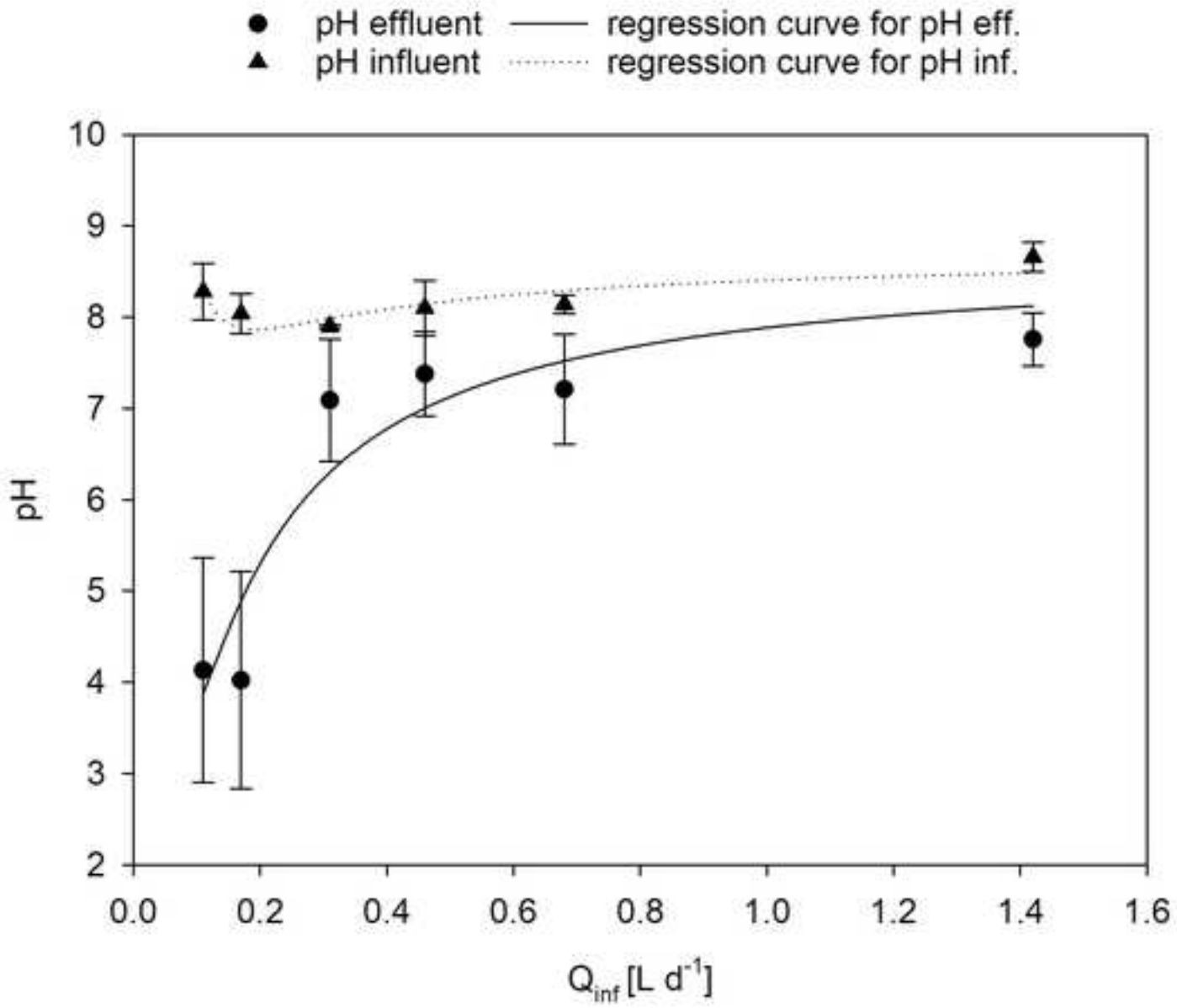

Figure 06

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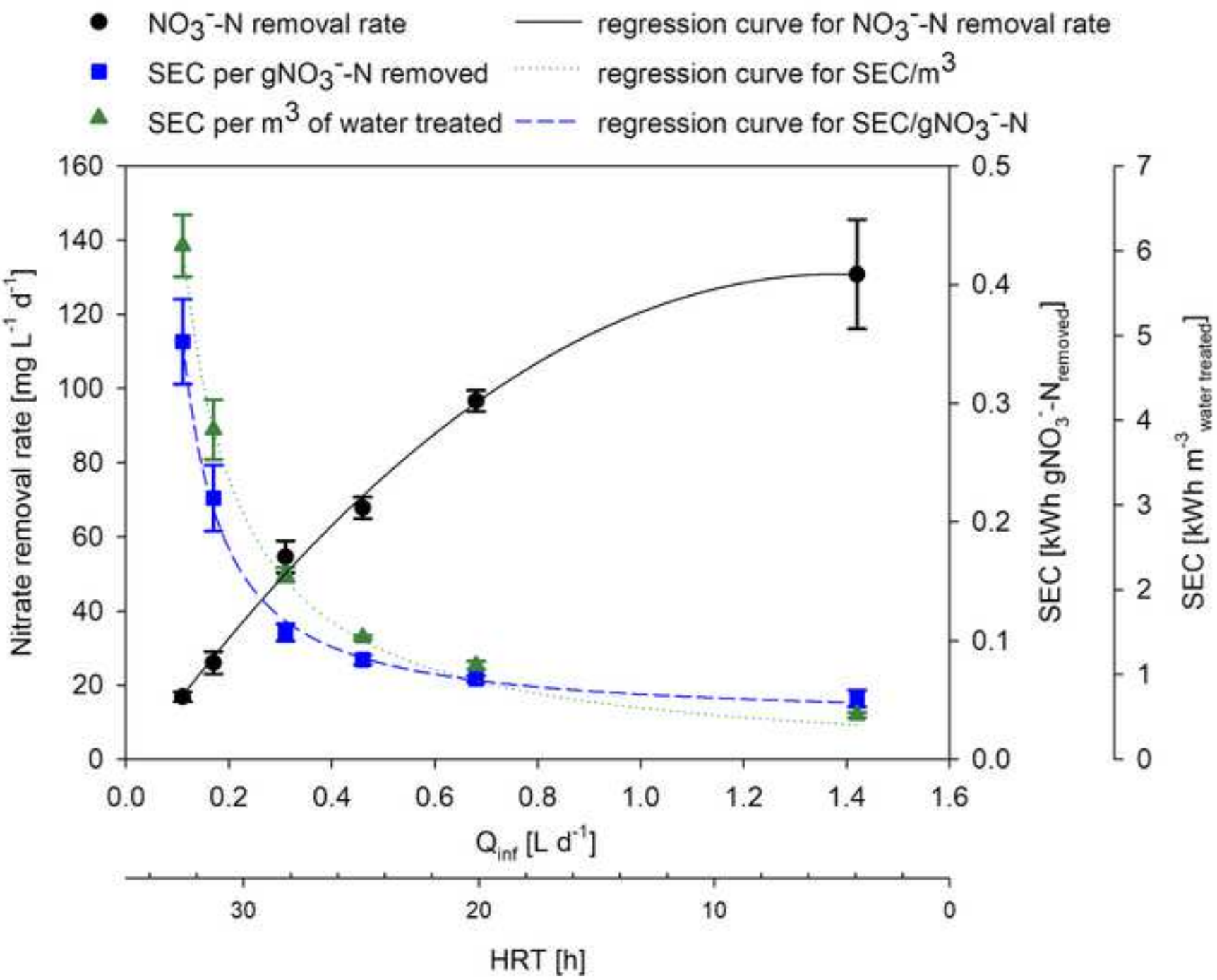

Figure 07

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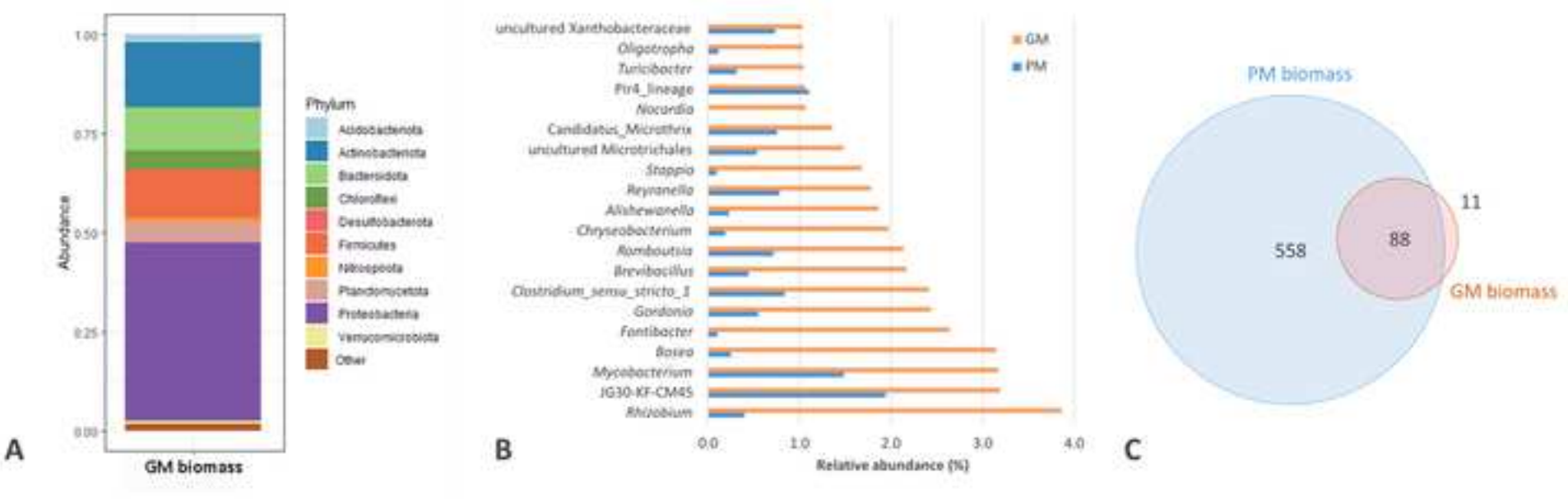

Declaration of interests

☒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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Author contribution statement

The manuscript was written through the contributions of all authors. G. Puggioni: Conceptualisation, Data curation, Investigation, Methodology, Writing – original draft; S. Milia: Conceptualisation, Methodology, Supervision, Funding acquisition, Writing – Review & Editing; V. Unali: Data curation, Investigation, Methodology; R. Ardu: Data curation, Investigation; E. Tamburini: Data curation, Writing - Review & Editing; N. Pous: Conceptualisation, Methodology, Supervision, Writing – Review & Editing; M. D. Balaguer: Conceptualisation, Writing – Review & Editing; S. Puig: Conceptualisation, Methodology, Supervision, Writing – Review & Editing; A. Carucci: Supervision, Funding acquisition, Writing – Review & Editing. All authors have given approval to the final version of the manuscript.