



# Electrifying secondary settlers to enhance nitrogen and pathogens removals

Alberto Botti<sup>a</sup>, Narcís Pous<sup>b</sup>, Hao-Yi Cheng<sup>c,d</sup>, Jesús Colprim<sup>b</sup>, Giulio Zanaroli<sup>a</sup>,  
Sebastià Puig<sup>b,\*</sup>

<sup>a</sup> Dept. of Civil, Chemical, Environmental and Materials Engineering (DICAM), University of Bologna, via Terracini 28, 40131 Bologna, Italy

<sup>b</sup> LEQUiA, Institute of the Environment, University of Girona, Carrer Maria Aurelia Capmany, 69, E17003 Girona, Spain

<sup>c</sup> State Key Lab of Urban Water Resource and Environment, School of Civil and Environmental Engineering, Harbin Institute of Technology Shenzhen, Shenzhen 518055, PR China

<sup>d</sup> CAS Key Laboratory of Environmental Biotechnology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, 100085, China

## ARTICLE INFO

### Keywords:

Bioelectrochemical system  
Wastewater  
Ammonium  
Nitrate  
Secondary treatments  
Disinfection

## ABSTRACT

Economic options to retrofit wastewater treatment plants (WWTPs) without tertiary treatments need to be explored. In this regard, bioelectrochemical systems (BES) can be hybridized with existing technologies, upgrading the removal performance of original techniques while avoiding replacement costs. Yet, few demonstrations of merged systems have been given. For the first time, in this work it was built a lab-scale model of a BES merged with a secondary settler, namely e-settler, to enhance the polishing performance of already existing WWTPs. In particular, to concomitantly increase nitrogen removal and perform wastewater (WW) disinfection, avoiding further tertiary treatments. In the e-settlers, nitrogen removal was increased through bioelectrochemical stimulation. Concomitant ammonium and nitrate removal without nitrite accumulation and a negligible amount of nitrous oxide emissions were observed. Ti-MMO as anode material showed a high disinfectant action. In conclusion, it was demonstrated how a simple bioelectrochemical set-up can upgrade existing WWTPs. The following step requires the study at a larger scale, identifying optimal operational and structural parameters for the *in-situ* application. The main limitations of the e-settlers were discussed, linking them to possible solutions that need to be deepened in a lab-scale model of conventional secondary treatments (activated sludge followed by secondary settler).

## 1. Introduction

Secondary effluents of wastewater treatment plants (WWTPs) are commonly characterized by the presence of total nitrogen (TN) [1,2]. High TN values can compromise the discharge in the environment and the reuse of these effluents. Indeed, Directive 91/271/EEC which regulates urban wastewater (WW) clearly states a maximum of 15 mg N-TN·L<sup>-1</sup> for discharging in sensitive areas (e.g. eutrophic areas) [3]. Water reuse from urban wastewater discharges is also promoted from the perspective of facing water scarcity and droughts [4]. A key parameter for water reuse is the pathogens content, which has to meet strict thresholds, specific for each European country [4]. For instance, the limit for urban uses ranges from 0.3 to 1.3 log(CFU·100 mL<sup>-1</sup>) of total coliforms (TC) in Greece and absence to 2 log(CFU·100 mL<sup>-1</sup>) of *E. coli* in Spain [5,6]. The more stringent Italian law requires a maximum threshold of 1 log(CFU·100 mL<sup>-1</sup>) of *E. coli* for any reuse applications

[7]. It becomes necessary to guarantee that secondary WWTP effluents match the target values to be either reused or released into the environment [3,8].

Technologies meant to polish the contaminant content in excess are defined as tertiary treatments (TT). Nowadays, even though 91.2 % of the European continent population has access to urban wastewater collecting and treatment systems, only 70 % can rely on WWTPs equipped with tertiary treatments. The remaining slice of the population has access to primary treatments (PT, 3.5 %), secondary treatments (ST, 19.5 %), and decentralized WWTPs (DEWATS, 7 %) [9]. PT and ST are often not sufficient to reduce the nitrogen content to environmentally acceptable values [10], while tertiary treatments are meant to meet the nitrogen discharge criteria and to furtherly reduce the pathogens content according to the water reuse or the discharge site [11]. Adding a treatment step inevitably increases costs and space requirements, which makes retrofitting solutions less attractive and highlights the need of

\* Corresponding author.

E-mail addresses: [alberto.botti3@unibo.it](mailto:alberto.botti3@unibo.it) (A. Botti), [narcis.pous@udg.edu](mailto:narcis.pous@udg.edu) (N. Pous), [chenghaoyihit@126.com](mailto:chenghaoyihit@126.com) (H.-Y. Cheng), [J.Colprim@lequia.udg.cat](mailto:J.Colprim@lequia.udg.cat) (J. Colprim), [giulio.zanaroli@unibo.it](mailto:giulio.zanaroli@unibo.it) (G. Zanaroli), [sebastia.puig@udg.edu](mailto:sebastia.puig@udg.edu) (S. Puig).

<https://doi.org/10.1016/j.cej.2022.138949>

Received 5 July 2022; Received in revised form 17 August 2022; Accepted 28 August 2022

Available online 1 September 2022

1385-8947/© 2022 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

finding alternative approaches [2]. Bioelectrochemical systems (BES) might contribute to enhancing the polishing power of WWTPs, without being necessary the implementation of additional tertiary treatments.

BES couple solid-state electrodes with bacteria, using the latter as a biocatalyst [12]. Electrodes can then act as electron donors/acceptors to stimulate the different microbial metabolisms, without the need for external chemical dosage. BES are considered a promising approach to reducing nitrogen content in WW, where microbial activity is used as a tool to reach the required quality standards for nitrate ( $\text{NO}_3^-$ ), nitrite ( $\text{NO}_2^-$ ) and ammonium ( $\text{NH}_4^+$ ). Ammonium is conventionally removed aerobically by a process named nitrification, which transforms  $\text{NH}_4^+$  to  $\text{NO}_3^-$  [10]. Nitrate is subsequently converted via denitrification, a heterotrophic or autotrophic anaerobic cascade mechanism that requires a source of electrons to reduce  $\text{NO}_3^-$  to dinitrogen gas ( $\text{N}_2$ ), via sequential steps that go through  $\text{NO}_2^-$ , nitric oxide (NO) and nitrous oxide ( $\text{N}_2\text{O}$ ) [13]. It is known that BES can prime biological nitrogen removal processes, such as  $\text{NO}_3^-$  reduction to dinitrogen gas or  $\text{NH}_4^+$  oxidation to  $\text{N}_2$  via anammox [14,15]. Autotrophic denitrification can be assisted by electrodes, which act as direct electron donors [16]. This concept has started to move toward engineering applications. For example, Sander *et al.* used a three-electrode system placed in a plug-flow reactor as a tertiary treatment to reduce the TN content of real WWTPs' secondary effluents. The nitrogen removal was mainly limited to denitrification, reducing the  $\text{NO}_3^-$  amount below  $0.5 \text{ mg N-NO}_3^- \cdot \text{L}^{-1}$  [17]. As for ammonium, a pilot-scale BES was coupled with a nitrifying reactor and used to treat real urban WW and centrate from anaerobic digestors with total-nitrogen removal efficiencies ranging between 10 and 95 % depending on the influent content ( $40\text{--}1460 \text{ mg N-TN} \cdot \text{L}^{-1}$ ) [18]. A similar set-up was applied to concomitantly remove COD,  $\text{NH}_4^+$  and  $\text{NO}_3^-$  from synthetic WW. The electrons supplied by the anodic oxidation of the organic matter were used to reduce the nitrate at the cathode, which was previously obtained by converting the ammonium in an external nitrifying reactor [19]. The concomitant nitrate and ammonium removals were observed also by integrating a BES with a membrane bioreactor to treat synthetic WW with high  $\text{NH}_4^+$  content [20]. Moreover, simultaneous nitrification and denitrification have been enhanced in the cathodic chamber of a bioelectrochemical system, when working with a synthetic medium [21]. In respect to the pathogens, the use of electrochemical oxidation (ECO) as disinfecting technique is becoming a feasible alternative to traditional methods (e.g. chemicals dosage or UV) [22,23]. Electrochemical oxidation can proceed via the anodic production of oxidant agents, such as chlorine when an anolyte (e.g. wastewater) contains chloride [24,25]. ECO as tertiary treatment showed an efficacy up to 99.5 % when disinfecting real wastewater containing *E. coli* ( $>5 \log(\text{CFU} \cdot 100 \text{ mL}^{-1})$ ) [26]. Electrochemical disinfection using Ti-MMO anode was demonstrated effective also for treating low chloride WW as stormwater ( $9 \text{ mg Cl}^- \cdot \text{L}^{-1}$ ) [27].

BES can become a reasonable stand-alone nitrogen removal technology soon, but their implementation in existing WWTPs might accelerate its adoption [14]. The replacement of existing infrastructures is not feasible due to the high costs invested in them, but the versatility of BES could allow their integration into already existing biological processes by the simply immersion of electrodes in the bioreactors. Sander *et al.* demonstrated how a simple bioelectrochemical configuration increased the polishing of real WW [17]. Yet, they studied the BES as a tertiary treatment and not retrofit secondary WW treatments. To the best of the authors' knowledge, there is only one paper showing the potential to merge BES in existing WWTPs for nitrogen removal. Tejedor-Sanz *et al.* adapted a three-electrode configuration to an activated sludge to increase the nitrogen removal rate of the system. The applied potential induced an anaerobic zone at the cathode and aerobic zone at the anode to concomitantly stimulate denitrification and nitrification [28]. However, the implementation in secondary settlers, where a natural redox stratification exists, could allow an easier implementation of BES-based applications. In addition, the previously studied systems were meant to enhance the sole nitrogen removal and the disinfectant power of the BES

was not assessed.

For the first time, a lab-scale model of a bioelectrochemical reactor coupled with a secondary settler, labelled as e-settler, was built to study how the immersion of polarized electrodes can enhance the polishing of real secondary wastewater. First, the usage of an immersed anode/cathode was explored as a method to stimulate nitrogen removal processes. Second, anode electrochemical oxidation will be explored to disinfect wastewater. The coupling of the two techniques in a simple configuration, which could allow an economic retrofit of existing WWTPs, has not been assessed previously. The set-up of the kit was meant to be placed in the upper part of the secondary settler to avoid the strict aerobic conditions of the activated sludge tank, which would compromise the growth of denitrifying bacteria as well as to avoid electrode clogging due to sludge accumulation [29].

## 2. Materials and methods

### 2.1. Reactors set-up

Two piston flow reactor replicates were built to study e-settlers, as depicted in Fig. 1. A Plexiglas tube (40 cm length, 2.5 cm inner diameter) was sealed to two PVC *t*-connectors (6.5 cm length, 2.5 cm inner diameter). The upper part was closed with a PVC cap (3 cm in length, 2.5 cm inner diameter). The length of the reactor was 55 cm, with a net volume of 170 mL. The cathode was made of a graphite-coated stainless steel mesh, prepared by Cheng *et al.* as described elsewhere [30]. Cathode features were: total cathodic area, TCA:  $468 \text{ cm}^2$ ; total cathodic volume, TCV: 24.5 mL; net cathodic volume, NCV: 2 mL; total cathodic length: 5 cm. A stainless steel wire was used as the current collector. Initially, graphite rods (total surface area:  $8.8 \text{ cm}^2$ ; Mersen Ibérica, Spain) were used as the anode. On day 64, graphite rods were

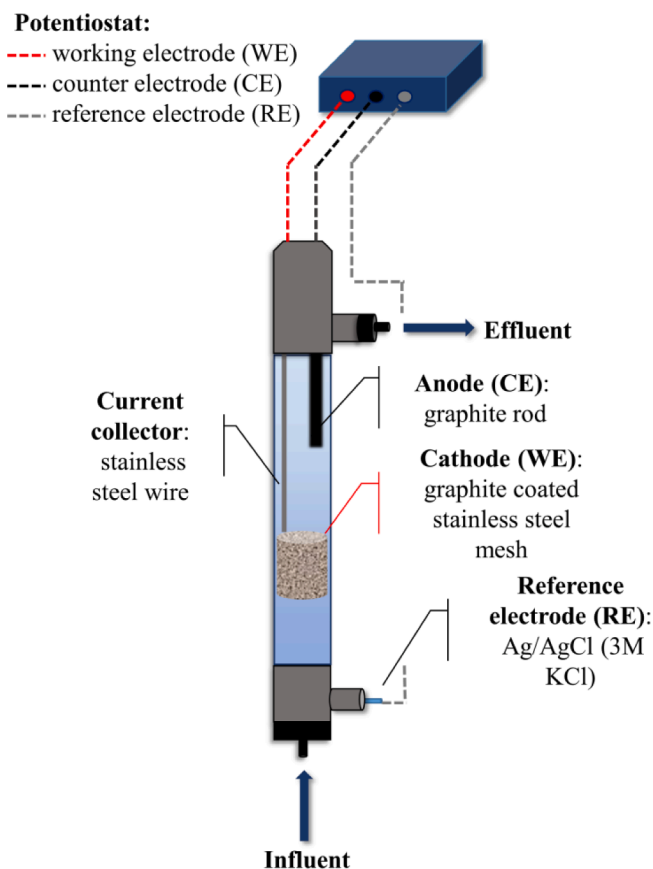


Fig. 1. Scheme of the lab-scale e-settler used for the experiment, details are reported in the text.

substituted with Ti-MMO (total surface area: 3.9 cm<sup>2</sup>; NMT electrodes, South Africa) to assess the anodic hypochlorite evolution as a disinfection action. An Ag/AgCl sat. KCl (+0.197 V vs standard hydrogen electrode, SHE, SE 11, Xylem Analytics Germany Sales GmbH & Co. KG Sensortechnik Meinsberg, Germany) was introduced at a height of 3 cm as the reference electrode. The distance between the cathode and the anode was set at 7.5 cm. The Influent was pumped with a peristaltic pump.

## 2.2. Wastewater characteristics

The effluent coming from the secondary settler of an urban WWTP (Quart, Girona, Spain) was used as the e-settlers' influent. Inorganic nitrogen composition is given as a range of values, observed due to the natural biological processes. Three different WW samplings were performed and addressed as low nitrogen wastewater (NO<sub>3</sub><sup>-</sup>-WW), used from day 1 to day 50, high nitrogen wastewater (NH<sub>4</sub><sup>+</sup>-NO<sub>3</sub><sup>-</sup>-WW), used from day 50 to day 85, and low nitrogen wastewater 2 (NO<sub>3</sub><sup>-</sup>-2-WW), used from day 85 to day 102. WW was stored in a refrigerated stirring tank at 4 °C to preserve its characteristics. Chemical physical parameters of NO<sub>3</sub><sup>-</sup>-WW were: BOD<sub>5</sub> (biological oxygen demand) 2 ± 1 mg O<sub>2</sub>·L<sup>-1</sup>; BOD<sub>30</sub> 11 ± 1 mg O<sub>2</sub>·L<sup>-1</sup>; COD (chemical oxygen demand) 20 – 76 mg O<sub>2</sub>·L<sup>-1</sup>; 18.7 – 31.5 mg N-NO<sub>3</sub><sup>-</sup>·L<sup>-1</sup>; 0 – 4.1 mg N-NO<sub>2</sub><sup>-</sup>·L<sup>-1</sup>; 0 – 8.9 mg N-NH<sub>4</sub><sup>+</sup>·L<sup>-1</sup>. Chemical physical parameters of NH<sub>4</sub><sup>+</sup>-NO<sub>3</sub><sup>-</sup>-WW were: BOD<sub>5</sub> 5.7 ± 0.5 mg O<sub>2</sub>·L<sup>-1</sup>; BOD<sub>30</sub> 23 ± 5 mg O<sub>2</sub>·L<sup>-1</sup>; COD 29.5 – 178 mg O<sub>2</sub>·L<sup>-1</sup>; NO<sub>3</sub><sup>-</sup> 19.3 – 40.8 mg N-NO<sub>3</sub><sup>-</sup>·L<sup>-1</sup>; NO<sub>2</sub><sup>-</sup> 0.12 – 4.2 mg N-NO<sub>2</sub><sup>-</sup>·L<sup>-1</sup>; NH<sub>4</sub><sup>+</sup> 0.17 – 33.7 mg N-NH<sub>4</sub><sup>+</sup>·L<sup>-1</sup>. Chemical physical parameters of NO<sub>3</sub><sup>-</sup>-2-WW were: COD 56.6–128 mg O<sub>2</sub>·L<sup>-1</sup>; NO<sub>3</sub><sup>-</sup> 32.9 – 37.1 mg N-NO<sub>3</sub><sup>-</sup>·L<sup>-1</sup>; NO<sub>2</sub><sup>-</sup> 0.03 – 0.67 mg N-NO<sub>2</sub><sup>-</sup>·L<sup>-1</sup>; NH<sub>4</sub><sup>+</sup> 0.16 – 1.8 mg N-NH<sub>4</sub><sup>+</sup>·L<sup>-1</sup>.

## 2.3. Operational mode

For the inoculation period, the reactors were poised at – 0.321 V vs Ag/AgCl sat. KCl in batch mode to promote the development of an electroactive population [31]. A 1:1 solution of wastewater and effluent from a denitrifying BES reactor [32], adjusted to a final nitrate concentration of 33 mg N-NO<sub>3</sub><sup>-</sup>·L<sup>-1</sup>, was used to inoculate the reactors. The media was recirculated at 25 L·d<sup>-1</sup> for 3 weeks.

After the inoculation period, the two reactor replicates were operated in continuous mode to study the functioning of the e-settlers. The influent used was a 9:1 solution of wastewater and effluent from a denitrifying BES reactor [32], and stored into a 10 L tank connected to the reactors, previously flushed with N<sub>2</sub> gas. After changing from inoculation mode to continuous mode, the current flow between the electrodes was negligible. For this reason, the potentiostat was switched to galvanostatic mode to guarantee a constant electron flow to prime biological denitrification. Data presented are reported as the average of the two replicates.

Two different operational phases can be distinguished. During phase I, wastewater with a low ammonium content (NO<sub>3</sub><sup>-</sup>-WW) was fed to the reactors. The composition of WW resembled a WWTPs with good nitrification performance, allowing studying the polishing power of the e-settlers in an ideal condition. During this phase, the effect of the current density and flow rate was studied. Each parameter was modified separately, to better identify the different contributions. The current applied was initially set to a value high enough to perform complete denitrification, 26 mA·L<sub>TCV</sub><sup>-1</sup>, and subsequently adjusted to 20 mA·L<sub>TCV</sub><sup>-1</sup>, to mild the electrode potentials. HRT, calculated on the cathode length, was set at 7.4 ± 0.6 h. Subsequently, the inflow's rate was increased step wisely, to a final HRT of 2.9 ± 0.2 h. Later on, the current applied was raised to 61 mA·L<sub>TCV</sub><sup>-1</sup>, maintaining the same HRT, aiming to increase nitrogen polishing.

During phase II, the composition of the influent presented a high concentration of ammonium (NH<sub>4</sub><sup>+</sup>-NO<sub>3</sub><sup>-</sup>-WW). The NH<sub>4</sub><sup>+</sup> content of the secondary effluent is representative of WWTPs with an inefficient aerobic stage, thus allowing us to assess the e-settler removal ability under

non-ideal conditions. Initially, HRT was set at 2.2 ± 0.1 h with a current of 98 mA·L<sub>TCV</sub><sup>-1</sup>, a theoretical value necessary to reduce the nitrate content. Yet, considering the only partial removal of TN, it was decided to increase the current flow to perform also denitrification of the NO<sub>3</sub><sup>-</sup> coming from NH<sub>4</sub><sup>+</sup> nitrification. Before doing so, anodes were replaced with Ti-MMO to evaluate the electrochemical anodic disinfection. The current was raised to 214 mA·L<sub>TCV</sub><sup>-1</sup>. Subsequently, wastewater composition changed again (NO<sub>3</sub><sup>-</sup>-2-WW) and the current was switched off to evaluate the open-circuit voltage (OCV) contributions (days 84–95). Finally, the potentiostat was turned on again to assess the possibility to restore the bioelectrochemical stimulation (days 95–102).

## 2.4. Abiotic tests

The abiotic contribution of the graphite-coated stainless steel mesh cathode to nitrogen removal was assessed in a two-compartment H-cell. This was composed of two 300 mL glass bottles separated by a cation membrane (CEM, CMI-7000, Membranes Int., USA). A graphite-coated stainless steel mesh was placed in the cathodic chamber (total cathodic volume: 39.25 mL; net cathodic volume: 3 mL; total cathodic area: 448 cm<sup>2</sup>) and stainless steel wire was used as the current collector. Graphite rods (18 cm<sup>2</sup>; Mersen Ibérica, Spain) were used as anode electrode. Ag/AgCl sat. KCl (+0.197 V vs standard hydrogen electrode, SHE, SE 11, Xylem Analytics Germany Sales GmbH & Co. KG Sensortechnik Meinsberg, Germany) was used as the reference electrode. The synthetic media described by [33] was used as abiotic media, with a concentration of 33 mg N-NO<sub>3</sub><sup>-</sup>·L<sup>-1</sup> and 2.7 mg N-NH<sub>4</sub><sup>+</sup>·L<sup>-1</sup>. The working electrode was poised at –0.3 V and –0.9 V vs Ag/AgCl sat. KCl as they were the potentials commonly found in the running e-settlers. Tests were run for a minimum of 7 days.

## 2.5. Analytical methods and calculations

A minimum of three samples of effluent and influent were taken and analysed from both reactors for each tested condition. The same procedure was kept for the abiotic test, analysing cathodic and anodic chambers. All liquid samples were analysed according to APHA standard water measurements [34] for nitrate (N-NO<sub>3</sub><sup>-</sup>), nitrite (N-NO<sub>2</sub><sup>-</sup>), and ammonium (N-NH<sub>4</sub><sup>+</sup>) by ionic chromatography (ICS 5000, Dionex, USA). The pH and electrical conductivity of the samples were measured with a pH-meter (pH-meter basic 20+, Crison, Spain) and a conductivity meter (EC-meter basic 30+, Crison, Spain), respectively. Nitrous oxide (N<sub>2</sub>O) was measured using an N<sub>2</sub>O liquid-phase microsensor (Unisense, Denmark). COD was measured via a colourimetric kit (Hach, Iowa, USA). BOD was determined according to the standard methods [34]. The hydraulic retention time was calculated considering the total cathodic length volume and the flow rate. All removal rates were calculated as reported in the [supplementary material](#) (equations S1-S5). The coulombic efficiency (CE) was calculated considering all reduction steps from nitrate to dinitrogen gas considering nitrite, nitrous oxide accumulation and ammonium nitrification (equation S6). The COD electron balance (COD-EB) was calculated considering COD removal and it was used to evaluate the maximum contribution from heterotrophic denitrification, as reported in the [supplementary material](#) (equation S7). The theoretical maximum COD-EB was calculated considering the effluent COD equal to 0, while the real maximum COD-EB was calculated considering the real effluent's COD. In both cases, COD was considered to be biodegradable and heterotrophically removed from denitrifiers bacteria. Total coliforms, E. coli and Enterococcus were analysed in an external laboratory following standard procedures for wastewater examinations (CECAM, Girona) [34].

### 3. Results and discussion

#### 3.1. Nitrogen removal performance of e-settlers treating low ammonium content wastewater ( $\text{NO}_3^-$ -WW)

After the inoculation period, the system was set on continuous mode to begin “phase I”. Real wastewater, labelled as  $\text{NO}_3^-$ -WW, was pumped into the reactors. At the initial conditions, HRT of  $7.4 \pm 0.6$  h and current applied of  $20 \text{ mA} \cdot \text{L}_{\text{TCV}}^{-1}$ , the nitrogen content decreased below the  $15 \text{ mg N} \cdot \text{L}^{-1}$  threshold, going from  $31 \pm 4 \text{ mg N} \cdot \text{L}^{-1}$  to  $2 \pm 3 \text{ mg N} \cdot \text{L}^{-1}$  of TN (Fig. 3), with a removal rate of  $94 \pm 17 \text{ g N} \cdot \text{m}_{\text{TCV}}^{-3} \cdot \text{d}^{-1}$  (Fig. 2). The flow rate was then increased (of  $5.1 \pm 0.6$  h HRT), observing a rise in the TN removal rate ( $115 \pm 16 \text{ g N} \cdot \text{m}_{\text{TCV}}^{-3} \cdot \text{d}^{-1}$ ), with an effluent presenting a TN concentration of  $3 \pm 3 \text{ mg N} \cdot \text{L}^{-1}$ . The HRT was further reduced to  $2.9 \pm 0.2$  h, reaching a TN removal rate of  $156 \pm 60 \text{ g N} \cdot \text{m}_{\text{TCV}}^{-3} \cdot \text{d}^{-1}$ . At this condition, e-settlers showed the highest nitrate removal rate during phase I, equivalent to  $146 \pm 62 \text{ g N} \cdot \text{NO}_3^- \cdot \text{m}_{\text{TCV}}^{-3} \cdot \text{d}^{-1}$ , and TN effluent standards were fulfilled ( $8 \pm 6 \text{ mg} \cdot \text{L}^{-1}$ ). Then, the applied current was increased and set to the theoretical flow required to fully reduce the nitrate content ( $61 \text{ mA} \cdot \text{L}_{\text{TCV}}^{-1}$ ). Consequently, the effluent TN decreased to  $4 \pm 3 \text{ mg N} \cdot \text{L}^{-1}$ .

During phase I,  $\text{NH}_4^+$  was fully removed, with neither  $\text{NO}_2^-$  nor  $\text{N}_2\text{O}$  accumulation (Table S7). To identify the contribution of the applied current to the nitrogen removal processes, CE was taken into account. It has to be reminded that the system worked under galvanostatic conditions: the current supplied was chosen by the operator, not as a result of the bioelectrochemical activity of the reactor. At the initial current density ( $20 \text{ mA} \cdot \text{L}_{\text{TCV}}^{-1}$ ), the value was adjusted to nitrogen availability, and CEs around 100 % were observed. As the HRT was decreased from 7 to 3 h while keeping the current density stable, the CE dropped to 37 % (Table 1), suggesting the co-occurrence of heterotrophic  $\text{NO}_3^-$  reduction [35]. The gradual decrease in CE revealed that electrochemical stimulation was partially contributing to the overall nitrogen removal. Then, when the current was raised to  $68 \text{ mA} \cdot \text{L}_{\text{TCV}}^{-1}$  to balance the theoretical nitrogen removal requirements, CE went up to 124 %. The succeeding increase of CE observed after incrementing the current might be related to an artefact of the mathematical expression. In order to better understand the role of heterotrophic denitrification on nitrogen depletion, COD was identified as an index to evaluate the organic matter content of WW, acting as an indigenous organic source of electrons. However, an

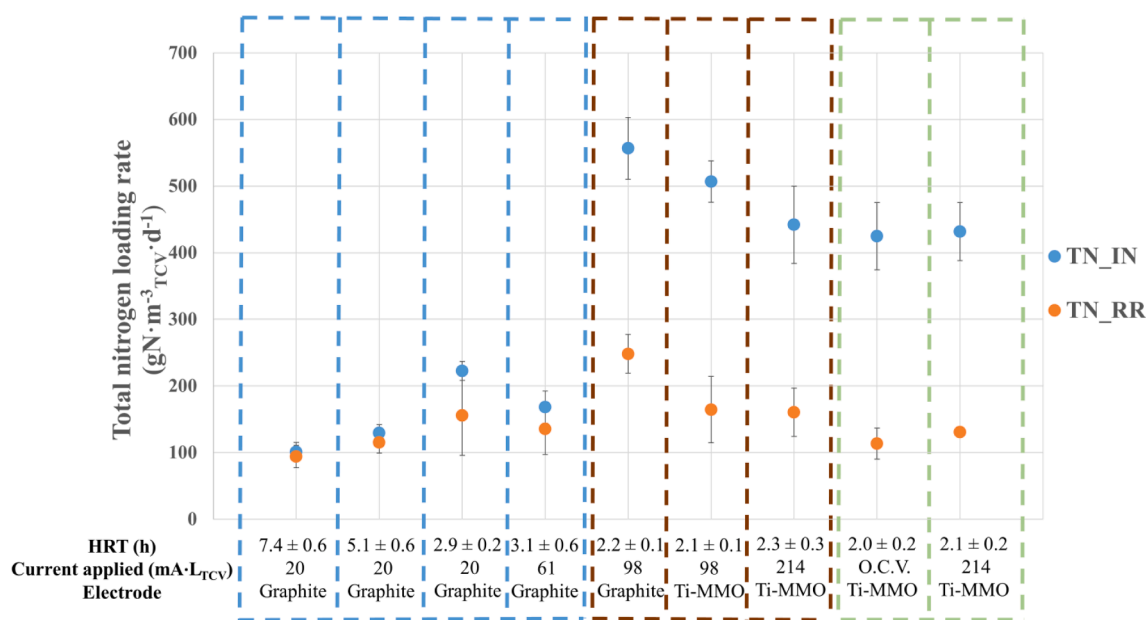
**Table 1**

Summary of wastewater treatment technologies for polishing of WW secondary flows.

	HRT (h)	Current applied ( $\text{mA} \cdot \text{L}_{\text{TCV}}^{-1}$ )	Anode	CE (%)	COD - EB (%)	Energy ( $\text{kWh} / \text{gN}_{\text{removed}}$ )
Phase I	$7.4 \pm 0.6$	20	Graphite	$63 \pm 24$	$82 \pm 31^{\text{T}}$	$1.5 \cdot 10^{-2} \pm 2 \cdot 10^{-3}$
				$45 \pm 6$	$64 \pm 16^{\text{T}}$	$1.2 \cdot 10^{-2} \pm 2 \cdot 10^{-3}$
			Graphite	$37 \pm 14$	$50 \pm 18^{\text{T}}$	$1.0 \cdot 10^{-2} \pm 4 \cdot 10^{-3}$
				$124 \pm 40$	$58 \pm 38^{\text{T}}$	$5 \cdot 10^{-2} \pm 2 \cdot 10^{-2}$
	$5.1 \pm 0.6$	20	Graphite	$100 \pm 12$	$83 \pm 51^{\text{T}}$	$610^{-2} \pm 1 \cdot 10^{-2}$
				$194 \pm 106$	$121 \pm 92^{\text{T}}$	$1.0 \cdot 10^{-1} \pm 3 \cdot 10^{-2}$
			Ti-MMO	$352 \pm 81$	$201 \pm 93^{\text{R}}$	$3.9 \cdot 10^{-1} \pm 7 \cdot 10^{-2}$
				-	-	-
O.C.V.	2.0	-	Ti-MMO	$170 \pm 89^{\text{R}}$	-	
				-	-	-
Validation test	2.1	214	Ti-MMO	$353 \pm 17$	$91 \pm 35^{\text{R}}$	$4.9 \cdot 10^{-1} \pm 2 \cdot 10^{-2}$
				-	-	-

T: theoretical COD-EB, based on total COD removal; R: calculated COD-EB, based on the measured COD removal.

increase of the COD in the effluent of the e-settlers was observed, due to the electrochemical oxidation of the graphite anode, which allowed only to speculate about the heterotrophic denitrification role in the system. Indeed, inlet COD ranged from 35 to  $64 \text{ mg O}_2 \cdot \text{L}^{-1}$ , while outlet COD presented values between 62 and  $89 \text{ mg O}_2 \cdot \text{L}^{-1}$  (Table S7). An electron balance was calculated considering that: i) all inlet COD was consumed by denitrifiers (theoretical COD-EB); ii) all inlet COD was biodegradable despite being the effluent of a secondary treatment. The results showed a hypothetical maximum contribution of heterotrophic denitrification that went from 82 % to 58 % (table 1), suggesting necessary electrochemical assistance. It has to be considered that COD measurements for this range of values can suffer from low accuracy. Moreover, the real contribution of heterotrophic denitrification could be negligible



**Fig. 2.** Inlet flows and removal rates of the total nitrogen in the e-settlers, at the different tested conditions: TN, total nitrogen; IN, influent; RR, removal rate; Dashed blue lines: phase I,  $\text{NO}_3^-$ -WW; Dashed brown lines: phase II,  $\text{NH}_4^+$ - $\text{NO}_3^-$ -WW; Dashed green lines: open circuit and validation tests,  $\text{NO}_3^-$ -WW.



considering that the BOD was around  $10 \text{ mg O}_2 \cdot \text{L}^{-1}$ . At the end of phase I, e-settlers demonstrated the ability to simultaneously remove ammonium and nitrate, under mild conditions of HRT and pollutant concentration.

### 3.2. e-settlers behaviour treating rich ammonium wastewater ( $\text{NH}_4^+ - \text{NO}_3^- - \text{WW}$ )

When phase I finished, the composition of WW changed. Phase II was characterized by effluent of secondary settlers with a high content of ammonium (up to  $31 \pm 3 \text{ mg N-NH}_4^+ \cdot \text{L}^{-1}$ , Table S7). At the initial conditions, HRT of  $2.2 \pm 0.1 \text{ h}$  and  $98 \text{ mA} \cdot \text{L}_{\text{TCV}}^{-1}$ , e-settlers reported the highest nitrogen removal rate observed,  $248 \pm 29 \text{ g N} \cdot \text{m}_{\text{TCV}}^{-3} \cdot \text{d}^{-1}$  (Fig. 2). The CE,  $100 \pm 12 \%$ , suggested a good correspondence between electrons supplied and reduced nitrogen (Table 1). However, the effluent composition was largely over the guideline value, being  $28 \pm 3 \text{ mg N} \cdot \text{L}^{-1}$  (Fig. 3). The supplied current was enough to reduce the initial  $\text{NO}_3^-$  but not for removing the whole of the inlet  $\text{NH}_4^+$  ( $31 \pm 3 \text{ mg N-NH}_4^+ \cdot \text{L}^{-1}$ ).

Before increasing the current density, graphite anodes were replaced with Ti-MMO to compare the disinfecting abilities of the two materials. The electrodes substitution led to a drop in the performance of the e-settlers. TN removal rate decreased to  $164 \pm 50 \text{ g N} \cdot \text{m}_{\text{TCV}}^{-3} \cdot \text{d}^{-1}$  (Fig. 1). Denitrification became less marked, with an effluent composition of  $21 \pm 13 \text{ mg N-NO}_3^- \cdot \text{L}^{-1}$  compared to  $20 \pm 2 \text{ mg N-NO}_3^- \cdot \text{L}^{-1}$  of the influent, while ammonium was still clearly reduced, going from  $23 \pm 3 \text{ mg N-NH}_4^+ \cdot \text{L}^{-1}$  in the influents to  $6 \pm 7 \text{ mg N-NH}_4^+ \cdot \text{L}^{-1}$  in the effluents (Table S7). To explain the variation of the e-settlers performance, a cathodic and anodic combined mechanism was considered. When the inlet was pumped into the reactors, wastewater was first exposed to the cathode, where denitrification took place transforming the available  $\text{NO}_3^-$  into  $\text{N}_2$ . As wastewater moved up, it reached the anode, where electrochemical oxygen promoted aerobic nitrification, which converts ammonium into nitrate [36]. At low ammonium contents or high HRTs, the nitrate content of the effluent was either negligible or could still be cathodically reduced thanks to back-diffusion, as observed during phase I. When the ammonium content was high (Phase II), ammonium was solely converted to nitrate, but the e-settlers were not able to completely remove it.

The reactors' performance decreased after the anode replacement

probably because Ti-MMO raised the electrochemical production of oxidant agents, which resulted to be harmful to the biocathode. The current density was then set to  $214 \text{ mA} \cdot \text{L}_{\text{TCV}}^{-1}$ , to verify that an increase in the current would not have positively primed the e-settlers performance in presence of the titanium anode. Indeed, after having raised the current, no significant changes were observed in terms of nitrogen polishing, with an average value of TN removal rate of  $160 \pm 36 \text{ g N} \cdot \text{m}_{\text{TCV}}^{-3} \cdot \text{d}^{-1}$  (Fig. 1). Similar effluent compositions were obtained compared to the previous test ( $20 \pm 9 \text{ mg N-NO}_3^- \cdot \text{L}^{-1}$ ;  $4 \pm 6 \text{ mg N-NH}_4^+ \cdot \text{L}^{-1}$ , Table S7). At these conditions, a high coulombic efficiency was observed,  $352 \pm 81 \%$ , indicating that the bacterial community was not able to utilise the whole of the current supplied to the system (Table 1). By replacing the graphite electrode with Ti-MMO, electrochemical anodic oxidation was avoided, allowing to estimate the real COD removal. The estimated COD-EB was  $390 \pm 96 \%$  (Table 1). Such high value suggested that the majority of the supplied electrons were delivered to other chemical reactions (e.g.  $\text{H}_2$  production). The CE and the electron balance inferred an inefficient nitrogen removal stimulation, possibly due to a high anodic oxygen production that limited the cathodic denitrification mechanism, and to high ammonium content that could not be completely polished in the studied set-up leading to an accumulation of nitrate, as depicted in Figure S5.

### 3.3. Control tests

After having assessed the nitrogen removal abilities of the e-settlers, wastewater was changed again ( $\text{NO}_3^- - \text{WW}$ ) and the electricity supply was switched off to identify the effect of the current on the microbial activity (OCV test). Without electrical stimulation, the TN removal rates dropped to  $113 \pm 23 \text{ g N} \cdot \text{m}_{\text{TCV}}^{-3} \cdot \text{d}^{-1}$  (Fig. 2), a value between 1.5 and 2 times lower than the ones observed with electricity supply. This was a proof of the influence of the applied current on the nitrogen removal processes. However, as the current was restored to  $214 \text{ mA} \cdot \text{L}_{\text{TCV}}^{-1}$ , the activity was not recovered ( $131 \pm 6 \text{ g N} \cdot \text{m}_{\text{TCV}}^{-3} \cdot \text{d}^{-1}$ ) (Fig. 2). Indicating that the absence of current negatively affected the microbial cathodic activity. This is in line with what was observed in a previous study, where long starvation periods ( $>10$  days) decreased the performance of the bioelectrochemical system [37].

Regarding abiotic contributions, no significant nitrogen removal was observed when working at the lowest cathodic potential reached during

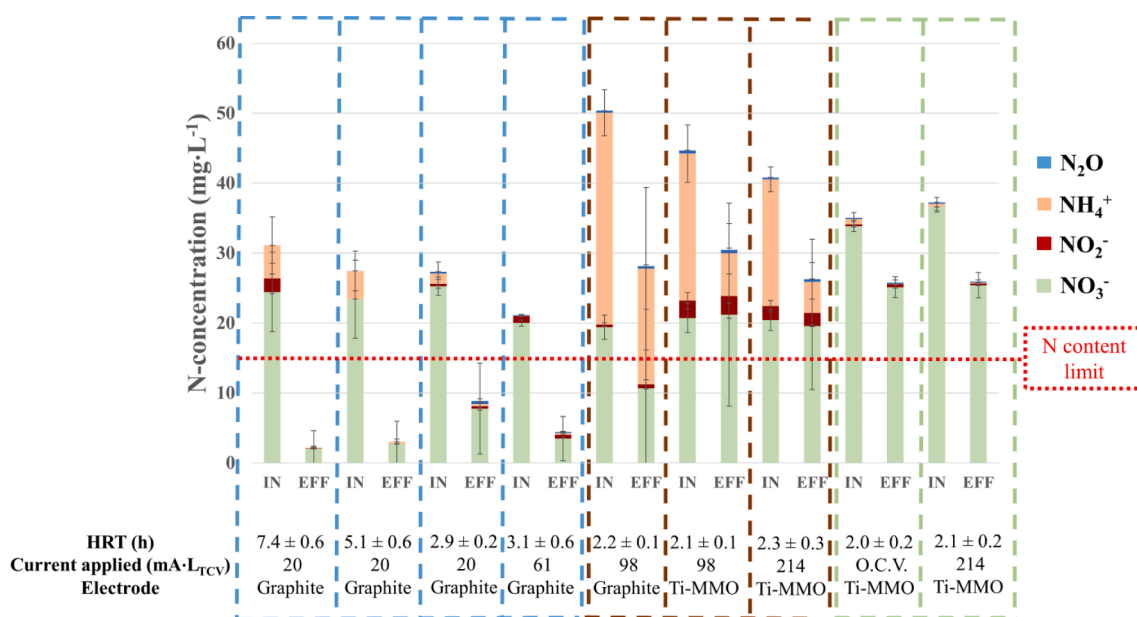


Fig. 3. Influent and effluent compositions of the e-settlers at the different tested conditions: IN, influent; EFF, effluent; Dashed blue lines: phase 1,  $\text{NO}_3^- - \text{WW}$ ; Dashed brown lines: phase II,  $\text{NH}_4^+ - \text{NO}_3^- - \text{WW}$ ; Dashed green lines: open circuit and validation tests,  $\text{NO}_3^- - 2 - \text{WW}$ .

the experiment,  $-0.9$  vs Ag/AgCl sat. KCl (Table S11). An increase in the TN content was detected, especially in terms of ammonium, which raised from 1 to 6 and 7 mg N-NH<sub>4</sub><sup>+</sup>·L<sup>-1</sup> in the cathodic and anodic chambers, respectively, after 7 days. Such a raise was possibly due to a trace of ammonia in the coating of the electrode, as a result of the manufacturing process [30].

### 3.4. Evaluating the removal of pathogens

The e-settlers concept was born as a technique to promote WW polishing. For this purpose, disinfection ability was also monitored to evaluate the treatment potential of this technology. The total content of coliforms of WW was characterized, reporting an influent concentration  $> 2 \log(\text{CFU} \cdot 100 \text{ mL}^{-1})$  (Table S12). The e-settlers equipped with graphite anodes showed a similar removal of the total coliforms content compared to the open circuit condition, resulting in an average effluent concentration of  $1.3 \pm 0.9 \log(\text{CFU} \cdot 100 \text{ mL}^{-1})$  for the graphite test and  $1.3 \pm 0.4 \log(\text{CFU} \cdot 100 \text{ mL}^{-1})$  for the open circuit test. Instead, e-settlers integrated with Ti-MMO anodes reduced the total coliforms to  $0.4 \pm 0.1 \log(\text{CFU} \cdot 100 \text{ mL}^{-1})$ . The better result obtained with the Ti-MMO anode is likely to be due to the higher anodic stability, which favors the formation of oxidizing agents rather than the oxidation of the anodic material itself. This hypothesis is in line with the data reported in the literature. Lai *et al.* compared graphite and Ti-MMO as anode materials [36]. Higher CO<sub>2</sub> production was detected when working with a graphite rod, which was linked to the degradation of the carbon electrode. Conversely, Ti-MMO promoted oxygen evolution, with an efficiency of 90 %. A comparative study of anode materials reported that Ti-MMO increased the active chlorine production up to 3 times more than graphite [38]. Bare titanium was demonstrated to be more effective than graphite for the electrochemical disinfection of water [39], probably due to the formation of oxidant agents. It has been demonstrated that sodium chloride at concentrations higher than  $0.2 \text{ g} \cdot \text{L}^{-1}$  enhanced the

electrochemical oxidation power of Ti-MMO anodes due to the evolution of active chlorine [40]. Considering the relevant NaCl amount in wastewater, which can range from 0.5 to a few g·L<sup>-1</sup> [41,42], e-settlers probably performed a WW disinfection via the presence of oxidant agents such as active chlorine.

### 3.5. Implications

Towards the development of technologies and smart solutions to upgrade already existing WWTPs, e-settlers appeared to be competitive compared to treatments based on conventional biological processes as well as to treatments equipped with bioelectrochemical systems (Table 2). The highest nitrogen removal rate observed in this experiment was  $248 \pm 29 \text{ g N} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$ , which is a value comparatively higher than those found in the literature for tertiary nitrogen polishing. Some of the conventional methods used as tertiary treatments rely on nature-based solutions. The usage of a bioreactor filled with plant substrates showed denitrification rates of  $17.5 \text{ g N} \cdot \text{NO}_3^- \cdot \text{m}^{-3} \cdot \text{d}^{-1}$  [2] while floating wetland removed nitrogen at a rate of  $12.2 \text{ g N} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$  [43]. The main strength of the e-settler is its compact configuration which minimize the space requirements. Moving to more technological/intensive treatments applicable as ST for nitrogen removal, fixed-film reactors showed a TN removal rate of  $29 \text{ g N} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$  [44] and membrane bioreactors had a TN removal rate of  $82 \text{ g N} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$  [45]. Those technologies are close to market or already being applied, while bioelectrochemical systems are still in a process of development. Taking into account this, the data obtained in the current work ( $248 \pm 29 \text{ g N} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$ ) were higher than the removal rates reported by Sander *et al.*, who worked with a plug-flow BES reactor as tertiary treatment removing  $150 \text{ g N} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$  from real secondary settler's effluents [17]. As for the small portfolio of studies about the integration of bioelectrochemical systems with already existing technology, BES were inserted into activated sludge for treating synthetic WW, showing a TN removal rate of  $18.7 \text{ g N} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$  [28].

**Table 2**  
Summary of wastewater treatment technologies for nitrogen removal.

Bioelectrochemical systems or technologies equipped with BES					
Technology	Nitrogen		Working volume (L)	Applied current or potential	Reference
	Influent type and composition (mgN·L <sup>-1</sup> )	Removal rate			
This study	Secondary settler's effluent 50 (TN): 31 (NH <sub>4</sub> <sup>+</sup> ); 19 (NO <sub>3</sub> <sup>-</sup> )	$248 \text{ gN} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$	0.17	$98 \text{ mA} \cdot \text{dm}^{-2} \cdot \text{CV}$	
Activated sludge + BES	Synthetic WW 37 (TN)	<sup>R</sup> $18.7 \text{ gN} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$	22	$-0.6 \text{ V vs Ag/AgCl } 3 \text{ M KCl } (70 \text{ mA} \cdot \text{dm}^{-2})$	[28]
BES + iron/sulfur mediated denitrification	Synthetic WW 35 (NO <sub>3</sub> <sup>-</sup> )	<sup>C</sup> $3900 \text{ gN} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$	0.3	$353 \text{ mA} \cdot \text{dm}^{-3}$	[48]
Wetland + BES	Urban WW 55 (TN): 47 (NH <sub>4</sub> <sup>+</sup> ); 1 (NO <sub>3</sub> <sup>-</sup> )	<sup>C</sup> $39.1 \text{ gN} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$	34	$0.3 \text{ V vs Ag/AgCl } (16.7 \text{ mA} \cdot \text{dm}^{-3})$	[46]
BES	Secondary settler's effluent 3.5 (TN): 0.5 (NH <sub>4</sub> <sup>+</sup> ); 3 (NO <sub>3</sub> <sup>-</sup> )	<sup>R</sup> $150 \text{ gN} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$	2	$-0.9 \text{ V vs Ag/AgCl } 3 \text{ M KCl } (25 \text{ mA} \cdot \text{dm}^{-2} \cdot \text{CV})$	[17]
BES	Primary treatment's effluent 40 (TN)	<sup>C</sup> $28 \text{ gN} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$	150	$1 \text{ V (cell voltage)}$	[18]
BES	Synthetic WW 49 (NH <sub>4</sub> <sup>+</sup> )	<sup>C</sup> $14 \text{ gN} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$	1.6	$50 \text{ mA} \cdot \text{dm}^{-2}$	[47]
Technologies not equipped with BES					
Bioreactor amended with organic matter	Secondary settler's effluent 17.7 (NO <sub>3</sub> <sup>-</sup> )	<sup>R</sup> $17.5 \text{ gN} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$	59	n.i.	[2]
Floating wetlands	Synthetic WW 15 (TN): 3.5 (NH <sub>4</sub> <sup>+</sup> ); 11.5 (NO <sub>3</sub> <sup>-</sup> )	<sup>C</sup> $12.2 \text{ gN} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$	224	n.i.	[43]
Fixed rope media	Primary treatment's effluent 24 (NH <sub>4</sub> <sup>+</sup> )	<sup>C</sup> $29 \text{ gN} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$	1890	n.i.	[44]
Membrane bioreactor	Primary treatment's effluent 57 (TN)	<sup>C</sup> $82 \text{ gN} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$	140	n.i.	[45]

TCC: total cathodic compartment.

R: reported in the paper.

C: calculated using the data reported in the paper.

Instead, coupling polarized electrodes with constructed wetlands resulted in stimulation of the nitrogen removal, with a rate of  $39.1 \text{ g N}_{\text{TCC}}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$  removed [46], and represents an improvement over other nature-based solutions [2,43]. Other BES-based studies have evaluated different configurations, that could be applied as secondary or tertiary treatments and would require a high degree of modifications of current WWTPs. For example, San-Martín *et al.* used a pilot-scale six-chambers BES to treat effluent from primary treatment and obtained a TN removal rate of  $28 \text{ g N}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$  [18]. A two chambers system used to treat synthetic WW was based on the coupling of electrochemical production of oxygen and hydrogen to stimulate nitrification and denitrification, showing a removal rate of  $14 \text{ g N}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$  [47]. High nitrogen removal rates,  $3900 \text{ g N}\cdot\text{m}_{\text{TCC}}^{-3}\cdot\text{d}^{-1}$ , were obtained using a BES, but the system was supplemented with sulphur and iron and high currents were applied (100 mA) [48].

A relevant point for the e-settler application is the durability of the system. In this experiment, this study aimed to tune the working parameters to increase nitrogen and pathogens removal whereas the durability of the BES kit was not deeply assessed (total operation of 102 days). First of all, electrode clogging should be evaluated and maintenance could be required (e.g. backwashing of trapped sludge). Placing the electrodes in the upper part of the secondary settler and optimizing the cathode dimension could decrease the clogging. It was demonstrated that in the bioelectrochemical systems for WW treatment, a smaller electrode dimensions decreased the occlusion [49]. Moreover, it has been suggested that the current itself can prevent the clogging of the electrodes when using BES for WW treatment, either by enhancing the degradation of the suspended particles [50] or by decreasing the agglomeration of the sludge particles [51]. A second parameter to be considered for system durability is the long-term stability of electrodes. The electrodes could partially lose their electrochemical characteristics, reducing the removal performance, e.g. as observed during Phase II. This work has explored a total of 3 electrodes: graphite anode, Ti-MMO anode and graphite-coated stainless steel cathode. Graphite anode worked as a sacrificial electrode, and thus a regular replacement would be required. When using a Ti-MMO anode, a longer life-span is expected. For example, the resistance of a Ti-MMO anode has been assessed during a long-term experiment (>200 days), using it for oxygen evolution in a marine environment [52]. Regarding the cathode, the relevant risk would come from the presence of anodic oxidizing agents. Before the replacement of the graphite rods with Ti-MMO, the bioelectrochemical systems had been operated for 64 days showing an increasing nitrogen removal rate by tuning the different operational parameters. During this period, e-settlers demonstrated their stability and room for improvement given by the optimization of the working conditions. Yet, the substitution of the anodes slightly decreased the e-settlers performance. At the last test with graphite, total nitrogen was removed at a rate of  $248 \text{ g N}\cdot\text{m}_{\text{TCV}}^{-3}\cdot\text{d}^{-1}$ , while at the first test with Ti-MMO, total nitrogen was removed at  $164 \text{ g N}\cdot\text{m}_{\text{TCV}}^{-3}\cdot\text{d}^{-1}$  (nitrogen removal rate decreased by 34 %). A larger anode–cathode distance, together with higher water upflow velocity could reduce the effect of oxidizing agents over the biocathode. Moreover, in scaled-up e-settlers a horizontal electrodes configuration would be used, as shown in the graphical abstract, which can allow a better control of the anode–cathode distance as it is homogeneous for the whole length of the electrodes. However, a compromise with smaller anode–cathode distances to ensure proper electrochemical stability would be required (i.e. minimization of ohmic losses). These factors could contribute to reduce the corrosion of the electrodes themselves but also provide higher protection over cathodic bacteria. After having optimized the working conditions, the performance of the scaled-up BES should be assessed in a long term to evaluate whether stability is preserved. In fact, during the operation of the studied e-settlers, mostly stable removal rates were observed for each tested condition. Deviations ranged between 6 and  $60 \text{ g N}\cdot\text{m}_{\text{TCV}}^{-3}\cdot\text{d}^{-1}$  according to the different tests performed, with an average removal rate variation of  $30 \text{ g N}\cdot\text{m}_{\text{TCV}}^{-3}\cdot\text{d}^{-1}$  (20 % of the mean value).

The optimization of the water upflow velocity and the anode–cathode distance would not only increase the system durability, but also the nitrogen removal rates.

Another important aspect of the application of the e-settler is how the nitrogen removal performance changes depending on the influent characteristics. In the studied set-up, when operating with  $\text{NO}_3^-$ -WW, the operational conditions ( $\text{NH}_4^+$  content; low HRT) were optimal to guarantee the removal of the converted ammonium. However, as the WW composition fluctuated to higher  $\text{NH}_4^+$  concentration ( $\text{NH}_4^+$ - $\text{NO}_3^-$ -WW) the system was not able to completely remove the nitrate produced through nitrification. To overcome this issue, it would be required to study the coupling of the e-settler with an activated sludge bioreactor. The ideal WW influent for e-settlers is mainly composed of  $\text{NO}_3^-$  and low dissolved  $\text{O}_2$ , to favor denitrifying bacteria. In the secondary treatment model, aeration can be improved by regulating the dissolved oxygen according to the ammonium content of the aerobic sludge effluent, which is a control system already demonstrated to be effective in real WWTPs [53]. In this manner, ammonium content can be maintained within the required limits, i.e.  $5 \text{ mg}\cdot\text{L}^{-1}$  for WW discharge [53] or  $0.5 \text{ mg}\cdot\text{L}^{-1}$  in case of water reuse for human consumption [54]. A further solution, not evaluated in the studied e-settler, to remove the  $\text{NH}_4^+$  content is via anammox. If WW contains anammox bacteria and the operational conditions can sustain their growth (e.g. anoxic environment), autotrophic anaerobic oxidation of ammonium mediated by electrodes could occur. In this regard, it has been shown how anammox bacteria can transfer electrons directly to electrodes [15] and how they can be used in BES to treat synthetic WW [55]. In parallel, HRT should be also adjusted according to the dimension of the ST model. HRT, calculated on the cathodic volume, was used as the index to have a flow rate comparable to real secondary settlers, which usually ranges from 1 to 3 h [10]. An application on a larger scale of the technique would mean adapting the electrochemical system to maintain the performance as high as possible while using an appropriate electrode dimension. Consequently, the HRT of the scaled-up technology should decrease, since it is not possible to think of a settler where the cathode occupies the entire space of the tank.

The BES kit is meant to be a versatile system that can be easily applied to existing plants. This retrofitting technology can play an important role in the perspective of water discharge and reuse, considering the relevant number of WWTPs unequipped with appropriate treatments for nitrogen and pathogens removal in the EU [9], the conspicuous amount of not safely treated household WW in the world (44 %) [56] and the environmental costs associated to the introduction of additional installations in existing WWTPs [57]. The e-settler technology should not replace large-scale tertiary treatments but upgrade the efficiency of the overall WWTP. In the case of small-scale plants (e.g. decentralized WWTPs) smart solutions to guarantee proper nitrogen removal and sanitation of waste streams are needed [58,59]. For instance, Singh *et al.* analyzed the polishing power of several DEWATS, currently widely employed in India [60]. The majority of the plants were equipped with settlers, anaerobic baffled reactors and collecting tanks, which were not always efficient resulting in a TN content in the effluent higher than  $15 \text{ mg}\cdot\text{L}^{-1}$  and a coliform amount over  $2.5 \log(\text{CFU}\cdot\text{L}^{-1})$ . The e-settler configuration is flexible (e.g. electrodes dimension) and it could be adapted to each plant. The use of electrochemical stimulation is expected to avoid the addition of chemicals, i.e. methanol, which is usually added to increase COD to enhance heterotrophic denitrification [10], or oxidant agents for disinfection. This effect will not only reduce the operational costs associated but also increase sustainability in the broader sense, as no transport of chemicals will be required and electricity could be generated in-situ through renewable energy.

#### 4. Conclusions

The e-settler concept aims to retrofit WWTPs, avoiding the additional

space required for the installation of conventional tertiary treatments. The potential of an e-settler was proved by coupling a bioelectrochemical system with a secondary settler. The applied current concomitantly increased the nitrogen removal rate and the disinfection power of the system. This is the first study that merges the ability of BES to stimulate nitrogen removal and perform electrochemical oxidation for WW disinfection while applying these techniques using a simple configuration suitable to retrofit existing WWTPs. For example, a similar set-up to the one used in this experiment was studied by Sander *et al* [17]. A three-electrode system, with graphite granules both as anode and cathode, was placed in a plug-flow reactor and used as a tertiary treatment for WW. The system increased the nitrogen removal of the treatment plant. But the so-constructed BES would require additional space to be implanted in an existing WWTPs and the system did not show disinfectant ability. Moreover, to the best of the authors' knowledge, the only other study about merging BES into existing WWTPs was conducted by Tejedor-Sanz *et al* [28] which placed a three-electrode system in an activated sludge bioreactor. Although the coupled technologies showed enhanced polishing power, they had some limitations: the aerobic conditions of the reactor required a membrane to guarantee low oxygen levels in the cathodic chamber; the anode was meant to supply the oxygen for nitrification and could not be used to perform electrochemical disinfection. Instead, the e-settler can merge the increased nitrogen removal and WW disinfection since it is placed in the upper part of the secondary settler. Given the high amount of untreated water discharged into the environment and the number of WWTPs not equipped with tertiary treatments, the simplicity of the BES kit could become a feasible solution to upgrade existing plants, especially considering small-scale plants (e.g. decentralized WWTPs).

Further studies are required to improve the system. High ammonium content ( $>30 \text{ mgN-NH}_4^+-\text{L}^{-1}$ ) could not be polished in the current e-settler, requiring better aeration in the activated sludge bioreactor. As for the electrochemical disinfection, the proximity of the two electrodes resulted in a diffusion of the oxidizing agents to the biocathode, requiring optimization of the anode–cathode distance. Although several parameters still need to be adjusted for a scale-up of the process, e-settler demonstrated as a feasible route to enhance the polishing action of secondary settlers.

### 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.

### Data availability

Data will be made available on request.

### Acknowledgements

This work was funded through: the ELECTRA project [grant agreement no. 826244], which was financially supported by the Horizon 2020 programme of the European Union; the NSFC-EU Environmental Biotechnology joint program (No. 31861133001); the Key Research and Development Project of Shandong Province (No. 2020CXGC011202). S. P is a Serra Hünter Fellow (UdG-AG-575) and acknowledges the funding from the ICREA Academia award. LEQUiA [2017-SGR-1552] has been recognized as consolidated research group by the Catalan Government. The research leading to these results has been conceived under the International PhD Program “Innovative Technologies and Sustainable Use of Mediterranean Sea Fishery and Biological Resources (www.FishMed-PhD.org). This study represents partial fulfilment of the requirements for the Ph.D. thesis of Alberto Botti. The authors acknowledge the support given by Sorigué S.A.U., which manages the Quart WWTP.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cej.2022.138949>.

## References

- [1] X. Li, H. Shi, K. Li, L. Zhang, Combined process of biofiltration and ozone oxidation as an advanced treatment process for wastewater reuse, *Front. Environ. Sci. Eng.* 9 (2015) 1076–1083, <https://doi.org/10.1007/s11783-015-0770-5>.
- [2] R. Díaz, J. García, R. Mujeriego, M. Lucas, A. Quick, Low-Cost Treatment Method for Secondary Effluent Nitrate Removal through Denitrification, *Environ. Eng. Sci.* 20 (2003) 693–702, <https://doi.org/10.1089/109287503770736195>.
- [3] C. Directive, Council Directive 91/271/EEC (1991), <https://doi.org/10.1039/AP9842100196>.
- [4] L.A. Sanz, B.M. Gawlik, Water Reuse in Europe, *Publ. Off. Eur. Union.* (2014) 1–51. <https://publications.jrc.ec.europa.eu/repository/handle/JRC92582>.
- [5] N. Pous, A. Barcelona, L. Sbardella, M. Hidalgo, J. Colomer, T. Serra, V. Salvadó, Zooplankton-based reactors for tertiary wastewater treatment: A pilot-scale case study, *J. Environ. Manage.* 278 (2021), 111538, <https://doi.org/10.1016/j.jenvman.2020.111538>.
- [6] A. Ilias, A. Panoras, A. Angelakis, Wastewater recycling in Greece: The case of Thessaloniki, *Sustain.* 6 (2014) 2876–2892, <https://doi.org/10.3390/su6052876>.
- [7] Ministerial Decree 185/2003, Italian Ministry of the Environment, 2003. <https://www.gazzettaufficiale.it/eli/id/2003/07/23/003G0210/sg>.
- [8] V. Dulio, B. van Bavel, E. Brorström-Lundén, J. Harmsen, J. Hollender, M. Schlabach, J. Slobodnik, K. Thomas, J. Koschorreck, Emerging pollutants in the EU: 10 years of NORMAN in support of environmental policies and regulations, *Environ. Sci. Eur.* 30 (2018) 1–13, <https://doi.org/10.1186/s12302-018-0135-3>.
- [9] Eurostat, Population connected to urban wastewater collecting and treatment systems, by treatment level, *Popul. Connect. to Urban Wastewater Collect. Treat. Syst. by Treat. Lev.* (2019). <https://ec.europa.eu/eurostat/databrowser/view/ten00020/default/table?lang=en>. (accessed 16 August 2022).
- [10] A.C. van Haandel, J.G.M. van der Lubbe, *Handbook of Biological Wastewater Treatment*, Second ed., Iwa Publishing, London, UK, 2012.
- [11] C.P. Gerba, L.L. Pepper, *Municipal Wastewater Treatment*, in: I. Pepper, C. Gerba, T. Gentry (Eds.), *Environ. Microbiol. Third Ed.*, 3rd ed., Elsevier Inc., Oxford, UK, 2015: pp. 583–606. <https://doi.org/10.1016/B978-0-12-394626-3.00025-9>.
- [12] K. Rabaey, J. Rodríguez, L.L. Blackall, J. Keller, P. Gross, D. Batstone, W. Verstraete, K.H. Nealson, Microbial ecology meets electrochemistry: Electricity-driven and driving communities, *ISME J.* 1 (2007) 9–18, <https://doi.org/10.1038/ismej.2007.4>.
- [13] P.R. Rout, M.K. Shahid, R.R. Dash, P. Bhunia, D. Liu, S. Varjani, T.C. Zhang, R. Y. Surampalli, Nutrient removal from domestic wastewater: A comprehensive review on conventional and advanced technologies, *J. Environ. Manage.* 296 (2021), 113246, <https://doi.org/10.1016/j.jenvman.2021.113246>.
- [14] M. Osset-Álvarez, L. Rovira-Alsina, N. Pous, R. Blasco-Gómez, J. Colprim, M. D. Balaguer, S. Puig, Niches for bioelectrochemical systems on the recovery of water, carbon and nitrogen in wastewater treatment plants, *Biomass and Bioenergy.* 130 (2019), 105380, <https://doi.org/10.1016/j.biombioe.2019.105380>.
- [15] D.R. Shaw, M. Ali, K.P. Taturi, J.A. Gralnick, J. Reimann, R. Mesman, L. van Niftrik, M.S.M. Jetten, P.E. Saikaly, Extracellular electron transfer-dependent anaerobic oxidation of ammonium by anammox bacteria, *Nat. Commun.* 11 (2020) 1–12, <https://doi.org/10.1038/s41467-020-16016-y>.
- [16] P. Clauwaert, K. Rabaey, P. Aelterman, L. De Schampheleire, T.H. Pham, P. Boeckx, N. Boon, W. Verstraete, Biological denitrification in microbial fuel cells, *Environ. Sci. Technol.* 41 (2007) 3354–3360, <https://doi.org/10.1021/es062580r>.
- [17] E.M. Sander, B. Viridis, S. Freguia, Bioelectrochemical nitrogen removal as a polishing mechanism for domestic wastewater treated effluents, *Water Sci. Technol.* 76 (2017) 3150–3159, <https://doi.org/10.2166/wst.2017.462>.
- [18] M. Isabel San-Martín, R. Mateos, B. Carracedo, A. Escapa, A. Morán, Pilot-scale bioelectrochemical system for simultaneous nitrogen and carbon removal in urban wastewater treatment plants, *J. Biosci. Bioeng.* 126 (2018) 758–763, <https://doi.org/10.1016/j.jbiosc.2018.06.008>.
- [19] B. Viridis, K. Rabaey, Z. Yuan, J. Keller, Microbial fuel cells for simultaneous carbon and nitrogen removal, *Water Res.* 42 (2008) 3013–3024, <https://doi.org/10.1016/j.watres.2008.03.017>.
- [20] H. Li, W. Zuo, Y. Tian, J. Zhang, S. Di, L. Li, X. Su, Simultaneous nitrification and denitrification in a novel membrane bioelectrochemical reactor with low membrane fouling tendency, *Environ. Sci. Pollut. Res.* 24 (2017) 5106–5117, <https://doi.org/10.1007/s11356-016-6084-8>.
- [21] B. Viridis, K. Rabaey, R.A. Rozendal, Z. Yuan, J. Keller, Simultaneous nitrification, denitrification and carbon removal in microbial fuel cells, *Water Res.* 44 (2010) 2970–2980, <https://doi.org/10.1016/j.watres.2010.02.022>.
- [22] Y. di Chen, X. Duan, X. Zhou, R. Wang, S. Wang, N. qi Ren, S.H. Ho, 2021. Advanced oxidation processes for water disinfection: Features, mechanisms and prospects, *Chem. Eng. J.* 409, 128207. <https://doi.org/10.1016/j.cej.2020.128207>.
- [23] S. Hand, R.D. Cusick, Electrochemical Disinfection in Water and Wastewater Treatment: Identifying Impacts of Water Quality and Operating Conditions on Performance, *Environ. Sci. Technol.* 55 (2021) 3470–3482, <https://doi.org/10.1021/acs.est.0c06254>.



- [24] Q. Fang, C. Shang, G. Chen, MS2 Inactivation by Chloride-Assisted Electrochemical Disinfection, *J. Environ. Eng.* 132 (2006) 13–22, [https://doi.org/10.1061/\(asce\)0733-9372\(2006\)132:1\(13\)](https://doi.org/10.1061/(asce)0733-9372(2006)132:1(13)).
- [25] C.R. Costa, P. Olivi, Effect of chloride concentration on the electrochemical treatment of a synthetic tannery wastewater, *Electrochim. Acta.* 54 (2009) 2046–2052, <https://doi.org/10.1016/j.electacta.2008.08.033>.
- [26] G. Pérez, P. Gómez, R. Ibañez, I. Ortiz, A.M. Urriaga, Electrochemical disinfection of secondary wastewater treatment plant (WWTP) effluent, *Water Sci. Technol.* 62 (2010) 892–897, <https://doi.org/10.2166/wst.2010.328>.
- [27] W. Feng, D.T. McCarthy, Z. Wang, X. Zhang, A. Deletic, Stormwater disinfection using electrochemical oxidation: A feasibility investigation, *Water Res.* 140 (2018) 301–310, <https://doi.org/10.1016/j.watres.2018.04.059>.
- [28] S. Tejedor-Sanz, T. Bacchetti De Gregoris, J.J. Salas, L. Pastor, A. Esteve-Núñez, Integrating a microbial electrochemical system into a classical wastewater treatment configuration for removing nitrogen from low COD effluents, *Environ. Sci. Water Res. Technol.* 2 (2016) 884–893, <https://doi.org/10.1039/c6ew00100a>.
- [29] W.W. Li, H.Q. Yu, Z. He, Towards sustainable wastewater treatment by using microbial fuel cells-centered technologies, *Energy Environ. Sci.* 7 (2014) 911–924, <https://doi.org/10.1039/c3ee43106a>.
- [30] D.C. Xu, S.Y. Zhai, H.Y. Cheng, A. Guadie, H.C. Wang, J.L. Han, C.Y. Liu, A. J. Wang, Wire-drawing process with graphite lubricant as an industrializable approach to prepare graphite coated stainless-steel anode for bioelectrochemical systems, *Environ. Res.* 191 (2020), 110093, <https://doi.org/10.1016/j.envres.2020.110093>.
- [31] N. Pous, S. Puig, M. Dolers Balaguer, J. Colprim, Cathode potential and anode electron donor evaluation for a suitable treatment of nitrate-contaminated groundwater in bioelectrochemical systems, *Chem. Eng. J.* 263 (2015) 151–159, <https://doi.org/10.1016/j.cej.2014.11.002>.
- [32] N. Pous, S. Puig, M.D. Balaguer, J. Colprim, Effect of hydraulic retention time and substrate availability in denitrifying bioelectrochemical, *Environ. Sci. Water Res. Technol.* 3 (2017) 922–929, <https://doi.org/10.1039/c7ew00145b>.
- [33] A. Ceballos-Escalera, N. Pous, P. Chiluitza-Ramos, B. Korth, F. Harnisch, L. Bañeras, M.D. Balaguer, S. Puig, Electro-bioremediation of nitrate and arsenite polluted groundwater, *Water Res.* 190 (2021), 116748, <https://doi.org/10.1016/j.watres.2020.116748>.
- [34] APHA, Standard Methods for the Examination of Water and Wastewater, 19th ed. Am. Public Heal. Assoc. Washing. DC, USA, 2005.
- [35] Y. Xiao, Y. Zheng, S. Wu, Z.H. Yang, F. Zhao, Bacterial Community Structure of Autotrophic Denitrification Biocathode by 454 Pyrosequencing of the 16S rRNA Gene, *Microb. Ecol.* 69 (2015) 492–499, <https://doi.org/10.1007/s00248-014-0492-4>.
- [36] A. Lai, F. Aulenta, M. Mingazzini, M.T. Palumbo, M.P. Papini, R. Verdini, M. Majone, Bioelectrochemical approach for reductive and oxidative dechlorination of chlorinated aliphatic hydrocarbons (CAHs), *Chemosphere.* 169 (2017) 351–360, <https://doi.org/10.1016/j.chemosphere.2016.11.072>.
- [37] Y. Ruiz, E. Ribot-Llobet, J.A. Baeza, A. Guisasaola, Conditions for high resistance to starvation periods in bioelectrochemical systems, *Bioelectrochemistry.* 106 (2015) 328–334, <https://doi.org/10.1016/j.bioelechem.2015.06.010>.
- [38] A. Khelifa, S. Moulay, F. Hannane, S. Benslimene, M. Hecini, Application of an experimental design method to study the performance of electrochlorination cells, *Desalination.* 160 (2004) 91–98, [https://doi.org/10.1016/S0011-9164\(04\)90021-5](https://doi.org/10.1016/S0011-9164(04)90021-5).
- [39] A.R. Rahmani, M.R. Samarghandi, D. Nematollahi, F. Zamani, 2019. A comprehensive study of electrochemical disinfection of water using direct and indirect oxidation processes, *J. Environ. Chem. Eng.* 7, 102785, <https://doi.org/10.1016/j.jece.2018.11.030>.
- [40] M.G. Tavares, L.V.A. da Silva, A.M. Sales Solano, J. Tonholo, C.A. Martínez-Huitile, C.L.P.S. Zanta, Electrochemical oxidation of Methyl Red using Ti/Ru 0.3Ti 0.7O 2 and Ti/Pt anodes, *Chem. Eng. J.* 204–205 (2012) 141–150, <https://doi.org/10.1016/j.cej.2012.07.056>.
- [41] S. Fontenot, S. Lee, K. Asche, The effects of chloride from waste water on the environment, City of Morris (2013).
- [42] M.F. Hamoda, I.M.S. Al-Attar, Effects of high sodium chloride concentrations on activated sludge treatment, *Water Sci. Technol.* 31 (1995) 61–72, [https://doi.org/10.1016/0273-1223\(95\)00407-E](https://doi.org/10.1016/0273-1223(95)00407-E).
- [43] L. Gao, W. Zhou, J. Huang, S. He, Y. Yan, W. Zhu, S. Wu, X. Zhang, Nitrogen removal by the enhanced floating treatment wetlands from the secondary effluent, *Bioresour. Technol.* 234 (2017) 243–252, <https://doi.org/10.1016/j.biortech.2017.03.036>.
- [44] W.A. Shewa, L. Sun, C. Gan, K. Bossy, M. Dagnew, Biological treatment of municipal wastewater using fixed rope media technology: Impact of aeration scheme, *Environ. Technol. Innov.* 27 (2022), 102387, <https://doi.org/10.1016/j.eti.2022.102387>.
- [45] H. Khasloo, A.H. Hassani, R. Mafigholami, R. Mahmoudkhani, Comparing the performance of the conventional and fixed-bed membrane bioreactors for treating municipal wastewater, *J. Environ. Heal. Sci. Eng.* 19 (2021) 997–1004, <https://doi.org/10.1007/s40201-021-00664-3>.
- [46] A. Aguirre-Sierra, T. Bacchetti-De Gregoris, A. Bernà, J.J. Salas, C. Aragón, A. Esteve-Núñez, Microbial Electrochemical Systems outperform fixed-bed biofilters for cleaning-up urban wastewater, *Environ. Sci. Water Res. Technol.* 2 (2011) 984–993, <https://doi.org/10.2134/jeq2002.1757>.
- [47] R.K. Goel, J.R.V. Flora, Sequential nitrification and denitrification in a divided cell attached growth bioelectrochemical reactor, *Environ. Eng. Sci.* 22 (2005) 440–449, <https://doi.org/10.1089/ees.2005.22.440>.
- [48] M. Zhu, M. Zhang, Y. Yuan, P. Zhang, S. Du, T. Ya, D. Chen, X. Wang, T. Zhang, Responses of microbial communities and their interactions to ibuprofen in a bio-electrochemical system, *J. Environ. Manage.* 289 (2021), 112473, <https://doi.org/10.1016/j.jenvman.2021.112473>.
- [49] S. Brunschweiler, E.T. Ojong, J. Weisser, C. Schwaferts, M. Elsner, N.P. Ivleva, R. Haseneder, T. Hofmann, K. Glas, The effect of clogging on the long-term stability of different carbon fiber brushes in microbial fuel cells for brewery wastewater treatment, *Bioresour. Technol. Reports.* 11 (2020), 100420, <https://doi.org/10.1016/j.biteb.2020.100420>.
- [50] H.M. Khalifabadam, M.P. Ginige, R. Sarukkalgie, A.S. Kayaalp, K.Y. Cheng, Bioelectrochemical system as an oxidising filter for soluble and particulate organic matter removal from municipal wastewater, *Chem. Eng. J.* 296 (2016) 225–233, <https://doi.org/10.1016/j.cej.2016.03.067>.
- [51] A. Ding, Q. Fan, R. Cheng, G. Sun, M. Zhang, D. Wu, Impacts of applied voltage on microbial electrolysis cell-anaerobic membrane bioreactor (MEC-AnMBR) and its membrane fouling mitigation mechanism, *Chem. Eng. J.* 333 (2018) 630–635, <https://doi.org/10.1016/j.cej.2017.09.190>.
- [52] S. Cappello, C. Cruz Viggì, M. Yakimov, S. Rossetti, B. Matturro, L. Molina, A. Segura, S. Marqués, L. Yuste, E. Sevilla, F. Rojo, A. Sherry, O.K. Mejeha, I. M. Head, L. Malmquist, J.H. Christensen, N. Kalogerakis, F. Aulenta, Combining electrokinetic transport and bioremediation for enhanced removal of crude oil from contaminated marine sediments: Results of a long-term, mesocosm-scale experiment, *Water Res.* 157 (2019) 381–395, <https://doi.org/10.1016/j.watres.2019.03.094>.
- [53] G. Bertanza, P. Baroni, S. Garzetti, F. Martinelli, Reducing energy demand by the combined application of advanced control strategies in a full scale WWTP, *Water Sci. Technol.* 83 (2021) 1813–1823, <https://doi.org/10.2166/wst.2021.109>.
- [54] C. Directive, Council Directive 98/83/EC (1998), <https://doi.org/10.1017/cbo9780511610851.055>.
- [55] A. Vilajeliu-Pons, C. Koch, M.D. Balaguer, J. Colprim, F. Harnisch, S. Puig, Microbial electricity driven anoxic ammonium removal, *Water Res.* 130 (2018) 168–175, <https://doi.org/10.1016/j.watres.2017.11.059>.
- [56] UN-Water, Summary Progress Update 2021 : SDG 6 — water and sanitation for all, Geneva, Switzerland, 2021. <https://www.unwater.org/new-data-on-global-progress-towards-ensuring-water-and-sanitation-for-all-by-2030/>.
- [57] M. Faragò, A. Damgaard, J.A. Madsen, J.K. Andersen, D. Thornberg, M. H. Andersen, M. Rygaard, From wastewater treatment to water resource recovery: Environmental and economic impacts of full-scale implementation, *Water Res.* 204 (2021), 117554, <https://doi.org/10.1016/j.watres.2021.117554>.
- [58] J. Yang, A. van Timmeren, D. Sidler, The sustainable implant: Decentralised sanitation and energy reuse (Desaer) in the built environment, *Constr. Innov.* 7 (1) (2007) 22–37.
- [59] R. Grommen, W. Verstraete, Environmental biotechnology: The ongoing quest, *J. Biotechnol.* 98 (2002) 113–123, <http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PA GE=reference&D=emed5&NEWS=N&AN=2002261686>.
- [60] A. Singh, M. Sawant, S.J. Kamble, M. Herlekar, M. Starkl, E. Aymerich, A. Kazmi, Performance evaluation of a decentralized wastewater treatment system in India, *Environ. Sci. Pollut. Res.* 26 (2019) 21172–21188, <https://doi.org/10.1007/s11356-019-05444-z>.