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Fluidized bed cathodes as suitable electron donors for bacteria to remove nitrogen and produce biohydrogen

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ABSTRACT

Microbial Electrochemical Fluidized Bed Reactors (ME-FBR) represent a new concept for promoting proper bacteria-electrode interaction and eventually efficient biocatalysis in Microbial Electrochemical Technologies (METs). In the current work we demonstrate how a fluidized cathode, a dynamic and discontinuous design of electrode, can be an effective electron donor for electroactive hydrogen-generating and nitrate reducing bacteria. Furthermore, the oxygen produced in the anodic reaction promoted ammonium oxidation to nitrate by nitrifying bacteria thus expanding the environmental applications of the system. By coupling both anodic and cathodic reactions, it was possible to simultaneously achieve nitrification–denitrification within one chamber and without external oxygen addition. Our proof-of-concept revealed the removal of 98% ammonium and *ca.* 29% of total nitrogen (31 g-N $m^{-3}_{reactor}$ d^{-1}) from an effluent with low organic matter under continuous mode. This study reveals for first time how fluidized beds can be integrated in METs not only as anodes but also as cathodes, broadening the opportunities and applications to bioremediation and bioelectrosynthesis processes.

1. Introduction

Electroactive bacteria can be cost-effective catalysts for promoting redox-based biotechnology [1]; for instance, they can be employed in environmental remediation for removing pollutants from soils, sediments and watercourses [2,3]. The use of this microbes in Microbial Electrochemical Technologies (METs) is gaining increasing attention as a sustainable alternative to conventional electrochemical systems. A cathode can be an effective electron donor for those bacteria able to perform extracellular electron transfer (EET). There have been reported a wide variety of reactions in which microorganisms use an electrode as donor for performing a reductive reaction: nitrate reduction [4,5], reductive dichlorination [6,7], carbon dioxide reduction [8–10], proton reduction [11], acetate reduction [12], etc. The electron transfer from electrode to bacteria can be direct or may require the presence of an exogenous or self-synthesized soluble redox mediator or even a redox enzyme [13] (mediated EET). For both direct and mediated EET scenario the electrode and the reactor design become critical factors for optimizing the electrochemical and microbial performance. Diffusion and migration processes of molecules within a reactor and a biofilm can highly limit the catalysis rate and thus the sustainability of the technology [1]. By creating a favorable scenario with proper mixing and electrode-bacteria contact, parameters like transport of substrates, mediators, ions and also bacteria across interfaces can be highly enhanced. Following this aim we previously proposed the use of fluidized bed electrodes as anodes in METs and, indeed, we demonstrated its viability as electron acceptors for either planktonic [14] and biofilm-forming bacteria [15]. The use of microbial fluidized electrodes provides high surface electrode area as well as an optimum substrate-bacteria-electrode interaction, and even mediated EET can also benefit from this design [16]. The potential of this electrode design can be set for driving specific microbial redox reactions. In addition to the anodic role, fluidized abiotic cathodes have already been used for improving electroreduction routes, providing better electrolyte access, and a solution for removing low concentration of electroactive species from effluents such as metal ions [17,18]. Here we investigate the feasibility of using the fluidized electrode in a ME-FBR as a cathode to stimulate reduction reactions mediated by bacteria such as hydrogen bioproduction and denitrification. Furthermore, we have performed a proof-of-concept test in this configuration for *in situ* nitrogen removal via simultaneous nitrification and denitrification reaction without supplying external oxygen.

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2. Methods

2.1. Reactor set-up and media growth

The configuration for the Microbial Electrochemical Fluidized Bed Reactor (ME-FBR) was previously reported for treating a brewery wastewater [15]. The reactor had a volume of 0.68 L and the fluidized bed consisted of 80 mL (43 g) of electroconductive activated carbon particles (Aquasorb) (0.63–1 mm of diameter), acting as working electrode (WE). We used a titanium mesh coated with Pt particles (3 × 10 cm) as counter electrode (CE) and a Ag/AgCl 3 M reference electrode (RE) (HANNA). A NEV3 potentiostat (Nanoelectra) was used for polarizing the fluidized bed, and a peristaltic pump (Heidolph 5006, Germany) was used for recirculating the electrolyte. A second and identical ME-FBR unit was constructed to serve as control for testing abiotic reactions. Both reactors were kept at 30 °C.

Bacteria were cultured in a basal medium with 50 mM of $NaHCO_3$, 0.5 g L^{-1} NH_4Cl, 0.6 g L^1 $NaH_2PO_4{\cdot}6H_2O$, 0.1 g L^1 KCl, 10 mL L^1 of a mixed vitamin solution and 10 mL L^1 of a mixed mineral solution, as previously described [14]. The reactor was kept anoxic by gassing the headspace with a mixture of N_2/CO_2 (80:20) (except when hydrogen measurements were performed).

2.2. Reactor operation under batch and continuous mode

The fluidized bed, which had been previously working as an anode in the ME-FBR, was polarized to −0.6 V, and the electrolyte velocity was maintained to 0.68 cm s^{-1}. Under this condition the distance between the top of the fluidized bed and the CE was of aprox. 15 cm.

For the assays at continuous mode we used the same basal medium but with a concentration of 6.4 mM NH_4Cl, 5 mM of sodium acetate and 8.3 mM of sodium nitrate. This influent was continuously bubbled with N_2/CO_2 (80:20) and stored at 4 °C. A peristaltic pump (Watson and Marlow 205S, United States) was used for feeding the ME-FBR from the bottom of the reactor. The outlet port was located aside at aprox. 4 cm from the very top. The effluent was collected in a tank, where the volume was daily registered. Effluent samples (5 mL) were freshly collected once a day, filtered and stored at −20 °C until they were analyzed.

2.3. Chemical analyses

COD was measured with a commercial kit from Merck Millipore (Germany) as previously reported [15]. Nitrate and nitrite were measured in a Dionex DX120 Ion Chromatograph (IC) equipped with a conductivity detector, a cation suppressor and an IonPac 4 × 250 mm AS9-HC column. Ammonium was measured in a Metrohm 861 Advance Compact IC equipped with a METROSEP C3 250 column of 4 mm × 250 mm.

Hydrogen and methane were detected on a Varian 3350 chromatograph equipped with a packed column (Porapack N 80/100) and a TCD detector with nitrogen as carrier gas (20 mL min^{-1}). The column was set to 80 °C and the injector to 110 °C. The gas flux in the reactor was stopped for aprox. 1 h before sampling to allow the produced gas to accumulate in the headspace. Dissolved oxygen was measured using a multi optical meter FireStingO2 from Pyro Science.

2.4. Data analyses

The linear velocity of the recirculating electrolyte was calculated with the flow rate of the recirculation pump (L min^{-1}) and the column internal diameter (46 mm) (flow rate/column section).

The coulombic efficiency (CE) was estimated by obtaining the percental ratio values between the consumed charge in the fluidized cathode (Qi) and the theoretical charge consumed to reduce the nitrate in the media. Qi was obtained by integrating the chronoamperometric curve (current response over time), which was corrected by subtracting the current baseline obtained before nitrate was added to the system. We considered that all the nitrate was reduced to dinitrogen gas (5 electron reaction).

The nitrification efficiency under continuous mode was calculated as the percentage of ammonium removed from the influent. Denitrification efficiency was calculated as the ratio (%) of nitrate removed and total nitrate available within he reactor. We considered the total nitrate available as the sum of the moles of nitrate in the feed plus the moles of nitrate produced from ammonium microbial oxidation (1:1 ratio). For this calculation we assumed the complete oxidation of ammonium to nitrate since the analyses revealed just trace levels of nitrite in the system.

3. Results and discussion

Inspired by the previous success of using fluidized anodes for promoting the microbial oxidation of organic pollutants [14,15], we hypothesize here that a novel, dynamic and discontinuous cathode could promote reduction reactions performed by electroactive bacteria in a fluidized bed reactor.

3.1. Fluidized electrode as electron donor for microbial hydrogen production And denitrification

Our initial experimental condition was a microbial community adapted to grow on the anode of a ME-FBR operated over a year. In order to promote an electron-accepting community, the polarization potential of the fluidized electrode was shifted from + 200 mV to −600 mV (vs Ag/AgCl, 3 M KCl), as elsewhere reported for conventional biocathodes [19–21]. Our hypothesis was that electroactive microorganisms able to perform outwards EET onto a fluidized anode, or a fraction of them, would be capable of perform inwards EET as well. After setting the potential to −600 mV, we maintained the ME-FBR for 5 days without the addition of external substrates to acclimate the microbial community to use an electrode as electron donor and sole energy source instead of organic substrates like acetate. After reaching a current baseline nitrate was spiked, leading to a fast current drop sustained within time, indicating that reduction reactions were triggered (Fig. 1). Analyses of the soluble nitrogen species in the ME-FBR media confirmed the reduction of nitrate, and the transitory presence of nitrite and ammonium as intermediates, which were further consumed in the system (Figs. 1B and S1). The two main pathways for microbial nitrate reduction are denitrification and dissimilatory nitrate reduction to ammonium. Nitrogen gas and ammonium are the end products of these metabolic processes, respectively, and both routes share nitrite as intermediate metabolite. We hypothesize that most of the ammonium produced must have been consumed through microbial assimilation processes as the media was limited in nitrogen during that experiment. Likewise, a part of it could have been oxidized to nitrate again through microbial nitrification. Overall, since no nitrogen species were found in the media after day 12 we hypothesized that nitrate was mostly removed via denitrification process to N_2. We anticipated that the nature of the microbial populations reducing nitrate was autotrophic since we did not add any carbon source to the media and thus most of the nitrate was consumed using the fluidized cathode as electron donor.

The integration of the chronoamperometry data revealed that a total of 17 mmol of electrons were consumed on the fluidized cathode. We estimated the theoretical charge needed to reduce all the nitrate according to the distribution of intermediates, obtaining a total of 17.3 mmol of electrons (calculations described in Materials and Methods section). As a result, a 85% of coulombic efficiency (ratio of nitrate reduced to N_2 coupled to current consumption to total nitrate reduced in the reactor) was observed for this process, suggesting that most of the nitrate was reduced bioelectrochemically.

Both the electrochemical and chemical data confirmed the viability

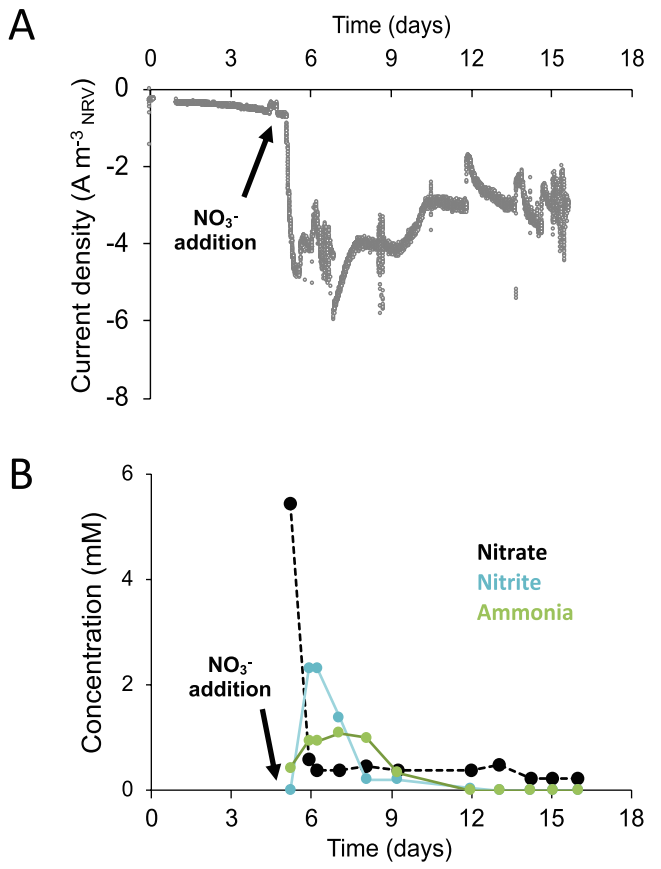

Fig. 1. Fluidized cathode serves as electron donor for microbial nitrate reduction. A. Current consumption evolution when the fluidized electrode was polarized to –600 mV (vs Ag/AgCl) and a pulse of nitrate was added to the ME-FBR media. B. Nitrogen species in species detected in media of the ME-FBR from nitrate pulse.

of using a fluidized cathode for serving as electron donor for microorganisms. This is to our knowledge the first time that an electrode design such as fluidized bed is shown to work in a microbial electrochemical system as a biocathode.

We observed that current was still consumed in the absence of nitrate or nitrite, which suggests the presence of alternative reduction reactions on the fluidized cathode (Fig. 1A). Thus, our next step was to identify the nature of this electrochemical reaction. We firstly explored the possibility that other reactions such as CO_2 and protons reduction reactions occurred under the potential applied (-0.6 V) to the fluidized cathode. These two reactions lead to methane [22] and hydrogen production , respectively, and can be mediated by electroactive bacteria as it has been reported before [11]. Therefore, we performed gas analyses of the headspace of our ME-FBR being the cathode polarized to -0.6 V and also at open circuit conditions in order to stablish the role of the fluidized cathode in these processes. Fig. 2A shows a production of hydrogen with 60–80% of gas composition (v/v) (counting with CO_2, H_2 and CH_4) and the absence of methane when the fluidized cathode was polarized. Hydrogen production notably dropped when the system was maintained at open circuit potential (OCP), demonstrating that the cathode was responsible for such hydrogen production. An abiotic control ran in parallel under the same polarization conditions showed that hydrogen production in the ME-FBR required the mediation of microorganisms in addition to the fluidized cathode as electron donor.

The hydrogen produced in the ME-FBR could serve as electron donor for the reduction of nitrate through hydrogenotrophic denitrification [23]. Nevertheless, both the direct and hydrogen-mediated reactions could be simultaneously occurring.

Next, we analyzed the presence of possible electron acceptors competitors in the medium generating a current consumption alongside with nitrate. The cathode is not the only reactive material in our ME-FBR; actually, the operating conditions very likely can lead to oxygen generation at the counter electrode (anode). To validate this hypothesis, we measured the dissolved oxygen (DO) concentration close to its putative generation source, *i.e.*, the counter electrode area. Results confirmed the presence of high levels (6–8 mg/L) of oxygen in that zone (top of the reactor) (Fig. 3B and C). Switching the electrochemical system to OCP led to a rapid decrease of oxygen to trace levels (inset of Fig. 3A) demonstrating a dependence of oxygen presence with the electrode polarization. Furthermore, the simultaneous analysis of DO at different reactors heights revealed the presence of an oxygen vertical gradient along the ME-FBR. In the proximity of the counter electrode, where oxygen is likely to be produced from water oxidation, the environment was clearly aerobic. In contrast, at the recirculating port column height, the media was microaerobic, with levels between 0.5 and 0.9 mg/L. As expected, the culture medium in contact with the fluidized cathode (sampled at the first third of the bed height) remained anoxic (DO below 0.2 mg/L). An oxygen gradient was also observed under abiotic conditions and only when the anode was polarized, indicating that oxygen was electrochemically generated on the counter electrode (Fig. S2).

The presence of oxygen could explain why methane was not generated from biological CO_2 reduction (electromethanogenesis) despite being more thermodynamically favorable than proton reduction reaction (standard reduction potential of -0.244 vs -0.414 V at pH 7, respectively). Indeed, operating conditions in presence of oxygen typically inhibit methanogenesis in a severe way and actually its electrolytically *in situ* production has been proposed as a method for reducing CH_4 production in METs [24].

3.2. Simultaneous microbial anodic and cathodic driven reactions in a single chamber MET: Proof of concept for nitrification–denitrification

This electrochemical production of oxygen in the culture media (anode) was coupled to current consumption at the cathode, and thus, was dependent of the reduction reactions (eg. hydrogen evolution and nitrate reduction). The presence of oxygen inspired us to take advantage of the electrochemical by-product for driving a key environmental reaction, microbial ammonium oxidation, within the same ME-FBR. We anticipated that the co-presence of oxygen and ammonium would promote microbial ammonium oxidation to nitrate or nitrite and these products would be further biologically reduced on the fluidized cathode, as we showed before. Actually, in previous assays (Fig. 1), ammonium from culture media eventually decreased, suggesting a microbial population oxidizing ammonium. We tested our hypothesis by simultaneously feeding ammonium and nitrate to the ME-FBR under continuous mode to perform a proof of concept regarding the technology capacity for removing nitrogen. The operating conditions and results of the nitrogen removal test are shown in Table 1. We observed that ammonium was successfully removed from the influent (93%) whereas nitrate reduction was the limiting step on this simultaneous nitrification/denitrification. An abiotic control showed that ammonium was not being oxidized abiotically on the anode (counter electrode) (Fig. S3), probing that ammonium oxidation to nitrate/nitrite corresponded to a biological reaction.

Our strategy was able to remove 28.6% of the total nitrogen of the influent at a COD/N ratio of 0.64 w/w, which is 5–10 fold lower in comparison to those ratios required for heterotrophic denitrification (3–6, w/w) [25]. We could not estimate the coulombic efficiency on the cathode specifically associated to nitrate reduction since the current consumption was due to three simultaneously competing reactions (the reduction of oxygen, protons and nitrate). Overall, we show the feasibility of removing nitrogen through a simultaneous nitrification/denitrification system by consuming a by-product (oxygen) at the counter

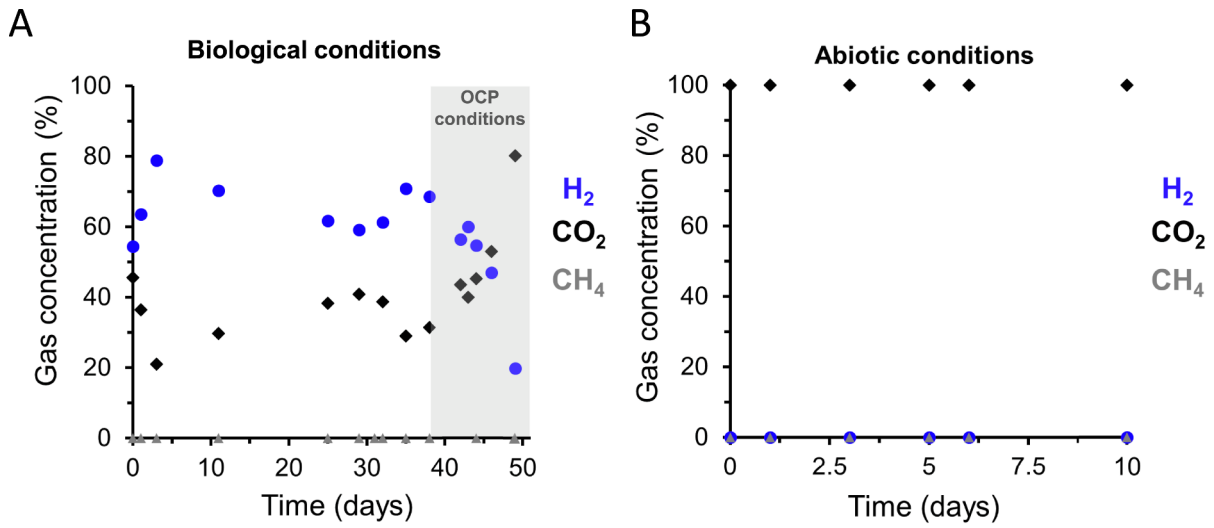

Fig. 2. Biological hydrogen is electrochemically produced on the fluidized cathode. Gas composition in the headspace of the ME-FBR under polarized conditions (A) and under polarized and abiotic conditions (B). The composition is given as relative composition based on hydrogen, carbon dioxide and methane.

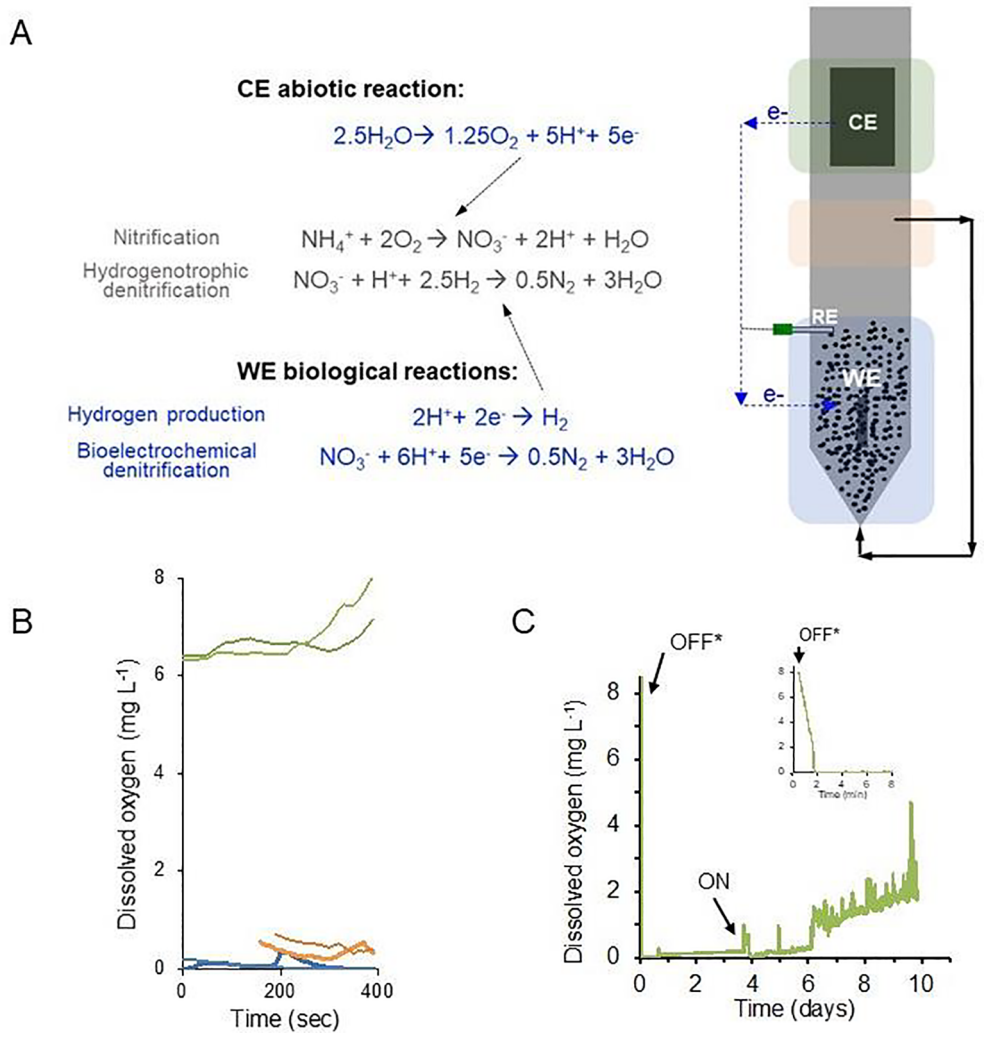

Fig. 3. Oxygen gradient generated by the anodic water oxidation reaction drives simultaneous ammonium oxidation and nitrate reduction in the same chamber. A. Schematic of the possible different reactions simultaneously occurring in the ME-FBR due to the presence of electrodes polarization. B. Dissolved oxygen measured with two probes in three different locations of the ME-FBR. Green line: counter electrode zone; Orange line: above recirculation port; Blue line: fluidized cathode zone (close to current collector). C. Oxygen evolution at the counter electrode zone when the polarization of the fluidized cathode was disconnected (from t = 0) and then was connected back again at a time of 3 days. The insight shows the fast drop of oxygen in the media when the electrodes were disconnected. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

electrode. To our knowledge, this strategy has rarely been demonstrated in previous studies within the field of METs. This proof of concept validates how using and coupling both anode and cathode reactions in the absence of a separating membrane can promote synergic microbial communities. We are currently exploring this application for removing nitrogen from water with low organic matter like the one found in groundwater close to manure and farming activity areas. For a better electrochemical performance of the system, employing ground

Table 1
Operating conditions and results of the nitrogen removal test at continuous mode in a ME.FBR fed with ammonium and nitrate as nitrogen sources.

Operating parameters		Reactor performance	
COD/N ratio	0.64	Total N removal (%)	28.6 ± 5.3
COD influent (mg L^{-1})	128	Nitrification (%)	95.3 ± 2.9
Hydraulic retention time (d)	2	Denitrification (%)	28.0 ± 8.5
N influent (mg L^{-1}) (6.3 mM NH_4^+ and 8.3 mM NO_3^-)	200	N removal rate (g $m^{-3}_{reactor}$ d^{-1})	31 ± 5
		Current consumption (mA)	−11 ± 4

waters with high conductivity is recommended to avoid a high ohmic drop. Likewise, the treatment of water containing low levels of pollutants might be a suitable niche of application as the current flow levels would be low, and thus the ohmic losses. By testing more efficient electrocatalytic materials, and reactor configuration (position of electrodes, column design) one could potentially optimize the system to minimize ohmic losses. We anticipate that studying the influence of operating parameters such as influent COD, HRT, cathode potential, one could greatly improve the nitrogen removal capacity of the ME-FBR.

4. Conclusions

We show for the first time the viability of using a fluidized cathode as electron donor for electroactive bacteria. The new configuration has potential applications within the field of nutrient-polluted water for removing nitrogen from low COD influents avoiding the costly procedure of supplying oxygen. The biological production of hydrogen let us to suggest a potential application on microbial electrosynthesis of organic compounds. The dispersed, particulate and dynamic nature of the fluidized cathode makes it an attractive scenario for cathodic bioelectrochemical reactions, especially if low concentrations of electroactive species play a role, such as a redox mediators, or a pollutant to be removed. Furthermore, we show a MET configuration in which both the cathodic and anodic reactions can be used for driving coupled microbial reactions, such as nitrification and denitrification.

CRediT authorship contribution statement

Tejedor-Sanz Sara: Conceptualization, Investigation, Data curation, Visualization, Supervision, Writing - original draft, Writing - review & editing. **Fernández-Labrador Patricia:** Data curation, Visualization, Writing - review & editing. **Manchón Carlos:** Data curation, Visualization, Writing - review & editing. **Esteve-Núñez Abraham:** Funding acquisition, Writing - review & editing.

Declaration of Competing Interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.elecom.2020.106759.

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