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Remediation of nitrate-contaminated groundwater in a denitrifying bioelectrochemical system

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ABSTRACT

Groundwater has become, in large parts of the world, the most important source of drinking water and its quality must be maintained. Nevertheless, groundwater pollution by anthropogenic nitrate is still being widely spread This study investigated the performance of a bioelectrochemical system in a laboratory scale to remove nitrate from groundwater in three different operational conditions: (i) open circuit, (ii) microbial fuel cell without externally applied potential, and (iii) microbial electrolysis cell with externally applied potential (0.3, 0.5, and 1.0 V). Initial nitrate concentration in the groundwater was 26.3 mg $N–NO_3^-$. The denitrifying bioelectrochemical systems (D-BES) reduced nitrate concentration to at least 8.3 mg $N–NO_3^-$, regardless of the operational condition, meeting the standards by the World Health Organization for water quality. The final nitrate concentration was even lower when D-BES was operated in microbial electrolysis cell (MEC) modes. MEC with 0.5 V reached nitrate concentration of 1.0 mg $N–NO_3^-$, being the best removal efficiency (96.2%). Additionally, there was no accumulation of nitrite and ammonium in the MEC modes, suggesting that energy currents might have stimulated the microbial community present in the D-BES. These results indicate that D-BES, especially under MEC operations, has the potential for nitrate bioremediation, with minimal maintenance and health risk.

Keywords: Bioelectrochemistry; Bioremediation; Contaminated groundwater; Nitrate removal; Wastewater

1. Introduction

The increasing demand for nitrogen fertilizers, crop irrigation with untreated wastewater, and the use of manure are some of the main causes of water pollution around the world. The primary reason is that in the environment, nitrogenous compounds might be transformed into nitrate, which is a rather mobile ion that easily reaches groundwater and surface water bodies [1]. Thus, nitrate is becoming a serious worldwide problem due to its risk to human health (high toxicity, mutagenicity, and carcinogenicity) [2].

Nitrate removal from groundwater is a major environmental challenge and currently, several physical, chemical, and biological technologies are implemented for satisfactory treatment [1,3,4]. Conventional technologies include *ex situ* methods such as reverse osmosis, electrodialysis, and ion exchange, which are pretty expensive, while *in situ* methods such as chemical and microbiological approaches require the addition of chemical reagents or electron donors (e.g., organic matter) which deteriorate water quality. However, these methods are not highly effective and/or might generate a relative amount of brine waste that needs afterward treatment and appropriate disposal [5].

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A relative emerging technology for simultaneous nitrate and organic matter (including some hazardous materials) removal from polluted groundwater is based on microorganisms to catalyze at least one or both oxidation/reduction processes, known as denitrifying bioelectrochemical systems (D-BES) [6,7]. In general, organic matter is oxidized by heterotrophic bacteria in the anode chamber and the electrons are transferred to the cathode electrode, where nitrate is reduced by autotrophic denitrifying bacteria to nitrous oxide (N_2O) and nitrogen gas (N_2), a mechanism known as microbial fuel cells (MFC) [7]. However, when a biodegradable fraction of organic matter is not adequate for supporting denitrification by microorganisms, an external potential can be applied at the cathode using a potentiostat at a desired reductive level, in combination with an abiotic anode. This mechanism is called microbial electrolysis cell (MEC). D-BES has the advantage of effective pollutant removal, easy control and operation, and environmental compatibility [6,8].

Some studies in the literature used bioelectrochemical methods for nitrate removal [6–10] and they confirmed that these biosystems had a rather promising prospect for water remediation. Tong and He [10] investigated nitrate removal from groundwater applying an electric force into anode produced by organic matter oxidation (MFC) and by an external voltage application of 0.8 V (MEC). The MFC mode achieved a nitrate removal rate of 154.2 ± 24.4 and MEC mode of 208.2 ± 13.3 g NO_3^- N m^{-3} d^{-1}. These results confirmed that D-BES is a promising technology for nitrate removal. However, few studies have compared results of D-BES treatment under diverse modes of operation using the same contaminated water sample.

This study investigated the feasibility to achieve nitrate removal from groundwater through a denitrifying BES in a laboratory scale. More precisely, the aim of the study was to compare three D-BES operations, which were open circuit (OC), MFC, and MEC with different external voltages. Also, influences of environmental parameters in the D-BES efficiency were elucidated, since little has been investigated so far. Moreover, advantages and disadvantages of each mode of operation were discussed to benefit upcoming works. Another novelty in the research was the use of granular graphite inoculated with biological sludge from a municipal wastewater treatment plant (WWTP) with the nutrients removed, as a support medium in the anodic and cathodic chambers.

2. Materials and methods

2.1. Experiment descriptions

A denitrifying BES with two acrylic chambers was constructed: one compartment was used as the anodic chamber (volume of 450 cm^3) and the other one as the cathodic chamber (volume of 600 cm^3). Both chambers were stocked with graphite granules of diameters between 1.0 and 1.7 mm. The working volume in each compartment was approximately 200 cm^3 or 0.2 L. Graphite electrodes (200 mm × 20 mm × 5 mm) were used in the anodic and cathodic chamber. An Ag/AgCl reference electrode was also used. Anodic and cathodic chambers were separated by a cation exchange membrane (Ultrex CMI-7000, Membrane International, EUA). Following the manufacturer procedures, the membrane was emerged in a 5% NaCl solution for 12 h to promote hydration and expansion. A laboratory-made acetate-enriched water (electron donor): CH_3COONa (450 mg L^{-1}), $NaHCO_3$ (152.5 mg L^{-1}), NH_4Cl (9.55 mg L^{-1}), $Na_2HPO_4{\cdot}2H_2O$ (340.7 mg L^{-1}), $KH_2PO_4{\cdot}2H_2O$ (150 mg L^{-1}), $CaCl_2{\cdot}2H_2O$ (1.75 mg L^{-1}), $MgSO_4{\cdot}7H_2O$ (11.25 mg L^{-1}), KCl (1.62 mg L^{-1}), and 0.1 mL L^{-1} microelements solution with composition of ethylenediaminetetraacetic acid (1,000 mg L^{-1}), $FeSO_4{\cdot}7H_2O$ (1,000 mg L^{-1}), $ZnCl_2$ (70.0 mg L^{-1}), $MnCl_2{\cdot}4H_2O$ (100 mg L^{-1}), H_3BO_3 (6 mg L^{-1}), $CaCl_2{\cdot}6H_2O$ (130 mg L^{-1}), $CuCl_2{\cdot}2H_2O$ (2 mg L^{-1}), $NiCl_2{\cdot}6H_2O$ (24 mg L^{-1}), $Na_2Mo_4{\cdot}2H_2O$ (36 mg L^{-1}), and $CoCl_2{\cdot}6H_2O$ (238 mg L^{-1}) were used to flow through the anodic chamber. The anode feeding was based on a phosphate-buffered saline (PBS) solution purged with nitrogen gas before using. On the other hand, a synthetic nitrate-polluted groundwater: $NaHCO_3$ (244 mg L^{-1}), $NaNO_3$–N (26.3 mg L^{-1}), $NaH_2PO_4{\cdot}2H_2O$ (545 mg L^{-1}), $KH_2PO_4{\cdot}2H_2O$ (240 mg L^{-1}), $CaCl_2{\cdot}2H_2O$ (2.8 mg L^{-1}), $MgSO_4{\cdot}7H_2O$ (18 mg L^{-1}), KCl (2.6 mg L^{-1}) [11], and 0.1 mL L^{-1} microelements solution (composition described above) were continuously flowing through the cathodic chamber. Both laboratory-made fluids flowed with a rate of 1.44 L d^{-1}. An internal recirculation loop (100 L d^{-1}) was placed in each chamber to maintain well-mixed conditions and to avoid gradient concentration and granular graphite obstruction. The system was kept in a climatized room with 23°C ± 2°C. Fig. 1 illustrates the D-BES set-up and its components used in this study.

The D-BES was inoculated with activated sludge from a WWTP located in the state of São Paulo, Brazil. The WWTP is of the activated sludge type. The removal of nitrogen is done by conventional biological route of nitrification and denitrification. The sludge from this WWTP was used to fill each chamber.

The activated sludge with 3,000 mg L^{-1} of volatile suspended solids (VSS) filled about 30% of the chambers' working volume. After inoculation, both chambers were filled out with a feeding solution according to the composition described in Tong and He [10] for 60 d to allow the microbial adaptation (acclimatization step). Then, the solution was changed by a synthetic nitrate-contaminated groundwater to perform the denitrifying D-BES experiment. Comparatively, the D-BES was operated under several different conditions: open circuit (OC), the MFC mode (without externally applied potential), and the MEC mode (with an externally applied potential). In the MEC mode, a power supply (Instrutherm, Brazil, FA-3003, 100 Ohms) was used to apply 0.3 V, 0.5 V, and 1.0 V vs. Ag/AgCl to the circuit. Those selected voltages are within the typical range of the applied voltage in an MEC (0.2–1.0V) [12]. The potential at the cathode was –0.305 V vs. SHE (–0.5 V vs. Ag/AgCl reference electrode), the value was chosen based on studies by Cecconet et al. [6], Molognoni et al. [24].

In OC and MFC modes, the anode was fed with groundwater containing 26.3 ± 0.3 mg NO_3–N L^{-1}. Each operation mode was performed for 20 uninterrupted days. Table 1 summarizes all experimental conditions.

Chemical oxygen demand (COD), pH, temperature, nitrogen series (nitrate NO_3^-–N, nitrite – NO_2^-–N, and ammonium

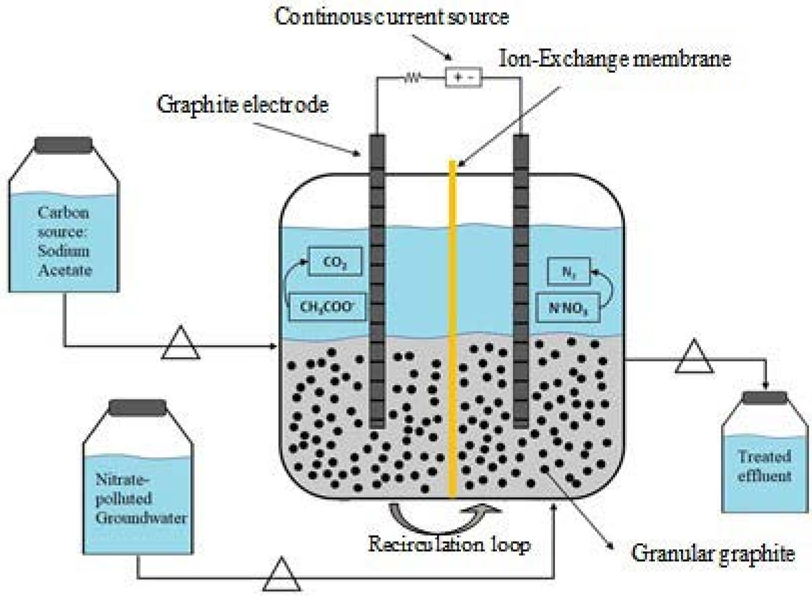

Fig. 1. Illustration of the denitrifying bioelectrochemical system used in this study.

Table 1
Experimental conditions of the denitrifying bioelectrochemical system

Run	Synthetic groundwater (mg NO_3 L^{-1})	Feed flow (mL min^{-1})	Recirculation flow (mL min^{-1})	Hydraulic retention time (hours)	Operation mode	External voltage (V)
Test 1	26.3 ± 0.3	1.0	73	8.3	OC	0.0
Test 2					MFC	0.0
Test 3					MEC	0.3
Test 4					MEC	0.5
Test 5					MEC	1.0

– NH_4^+–N), total organic carbon (TOC, Shimadzu, China, 5000A), chlorides, and electrical conductivity analyses were regularly done according to the Standard Methods for the Examination of Water and Wastewater [13]. Nitrate and nitrite were quantified by ion chromatography (Dionex-100, column ASCR2_mm and CSCR2_mm).

All the chemical analyses were performed in triplicates. The conductivity was measured by a benchtop conductivity meter (Mettler–Toledo, Columbus, OH, USA). Carbon and nitrogen volume-loading rates were calculated using carbon and nitrogen concentrations by hydraulic retention time (HRT).

The cell potential (V) in MFC mode was monitored every 60 s using a multimeter (Minipa, Brazil) connected to a data acquisition system (Arduino Uno, Italy). Ohm's law as in Eq. (1) was used to calculate the current density across the 100 Ω load resistor.

$$V = I \times R \tag{1}$$

Power density (W m^{-3}) was calculated using Eq. (2).

$$P_{an} = R \times \frac{I^2}{V_{ca}} \tag{2}$$

where I is the current flowing through the resistor in ampere and V_{an} is the volume of the cathode chamber in cubic meter.

2.2. Statistic model

The Snedecor's F distribution model was used to evaluate carbon removal by MEC modes and compare the nitrogen loading rates (NLR).

3. Results and discussion

At the biological sludge acclimatization step, D-BES achieved both nitrate and COD removals about 60% and 75%, respectively. It suggests good system stability, since the heterotrophic bacteria community was metabolically active and in high numbers (data not shown). After this initial step, to further understand the effect of the operating conditions, the D-BES was examined in the MEC mode with 0.3, 0.5, and 1.0 V, in an MFC mode without any additional potential, and in an OC, comparatively. The acetate-enriched groundwater that fed the anode chamber showed pH values 7.0 ± 0.1 and conductivity of 931 ± 57.3 µS cm^{-1}, such results are similar to those found in the literature. Pous et al. [8] described pH 7.7 ± 0.1 and conductivity 984 ± 119 µS cm^{-1}

for similar synthetic groundwater. Table 2 summarizes the physical–chemical and electrical results obtained during nitrate-polluted groundwater treatment in different D-BES operation modes.

All D-BES modes showed a slightly lower pH value in the anode compared to the cathode and the final effluents. Even though a PBS solution was used in the anode chamber. It happened because the oxidation reaction at the anode can be represented by acetate degradation as $CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + 9H^+ + 8e^-$. Besides electrons, protons are released by the microbial degradation of the organic substrate in the anode chamber and its accumulation leads to acidification [14,15]. On the other hand, hydroxyl ions (OH^-) are produced in the cathodic reactions, where the pH of the catholyte was naturally maintained at 7.1 ± 0.1. Even though a large number of studies on bioelectrochemical denitrification have been reported, in only one case the authors dealt with actual groundwater [16]. Thus, other researches should be conducted on the matter, taking into consideration, the strong influence that pH exerts on cathodic denitrification [17]. It is noteworthy that the catholyte composition was based on Puig et al. [11]. However, after experimental planning, we noticed a little mistake in the original recipe of the catholyte. Probably, NaH_2PO_4 was wrong used instead of Na_2HPO_4, since there is no sense employing NaH_2PO_4 and KH_2PO_4 in the same fluid composition. Unfortunately, although this mistake was replied by this work, we suggest paying attention to this issue in oncoming studies.

Conductivity of effluents were rather higher than those values verified in the anode and cathode chambers, regardless of the operation mode. It is hypothesized that concentration of nitrate in groundwater affects the conductivity and consequently the electricity generation. Power and current density are also of interest, as the process is bioelectrochemical, and therefore both parameters were continuously recorded throughout the study [18]. The current density values were 8.2, 26.0, 95.9, 167.8, and 746.3 mA for OC, MFC, MEC 0.3, 0.5, and 1.0 V, respectively. Thus, these current densities generated a power density of 0.6 mW for OC, 2.0 mW for MFC, 28.8 mW for MEC 0.3 V, 84.1 mW for MEC 0.5 V, and 745.1 mW for MEC 1.0 V. These results showed that there was a strong correlation between nitrate removal efficiencies and parameters such as pH, conductivity, current density, and power density. In addition, it is seen that to promote a high nitrogen removal efficiency is necessary proportional power demand. Table 3 shows the performance of the nitrifying D-BES reactor to remove carbon and nitrogen under different operation modes and corroborates the results of pH, conductivity, current, and power discussed previously.

The organic loading rate (OLR) applied in the anode chamber had an average of 179, 169, 160, 158, and 152 mg COD L^{-1} d^{-1} that resulted in OLR rates of 30, 20, 4, 4, and 6 mg COD L^{-1} d^{-1} for the treated effluent under OC, MFC, MEV 0.3, 0.5, and 1.0 V modes, respectively. Statistical analysis considering 1 and 60 degrees of freedom and 5% significance level showed a smaller *f*-value (0.03) than the critical *f*-value (4.00). It confirms that there are no statistically significant differences in carbon removal by different MEC voltages used here. Fig. 2 shows COD variation in the anodic chamber and in the treated effluent under different D-BES operation modes. In fact, external voltage supply to the D-BES promoted the best organic matter oxidation rates, indicating high electron release to nitrate reduction. In general, MEC increased at least 20% consumption of organic matter available in the system compared with OC and MFC modes. Thus, better results for nitrogen removal by MEC modes were expected than by OC and MFC.

Statistical analysis of NLR applied to influents showed that the *f*-value was smaller than the critical *f*-value, suggesting that the initial nitrogen rates were statistically the same for all modes of operation. However, NLR values in the treated effluents showed higher *f*-value (4.58) than the critical *f*-value (4.00) using the same parameters. It shows that each mode of operation had different efficiency of nitrogen removal. In other words, even the very similar NRL values obtained by MEC with different voltages are statistically different. The D-BES nitrogen removal efficiency of 96.3% was obtained by MEC 0.5 V, which treatment showed almost total organic oxidation. An efficiency slightly lower (90.6%) was recorded by MEC 1.0V, followed by MEC 0.3V (87.9%), MFC (77.6%), and OC (67.8%). Compared with the modes without electrical stimulation (OC and MFC),

Table 2
Main electrical and physical–chemical parameters of the D-BES in different operation modes

Parameters	OC	MFC	MEC 0.3 V	MEC 0.5 V	MEC 1.0 V
pH					
Cathode	7.1 ± 0.1	7.1 ± 0.1	7.1 ± 0.1	7.0 ± 0.1	7.1 ± 0.1
Anode	6.7 ± 0.2	6.6 ± 0.1	6.5 ± 0.1	6.6 ± 0.2	6.5 ± 0.1
Effluent	7.5 ± 0.2	7.5 ± 0.1	7.4 ± 0.2	7.3 ± 0.1	7.2 ± 0.1
Conductivity (μS cm^{-1})					
Cathode	951 ± 89.6	981 ± 49.2	971 ± 65.4	958 ± 75.2	955 ± 64.3
Anode	931 ± 57.3	951 ± 37.4	941 ± 43.2	945 ± 52.1	938 ± 39.2
Effluent	1,071 ± 46.6	1,064 ± 35.3	1,148 ± 29.8	1,138 ± 47.4	1,202 ± 31.4
Current density (mA)	8.2 ± 3.0	26.0 ± 10.3	95.9 ± 2.3	167.8 ± 4.2	746.3 ± 150.5
Power density (mW)	0.6 ± 0.2	2.0 ± 1.3	28.8 ± 0.8	84.1 ± 2.1	745.1 ± 148.9

OC: open circuit; MFC: microbial fuel cells; MEC: microbial electrolysis cell.

Table 3
Nitrogen and carbon parameters under different D-BES operations

Parameters	OC	MFC	MEC 0.3 V	MEC 0.5 V	MEC 1.0 V
$OLR_{anode\ influent}$ (mg COD L^{-1} d^{-1})	179 ± 50	169 ± 26	160 ± 30	158 ± 19	152 ± 17
$NLR_{cathode\ influent}$ (mg NO_3^- L^{-1} d^{-1})	60 ± 20	61 ± 5	62 ± 2	63 ± 2	63 ± 2
$OLR_{anode\ effluent}$ (mg COD L^{-1} d^{-1})	30 ± 30	20 ± 12	4 ± 4	4 ± 4	6 ± 6
$NLR_{cathode\ effluent}$ (mg NO_3^- L^{-1} d^{-1})	20 ± 10	10 ± 10	8 ± 4	2 ± 2	6 ± 4
$C_{removed}$ (%)	84.7 ± 19.3	87.3 ± 8.0	97.4 ± 3.6	97.8 ± 2.3	96.2 ± 4.0
$N_{removed}$ (%)	67.8 ± 15.4	77.6 ± 6.3	87.9 ± 6.1	96.3 ± 4.6	90.6 ± 6.9
COD/N influent (mg mg^{-1})	2.2 ± 0.7	2.1 ± 0.4	1.9 ± 0.4	1.9 ± 0.3	1.8 ± 0.2
TOC (mgC L^{-1})					
Cathode	23.1 ± 2.1	25.6 ± 2.2	25.5 ± 0.9	27.5 ± 1.6	28.0 ± 1.6
Anode	76.2 ± 3.9	72.5 ± 8.6	73.4 ± 1.3	73.0 ± 1.3	71.4 ± 2.1
Effluent	34.8 ± 6.0	33.5 ± 9.9	27.3 ± 13.5	25.1 ± 11.4	25.4 ± 9.5

OC: open circuit; MFC: microbial fuel cells; MEC: microbial electrolysis cell.

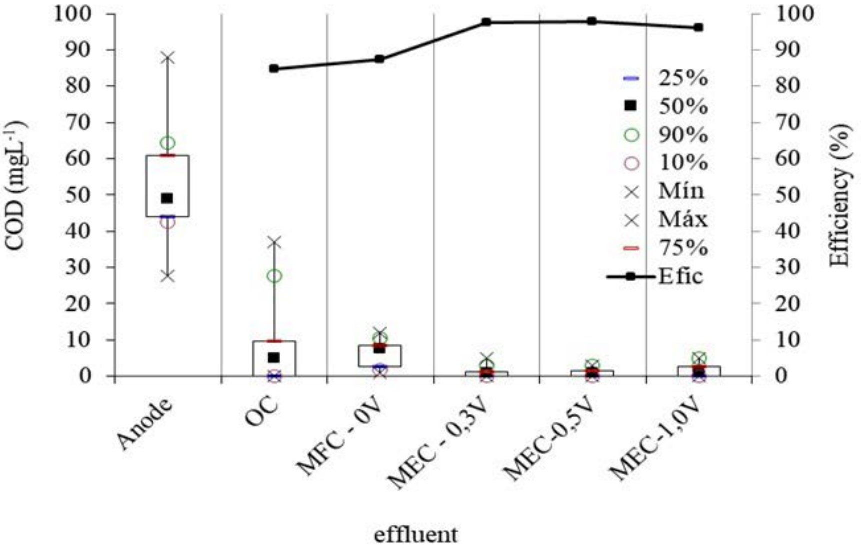

Fig. 2. COD variation in the anode chamber and treated effluent under different operation modes: OC: open circuit; MFC: microbial fuel cells; MEC: microbial electrolysis cell.

the activity of denitrifying bacteria and nitrogen removal must have increased in MEC, regardless of external voltage applied.

Biological denitrification and dissimilatory nitrate (NO_3–N) reduction are the two main pathways of nitrate related to the biological nitrogen cycle, being nitrite (NO_2–N) and ammonium (NH_4–N) the main reduction intermediates [19]. Thus, NO_2–N, NH_4–N as well as total nitrogen were also measured in both catholyte and final effluent. Table 4 presents the mean values for nitrogen series (nitrate, nitrite, and ammonium) under the different operation modes.

As shown in Table 4, MEC 0.5 V mode was the most effective treatment with a final nitrate concentration of 1.0 mg N–NO_3^- L^{-1} and removal efficiency of 96.2%. The other removal nitrate efficiencies were 90.5%, 88.2%, 78.3%, and 68.4% for MEC 1.0 V, MEV 0.3 V, MFC, and OC mode, respectively. Clearly, these results indicate that there was an accurate voltage between 0.5 and 1.0 V that would promote a better nitrate removal than those achieved in this study. Tong and He [10] investigated MEC for water treatment, the authors reported that the best nitrate removal was obtained in a voltage of 0.8 V. Here, better performance with 0.5 V than 0.3 and 0 V (MFC and OC) was because a higher power input increases current generation (Table 2), as widely spread in other D-BES studies. Surprisingly, although the 1.0 V condition leads to much more current density production than 0.5 V, the results of nitrate removal at this voltage were a little less efficient; but the exact reason is not currently clear. Pous et al. [8] also demonstrated that the denitrification rate increased changing the input voltage into the system, where −123 mV vs. SHE was the best voltage using water as anode electron

Table 4
Nitrate, nitrite, ammonium, and total nitrogen concentrations in the groundwater under different D-BES operation modes

Parameters	OC	MFC	MEC 0.3 V	MEC 0.5 V	MEC 1.0 V
N-NO_3^- (mg N L^{-1})					
Cathode	26.3 ± 0.3	26.3 ± 0.3	26.3 ± 0.3	26.3 ± 0.3	26.3 ± 0.3
Effluent	8.3 ± 4.1	5.7 ± 1.6	3.1 ± 1.6	1.0 ± 1.0	2.5 ± 1.8
N–NO_2^- (mg N L^{-1})					
Cathode	<0.2	<0.2	<0.2	<0.2	<0.2
Effluent	1.1 ± 0.7	<0.2	<0.2	<0.2	<0.2
N–NH_4^+ (mg N L^{-1})					
Cathode	10.0 ± 2.5	9.5 ± 1.5	9.8 ± 1.5	10.0 ± 1.3	9.5 ± 2.0
Effluent	<0.2	<0.2	<0.2	<0.2	<0.2
Total nitrogen (mg N L^{-1})					
Cathode	36.3 ± 2.0	35.8 ± 1.5	36.1 ± 2.0	36.3 ± 1.7	35.8 ± 2.5
Effluent	9.4 ± 3.9	5.7 ± 0.4	3.1 ± 1.6	1.0 ± 1.0	2.5 ± 1.8

OC: open circuit; MFC: microbial fuel cells; MEC: microbial electrolysis cell.

donor. Furthermore, the present study showed that nitrogen series removal was increased steadily with the increase of applied voltages up to 0.5 mV vs. Ag/AgCl.

First intermediate of dissimilatory nitrate reduction, nitrite was not detected in the effluents from MFC, MEC 0.3 V, MEC 0.5 V, and MEV 1.0 V modes, but present in the effluent from OC mode (1.1 ± 0.7 mg N–NO_3^- L^{-1}). Nitrite concentration of 1.1 ± 0.7 mg N–NO_3^- L^{-1} is classified as a concentration risk for human health (>0.91 mg N–NO_3^- L^{-1}), according to the World Health Organization (WHO) [20]. Accumulation of nitrite may be due to a variety of reasons, such as (i) system's inability to easily adapt to higher nitrate loads, (ii) slower kinetics of nitrite compared to nitrate reduction, (iii) limitation of electrons necessary for denitrifying bacteria activity to complete nitrate reduction [6]. Differential removal rates of nitrogen forms were also observed in other studies [4,6,25]. Surprisingly, ammonium was detected in high amounts in the cathode chamber. It is rather hypothesized that ammonia moved through the ion-exchange membrane and reached the cathodic chamber since the dissimilatory reduction is extremely rare in D-BES' cathodes. Finally, ammonium was not present in the final effluent from all D-BES modes.

The results suggest that the applied voltage was the key influencing factor in this denitrifying bioelectrochemical system, especially the voltage of 0.5 V. Proper microcurrents have been shown to stimulate bacteria community in D-BES by increasing enzyme activity and metabolism, promoting bacterial growth and reproduction, and improving cell membrane permeability [21]. Different microorganisms were detected on the surface of biocathodes: phylum proteobacteria have been reported as abundant or dominant in several works [1,16,22]. Vilar-Sanz et al. [22] reported a monospecific biofilm nitrate reduction; however, the nitrite-reducing alphaproteobacteria strains were not able to reduce nitrate, suggesting that a mixed bacteria community is necessary to complete a nitrate denitrification pathway to nitrogen gas.

It is important to highlight that the nitrate concentrations in treated effluents under all different modes of operation were lower than 11.3 mg N–NO_3^- L^{-1}, which is the maximum concentration recommended for drinking-water quality by the WHO [20]. In addition, all effluents also showed NH_4–N concentration below 0.2 mg L^{-1}, meeting the Grade III requirements of "Standards for Groundwater Quality" [23]. Therefore, almost all D-BES modes of operation were able to fulfill the nitrate, nitrite, and ammonium limits imposed by most of the worldwide regulations. The exception was the effluent from OC mode, which showed high nitrite concentration.

A comparison with similar studies available in Literature that used D-BES with two chambers is summarized in Table 5. The present study showed equivalent results in terms of nitrate removal using the D-BES-MEC mode with 0.5 V. D-BES operation showed advantages of short HRT and high nitrate removal efficiency.

The MEC mode (0.5 V), in terms of nitrate removal, showed similar results to those from Cecconet et al. [6]; while taking into consideration previous works with similar influent nitrate concentrations, the MEC mode (0.5 V) outperformed the double-chamber D-BES treating actual groundwater described by Pous et al. [4] and nitrate-contaminated drinking water studied by Szekeres et al. [26].

4. Conclusions

This study proved the feasibility of using denitrifying bioelectrochemical systems for nitrate-contaminated groundwater treatment by open circuit, MFC, and MEC modes. All configurations resulted in a nitrate concentration accepted by the WHO guidelines for drinking water quality. MEC operations were statistically better than OC and MFC modes, especially with a voltage supply of 0.5 V, reaching the highest removal efficiency (96.3%) and the lowest nitrate concentration in the treated effluent (0.96 mg N–NO_3^- L^{-1}). Some aspects of bioelectrochemical systems performance

Table 5
Comparison of nitrate removal in various D-BES with two chambers

Influent type	Volume (L)	Initial nitrate concentration (mgNO$_3$ L^{-1})	HRT (h)	Removal efficiency (%)	Reference
Synthetic contaminated groundwater	0.2	26.3	8.3	96.2	This study
Synthetic contaminated groundwater	0.675	11–45	16.2–32.4	96.3	[6]
Actual contaminated groundwater	0.600	33.11	11.25	90	[4]
Synthetic contaminated groundwater	0.675	23.33	24	99	[24]
Synthetic contaminated water	0.700	50	–	78	[25]
Synthetic contaminated water	0.270	25	3.3	92	[26]
Synthetic co-contaminated groundwater	0.350	30	8.0	85	[27]
Synthetic contaminated water	1.435	40	14.3–3.2	>95%	[18]
Synthetic contaminated water	2.0	45.2	9.5	>99%	[28]

still need to be evaluated by further studies, such as long-term and real-scale implementation. However, recent studies show that bioelectrochemical systems are robust and can reduce nitrate from water even at high loading. Therefore, D-BES is a promising economical and efficient technology for nitrate-contaminated groundwater treatment.

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