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**Cathode potential and anode electron donor evaluation for a suitable
treatment of nitrate-contaminated groundwater in bioelectrochemical
systems**

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Abstract

Several regions around the world present high levels of nitrate in groundwater. Due to its toxicity, nitrate must be removed before the groundwater is used as drinking- water. This study assessed how a denitrifying bioelectrochemical system could be operated to treat nitrate- polluted groundwater. It evaluated the cathode potential (from +597 to -703 mV vs SHE) and the anode electron donor (acetate and water). Similar trends were found regardless of the anode electron donor. The nitrate removal rate increased from 1.05 to 5.44 $\text{mgN-NO}_3^- \cdot \text{L}_{\text{NCC}}^{-1} \cdot \text{h}^{-1}$ when the cathode potential was lowered from +597 to -403 mV vs SHE, where it stabilized. The nitrate reduction end- products (nitrite, nitrous oxide and dinitrogen gas) also changed with the different potentials of the cathode electrode. The World Health Organization nitrates and nitrites standards for drinking- water were reached at cathode potentials between -103 and -203 mV vs SHE. The highest rate of nitrate conversion to N_2 (2.59 $\text{mgN-NO}_3^- \cdot \text{L}_{\text{NCC}}^{-1} \cdot \text{h}^{-1}$, 93.9%) occurred at -123 mV using water as anode electron donor, with an estimated operational cost similar to conventional technologies ($0.68 \cdot 10^{-2} \text{ kWh} \cdot \text{gN-NO}_3^-_{\text{removed}}^{-1}$). The long-term stability of proposed operation was demonstrated during 96 days, and the rate of nitrate conversion to N_2 even increased to 4.09 $\text{mgN-NO}_3^- \cdot \text{L}_{\text{NCC}}^{-1} \cdot \text{h}^{-1}$. A carbon- free operation for a bioelectrochemical system has been developed to treat nitrate- polluted groundwater at a competitive cost.

Keywords: Biocathode, bioremediation, denitrification, microbial electrolysis cell, microbial fuel cell, nitrogen.

1. INTRODUCTION

The presence of nitrate in groundwater has become a worldwide concern because of its toxicity to human health [1-3]. The Environmental Protection Agency (EPA) considers that only removal techniques such as reverse osmosis, electrodialysis and ion exchange are able to reduce nitrate content below its guideline values [4]. However, reverse osmosis, electrodialysis technologies imply high energy costs between 0.69 and 2.09 kWh m⁻³groundwater [5], while ion exchange requires an extra cost for resin regeneration [6]. In all three cases, they also generate waste brine that is difficult to dispose of because nitrate is only separated from groundwater, not treated [7].

Biological denitrification could overcome these drawbacks by allowing a complete treatment of nitrate to produce harmless dinitrogen gas (N₂). Groundwater is characterized by the absence of organic matter. Therefore, conventional heterotrophic denitrification has to be performed by adding an organic carbon source [8,9]. However, this generates an excess of sludge, and the dose of organic matter increases the treatment cost. Moreover, the presence of organic carbon compounds is forbidden in drinking- water. Therefore, unconventional strategies for biologic denitrification need to be explored. Bioelectrochemical systems (BES) are capable of treating nitrates in an autotrophic denitrifying biocathode [10]. The main challenge of reducing nitrate to N₂ is the accumulation of denitrification intermediates such as nitrite (NO₂⁻) and nitrous oxide (N₂O). NO₂⁻ in drinking- water is more toxic for human health than nitrate (standard value of 0.91 mgN-NO₂⁻·L⁻¹; [11]), and the emission of N₂O, a high- impact greenhouse gas, should be avoided [12]. Biocathodes used in BESs are able to perform the entire autotrophic denitrificative pathway sequentially from NO₃⁻, NO₂⁻ and allow N₂O reduction to N₂ [13]. The single reductions of nitrite and nitrous oxide using a

biocathode were also demonstrated by Puig et al [14] and Desloover et al [15], respectively.

Previous studies [16,17] have demonstrated that nitrate could also be removed from low ionic strength waters. In these studies the BES was operated as a microbial fuel cell (MFC). In a MFC anode and cathode reactions are thermodynamically spontaneous, and no power input is required. Although nitrate could be removed, full denitrification was not reached, with N_2O emissions accounting the 50% of nitrate removed. The accumulation of denitrification intermediates can be caused by a limitation of the electrons necessary for denitrifying bacteria to completely reduce nitrate to N_2 [18]. In a denitrifying MFC, the electron availability depends on the electron transport from the anode electron donor to the cathode denitrifying bacteria. The overall electron transport is hindered by MFC overpotentials that are mainly caused by electrode materials, bacterial metabolic losses, membrane characteristics and ionic strength [19]. When water with low ionic strength such as groundwater is treated, the overpotentials related to ion-transport (membrane transport, ohmic and pH gradient losses) increase. Puig et al [16] observed that overpotentials related to ion- transport could be increased up to 80% by reducing water conductivity from 4000 to 1000 $\mu\text{S s}^{-1}$. For groundwater treatment, if conductivity is artificially increased through added chemicals such as NaCl, they must be discarded so that they do not impair water quality. To remove the nitrate from groundwater at higher rates and produce fewer intermediates, other strategies should be followed. If an external power is applied to the BES, the cell is known as a microbial electrolysis cell (MEC). To promote denitrification in BES, the cell can be operated as an MEC instead of an MFC.

This study aimed to improve BES performance in treating nitrate- polluted groundwater and overcome its main drawbacks (low conductivity and accumulation of

intermediates). This study focused on the influence of the cathode potential and examined the use of organic matter and water as anode electron donors. The metrics for the operation and process assessments were: i) nitrate removal rates; ii) complete denitrification achieved and iii) energy requirements. At the end of the study, the long-term stability of the proposed operation was tested.

2. EXPERIMENTAL SECTION

2.1. Experimental set-up

The BES consisted of an anode and a cathode placed on opposite sides of a single methacrylate rectangular chamber [16]. The anode and cathode chambers were filled with granular graphite (diameter 1.5-5 mm, EnViro-cell, Germany), which decreased the compartment volumes to 450 and 600 mL (net anode and cathode compartments NAC and NCC volumes), respectively. Two thinner graphite electrodes (130 x 6 mm, Sofacel, Spain) were used as anode and cathode current collectors. A cation exchange membrane (CEM, Nafion[®] 117, Dupont, USA) was placed between the anode and cathode frames. At the steady state, influents were continuously fed at a flow rate of 1.12 ± 0.07 and 1.28 ± 0.16 L d⁻¹ in the anode and cathode compartments, respectively. An internal recirculation loop (105 L d⁻¹) was placed in each compartment. The system was thermostatically controlled at 22.5 ± 0.5 °C.

2.2. Influent characteristics

Nitrate contaminated groundwater from the village of Ordis (42°13'13''N, 2°54'31'', Girona, N.E. Spain) was treated in the cathode of the BES. The groundwater was purged with dinitrogen gas prior to being fed into the cathode to ensure anoxic conditions. The groundwater contained 33.11 ± 2.55 mg N-NO₃⁻ L⁻¹ and 0.11 ± 0.22 mg N-NO₂⁻ L⁻¹. These values were higher than the limits given by the World Health Organization (WHO):

11.29 mg N-NO₃⁻ L⁻¹ (50 mg NO₃⁻ L⁻¹) and 0.91 mg N-NO₂⁻ L⁻¹ (3 mg NO₂⁻ L⁻¹) [11]. The inorganic carbon content of the groundwater was 56.4±2.4 mg C-IC L⁻¹. The average pH was 7.8±0.2, with a conductivity of 918±31 μS cm⁻¹. Neither ammonium (N-NH₄⁺) nor organic matter (TOC) were detected in the groundwater.

Two different electron donors were evaluated at the anode: acetate, as an example of an organic carbon source, and water. Firstly, the anode compartment was fed with acetate- enriched water with a chemical oxygen demand (COD) concentration of 297±60 mg COD L⁻¹, a pH of 7.7±0.1 and a conductivity of 984±119 μS cm⁻¹.

In the second round of tests, the anode was fed with tap water without added organic matter. In consequence, the organic matter content was found below the detection limit in both COD (< 30 mg COD L⁻¹) and TOC (< 0.01 mgC L⁻¹) analyses. The conductivity was of 817±145 μS cm⁻¹ with a pH of 8.0±0.4. The inorganic carbon content was 48.7±2.4 mg C-IC L⁻¹.

2.3. BES operation

In a previous study [17], a BES was operated during 97 days to treat 1.21 L d⁻¹ of nitrate-polluted groundwater (28.32±6.15 mg N-NO₃⁻ L⁻¹) at the biocathode. The microbial community established in the biocathode was dominated by *Betaproteobacteria*, *Candidatus Nitrotoga arctica* and *Thauera* sp. species. That work used acetate- enriched water as an electron donor with an anode that was fed at 1.23 L d⁻¹ and operated at a chemical oxygen demand concentration of 283±75 mg COD L⁻¹. Once the BES reached the steady state, the experiments for the present study started.

The nitrate removal and the end- products obtained were evaluated at different fixed cathode potentials (Table 1). Firstly, the anode chamber was operated with acetate- enriched water (tests 2 to 7) and the cathode was fed with the nitrate- polluted

groundwater. In the first test (test 1), the BES was operated without cathode potential control (MFC operation, data extracted from Pous et al [17]). Five tests were performed by varying the cathode potential from +597 to -103 mV vs SHE (tests 2 to 6). A control test (test 7) was conducted at open cell voltage (OCV).

During the second run of experiments, the BES was operated using acetate- free water at the anode. The cathode was fed with the nitrate- polluted groundwater again. Eleven tests (tests 8 to 18) were performed by poisoning the cathode potential at different values from +97 to -703 mV vs SHE. The daily flow was incremented to 1.85 L d⁻¹ on test 14 and to 2.69±0.05 L d⁻¹ on tests 15 to 18 due to the high nitrate removal performance.

For all of the tests, the BES was operated during at least three times the cathode hydraulic retention time before analyzing the influent and effluent concentrations, pH, conductivity, current production and anode potential. Samples were taken for three consecutive days and the results were expressed as the mean value plus the standard deviation of the measurements.

After test 18, the system was operated at the conditions of test 12 for 96 days to examine its behavior over the long- term.

2.4. Analytical methods and calculations

Standard wastewater measurements of the chemical oxygen demand (COD), total organic carbon (TOC), inorganic carbon (IC), ammonium (N-NH₄⁺), nitrites (N-NO₂⁻) and nitrates (N-NO₃⁻) were taken and analyzed according to the recommendations of the American Public Health Association (APHA) [20]. The pH and conductivity were measured with a pH- meter (pH- meter basic 20⁺, Crison, Spain) and an EC-meter (EC-meter basic 30⁺, Crison, Spain), respectively. For the biocathode, the nitrate loading

rate ($\text{mg N-NO}_3^- \text{ L}^{-1}_{\text{NCC}} \text{ h}^{-1}$) was calculated as the daily influent/effluent of nitrate concentration per hydraulic retention time. Finally, the nitrate removal rate ($\text{mg N-NO}_3^- \text{ L}^{-1}_{\text{NCC}} \text{ h}^{-1}$) was calculated as the difference between the influent and effluent nitrate loading rates.

Considering the possibility of the simultaneous presence of nitrate and nitrite in drinking- water, the WHO recommends using a quality ratio (QR) that involve both the concentration and the guideline value for nitrate and nitrite [11]. In order to consider water as safe drinking- water in terms of nitrate and nitrite, the QR should not exceed a value of one according to equation 1:

$$QR = \frac{C_{NO_3^-}}{11.29} + \frac{C_{NO_2^-}}{0.91} \leq 1 \quad (\text{eq. 1})$$

where, $C_{NO_3^-}$ is the nitrate concentration ($\text{mgN-NO}_3^- \text{ L}^{-1}$) and $C_{NO_2^-}$ is the nitrite concentration ($\text{mgN-NO}_2^- \text{ L}^{-1}$).

For tests 8 to 18, when no acetate was fed at the anode, the concentration of N_2O in the liquid- phase was measured using a N_2O liquid- phase microsensor (Unisense, Denmark). The total amount of N_2O at the cathode effluent was calculated from a two-step calculation: 1) the N_2O in the gas- phase was calculated from the liquid- phase concentration as shown in S1, 2) the total amount of N_2O per volume of groundwater treated was determined by applying equation 2:

$$nN_2O = nN_2O_{gas} + nN_2O_{liquid} \quad (\text{eq. 2})$$

where nN_2O is the total amount of nitrous oxide produced per volume of groundwater treated ($\text{mole N L}_{\text{groundwater}}^{-1}$). To close the nitrogen mass balance, the presence of nitric oxide (NO) was considered negligible [13] and the remaining nitrogen was considered as the sum of nitrate assimilation and N_2 gas produced.

The cathode and the anode potentials were monitored with an Ag/AgCl reference electrode (0.197 V vs standard hydrogen electrode (SHE), model RE-5B BASi, United Kingdom). For tests 2 to 6 and 8 to 18 the cathode potential was fixed using a potentiostat (model SP50, Bio-logic, France).

3. RESULTS AND DISCUSSION

3.1. Effect of the cathode potential in a denitrifying BES

Previous experiments have demonstrated the capacity of the BES to reduce nitrate in the groundwater (test 1, [17]). At the steady- state, the cathode potential stabilized at -13 ± 9 mV vs SHE, achieving a nitrate removal efficiency of 64% and a nitrate removal rate of $1.98 \text{ mgN-NO}_3^- \text{ L}_{\text{NCC}}^{-1} \text{ h}^{-1}$. However, N_2O was the main product of the denitrifying cathode, and the standard of drinking- water quality was not reached in terms of nitrates and nitrites. It was necessary to improve the water quality as well as the nitrate removal rate. To increase the nitrate removal efficiency, the cathode electrode was poised at different potentials from +597 to -703 mV vs SHE (Table 1). At the start, the anode compartment was fed with acetate (from tests 1 to 7) because that is the most common feed for the conventional anode used in denitrifying BES [10,13]. Considering that groundwater is characterized for the absence of organic matter, from tests 8 to 18 only water was provided as an anode electron donor. The results obtained in terms of nitrate removal rate and demanded current are shown in Figure 1 and discussed in section 3.1.1, while the percentage of nitrogen species at the effluent is shown in Figure 2 and discussed in section 3.1.2. A complete dataset of the different tests can be found at supplementary information.

3.1.1. Nitrate removal rate and current demand

The nitrate removal rate in the biocathode was strongly influenced by the cathode potential (Figure 1), and it showed negligible differences when acetate or water were

used as an anode- electron donor. In tests where acetate was used as the donor (tests 1 to 6), the nitrate removal rate increased from 1.05 to 2.12 $\text{mgN-NO}_3^- \text{ L}_{\text{NCC}}^{-1} \text{ h}^{-1}$ (38 to 80% nitrate removal efficiency) when the cathode potentials were lowered from potentials of +597 to -103 mV vs SHE. Then, only water was fed to the anode (tests 8 to 18) and the biocathode showed an increase of nitrate removal rate from 0.96 to 5.44 $\text{mgN-NO}_3^- \text{ L}_{\text{NCC}}^{-1} \text{ h}^{-1}$ (32 to 86% nitrate removal efficiency) when the cathode potential was lowered from +97 to -403 mV vs SHE. The nitrate removal rate stabilized at cathode potentials below -403 mV vs SHE, reaching a maximum removal rate of 6.16 $\text{mgN-NO}_3^- \text{ L}_{\text{NCC}}^{-1} \text{ h}^{-1}$ at -703 mV vs SHE (99% nitrate removal efficiency). Therefore, above -403 mV vs SHE, the lower the cathode potential was poised, the higher the nitrate removal rate achieved.

An increase of the nitrate removal rate with a decrease of the cathode potential to a minimum of -200 and -400 mV vs SHE has been reported previously by Viridis et al [13] and by Cheng et al [21] using synthetic wastewater. However, in the present study: i) a wider range of potentials, from +597 to -703 mV vs SHE, was studied; ii) real groundwater was treated at the cathode; iii) the entire denitrification pathway was evaluated (nitrate, nitrite and nitrous oxide reduction) (section 3.1.2), and iv) two different anode feeds were used (water and acetate) (section 3.2). The wider range of potentials evaluated allowed nitrate removal rate to increase only until a cathode potential of -403 mV vs SHE. At cathode potentials of -403 mV vs SHE and lower, the nitrate removal rate reached a plateau.

The influence of the electrochemical potentials in the removal activity of BES is related to the energy gained by bacteria [22]. The major difference between the cathode electrode potential and the formal redox potential (sequential reductions from NO_3^- to N_2), the major is the energy gained for bacteria and therefore the nitrate removal rate

can be increased. This hypothesis was valid at a range of cathode potentials from +597 to -403 mV vs SHE, from where the nitrate removal rate stabilized until a potential of -703 mV vs SHE.

The bioelectrochemical nature of the process described above could be demonstrated with the current that was demanded by the biocathode (Figure 1B). When the cathode potentials were lowered from potentials of +597 to -103 mV vs SHE in the presence of acetate at the anode (tests 1 to 6), the increase in the nitrate removal rate was accompanied with the growth of current demand from 0.3 mA to 9.4 mA. When water was used as anode electron donor (tests 8 to 18) and the cathode potential was shifted from +97 to -703 mV vs SHE, the current demand increased from 1.5 to 23.5 mA. However, the current demand behavior was not constant in the whole cathode potential window. From +97 to -203 mV vs SHE, the current increased exponentially from 1.5 to 12.6 mA. In contrast, from -303 to -703 mV vs SHE, the current showed grew linearly from 15.8 to 23.5 mA.

In the OCV experiment (test 7), the nitrate removal rate was $0.23 \text{ mgN-NO}_3^- \text{ L}_{\text{NCC}}^{-1} \text{ h}^{-1}$ (10% of nitrate removal efficiency), indicating a low nitrate consumption by non-electrotroph bacteria.

3.1.2. Nitrate reduction end- products (NO_2^- , N_2O , N_2 + N-assimilation)

In a denitrification process, not only the nitrate removal rate but also the nitrate reduction end- products (NO_2^- , N_2O , N_2) should be considered, with N_2 as the desired end- product. Although N_2 concentrations could not be evaluated in this work, NO_3^- , NO_2^- , N_2O and NH_4^+ were analyzed in both anode and cathode chambers. Ammonium was not detected in either anode or cathode effluent, suggesting that the dissimilatory nitrate reduction to ammonium did not take place. No presence of nitrate, nitrite or

nitrous oxide was detected at the effluent of the anode in any test, suggesting no diffusion of these species from the cathode to the anode. As a result, the authors consider that nitrate was solely consumed at the cathode. The percentage of nitrate, nitrite and nitrous oxide at the effluent of the cathode at each test are shown in Figure 2.

When acetate was used as anode electron donor (tests 1 to 6; Figure 2A), the nitrite percentage at the effluent decreased from 4.0% to 0.4% by drifting the cathode potential from +597 to -103 mV vs SHE. When water was used as an anode electron donor (tests 8 to 18; Figure 2B), the behavior was similar. Nitrite accumulation was depleted from 1.9 to 0.0% when the cathode potential was changed from +96 to -53 mV vs SHE. From here down, nitrite was not accumulated until -303 mV vs SHE. From -303 to -703 mV vs SHE, the NO_2^- increased to percentages between 30.2 and 49.5%. Regarding N_2O accumulation, the percentage at the effluent was between 3.7 and 5.5% at cathode potentials higher than -123 mV vs SHE. The percentage increased up to 18.8% and 18.4% at -203 and -303 mV vs SHE, respectively. At potentials between -403 and -703 mV vs SHE, the percentage of N_2O at the effluent decreased to values between 7.1 and 12.6%.

The variation of nitrate reduction end-products (NO_2^- and N_2O) has been reported previously from +100 to -200 mV vs SHE [13], where a decrease on intermediates accumulation were observed when the cathode potential was lowered using synthetic wastewater as medium. In the present study, the cathode potential range was larger (from +597 to -703 mV vs SHE), and contaminated groundwater was treated. The use of groundwater implies the use of restrictive water because of its low ionic strength ($<1.5 \text{ mS cm}^{-1}$) and low nutrient availability (no organic carbon or phosphates were detected). The study of a wider range of cathode potentials revealed unexpected results. When the cathode electrode was polarized at potentials lower than -303 mV vs SHE, the reduction

of nitrite and nitrous oxide was negatively destabilized. As a consequence, an accumulation of nitrite and nitrous oxide was observed at those potentials.

As mentioned above, no ammonium was produced and no nitrate diffused to the anode. Therefore, all reduced NO_3^- that was not accumulated as NO_2^- or N_2O , had to be finally reduced to N_2 or assimilated by bacteria. The sum of N_2 and NO_3^- assimilation at the effluent reached its highest value at -123 mV vs SHE (84.1%). At this potential, 93.9% of reduced nitrate was converted to N_2 or assimilated by bacteria. Only 6.1% of reduced nitrate was in a form with N_2O as an intermediate product. Such a low N_2O accumulation in a denitrifying BES is not common. In a recent article, Van Doan et al [23] described that 70% of reduced nitrate was accumulated as nitrous oxide by operating a BES at a constant current. A constant current gives a limited amount of electrons. By supplying a limited amount of electrons, electron competition between the different denitrifying enzymes can end- up producing an accumulation of N_2O [18]. On the contrary, the study presented here demonstrates that N_2O accumulation can be sharply reduced by controlling the cathode potential instead of controlling the current supplied. The use of a poised electrode gives unlimited source electrons for the denitrifying community. Hence, the electron competition between the different denitrifying enzymes can be lessened and the N_2O accumulation can be avoided.

3.2. Anode electron donor behavior in the denitrifying BES

The activity of the anodic compartment is a key factor for evaluating the feasible utilization of a denitrifying BES to treat nitrate- polluted groundwater. Taking into account that groundwater is characterized for the absence of organic matter, two different anodic feeds were considered: i) acetate- enriched water (tests 1 to 6) and ii)

water (tests 8 to 18). The objective was to check the influence of using an acetate-oxidizing anode or a water-oxidizing anode.

The activity of anode compartment was followed via the anode potential measured at each test (Table 2). Considering first the tests where acetate was fed at the anode (tests 1 to 6), it could be deduced that when cathode potentials higher than 0 mV vs SHE were applied, acetate was the sole anode electron donor. However, at cathode potentials lower than -100 mV vs SHE, the anode potential rose to +1000 mV vs SHE. At that higher potential, the oxidation of water to oxygen ($E^{\circ}(\text{H}_2\text{O}/\text{O}_2) = +776 \pm 31$ mV vs SHE) is more likely to occur than acetate oxidation to carbon dioxide ($E^{\circ}(\text{CH}_3\text{COO}^-/\text{CO}_2) = -173 \pm 27$ mV vs SHE). When the cathode potential was poised at values lower than 0 mV vs SHE, the biocathode required higher electron flux. However, the oxidation of acetate at the anode was not able to provide the current needed for the nitrate reduction at the cathode. As a consequence, the electron donor at the anode changed from acetate to water. Moreover, in all tests, the anode coulombic efficiencies were lower than 15%, which suggested that acetate was mainly consumed by non-electroactive bacteria.

The pHs of each compartment were also checked (Table 2). As the cathode potential was lowered from +597 to -203 mV vs SHE, the increase current demand increased, resulting on a decrease of the anode pH regardless the anode fed used (acetate or water).

Focusing on tests with presence of acetate at the anode, it could be observed that when the cathode potential was poised at values higher than 0 mV vs. SHE, the bioelectrochemical activity was low (current demand below 3 mA), which produced little changes on the anodic pH (0.7 units of pH). As the cathode potential was drifted to potentials lower than -103 mV vs SHE, the bioelectrochemical activity grew (current demand increased to 9.4 mA), which provoked a decrease of the anodic pH regardless

the anode fed used. Considering the low pHs observed at the anode when water was being used as electron donor, it can be assumed that water oxidation was an abiotic process. On the contrary, the pH at the effluent of the cathode remained almost stable or slightly increased, allowing the biological reduction of nitrate. The combination of biological denitrification at the cathode and abiotic oxidations at the anode could improve the denitrifying BES capabilities. For example, abiotic anodes could support disinfection processes, as chlorine evolution [24].

3.3. Driving the BES performance to achieve the standard of drinking-water quality

In order to accomplish the legislation in terms of nitrates and nitrites, drinking- water must present a QR value below or equal to 1. Figure 3 shows the QR at the effluent of the cathode in all tests.

In spite of the different anode fed used (acetate or water), similar QR trend was observed. The quality ratio stabilized approximately 3 at cathode potentials higher than +196 mV vs SHE. By lowering the cathode potential to -103 mV vs SHE, the QR diminished until reaching the standards (values less than 1). At a cathode potential of -103 mV vs SHE, the QR was 0.64 and 0.87 for acetate and water as anode fed, respectively. The standards of quality were accomplished at cathode potentials between -103 and -203 mV vs SHE, with the lowest QR of 0.31 at -123 mV vs SHE. Therefore, we propose that the biocathode of a denitrifying BES, for the treatment of nitrate-polluted groundwater, should be poised at a potential between -103 and -203 mV vs SHE. At these low cathode potentials, the anode electron donor was always water, even when acetate was fed to the anode compartment. Hence, the use of acetate at the anode was useless when biocathode demanded higher currents; the acetate- oxidizing reaction

was not able to fulfill the biocathodic requirements. Moreover, for groundwater treatment, the use of acetate would imply not only the addition of chemicals, but also an additional care of the anode microbial community and a possible contamination of cathode groundwater due to acetate flux through the membrane [25]. For these reasons, we consider that the use of acetate can be avoided in a BES treating groundwater through the input of external energy.

3.4. Economic perspectives on treating nitrate contaminated groundwater

The operational cost related to energy consumption was calculated at the best performance using water (test 12) as anode electron donor. Table 3 presents the energy consumption for the nitrate- polluted groundwater treatment using BES and compares it with other technologies, such as electro dialysis or membrane bioreactor. Two different methods of calculation were used: i) from Gibbs free energy and ii) from specific power consumption [26].

The energy consumed in the BES proposed in our study was $1.27 \cdot 10^{-2}$ or $0.68 \cdot 10^{-2}$ kWh $\text{gN-NO}_3^- \text{ removed}^{-1}$ (0.37 and 0.20 kWh $\text{m}^{-3} \text{ treated}$). If energy consumption is compared to other technologies that are able to reduce NO_3^- to N_2 , the use of BES with the control of the cathode potential presented lower energy consumption. A special mention should be made of the biofilm- electrode reactor technology (BER) [27]. In a BER, an external power input is used to produce hydrogen in the cathode, which is subsequently used by bacteria to reduce nitrates. Power input is also applied in the BES presented in this article, but no H_2 production was detected. Thus, nitrate reduction was not mediated through hydrogen oxidation. In the BES presented in here, the cathode potential is the parameter that is proposed to be controlled, while in the BER, the external power voltage is fixed. Hence, BER controls the voltage difference between anode and cathode, whereas here we propose to control the potential of the cathode chamber where

the desired reaction takes place. According to the results, the control of the cathode potential allowed the control of nitrate reduction end-products and it implied a lower energy consumption ($0.68 \cdot 10^{-2}$ and $1.27 \cdot 10^{-2}$ kWh gN-NO₃⁻ removed⁻¹) with respect to the BER ($7.00 \cdot 10^{-2}$ kWh gN-NO₃⁻ removed⁻¹ [27]).

In addition, BES showed similar energy requirements as those observed in a conventional technique such as electrodialysis (between $0.40 \cdot 10^{-2}$ – $4.95 \cdot 10^{-2}$ kWh gN-NO₃⁻ removed⁻¹ according to El Midaoui et al [28] and Ortiz et al [29]). By supplying a similar amount of energy, the denitrifying BES was able to diminish the nitrate content below its guideline value. Moreover, NO₃⁻ was reduced without producing harmful products (NO₂⁻, N₂O or NH₄⁺).

3.5. Long-term activity of denitrifying-BES

Taking into account the nitrate treatment performance and the associated energy requirements, we propose that a denitrifying BES for the treatment of nitrate-polluted groundwater could be operated at a poised cathode of -123 mV vs SHE and the anode fed with water (test 12). The BES was operated under conditions expressed at test 12 for 96 days after test 18 to demonstrate the robustness of the technology. During the first 48 days groundwater was fed a 1.3 L d⁻¹, while from day 49th to 96th, the flow was doubled to 2.6 L d⁻¹. Figure 4 presents the results obtained.

Despite the different cathode potentials tested earlier, when the cathode potential was again poised at -123 mV vs SHE, the denitrifying activity quickly recovered (nitrate removal rate of 2.09 ± 0.29 mgN-NO₃⁻ L⁻¹_{NCC} h⁻¹), and the quality ratio was accomplished (0.92 ± 0.14). From day 18th to 48th, the overall denitrifying performance was even improved, the nitrate removal rate increased to 2.72 ± 0.20 mgN-NO₃⁻ L⁻¹_{NCC} h⁻¹, delivering a 3.5% of nitrate at the effluent, and the QR decreased to 0.10 ± 0.05 .

Moreover, no nitrite or nitrous oxide was detected, indicating that nitrate was successfully reduced without accumulating intermediate products.

From day 49th to 96th, the influent flow was doubled from 1.3 to 2.6 L d⁻¹. Nitrate removal rate increased to 4.74 ± 0.39 mgN-NO₃⁻ L⁻¹_{NCC} h⁻¹ and the QR was still accomplishing the standards (0.12 ± 0.27). The nitrate conversion rate to N₂ was 4.09 ± 0.33 mgN-NO₃⁻ L⁻¹_{NCC} h⁻¹.

Therefore, the long- term stability of the proposed parameters (cathode potential poised at -123 mV vs SHE and water as anode electron donor) was demonstrated.

5. CONCLUSIONS

The usage of a denitrifying biocathode, at a fixed potential, allowed nitrate- polluted groundwater treatment, reaching the standards of nitrates and nitrites for drinking- water at a competitive cost. The lowest accumulation of intermediates (NO₂⁻, N₂O) was observed at -123 mV vs SHE and using water as anode electron donor. The long- term stability of the proposed operation was demonstrated during 96 days, reaching a nitrate conversion rate to dinitrogen gas of 4.09 mgN-NO₃⁻ L⁻¹_{NCC} h⁻¹.

Water, rather than acetate, was the most reasonable anode electron source because it: i) has a similar cost; ii) does not require the addition of chemicals no caring for the anode microbial community; iii) does not have the potential to poison groundwater (as acetate does by diffusion through membrane).

A carbon- free technology for the biologic treatment of nitrates in groundwater at a competitive cost has been developed.

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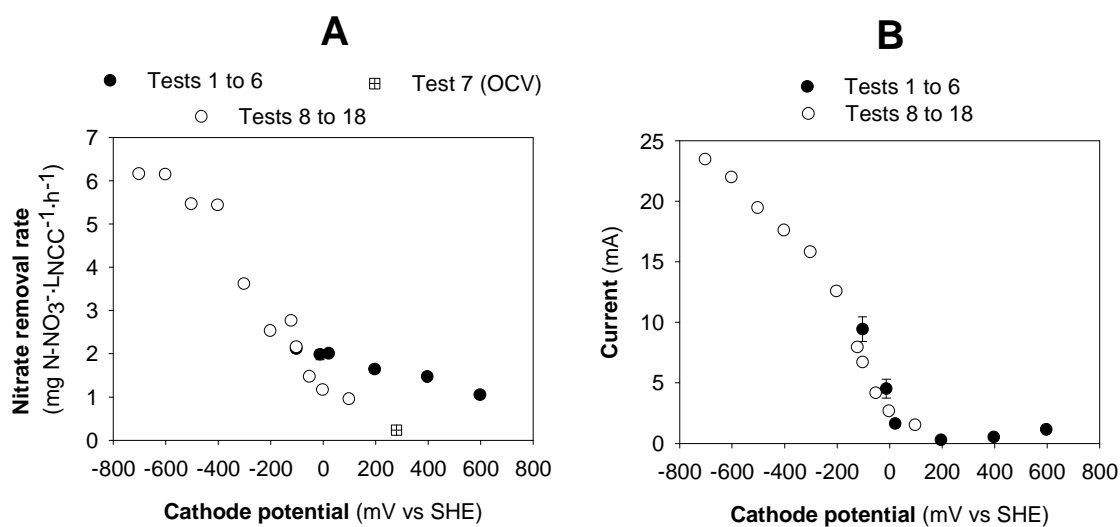


Figure 1. **A)** Nitrate removal rate performance at different cathode potentials. Error bars represent standard deviations of replicate samples (n=3). **B)** Current demand at different poised cathode potentials. Error bars represent standard deviations of replicate samples (n=3).

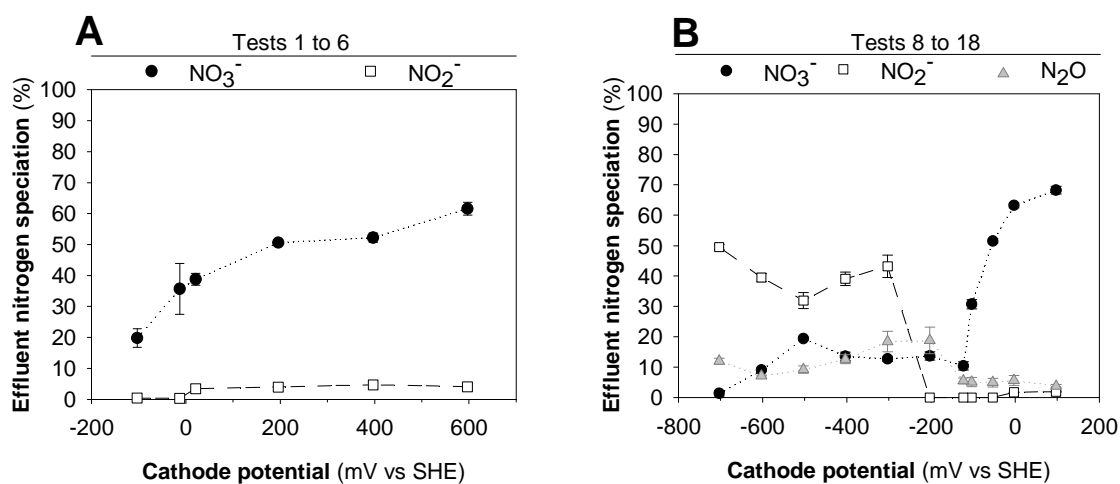


Figure 2. A) Percentages of nitrate and nitrite at the effluent at different cathode potentials treating nitrate- contaminated groundwater with acetate fed at the anode (tests 1 to 6). Error bars represent standard deviations of replicate samples (n=3). B) Percentages of nitrogen compounds at the effluent when different cathode potentials were applied and water was used as anode electron donor (tests 8 to 18). Error bars represent standard deviations of replicate samples (n=3).

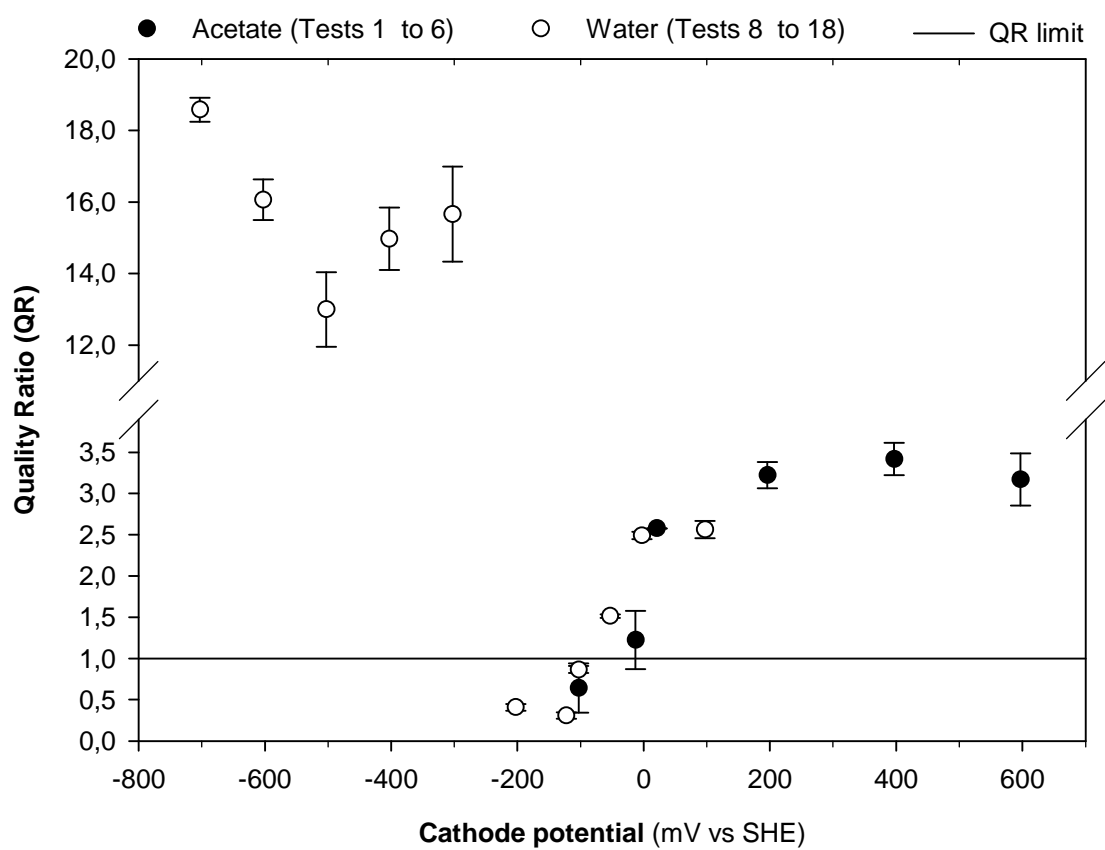
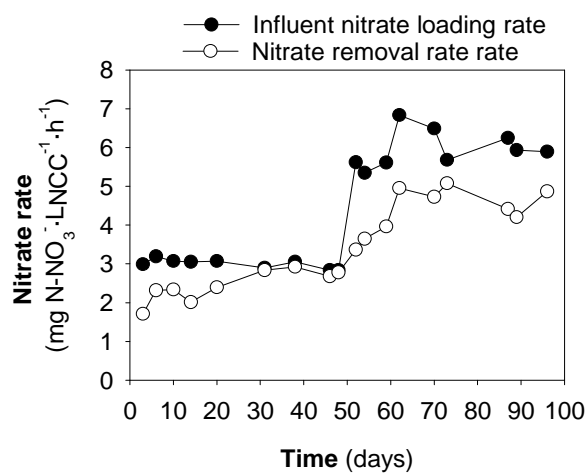


Figure 3. Quality ratio at the effluent depending on the cathode potential and the anode fed: acetate (tests 1 to 6) and water (tests 8 to 18). Error bars represent standard deviations of replicate samples (n=3).

Figure 4. Long- term performance of BES at cathode potential of -123 mV vs SHE and using water as anode fed.



		Day of operation		
		0 -17	18 - 48	49 - 96
Nitrogen speciation at the effluent (%)	NO ₃ ⁻	32.0±8.3	3.5±1.7	26.8±8.4
	NO ₂ ⁻	0.2±0.2	0.0±0.0	0.1±0.3
	N ₂ O	4.4±2.9	0.0±0.0	4.5±5.2
QR		0.92±0.14	0.10±0.05	0.12±0.27
Current (mA)		8.5±2.2	17.8±0.6	24.6±2.5

Table 1. Experimental conditions of each test. Legend: (*) states for the conditions applied during the long- term performance evaluated after the test 18.

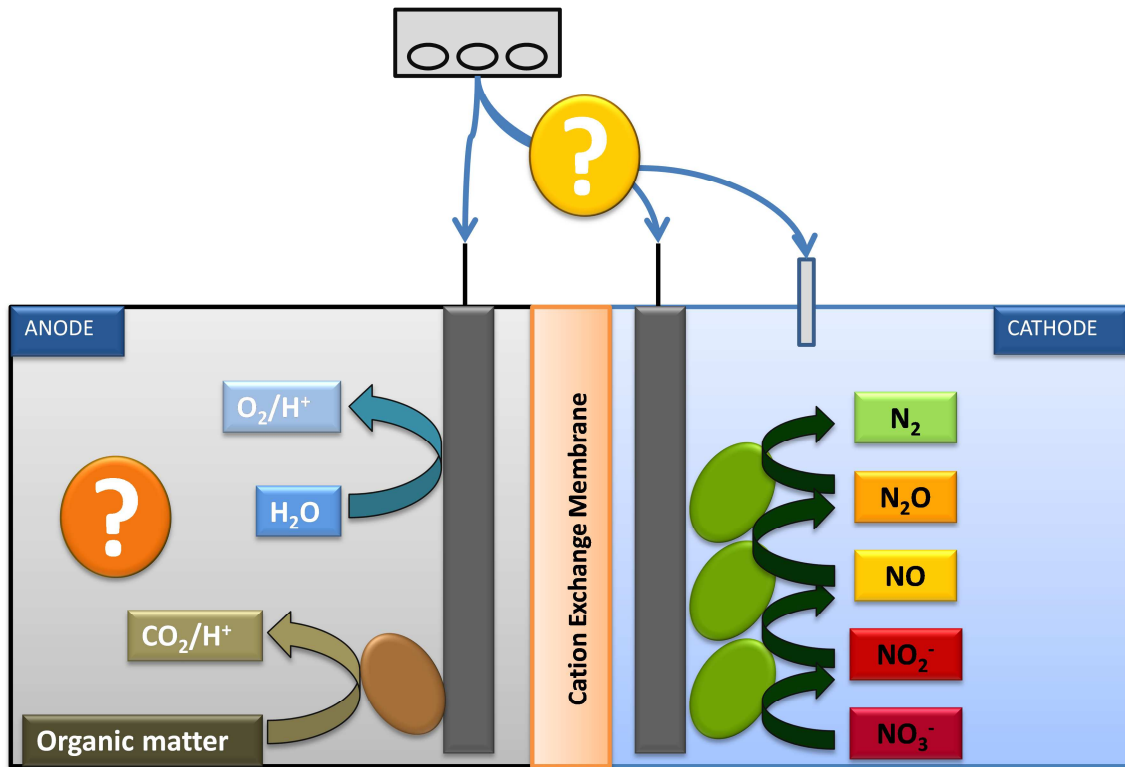
Experiment	Electron donor (Anode)	Cathode potential control	Cathode potential (mV vs SHE)	Cathode flow (L.d ⁻¹)
Test 1		No, free	-13±9	1.35
Test 2			+597	1.27
Test 3			+397	1.30
Test 4	Acetate	Yes	+196	1.33
Test 5			+21	1.33
Test 6			-103	1.33
Test 7		No, OCV	+279	1.26
Test 8			+97	1.38
Test 9			-3	1.38
Test 10			-53	1.32
Test 11			-103	1.41
Test 12(*)			-123	1.34
Test 13	Water	Yes	-203	1.26
Test 14			-303	1.85
Test 15			-403	2.67
Test 16			-503	2.76
Test 17			-603	2.68
Test 18			-703	2.64

Table 2. Anode potential and pH measured at the effluent of the anode and the cathode at each test. The results are presented as the mean \pm standard deviations of replicate samples (n=3).

Experiment	Anode potential (mV vs SHE)	pH anode effluent	pH cathode effluent
Test 1	-191 \pm 51	7.95 \pm 0.21	7.75 \pm 0.39
Test 2	-579 \pm 19	7.71 \pm 0.15	7.77 \pm 0.21
Test 3	-471 \pm 21	7.99 \pm 0.15	8.06 \pm 0.16
Test 4	-420 \pm 4	7.80 \pm 0.23	8.15 \pm 0.21
Test 5	-290 \pm 1	7.25 \pm 0.05	8.45 \pm 0.15
Test 6	+1010 \pm 38	6.61 \pm 0.10	8.56 \pm 0.09
Test 8	+967 \pm 4	6.73 \pm 0.04	8.15 \pm 0.18
Test 9	+984 \pm 2	6.73 \pm 0.19	8.15 \pm 0.27
Test 10	+965 \pm 5	6.26 \pm 0.08	8.17 \pm 0.33
Test 11	+966 \pm 4	5.80 \pm 0.09	8.18 \pm 0.06
Test 12	+1072 \pm 7	5.45 \pm 0.29	8.19 \pm 0.19
Test 13	+1179 \pm 4	4.27 \pm 0.26	8.22 \pm 0.36
Test 14	+1196 \pm 6	3.99 \pm 0.09	8.23 \pm 0.11
Test 15	+1240 \pm 5	3.42 \pm 0.08	8.25 \pm 0.05
Test 16	+1264 \pm 2	3.94 \pm 0.07	8.23 \pm 0.24
Test 17	+1237 \pm 8	3.57 \pm 0.14	8.24 \pm 0.06
Test 18	+1274 \pm 3	3.13 \pm 0.15	8.25 \pm 0.17

Table 3. Comparison of energy consumption and characteristics for nitrate- polluted groundwater treatment for this study (test 12), conventional treatments and other technologies found in the bibliography. In references where energy consumption was not directly mentioned, it was calculated from Gibbs free energy and Specific power consumption. Legend: Not mentioned (n.m.).

Reference	Energy consumption (kWh gN-NO ₃ ⁻ removed ⁻¹)		Energy consumption (kWh m ⁻³ treated)		Nitrate removal pathway	Addition of chemicals?
	From Gibbs free energy	Specific power consumption [26]	From Gibbs free energy	Specific power consumption [26]		
This study (test 12)	1.27·10 ⁻²	0.68·10 ⁻²	0.38	0.20	Reduction to N ₂	No
3D electrode biofilm reactor [30]	3.35·10 ⁻²	1.46·10 ⁻²	1.01	0.44	Reduction to N ₂	Yes, ethanol
Biofilm-electrode reactor [31]		0.16·10 ⁻²		0.08	Reduction to N ₂	Yes, methanol
Biofilm electrode reactor + sulfur denitrification [32]	4.79·10 ⁻²	1.50·10 ⁻²	1.00	0.31	Reduction to N ₂	Yes, sulphur and anthracite granules
Biofilm- electrode reactor [27]		7.00·10 ⁻²		1.12	Reduction to N ₂	No
Membrane Bioreactor [33]		2.04·10 ⁻²		0.30	Reduction to N ₂	Yes, organic matter source
Electrodialysis [29]	4.95·10 ⁻² – 1.01·10 ⁻²		0.92 – 1.69		Separation in a waste brine	No
Electrodialysis [28]	0.40·10 ⁻² – 0.80·10 ⁻²		0.04 – 0.11		Separation in a waste brine	No
Electrodialysis [5]	n.m.		0.69 – 1.32		Separation in a waste brine	No
Reverse Osmosis [5]	n.m.		1.03 – 2.09		Separation in a waste brine	No



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Highlights

- Anode feed and cathode potential were evaluated for nitrate treatment in groundwater.
- Cathode potential shifted nitrate removal rate and intermediates accumulation.
- The lowest accumulation of NO_2^- and N_2O was observed at -123 mV vs SHE.
- Water, rather than acetate, was the most reasonable anode electron source.
- Carbon- free treatment of nitrate- polluted groundwater at a competitive cost.

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