Electro-bioremediation of nitrate contaminated saline groundwater

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Abstract Sustainable exploitation of coastal aquifers is often hindered by the presence of contaminants and high salinity levels. This study proves for the first time the electro-bioremediation of nitrate contaminated saline groundwater. Two 3-chamber bioelectrochemical systems (BES) were operated in potentiostatic mode for the treatment of a synthetic medium mimicking saline groundwater from the Nitrate Vulnerable Zone of Arborea (Sardinia, Italy). This groundwater is characterised by high nitrate concentrations (>33 mgNO_3^--N/L) and conductivity (>3.5 mS/cm). The electrochemically active microbial community grown on the surface of the biocathode was able to achieve nitrate removal rates and efficiencies of 6.8±0.4 mg/L·d and 87±2%, respectively. Conductivity significantly decreased (from 4.11±0.2 to 0.17±0.2 mS/cm) by enhancing the electromigration of ions from the middle chamber to the peripheral anodic and bio-cathodic chambers. Under the applied conditions, WHO (World Health Organization) drinking water threshold limits for nitrate (11.3 mgNO_3^--N/L) and conductivity (2.5 mS/cm) were satisfied. Moreover, high chloride migration was observed (63±7 mg/L·d), suggesting its possible recovery as chlorine in the anodic chamber using a specific electrode (Ti-MMO), within a circular economy-based approach. The results pave the way to the potential development of a new electro-bioremediation technology for water recovery (i.e., simultaneous denitrification and desalination) and sustainable chemicals production (i.e., chlorine).

Keywords: conductivity, denitrification, desalination, microbial electrochemical technology, value-added products.

1. Introduction

The World Health Organization (WHO) has predicted that half of the world’s population will live in water-stressed areas by 2025 (WHO, 2019). Groundwater represents an essential resource for human life, being crucial for both drinking water and agriculture irrigation. It has been esteemed that 43% of the total amount of water used for irrigation has an underground origin. In this regard, it becomes a priority to preserve groundwater quality and adapt it to human consumption. In those areas where both natural conditions and human activities have caused aquifers degradation (EEA, 2016), sustainable treatments that can lead to water reclamation must be implemented. Bioelectrochemical systems (BES) are certainly among the most innovative and promising technologies to achieve this goal (Ceballos-Escalera et al., 2021; Pous et al., 2017), and their development towards full-scale application can potentially overtake the disadvantages of traditional treatments (i.e., electrodialysis, ion exchange, and reverse osmosis), namely high energy requirements or the production of concentrated waste brines (Shrimali et al., 2001). This study assesses the feasibility of a three-chamber BES specifically designed for the simultaneous removal of nitrates and salinity from a synthetic medium mimicking saline groundwater from the Nitrate Vulnerable Zone of Arborea (Sardinia, Italy). The experiments were carried out in order to verify the proof-of-concept of the system, and to provide useful information for the process optimization in view of the forthcoming step of the study, the continuous operation mode.

2. Materials and Methods

2.1 Bioelectrochemical cells set-up

The experimentation was carried out in two bioelectrochemical cells (**Figure 1**) made of transparent Plexiglas and consisting of 3 chambers: bio-cathodic chamber (8x8x2 cm), anodic chamber (8x8x2 cm), and middle “desalination” chamber (8x8x0.5 cm). A Cation-

Exchange Membrane (CEM, 64 cm^2, CMI7000-S, Membrane International Inc., USA) and an Anion-Exchange Membrane (AEM, 64 cm^2, AMI 7001-CR, Membranes International Inc., USA) were used to separate the middle chamber from the cathodic and anodic ones, respectively. Carbon felt (thickness 1.12 cm, degree of purity 99.9%, AlfaAesar, Germany) was used as bio-cathode and connected to a stainless-steel mesh, which worked as the current collector; a reference electrode (Ag/AgCl, mod. MF2052, BioAnalytical Systems, USA) was placed in the same chamber. Titanium mesh coated with mixed metals oxide (Ti-MMO) was used as anode (NMT-Electrodes, South Africa), and connected to a titanium wire (thickness 0.75 mm, degree of purity 99.98%, AlfaAesar, Germany) which worked as the current collector. Cathode, anode, and reference electrodes were connected to a multichannel potentiostat (IviumNstat, Ivium technologies, NL). Experiments were conducted at room temperature (25±2 °C).

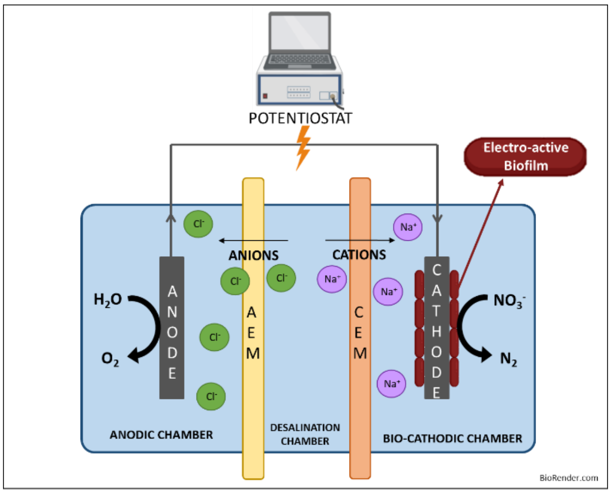

Figure 1. Schematic diagram of the cells.

2.2 Inoculum and media characteristics

The bio-cathodic chambers were inoculated with a mixture (< 100 mg_{TSS}/L) of electro-active biomass coming from a parent BES (Laboratory of Chemical and Environmental Engineering LEQUiA, University of Girona, Spain) and supernatant of activated sludge liquor drawn from the municipal wastewater treatment plant of Cagliari (Italy).

The bio-cathodic chambers were batch fed with Medium A and inoculum (50:50 ratio, v:v). Medium A contained 216.6 mg/L KNO_3, 10.0 mg/L NH_4Cl, 4.6 mg/L KH_2PO_4, 11.5 mg/L K_2HPO_4, 350.0 mg/L $NaHCO_3$, 2000 mg/L NaCl, and 100 µL/L trace element solution (Patil et al., 2010). The anodic chamber and the middle desalination chamber were filled with Medium B, which had the same composition as Medium A to avoid conductivity and pH gradients between the chambers, but without KNO_3 and NH_4Cl. The resulting pH and electric conductivity of both media were 7.5±0.6 and 4.1±0.5 mS/cm, respectively. Both media were prepared using distilled water, and Medium A was flushed with N_2 gas for 15 minutes to avoid any presence of oxygen.

2.3 Experimental procedure

Table 1 summarizes the experimental procedure followed in this study. The medium in the bio-cathodic chamber was periodically supplied with nitrate by dosing a proper amount of KNO_3 solution (0.2 M) when nitrate concentration dropped below 5 $mgNO_3^-$-N/L, in order to bring it up to 30 $mgNO_3^-$-N/L (each NO_3^--N supply corresponded to a new batch cycle in the bio-cathodic chamber). The media in the anodic and middle chambers were replaced once during the experiment (corresponding to a new batch cycle in each compartment). A potential of -0.500 V vs Ag/AgCl (-0.303 V vs SHE) was poised at the bio-cathode, in agreement with previous studies (Pous et al., 2015). The resulting currents were recorded every five minutes.

Table 1. Experimental procedure.

Cell chamber	**Batch cycles**	**Duration of each cycle days (min-max)**
Bio-cathodic	5	6-9
Middle	2	24-30
Anodic	2	24-30

2.4 Analytical methods

Samples from the cathodic, anodic, and middle chambers were taken and analysed three times per week. Liquid samples were periodically analysed for quantification of chloride (Cl^-), nitrite (NO_2^--N), and nitrate (NO_3^--N) using an ion chromatograph (ICS-90, Dionex-Thermofisher, USA) equipped with an AS14A Ion-PAC 5 µm column. Samples were filtered (acetate membrane filter, 0.45 µm porosity) and properly diluted with distilled water. The concentrations of the main cations, namely sodium (Na^+) and potassium (K^+) were determined using an ICP/OES (Optima 7000, PerkinElmer, USA): samples were filtered (acetate membrane filter, 0.45 µm porosity), acidified (1% (v/v) of Nitric Acid) and diluted with grade 1 water. pH and electrical conductivity were measured using Hanna instrument benchtop meter (mod. HI5522, Italy).

3. Results and discussion

3.1 Denitrification and desalination performances

The performances of the bioelectrochemical cells were evaluated in terms of denitrification in the bio-cathodic chamber and conductivity reduction in the middle chamber. **Figure 2** compares the average nitrate concentrations in the bio-cathodic chamber and the conductivity in the middle chamber at the beginning and end of the batch cycles. These results show that simultaneous bioelectrochemical removal of nitrate and reduction of salinity can be achieved with such cell configuration: the concentration of nitrate in the bio-cathodic chamber and the conductivity in the middle chamber significantly dropped between the beginning and the end of each batch cycle, from 30.8±2.8 to 5.8±1.1 $mgNO_3^-$-N/L and from 4.11±0.2 to 0.17±0.2 mS/cm, respectively.

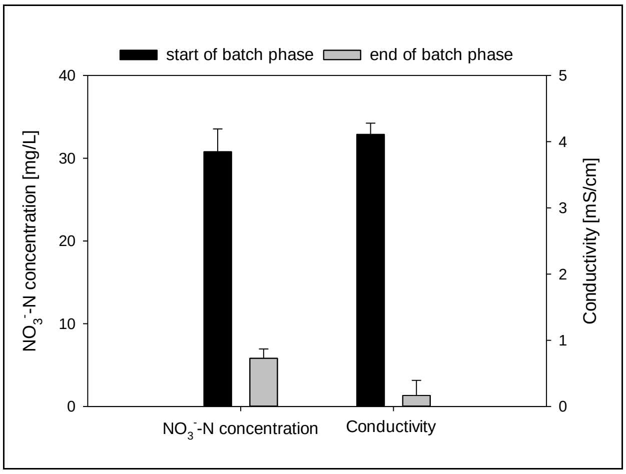

Figure 2. Average values of nitrate concentrations in the bio-cathodic chamber and of conductivity in the middle chamber at the beginning and at the end of batch cycles.

Regarding nitrate, drinking water threshold limits in the bio-cathodic chamber were reached after only 4 days in each batch cycle, corresponding to a nitrate removal rate of about 7 mg/L·d and removal efficiency of about 90%. No nitrite production was observed throughout the experiment. Such rates were lower than those reported in previous studies concerning nitrate removal using BES (Kondaveeti et al., 2013), likely due to the much more complex configuration of the system under study for the simultaneous removal of nitrate and salinity, which still has a strong potential for further optimization. High values of coulombic efficiency related to nitrate removal were observed in our study, often above 100%, which can be explained by possible oxygen intrusion. Oxygen represents a preferential electron acceptor for the electro-active biofilm compared to nitrate, resulting in a higher current in the system and, consequently, in values of coulombic efficiency higher than 100%.

The conductivity in the middle chamber dropped below 2.5 mS/cm after 13 days, corresponding to a desalination efficiency of about 60%. At the end of each middle chamber batch cycle, the conductivity was below 0.2 mS/cm, as confirmed by the significant reduction not only in chloride concentration (i.e., removal efficiencies of about 95%) but also in the concentration of cationic species, particularly sodium and potassium (i.e., removal efficiencies of up to 95% and about 70%, respectively).

A summary of the main results achieved in this study is shown in **Table 2**.

Electricity-driven migration of ions across membranes was observed, resulting in the accumulation of anionic species in the anodic solution and cationic species in the cathodic solution. The accumulation of chlorides in the anodic chamber was particularly interesting, reaching end-of-batch concentrations of 2.7±0.9 gCl^-/L (starting from 1.5±0.3 gCl^-/L), with a consequent increase in conductivity (7.3±2.7 mS/cm). This result is encouraging regarding the possibility of converting the accumulated chloride into a valuable form, such as free chlorine (Cl_2) which is a strong disinfectant widely used in water treatment. In fact, Batlle-Vilanova et al. (2019) showed that the use of Ti-MMO anode electrodes promotes chlorine evolution. In practical terms, the results achieved in this study suggest that operating the anodic chamber in batch mode could promote the accumulation of chlorides and their conversion into free chlorine.

Table 2. Best nitrate and salinity removal performances observed during experimentation.

Parameter	**Results achieved**	**Days of experimentation**
Bio-cathodic nitrate removal efficiency	87±2 [%]	4
Bio-cathodic nitrate removal rate	6.8±0.4 [mg/L·d]	4
Middle chamber desalination efficiency	57±12 [%]	13
Chloride removal rate from middle chamber	63±6.8 [mg/L·d]	13

Despite the observed decrease in salinity, the current density achieved during experimentation (about 0.1 A/m^2) was low compared to that observed in previous studies operating with MDCs (Microbial Desalination Cells). Kim et al. (2013) reported current densities for MDCs range from 0.7 to more than 8.4 A/m^2. In this sense, the switch to continuous mode operation in the bio-cathodic and middle chambers appears particularly convenient. Firstly, it could increase the nitrate removal kinetics at the bio-cathode, consequently increasing current density and thus further enhancing the electromigration of ionic species through the membranes, which would result in the reduction of conductivity in the middle chamber.

3.2 pH trend

The pH is a parameter of fundamental importance for the proper functioning of the system, in particular, to ensure optimal conditions for denitrifying activity of the cathode biofilm, but it is also one of the most important water quality parameters. The optimal pH can vary for different drinking water supplies but is often in the range of 6.5-9.5 (WHO, 2003).

Figure 3 shows the average pH in the different cell chambers at the beginning and at the end of batch cycles. During the operation of the cells, a clear increase in pH was observed in the bio-cathodic chamber (from 7.4±0.5 to 9.0±0.5), a decrease occurred in the anodic chamber (from 7.4±0.1 to 2.8±1.1), while in the central chamber a less marked decrease was observed (from 7.7±0.3 to 4.9±0.5). Bacteria normally require a pH close to neutral for optimal growth; however, reduction reactions on the cathode electrode result in an alkaline environment, being a source of overpotentials (Puig et al., 2010). Generally, in two-chamber bioelectrochemical systems for nitrate removal, the chambers are separated by a cation-exchange membrane that allows protons diffusion from the anode to the cathode and counter-balances pH variations. In the system under study, instead, the flow of protons to the bio-cathodic chamber is tougher due to the longer distance between the anode and the cathode, because of the central chamber. Therefore, if protons are not replenished through the membrane and if a pH control system is not implemented, the pH will increase undisturbed in the cathodic chamber, as reported by Rozendal et al. (2006). In this sense, continuous feeding to the bio-cathodic chamber may potentially balance pH increment, ensuring optimal values for denitrifying biomass activity.

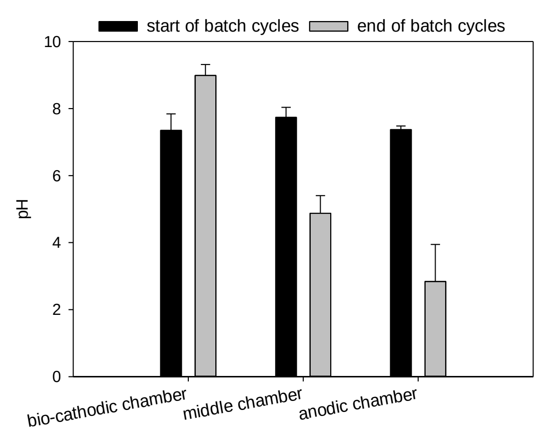

Figure 3. Average pH values at the beginning and at the end of batch cycles.

It will be essential for the next step of the study (continuous mode operation) to monitor pH trends, in order to assess whether a pH control system in the bio-cathodic chamber needs to be implemented to ensure optimal conditions for the denitrifying electro-active biofilm.

4. Conclusions

A 3-chamber BES was tested as a proof-of-concept for the electro-bioremediation of nitrate contaminated saline groundwater. The possibility of exploiting the electro-active biofilm grown on the bio-cathode for the removal of nitrate and, at the same time, for the reduction of salinity was successfully assessed, and WHO threshold limits for drinkable water were satisfied. Moreover, batch experiments showed that significant chloride accumulation can be achieved in the anodic chamber, which can be subsequently recovered as chlorine, a valuable disinfectant. Results are promising, and provide useful information for process optimization, paving the way to the application of BES to contaminated groundwater under a synergic circular economy-based approach.

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