

Bioelectrochemical Nitrate Reduction in a Continuous Flow Microbial Electrolysis Cell

Hafsa Yaqoubi^{ab}, Geremia Sassetto^a, Marco Petrangeli Papini^a, Marco Zeppilli^{a*}, Mustapha Belfaquir^b

^aDepartment of Chemistry, University of Rome Sapienza, Piazzale Aldo Moro 5, 00185 Rome, Italy

^bDepartment of Chemistry, Ibn Tofail University, Laboratory of advanced material and process engineering, Campus Universitaire, Kénitra BP 133, Morocco
marco.zeppilli@uniroma1.it

High Nitrate concentrations in groundwater present considerable public health hazards. This study examines a dual-chamber Microbial Electrolysis Cell (MEC) reactor, consisting of two cylindrical borosilicate glass chambers (cathode and anode), for nitrate reduction from synthetic groundwater, using enriched mixed cultures from Venice Lagoon sediments. Different cathodic potentials were tested, with -450 mV vs. SHE used during batch mode and -450 mV, -550 mV, and -750 mV vs. SHE potentials, with three different Hydraulic Retention Times of 0.63, 1.35, and 0.38 days, tested during continuous flow mode. The highest Denitrification Rate of $72.58 \pm 19.28 \text{ mgL}^{-1}\text{d}^{-1}$ was achieved at -750 mV vs. SHE with a low HRT of 0.38 day. Comparisons between biotic and abiotic conditions at -750 mV vs. SHE and 0.63 day HRT showed slightly higher rates in biotic environments ($59.01 \pm 9.88 \text{ mgL}^{-1}\text{d}^{-1}$ vs. $45.41 \pm 1.17 \text{ mgL}^{-1}\text{d}^{-1}$). The study highlights the potential of MEC technology for efficient nitrate reduction in groundwater.

1. Introduction

Nitrate (NO_3^-), commonly found in soil, water, and food due to nitrogen-based fertilizers in agriculture (Schröder et al., 2004), poses significant risks to groundwater quality and human health, particularly causing Methemoglobinemia in infants and pregnant women (Hmelak Gorenjak and CenČič, 2013). Nitrate can additionally contaminate groundwater through human induced processes, including industrial wastewater discharge and urban runoff (Wakida and Lerner, 2005). The drinking water guideline for nitrate is 50 mgL^{-1} as nitrate ion (11 mgL^{-1} as nitrate-nitrogen). To safeguard water quality and public health, nitrate-contaminated groundwater must be treated using advanced methods such as reverse osmosis, electrodialysis, ion exchange and biological approaches (Marzulli et al., 2023; Vuppala et al., 2019; Archana et al., 2012). Bioelectrochemical systems (BES) offer an energy efficient (Ceconet et al., 2020) it was also demonstrated that BES effectively removes nitrate from groundwater, achieving optimal performance at 0.8 V (Tong and He, 2013). Studies have demonstrated their efficacy, such as simultaneous nitrate and arsenite removal using microbial electrochemical technology (Ceballos-Escalera et al., 2021), the impact of process parameters on denitrifying biocathodes with the eClamp method, and innovative tubular designs for denitrification (Pous et al., 2017). However, these processes need proper process design and management, thus preventing membranes fouling or break (Stoller et al., 2019).

In this context, this study investigates nitrate reduction in a dual-chamber Microbial Electrolysis Cell (MEC) reactor, evaluating the effects of varying cathodic potentials (-450 mV, -550 mV, -750 mV vs. SHE) and Hydraulic Retention Times (HRTs) on reductive denitrification rates (DRs) and Coulombic Efficiency (CE %). A mixed enriched culture was introduced to promote anaerobic denitrification in the cathode chamber for effective electro-bioremediation, a method previously applied to Chlorinated Aliphatic Hydrocarbons (CAHs) (Aulenta et al., 2009; Zeppilli et al., 2019). In the cathode chamber, conductive graphite granules were used to facilitate electron transfer for nitrate reduction, while silica beads in the anode chamber, and they were separated by a

Cation Exchange Membrane (CEM). The findings provide valuable insights into optimizing nitrate removal using BES reactors.

2. Materials and methods

2.1 Dual chamber MEC reactor configuration

Two nearly resembling cylindrical borosilicate glass chambers, divided by a Nafion® 117 Cation Exchange Membrane (CEM), constructed the structure of the MEC reactor (Figure 1). The cathode and anode chambers had total empty volumes of about 0.82 L and 0.95 L, respectively. An aluminum clamp and a Viton® seal, which also guaranteed air tightness, kept the two chambers in place. Prior to being used, the CEM was pretreated by boiling successively in H_2O_2 (3% v/v), distilled water, 0.5 MH_2SO_4 , and finally in distilled water again, each for 2h. The anodic chamber and cathode chamber were filled with Silica beads and conductive graphite granules, respectively, with a diameter of 1 to 4 mm, in order to guarantee the external electric connections. The graphite granules and silicate were used after being submerged in a 37% HCl solution for 24 hours, completely washed in distilled water, and then dried at 100 °C for a whole night. Each chamber had a specified electrode surface area of approximately $1290 \text{ m}^2 \text{ m}^{-3}$ from graphite rod connector ensured the electric connection to a potentiostat (Marchetti et al., 2025), and an Ag/AgCl reference electrode (+0.2 V vs. standard hydrogen electrode, SHE) (Amel, Milan, Italy) was placed in the cathode chamber. All voltages are reported with respect to Standard Hydrogen Electrode (SHE). The cathode electrode (Working Electrode), anode electrode (Counter Electrode), and reference electrode were linked to a potentiostat Amel Model 549 (Milan, Italy) in order to set the cathode potential at the appropriate value.

2.2 Dual chamber MEC reactor operating conditions

Before starting the batch process, a tracer test was carried out for each compartment of the cathode and the anode, the description was clarified in details on this previous work (Zeppilli et al., 2024). The mixed culture utilized as inoculum consisted in an enriched dechlorinating culture from Venice Lagoon sediments (Aulenta et al., 2009). Notably, as was reported in a previous research (Yaqoubi et al., 2025), the enrichment culture used was composed of 75% *D. Mccartyi*. A quantity of 1 liter, roughly, comprising an enriched culture, was drawn from the bioreactor and subsequently introduced into the cathode chamber of the experimental reactor, serving as the initial step in the inoculation process preceding continuous operation.

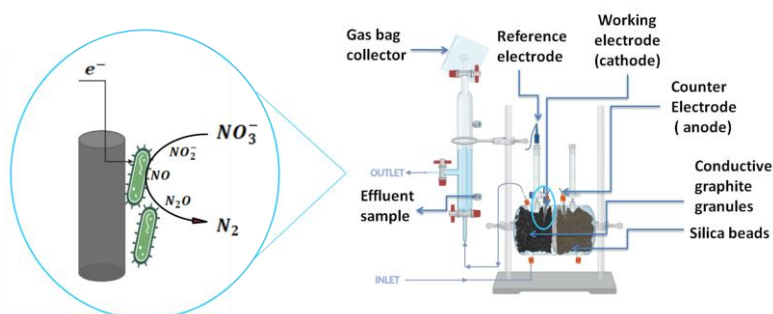


Figure 1: Schematic Illustration of the dual chamber MEC Reactor and Bioelectrochemical Nitrate Reduction in the Cathode Chamber.

2.3 Batch processing of the MEC reactor

For batch processing in a closed system, a peristaltic pump was employed to maintain continuous recirculation of the solution at a flow rate of 4 L d^{-1} (i.e., closed-loop mode). To sustain microbial growth, a cathode potential of -0.45 V vs. SHE was applied to the cathode cell. Several days after the introduction of the mixed culture, nitrate solution was initially injected daily to achieve a concentration of approximately 50 mg L^{-1} . After three days, when complete nitrate removal was observed, the concentration was adjusted by injecting a higher nitrate dose of approximately 100 mg L^{-1} . Specifically, 100 mL of nitrate solution with an initial concentration of 820 mg L^{-1} was introduced into the cathode cell to achieve a final concentration of 100 mg L^{-1} of nitrate. The system was then operated in batch mode for approximately 8 days to monitor the denitrification reaction.

2.4 Continuous flow reactor set-up

The cathode chamber of the MEC reactor was continuously supplied with an anaerobic medium throughout the operating period. The medium contained (mg L^{-1}): NH_4Cl , 0.3 ; $\text{MgCl}_2 \cdot \text{H}_2\text{O}$, 0.5 ; KH_2PO_4 , 0.2; NaCl , 1 ; KCl , 0.3; NaHCO_3 , 2.5; $\text{CaCl}_2 \cdot \text{H}_2\text{O}$, 0.015 (Matturro et al., 2018); 1 mL L^{-1} of Metal solution; Vitamin solution 10 mL L^{-1} , and nitrate at an approximate concentration of $100 \text{ mg NO}_3^- \text{ L}^{-1}$, all dissolved in tap water. To achieve anaerobic conditions, the solution underwent meticulous flushing with a mixture of carbon dioxide (30% mol/mol) and nitrogen (rest 70 %) contained in a pressurized cylindrical gas vessel. This process involved inserting a needle into the top cap of the solution preparation tank and allowing the gas to displace oxygen for duration of 30 minutes. The inlet solution was stored in a 10 L collapsible Tedlar® bag (Supelco, Cerritos, CA, USA), and the same type of bag was utilized for the outlet. During the initial 13 days of operation, the MEC reactor functioned continuously at potentials of -450 mV vs. SHE and -550 mV vs. SHE, while maintaining a flow rate of 1.3 L d^{-1} corresponding to an HRT of 0.63 day. The operation of the MEC reactor was interrupted for one month, due to the summer holidays. Following this interruption, for the subsequent 160 days, the operation was sustained at -750 mV vs. SHE, with The HRT of 0.63 d. Afterward, for a period of 15 days, the flow rate was adjusted to 0.6 L d^{-1} resulting in an HRT of 1.35 day. In the final experimental period of the continuous flow operation, the flow rate was adjusted to 2.2 L d^{-1} resulting in a lower HRT of 0.38 day. Throughout the operation, the mineral medium solution, as previously described, was consistently used. However, it is important to note that during the last 50 days, ammonium chloride (NH_4Cl) was intentionally excluded from the solution to prevent nitrification, despite the fact that the cathode was maintained under anoxic conditions, slight oxygen intrusion may have occurred, creating microaerobic zones that supported nitrification and contributed to the nitrate production observed at the final stage.

2.5 Abiotic MEC reactor test

During this last phase of the operational period, duration of 15 days was allocated to conducting abiotic test. The dual chamber MEC reactor was disassembled to replace graphite granules in the cathode chamber with newly prepared ones and to install a new graphite rod cathode electrode serving as the working electrode for evaluation. A continuous flow system was established following the previously described procedure. The mineral medium used in the abiotic test closely matched that of the biotic continuous flow system, with the intentional exclusion of ammonium salts. The HRT for the abiotic test was maintained at 0.63 day and potential of -750 mV vs. SHE was applied.

2.6 Sampling and analytical methods

Sampling was conducted from two distinct points within the MEC reactor. The influent sample was directly extracted from the inlet bag, while the effluent sample was collected from the glass flask connecting both the cathode chamber and the outlet bag, as depicted in Figure (1). Liquid samples (1.5 mL) were taken using sterile disposable plastic syringes, filtered ($0.45 \mu\text{m}$), and analyzed for nitrate anions using ion chromatography (0.5 mL sample, Dionex DX-100, Ionpac As9-Sc column, conductivity detector) (Zeppilli et al., 2024). For the ions $\text{N} - \text{NH}_4^+$ was determined by Nessler reagent method and the pH values were determined using a pH electrode (SI Analytics™ pH Combination Electrode A 7780 IDS).

2.7 Calculations

For The continuous flow reactor Denitrification Rate was calculated in unit of $\text{mg L}^{-1} \text{ d}^{-1}$ as follows (Eq (1)). The DR in batch mode (DR_{Batch}) was calculated as the average of all slopes measured each day. Each slope represents the nitrate removal rate over a specific time interval and is determined by dividing the change in nitrate concentration ($\Delta C_{NO_3^-}$) by the corresponding time difference (Δt_i). The final daily rate was obtained by averaging all these slopes, as shown (Eq (2)):

$$DR_c = \frac{C_{NO_3^- in} - C_{NO_3^- out}}{v} \times Q \quad (1) \quad DR_{batch} = \frac{1}{n} \sum_{i=1}^n \frac{\Delta C_{NO_3^-}}{\Delta t_i} \quad (2)$$

Let $C_{NO_3^- in}$ and $C_{NO_3^- out}$ represent the average nitrate mass concentrations (mg L^{-1}) for the influent and effluent, respectively. Q denotes the flow rate (L d^{-1}), and v corresponds to the volume of the cathode chamber of MEC reactor. The Coulombic Efficiency (CE %) was calculated starting from the DR using the following equation Eq (3):

$$CE \% = \frac{DR \times v}{\frac{62.0049}{\text{average}} \times 5 \times \frac{F}{86400}} \times 100 \quad (3)$$

Here, the constant $62.0046 \text{ gmole}^{-1}$ signifies the molar mass of nitrate. The parameter I signifies the average current within the cathode chamber, measured in milliamperes (mA), and F represents Faraday's constant (96485 Cmol^{-1}). Moreover, the numerical value '5' holds importance in denitrification; it represents the number of moles of electrons needed for the denitrification intermediate. This is explained further in the overall denitrification reaction (Eq 4). The equation below (Eq 5) assesses the specific Removal Efficiency (%) of the target nitrate:

$$\text{NO}_3^- + 6\text{H}^+ + 5\text{e}^- \rightarrow \frac{1}{2}\text{N}_2 + 3\text{H}_2\text{O} \quad (4) \quad RE (\%) = \frac{C_{\text{in}} - C_{\text{out}}}{C_{\text{in}}} * 100 \quad (5)$$

3. Results and discussion

The batch process spanned 8 days, succeeded by the continuous mode operation. Initially, the potential was set at -450 mV vs. SHE to stabilize the system and support the initial development of the biofilm. The batch experiments achieved DR_{batch} of $56.94 \pm 29.66 \text{ mgL}^{-1}\text{d}^{-1}$, with nitrate initially injected at 56 mgL^{-1} . In each daily cycle marked by vertical dashed lines in Figure (2a) nitrate levels declined steadily, reaching depletion after about three days. Following complete consumption, nitrate was reinjected to approximately 120 mgL^{-1} , which rapidly reduced to 100 mgL^{-1} before stabilizing, and then declined sharply to around 80 mgL^{-1} later in the cycle. The system shifted to continuous mode at an HRT of 0.63 (d) (Figure 2b), the DRs at applied potentials of -450 mV and -550 mV were observed to be $17.86 \pm 9.35 \text{ mgL}^{-1}\text{d}^{-1}$ with CE% of $115.94 \pm 2.49\%$ and $21.73 \pm 6.18 \text{ mgL}^{-1}\text{d}^{-1}$ with CE% of $113.39 \pm 2.68\%$, respectively (Table 1). This overestimation in CE% may possibly be the result of side reactions involving intermediate products like nitrite rather than the complete conversion of nitrate to nitrogen.

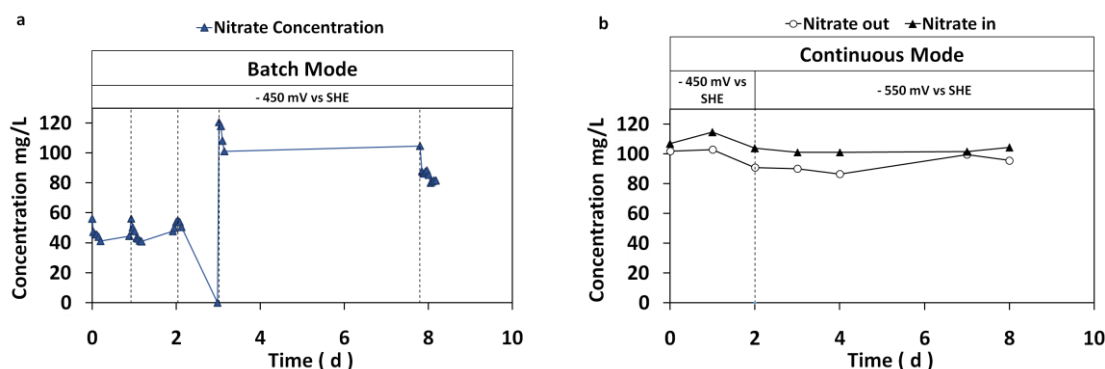


Figure 2: Variation in nitrate concentration during sequential operation: Batch mode initiation (a) followed by continuous mode (b) by varying the applied potential.

To further optimize reactor performance, a higher applied potential of -750 mV vs. SHE was tested (Figure 3a). However, an anomaly was observed where effluent nitrate concentrations exceeded those in the influent. This unexpected increase was likely due to ammonium oxidation, attributed to the presence of ammonium salts in the feeding solution. Although the inlet solution was carefully deoxygenated, minor oxygen intrusion at the cathode chamber may have created microaerobic zones that facilitated limited nitrification, leading to nitrate formation. Despite precautions to prevent nitrification by removing ammonium from the influent, these findings suggest that even slight oxygen contamination can influence nitrogen transformation pathways in the system. Following this adjustment, nitrate reduction improved, with a slight decrease in nitrate levels at HRT 0.63 d . Over 30 days, the DR_c was $59.01 \pm 9.88 \text{ mgL}^{-1}\text{d}^{-1}$ corresponding to CE %, i.e. the fraction of electrons involved in nitrate reduction, of $98.68 \pm 3.57\%$. Moreover, using higher HRT of 1.35 (d) , the DR_c decreased to $26.99 \pm 9.02 \text{ mgL}^{-1}\text{d}^{-1}$ corresponding to the CE% of $55.36 \pm 2.72\%$. In contrast, the decrease of the HRT to 0.38 (d) yielded a substantial increase in DR_c to $72.58 \pm 19.28 \text{ mgL}^{-1}\text{d}^{-1}$ with CE% of $128.87 \pm 7.71\%$. Notably, the persistent high nitrate concentration in the effluent and CE% values exceeding 100% suggest that incomplete denitrification may be occurring. This overestimation of electron utilization is likely due to the accumulation of intermediate nitrogen species (e.g. nitrite or nitrous oxide). However, the abiotic (blank) test determines the contribution of the mixed microbial culture versus purely electrochemical reactions. The results showed at Figure (3b) and Table (2) that the nitrate removal efficiency in the presence of microbes ($35.52 \pm 2.35\%$) was only slightly higher than that achieved by electrochemical reactions alone ($28.63 \pm 3.39\%$). This indicates that, under the applied conditions, the electrochemical process was the primary driver of nitrate reduction, with the microbial community playing only a secondary role.

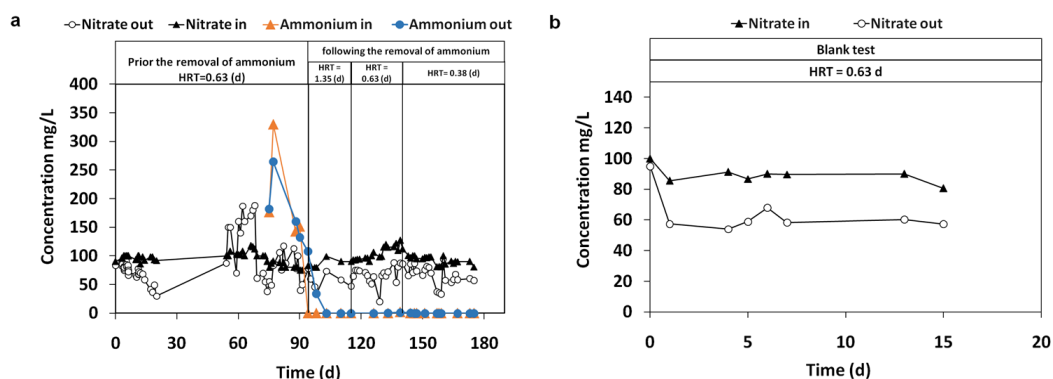


Figure 3: Variation in nitrate and ammonium concentration in mgL^{-1} for the influent and effluent at the applied potential -750 mV vs. SHE and different HRTs (a), and variation in nitrate concentration mgL^{-1} during the abiotic test (b)

In summary, the enriched mixed culture utilized in this study was evaluated for its effectiveness in nitrate reduction for contaminated synthetic groundwater, even if their contribution in this experiment was marginal as indicated by the abiotic test results. However, further optimization of microbial conditions is essential to maximize system efficiency. Selecting appropriate bacterial strains for nitrate reduction (e.g., *Pseudomonas*, *Bacillus*, *Paracoccus*, *Ferrobacillus* and *Thiobacillus*) could significantly enhance biological nitrate removal. Furthermore, the findings indicated that reducing the HRT and increasing the applied potential led to an increase in the denitrification rate, highlighting the potential for optimizing system performance through adjustments in operational parameters

Table 1: Comparative analysis of parameters, evaluating denitrification rate and energetic consumption in biotic and abiotic processes

Batch/Continuous Mode	Applied potential mV vs. SHE	Biotic/Abiotic process	HRT (days)	DRs $\text{mgL}^{-1}\text{d}^{-1}$	CE %	I average (mA)
Batch mode	-450	Biotic process	-	56.94 ± 29.66	-	-
	-450	Biotic process	0.63	17.86 ± 9.35	115.94 ± 2.49	-0.65 ± 0.24
	-550	Biotic process	0.63	21.73 ± 6.18	113.39 ± 2.68	-1.50 ± 0.19
			1.35	26.99 ± 9.02	55.36 ± 2.72	-3.6 ± 0.24
Continuous Mode	-750	Biotic process	0.63	59.01 ± 9.88	98.68 ± 3.57	-4.42 ± 0.20
			0.38	72.58 ± 19.28	128.87 ± 7.71	-4.16 ± 0.18
		Abiotic process	0.63	45.41 ± 1.17	67.85 ± 1.27	-4.94 ± 0.74

Table 2: Evaluation of nitrate removal efficiency in biotic and abiotic processes at HRT of 0.63 d and applied potential of -750 mV vs. SHE .

Removal efficiency (%) in biotic process	$35.52 \% \pm 2.35$
Removal efficiency (%) in abiotic process	$28.63 \% \pm 3.39$

4. Conclusion

The comparison between biotic and abiotic environments highlighted the role of applied potential in enhancing microbial activity, even though the results were not impressive. The selected inoculums provided limited improvements, suggesting the need to optimize biofilm activity and conduct a detailed analysis of the intermediate products formed during denitrification. Furthermore, the study demonstrated that DRs increased with shorter HRTs, ranging from $26.99 \pm 9.02 \text{ mgL}^{-1}\text{d}^{-1}$ at 1.35 (d) to $72.58 \pm 19.28 \text{ mgL}^{-1}\text{d}^{-1}$ at 0.38 (d), under an applied potential of -750 mV vs. SHE . Future studies will focus on refining biofilm management strategies and elucidating the role of these intermediates in order to achieve more complete nitrate conversion and reliable electron accounting.

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