



A review of autotrophic denitrification for groundwater remediation: A special focus on bioelectrochemical reactors

Eduardo Ortega-Martínez^a, Javiera Toledo-Alarcón^{a,*}, Edel Fernández^a, José Luis Campos^a, Ricardo Oyarzún^{b,c,d}, Claudia Etchebehere^e, René Cardeña^f, Angela Cabezas^f, László Koók^g, Péter Bakonyi^g, José Antonio Magdalena^{h,i}, Eric Trably^h, Nicolas Bernet^h, David Jeison^{j,*}

^a Facultad de Ingeniería y Ciencias, Universidad Adolfo Ibáñez, Av. Padre Hurtado 750, Viña del Mar 2520000, Chile

^b Departamento Ingeniería de Minas, Universidad de La Serena, Benavente 980, La Serena, Chile

^c Centro de Recursos Hídricos para la Agricultura y Minería, ANID Fondap 15130015, Concepción, Chile

^d Centro de Estudios Avanzados en Zonas Áridas, La Serena, Chile

^e Microbial Ecology Laboratory, Department of Microbial Biochemistry and Genomic, Biological Research Institute "Clemente Estable", Avenida Italia 3318, CP: 11600, Montevideo, Uruguay

^f Instituto Tecnológico Regional Centro Sur, Universidad Tecnológica, Francisco Antonio Maciel S/N, Durazno 97000, Uruguay

^g Research Group on Bioengineering, Membrane Technology and Energetics, University of Pannonia, Egyetem u. 10, 8200 Veszprém, Hungary

^h INRAE, University of Montpellier, LBE, 102 Avenue des Etangs, 11100 Narbonne, France

ⁱ Vicerrectorado de Investigación y Transferencia de la Universidad Complutense de Madrid, 28040 Madrid, Spain

^j Escuela de Ingeniería Bioquímica, Pontificia Universidad Católica de Valparaíso, Avenida Brasil 2085, Valparaíso, Chile

ARTICLE INFO

Editor: Deepak Pant

Keywords:

Autotrophic denitrification
Bioremediation
Bioelectrochemical systems
Investment costs
Groundwater recuperation
Microbial community

ABSTRACT

Groundwater is an important resource that can help in climate change adaptation. However, the pollution of these aquifers with nitrate is a widespread problem of growing concern. Biological denitrification using inorganic electron donors shows significant advantages in treating nitrate-polluted groundwater where organic matter presence is negligible. However, mass transfer limitations and secondary contamination seem to be the major hindrance to spread the use of these technologies. This could be solved by the use of bioelectrochemical systems (BES), which emerge as an attractive technology to solve these problems due to the reported low energy demand and high denitrification rates. However, technical and operational issues must be considered to replicate these results at full-scale. This review summarizes the biological basis of autotrophic denitrification and the key aspects of its application in bioelectrochemical systems. In addition, an estimation of the capital costs required for the implementation of a BES considering different population sizes and initial nitrate concentration in the groundwater is made.

1. Introduction

Groundwater is one of the largest reservoirs of water on earth [1]. It is estimated that the total groundwater volume in the upper 2 km of continental crust is approximately 22.6 million km³ [2,3]. However, more than 80% is sequestered below 500 m, and in such situations, it is not an active component of the hydrological cycle [1]. In fact, it is estimated that only 0.35 million km³ is actively contributing to the hydrological cycle, which is 3.5 times more than the total amount of surface water [2,3]. Groundwater is currently used worldwide to supply almost half of the world's drinking water. About 40% is used for irrigation and close to 33% for industrial processes [4]. Even though

groundwater is such an important resource, its quality is increasingly compromised [5].

Groundwater contaminants can occur naturally, such as arsenic and fluoride; or can be introduced by anthropogenic activities, as a result of human settlements human activities, industrial activities, and use of fertilizers in agriculture [5,6]. Nitrate is one of the most common pollutants found in groundwater aquifers. Nitrate derives mainly from fertilizers, and human or animal wastes [6]. Nitrate is responsible for the blue baby syndrome that fatally affects many infants, by reducing the oxygen-carrying function of hemoglobin in the body [7]. Also, it has been related to gastric cancer, when groundwater with high nitrate concentrations is commonly ingested [8]. To prevent human health

* Corresponding authors.

E-mail addresses: javiera.toledo.a@uai.cl (J. Toledo-Alarcón), david.jeison@pucv.cl (D. Jeison).

<https://doi.org/10.1016/j.jece.2023.111552>

Received 28 August 2023; Received in revised form 7 November 2023; Accepted 20 November 2023

Available online 30 November 2023

2213-3437/© 2023 Elsevier Ltd. All rights reserved.

problems, the World Health Organization (WHO) guidelines recommend 11 mg N-NO₃⁻/L (50 mg/L of NO₃⁻) as the maximum level for drinking water [6,7]. However, new evidence shows that this limit is still very high, as low nitrate concentrations such as 0.87 mg N-NO₃⁻/L (3.87 mg/L of NO₃⁻) have been strongly correlated as a risk factor for several diseases, such as thyroid and neural tube defects, and recently to prostate and colorectal cancer due to secondary formation of N-Nitroso compounds [6,7,9,10]. Additionally, nitrate consumption, even at levels below regulatory limits, has been recently associated with risks of adverse pregnancy outcomes, specially the risk of small-for-gestational-age (SGA) [11]. However, the current literature is scarce and still does not provide sufficient evidence of the real effect of nitrate [12–14].

Groundwater pollution with nitrate has been a topic of deep concern worldwide [15–18]. Recently Abascal et al. [15] reported that from a study universe of 292 regions around the world, more than 20% have average concentrations exceeding 11 mg N-NO₃⁻/L (50 mg/L NO₃⁻), with some exceeding 45 mg N-NO₃⁻/L (200 mg/L NO₃⁻), in Spain, Malta, Congo, and Palestine. The distribution of contaminated areas was set in the following order: Africa > Asia > Europe > Americas, being the use of fertilizers and pesticides a common source factor in all continents. Situation becomes of special concern in areas with rock aquifers, where nitrate was found at depths of more than 350 m [6]. Intensive groundwater pumping could also potentially pollute deep aquifers [19,20]. Moreover, the presence of nitrate aids the mobilization of trace metals such as Mn, Ni, As, Cd, and U, further polluting an aquifer [21–23] and endangering its endemic biodiversity [24], a problem often neglected.

To remediate groundwater nitrate pollution, several strategies have been implemented [15,25,26]. The most common is the dilution of a high nitrate water source with another containing a low concentration of nitrate, to obtain drinking water that complies with regulations. However, this can only be considered a temporary solution, since it requires a nearby source of water low in nitrate [25,26]. Other methods are based on separation techniques such as ion exchange, reverse osmosis, and electrodialysis [15,25,26]. These systems are highly efficient with separation efficiencies close to 90% [25]. Abascal et al. [15] reported that of 75 nitrate treatment facilities, 55% use one or more of these technologies, with capacities between 18 m³/day to 37,854 m³/day. However, using these technologies generates a highly concentrated nitrate waste that represents between 15% and 30% of the treated water [27–29]. This waste is commonly discharged into the sea, used for irrigation, or diluted in the same well from which it was obtained [30,31]. However, the high risk of eutrophication has considerably restricted these disposal practices, so additional treatments are necessary for sustainable waste management [16,25,26,32–35]. In this context, biological treatments are especially relevant by safely transforming nitrate into gaseous nitrogen, without generating a concentrated waste that must be treated further [32,36,37].

Autotrophic denitrification can contribute to the development of more adequate and sustainable process for nitrate removal from groundwaters. In comparison to separation processes, nitrate is converted to inert nitrogen gas, eliminating the risk of recontamination because of inadequate disposal of concentrates. Also, in comparison to heterotrophic denitrification processes, the addition of organic matter that can further pollute water isn't needed. It is expected then that initiatives dealing with the application of autotrophic denitrification for the removal of nitrate will become more common in the near future.

This review aims to provide the key biological fundamentals of autotrophic denitrification, with a special focus on the application of bioelectrochemical systems (BES). Relevant concepts associated with groundwater characteristics, operational parameters, and BES configuration are addressed. Special attention is given to the application of BES for groundwater remediation, analyzing the main challenges for their industrial use at large scale.

2. General aspects of biological nitrate removal in groundwater

Biological systems stand out because they can achieve high nitrate removal efficiencies, are simple to operate, and have a low operational costs [16,25,26]. In fact, nitrate removal by biological denitrification-based technologies is cheaper than reverse osmosis or ion exchange [25,26].

Biological nitrate removal uses denitrifying bacteria that utilize an organic (heterotrophic denitrification) or inorganic (autotrophic denitrification) electron donor, to reduce nitrate to gaseous nitrogen as shown in Table 1 [32,36,38]. Heterotrophic denitrification is suitable for treating wastewater with a high carbon/nitrogen (C/N) ratio, considering the carbon source is organic. However, when the C/N ratio is lower than 2.47, as in groundwater, the low presence of organic matter must be supplied externally increasing operating costs [39].

As seen in Table 1, there are different compounds that can be used as electron donors. In heterotrophic denitrification, methanol, and ethanol are the most widely used organic compounds for their efficiency in terms of nitrate removal [40]. However, the standardized cost of these electron donors per amount of removed N-NO₃⁻ is higher than those of the inorganic electron donors used in autotrophic denitrification. On the other hand, the addition of methanol or ethanol should be carefully controlled, as its excess could lead to secondary pollution [41]. In addition, compared to autotrophic denitrification, the heterotrophic denitrification process produces larger amounts of biomass [42–44]. Strohm et al. [45] estimated that the heterotrophic denitrification process produces 5–6 times more biomass than the autotrophic denitrification process [45]. This would suppose an increase in the operating costs associated with sludge disposal by 2.5 times, when comparing heterotrophic denitrification process with autotrophic one [41]. For all of the above, autotrophic denitrification is gaining more and more interest in the treatment of nitrate-contaminated groundwater.

Although many inorganic electron donors have been used in autotrophic denitrification processes (see Table 1), H₂ and sulfur are the most used ones on an industrial scale [40]. However, they also present some characteristics that hinder the spread of their application at full scale [36]. In the case of H₂, its main obstacle is its low solubility in the liquid phase, which reduces its bioavailability for the autotrophic denitrification [46]. As a result, different strategies have been proposed to increase the H₂ mass transfer rate, such as the use of high pressure or the use of membranes, which overall increases the operational costs of the process [47–49]. Additionally, the potential flammability and explosibility of H₂ also generate additional costs associated with safe transport and storage [50]. On the other hand, sulfur denitrification has the main drawback of producing sulfate as a byproduct [46]. Since sulfate production is about 7.54 g SO₄²⁻/g N-NO₃⁻, the maximum nitrate concentration that can be removed would be 33 mg N-NO₃⁻/L to comply with drinking water regulations (U.S. Environmental Protection Agency, EPA: 250 mg SO₄²⁻/L). Then, its application would be limited to only some groundwater [51]. In fact, the same drawback could be attributed to sulfide, thiosulphate [46]. Metal sulphide-minerals such as pyrrhotite [52], sphalerite [53], pyrite [54], among others have also been used as electron donors. However, low denitrification rates and the potential leaching of heavy metals have been reported as the main drawbacks [55]. Also, although sulphate generation is lower than sulphur based denitrification (3.81 – 4.99 g SO₄²⁻/g N-NO₃⁻), is still a matter of concern that may limit its practical applicability [55].

Zero valent iron (Fe⁰) could also be used as electron donor [56]. The corrosion of Fe⁰ will release hydrogen, that could be used by hydrogenotrophic denitrifying bacteria to remove the nitrate. The main advantage of this process is the in situ hydrogen production. However, abiotic reduction of Fe⁰ and N-NO₃⁻, can co-occur, resulting in excess production of N-NH₄⁺ which may limit its practical applicability [46].

In general, the main obstacles to large-scale autotrophic denitrification are associated with (i) the low mass transfer rate due to the low solubilization of electron donors and (ii) the formation of by-products

Table 1
Denitrification reactions using different electron donors, their costs and sludge generated per amount of nitrate removed.

Heterotrophic denitrification				
Electron donor	Stoichiometry	Electron donor cost(US\$/kg)	Standardized electron donor cost (US\$/kg N-NO ₃ ⁻ Removed)	Sludge generated* (kg/kg N-NO ₃ ⁻ Removed)
Acetic Acid	0.85 CH ₃ COOH + NO ₃ ⁻ + H ⁺ → 0.10 C ₅ H ₇ O ₂ N + 0.45 N ₂ + 1.19 CO ₂ + 2.12 H ₂ O	0.57	2.08	0.81
Ethanol	0.69 C ₂ H ₅ OH + NO ₃ ⁻ + H ⁺ → 0.14 C ₅ H ₇ NO ₂ + HCO ₃ ⁻ + 0.67 CO ₂ + 2.07 H ₂ O + 0.43 N ₂	1.10	2.00	1.13
Autotrophic denitrification				
Electron donor	Stoichiometry	Electron donor cost(US\$/kg)	Standardized electron donor cost (US\$/kg N-NO ₃ ⁻ Removed)	Sludge generated* (kg/kg N-NO ₃ ⁻ Removed)
Hydrogen	3.03 H ₂ + NO ₃ ⁻ + H ⁺ + 0.23 CO ₂ → 0.05 C ₅ H ₇ NO ₂ + 0.48 N ₂ + 3.37 H ₂ O	2.65	1.13	0.40
Methane	0.78 CH ₄ + NO ₃ ⁻ + H ⁺ → 0.51 CO ₂ + 1.87 H ₂ O + 0.47 N ₂ + 0.053 C ₅ H ₇ NO ₂	0.33	0.29	0.43
Sulphur	1.10 S + NO ₃ ⁻ + 0.76 H ₂ O + 0.4 CO ₂ + 0.086 NH ₄ ⁺ → 0.04 C ₅ H ₇ NO ₂ + 0.48 N ₂ + 0.98 SO ₄ ²⁻ + 0.96 H ⁺	0.10	0.26	0.32
Sulphide	HS ⁻ + 1.23 NO ₃ ⁻ + 0.573 H ⁺ + 0.438 HCO ₃ ⁻ + 0.027 CO ₂ + 0.093 NH ₄ ⁺ → 0.093 C ₅ H ₇ O ₂ N + 0.866 H ₂ O + 0.614 N ₂ + SO ₄ ²⁻	0.13	0.25	0.61
Thiosulphate	NO ₃ ⁻ + 0.86 S ₂ O ₃ ²⁻ + 0.03 CO ₂ + 0.45 HCO ₃ ⁻ + 0.11 H ₂ O + 0.095 NH ₄ ⁺ → 0.5 N ₂ + 1.724 SO ₄ ²⁻ + 0.095 C ₅ H ₇ NO ₂ + 0.375 H ⁺	0.24	1.55	0.77
Pyrite	NO ₃ ⁻ + 0.364 FeS ₂ + 0.116 CO ₂ + 0.821 H ₂ O + 0.023 NH ₄ ⁺ → 0.5 N ₂ + 0.728 SO ₄ ²⁻ + 0.023 C ₅ H ₇ NO ₂ + 0.364 Fe(OH) ₃ + 0.48 H ⁺	0.20	0.14	0.19
Zero-Valent Iron	Fe ⁰ + 0.4 NO ₃ ⁻ + 1.2 H ₂ O → Fe ²⁺ + 0.2 N ₂ + 2.4 OH ⁻	0.32	3.19	n.a.

* Calculated based on the stoichiometry of the reaction.

n.a.: not available

associated with microbial metabolism. These problems could be solved if autotrophic denitrification is carried out by means of bio-electrochemical systems (BES) [46]. In these systems, electrons could be provided to autotrophic denitrifiers either by the oxidation of organic matter in the anodic chamber or by an external power supply [57]. At the cathode surface microorganisms can consume electrons directly or can be mediated through H₂ production [58]. Thus, the production of electrons or H₂ on demand in the cathode chamber cleverly solves the problems of mass transfer, safety associated with the use of H₂, and the generation of the by-product that could lead to secondary contamination, as occurs when sulfur is used as electron donor [46]. However, although BESs have been successfully tested with real groundwater reaching technology readiness levels (TRL) of 4 [59], bio-electroremediation technologies are still at a TRL 3, as the performance observed at the laboratory scale has not yet been implemented at the pilot scale [60].

3. Biological basis of autotrophic denitrification

3.1. Pathway involved in nitrate reduction

Biological denitrification is a stepwise process where nitrate (NO₃⁻) is sequentially reduced to dinitrogen gas (N₂) via the three intermediates nitrite (NO₂⁻), nitric oxide (NO), and nitrous oxide (N₂O) [61]. The reactions involved are the following:

1. NO₃⁻ + 2 H⁺ + 2 e⁻ → NO₂⁻ + H₂O
2. NO₂⁻ + 2 H⁺ + e⁻ → NO + H₂O
3. 2 NO + 2 H⁺ + 2 e⁻ → N₂O + H₂O
4. N₂O + 2 H⁺ + 2 e⁻ → N₂ + H₂O

Several enzymes are involved in the nitrate reduction to N₂. The first step is catalyzed by molybdenum-containing nitrate reductases, which can be of two dissimilatory classes: respiratory membrane-bound (NAR) and periplasmic (NAP). In the second step, nitrite is further reduced to nitric oxide, and the reaction can be catalyzed by two different enzymes that have the same function, cytochrome cd1 (NIR S) or copper-containing enzymes (NIR K). Denitrifying microorganisms generally have either NIR S or NIR K, although there is evidence of a few with both enzymes. In the third step, nitrous oxide is produced due to the conjugation of two nitric oxide molecules. Nitric oxide reductase (NOR) which contains heme c, heme b, and non-heme iron cofactors, catalyzes this step. The fourth and final step corresponds to the reduction of nitrous oxide to dinitrogen and is catalyzed by a copper-containing enzyme (nitrous oxide reductase, NOS) [62,63] (See Fig. 1).

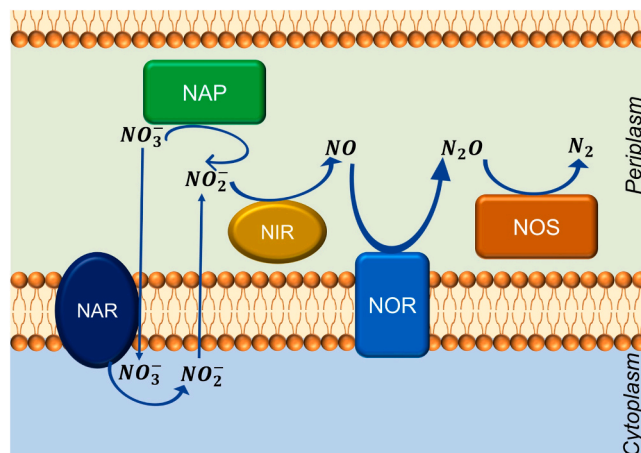


Fig. 1. Scheme showing the distribution of the denitrifying enzymes in the cell.

3.2. Pathways involved in conventional inorganic electron donor utilization

As mentioned earlier, H_2 and reduced sulfur compounds are the most used inorganic electron sources. In order to provide the electrons to the nitrate reduction chain, electron donors must be oxidized. For H_2 , the key redox enzymes are hydrogenases, which are involved in the production and consumption of H_2 . In bacteria, the most common are those with an active center associated with two metal atoms, which can be iron-iron or nickel-iron [64,65]. Albina et al. [65] explain that hydrogenase production in *Ralstonia eutropha* is controlled by a regulatory hydrogenase, which forms a tight complex with a histidine protein kinase acting as a H_2 sensor. When H_2 is present, the regulatory hydrogenase enhances the production of membrane-bound and cytoplasmic hydrogenases. More than one regulatory pathway exists in other microorganisms. For instance, *Cyanobacterium Synechocystis* has two positively acting regulators (LexA and AbrB1) and a repressor (AbrB2). [65].

The enzymes involved in sulfur oxidation (Sox) pathways present in different microorganisms be classified into two groups: Sox-dependent enzyme complex and Sox-independent enzyme system. The first one is regulated by a conserved Sox operon and includes the SoxA, SoxB, and Sox(CD)₂ enzymes, which allow the direct oxidation of various reduced inorganic sulfur contaminants (e.g., sulfide and thiosulfate) to sulfate [66]. Whereas the Sox-independent enzyme system contains a set of distinctive oxidases and hydrolases that are responsible for the oxidation of thiosulfate, sulfide, and elemental sulfur. Thiosulfate oxidation can occur in some obligate chemolithotrophic bacteria belonging to β - and γ -proteobacteria, through the formation of polythionate as intermediate with the enzyme thiosulfate dehydrogenase (also called thiosulfate quinone oxidoreductase). Later, this intermediate can be oxidized to sulfite through the cytoplasmic enzyme polythionate hydrolase [66,67]. Sulfide oxidation to elemental sulfur can be mediated either by the membrane enzyme sulfide quinone reductase, or by the periplasmic enzyme favocytochrome c reductase. Elemental sulfur produced in the periplasm can be stored as inclusion bodies and then transported to the cell interior with the help of a sulfur-bearing enzyme system. Once in the cytoplasm, they can be oxidized to sulfite by the action of the enzyme reverse-acting dissimilatory sulfite reductase. Finally, sulfite oxidation to sulfate is carried out by two types of sulfite dehydrogenases: (i) the cytoplasmic enzyme sulfide acceptor oxidoreductase; and (ii) a combination of the membrane enzyme adenylylsulfate (APS) reductase and the cytoplasmic enzyme adenosine triphosphate (ATP) sulfurylase [66,67].

Sulfate is always the end product of complete sulfur oxidation. Although full oxidation of sulfide is thermodynamically preferable, under some conditions there may be an accumulation of elemental sulfur during partial oxidation due to nitrate restriction or an excess electron source [68–70].

3.3. Cathode surface as electron source in BES

Architecturally BESs are composed of three main components. The anode and the cathode, where oxidation and reduction reactions occur, and a membrane that separates both compartments. As shown in Fig. 2, autotrophic denitrification occurs in the cathode. BESs can operate as microbial electrolysis cells (MECs) by utilizing the electrons provided through an external power source, or as microbial fuel cells (MFCs), by utilizing the electrons generated through the oxidation of organic matter in the anode chamber (Fig. 2) [57]. The provided electrons can then be used directly or indirectly in the cathode. Indirect uses of electrons include the synergic production of reduced molecules (such as H_2) or electro-stimulation of microbial metabolism [71–73]. In these cases, the denitrification process could occur through the mechanisms described in the previous section. Oxygen and carbon dioxide produced in the anode is released avoiding the permeation of these gases through the membrane [71–73].

The capture of electrons directly from the cathode surface requires electroactive denitrifying bacteria, i.e., bacteria that have extracellular electron transfer mechanisms. However, to achieve complete nitrate reduction to dinitrogen and to avoid intermediate accumulation, a more complex microbial community is needed, not only composed of electroactive bacteria [74]. Although the exact mechanisms of electron transfer during denitrification in BES are still unknown, microbial communities with a significant abundance of *Geobacter* (model electroactive bacteria) have been reported to be associated with high nitrate removals [75,76]. This genus is known to use conductive pili and proteins associated with the outer membrane as cytochromes, as direct electron transfer mechanisms [77,78]. Recently Zhao et al. [75] reported that *Geobacter* and *Afpia* were the dominant genera in autotrophic denitrification reactors, evidencing possible symbiotic growth. This, is under the hypothesis that an outer membrane cytochrome c and formate dehydrogenase were potentially involved in cathodic electron uptake in *Geobacter* [75].

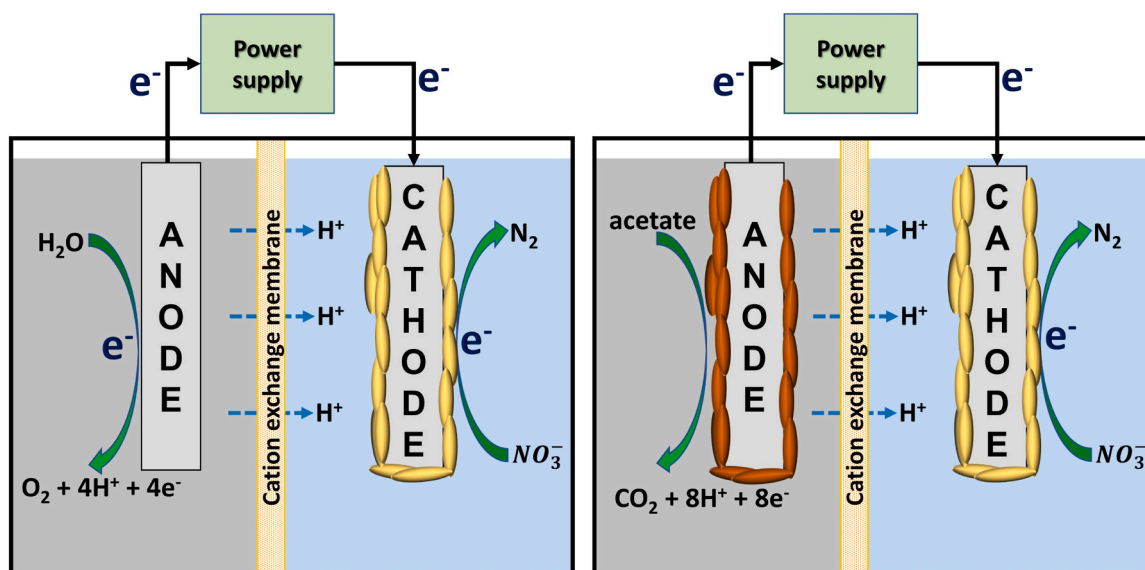


Fig. 2. Autotrophic denitrification in BESs as a MEC (left) or MFC (right) configuration.

3.4. Microorganisms involved in autotrophic denitrification

Bacteria capable of performing autotrophic denitrification have been isolated from diverse ecosystems, including natural and anthropogenic habitats such as soil, freshwater, groundwater, wastewater treatment systems, and microbial fuel cells. A complete revision of the microorganisms described that perform autotrophic denitrification and the ecosystems where they were isolated can be found in the [supplementary materials](#) (See [Table S1](#)).

In general, these microorganisms are taxonomically diverse and distributed within α -, β -, γ -, and ϵ -Proteobacteria. Some genera can perform both autotrophic and heterotrophic denitrification (such as *Acidovorax*, and *Paracoccus*), while others can only perform autotrophic denitrification (such as *Ferrigenium*, and *Thiobacillus*). Most of these isolates were retrieved from investigations in which bioremediation was the main objective.

More recently, molecular methods have been used to describe species without isolation by assembling genomes from metagenomic data (Assembled Genomes). This is very useful to know the potential metabolic pathways of species that cannot be isolated or cultured in laboratory conditions. Using this technique, Mao et al. [79] reconstructed a genome from metagenomic data obtained from a hydrogenotrophic consortium. The metagenome-assembled genome was classified within the genus *Thauera*, a genus with a very versatile metabolism, able to use a wide spectrum of recalcitrant compounds. The authors found an [NiFe]-hydrogenase catalyzing molecular H_2 oxidation in a nitrate-rich solution. They also found genes from key enzymes for autotrophic CO_2 -fixation and heterotrophic acetate assimilation metabolism, which would show the mixotrophic capability of this strain [79].

In BES, some species have been demonstrated to use the cathode directly as an electron donor (see [Table 2](#)). For example, six strains were isolated from a biocathode, classified within *Rhizobiales* in the α -Proteobacteria. The authors tested the reduction of nitrite associated with the cathode and confirmed the electron-driven nitrite reduction in four of them. However, no electrode-driven nitrate or nitrous oxide reduction could be detected for any of them. Although, according to cyclic voltammetry results, all bacteria showed electroactivity to some extent. The experimental demonstration that the cathode is the direct electron donor is challenging because electrochemical analysis, chemical, and microbiological tests are needed [80].

When BESs are used for nitrate removal from groundwater, diverse communities grow on the cathode, and bacteria described as autotrophic denitrifiers occur, as well as others with unknown roles (see [Table 2](#)). Different molecular biology tools, based on 16 S rRNA gene microbial communities' characterization, were used to analyze these communities. For example, Nguyen et al. [81] analyzed the microbial communities from a cathode that eliminates nitrate from groundwater. According to the 16 S rRNA gene DGGE results, the community comprised *Thiobacillus denitrificans*, *T. thioparus* (S-compounds denitrifying microorganisms), and *Paracoccus denitrificans* [82]. More recently, Yao et al. [83], studied the microbial community from a system combining heterotrophic denitrification using biodegradable polymers and electrochemical H_2 autotrophic denitrification to eliminate nitrate from groundwater. Using 16 S rRNA gene massive amplicon sequencing, they found that the communities comprised key crucial denitrifying bacteria: *Dechloromonas*, *Thauera*, and *Hydrogenophaga*. Using metagenomic sequencing, the authors also studied the genes involved in the nitrogen metabolic pathway; the results revealed that the conversion of NO to N_2O was the rate-limiting step in the overall denitrification pathway [83].

4. Critical issues involved in autotrophic denitrification in BES

In 1993 a process of nitrate removal by hydrogenotrophic denitrification was proposed inside a water electrolyzer. In this study, 0.165 kW-h was required to remove 10 mg N- NO_3^-/L in a 2.4 L reactor

(83 kW-h/m³) [94], which is around 20 times more than the energy required by reverse osmosis to treat the same amount of water (from 3.5 to 4.5 kW-h/m³) [95]. Since then, important advances have been made, such as the discovery of the capacity of certain microbes to reduce nitrate using electrons directly from the electrode [96], where microorganisms that degrade organic matter in the anode chamber can also produce these electrons [97]. Both aspects have made it possible to reduce the energy consumption of these systems [98]. In fact, the energy consumption in MFC and MEC configurations is estimated at around 0.04 and 0.251 kW-h/m³, respectively [98], making them competitive against traditional systems.

However, progress in system optimization is needed to improve nitrate removal rates, in order to move this technology to an industrial scale. According to Bi et al. [46], nitrate removal rates by conventional autotrophic denitrification can reach up to 6.2 g N- NO_3^-/L -d while in BES only 0.87 g N- NO_3^-/L -d, as seen in [Table 3](#). Critical factors that have hindered the scale-up of this technology to date are discussed below, considering groundwater characteristics, key operational parameters in BES, as well as the BES configuration.

4.1. Groundwater characteristics

4.1.1. C/N ratio

Groundwater is characterized by low organic matter content. More than 400 groundwater samples from eight EU were analysed resulting in an average concentration of 2.7 mg/L total organic carbon [105]. Such a low amount of organic carbon highlights the importance of autotrophic nitrate removal strategies versus the heterotrophic alternative in groundwater remediation where high carbonate contents are expected. In this context, Huang et al. [106] evaluated $NaHCO_3$ as an inorganic carbon source at different C/N ratios of 2.0, 2.7, and 3.5 during nitrate removal from synthetic groundwater using a BES. These authors confirmed the important role of autotrophic denitrification in reducing nitrate at low C/N ratios (C/N 2.0, nitrate removal 72.74%). Nonetheless, they indicated that higher C/N ratios (2.7 and 3.5) were detrimental to autotrophic denitrification (nitrate removal of 62.28% and 50.23%, respectively). In fact, when higher C/N ratios are applied, heterotrophic and autotrophic bacteria can coexist to enhance nitrate removal [74]. Another investigation assessed the feasibility of autotrophic denitrification using a MEC, at different nitrate loads ranging from 11–45 mg N- NO_3^-/L (50–200 mg NO_3^-/L) and C/N ratios in the range of 3 – 3.5 [102]. In this case, results showed a capacity to remove nitrate with a maximum efficiency of $96.3 \pm 1.5\%$ but nitrite accumulation was reported for nitrate load over 23 mg N- NO_3^-/L .

4.1.2. Conductivity

Another important characteristic of groundwater is its low conductivity or ionic strength (< 1 mS/cm). This is an important parameter because might affect the BES efficiency by increasing energy losses or overpotentials in the form of (i) high ohmic resistance, (ii) transport losses such as ion transport between electrodes or slow proton transport inside the biofilm, and (iii) resistance of the membrane because of the pH gradient between compartments [107–109]. In fact, it was shown that low ionic strength groundwaters strongly limited the nitrate removal efficiency (between 4% and 20% of nitrogen removed) when carrying out autotrophic denitrification in MFC [101]. These authors identified the cathodic overpotential as the main factor of energy loss reaching up to 83 – 90% of the total losses, which evidenced the high energy consumed by microbes in the reactions catalyzing denitrification. Thus, the presence of certain salinity levels in groundwater could effectively favor the BES performances. However, salinity levels above 30 mS/cm may strongly and negatively affect bacterial activity [110]. Despite the importance of conductivity, no systematic research has been done to have complete clarity on how to manage it, thus future research efforts should be directed along these lines.

Table 2

Microbial communities detected in autotrophic nitrate removal investigations by means of bioelectrochemical systems.

Reactor architecture	Inocula	Feeding	Method	Dominant associated microbiome on cathode	Ref.
As ³⁺ and NO ₃ ⁻ tubular removal reactor with a cation exchange membrane (CMI-7000) Anode: Granular graphite Cathode: Graphite rods	79% of synthetic groundwater, 20% of effluent from a denitrifying BES reactor (Pous et al. [84]) and 1% of an enriched autotrophic arsenite oxidizing culture	Synthetic groundwater (28 ± 6 mg N-NO ₃ ⁻ /L, 5.0 mg As ³⁺ /L, 1.0 ± 0.1 mS/cm, pH of 8.0 ± 0.2) with 10% of effluent from a denitrifying BES reactor composed of Proteobacteria phylum (78%) being <i>Acidithiobacillus</i> sp., the most abundant genus.	Illumina MiSeq, 515 F and 806 R V4 region.	<i>Sideroxydans</i> (80–54%), <i>Denitratisoma</i> (4–5%), and <i>Achromobacter</i> (2–5%), <i>Sideroxydans lithotrophicus ES-1</i>	[85]
BES reactors connected: Anode (carbon felt) of Sediment Microbial Fuel Cell (SMFC) and Cathode (stainless-steel mesh) of Biofilm Electrode Reactor (BER)	SMFC: River sediment BER: Groundwater	Synthetic groundwater (around 30 mg N-NO ₃ ⁻ /L) at pH of 6.8 with dissolved oxygen concentration under 0.5 mg/L	V3-V4 Illumina MiSeq platform primers Nobar_341F/ Nobar_805R	<i>Hyphomicrobium</i> , <i>Terrimicrobium</i> , <i>Tepidisphaera</i> , <i>Prostheobacter</i> , <i>Diaphorobacter</i> , <i>Sediminibacterium</i> , <i>Pseudoxanthomonas</i> , <i>Bacillus</i> , and <i>Pirellula</i>	[86]
Two-chamber cell Anode and cathode: Graphite felt. Four sand/medium ratios (saturated aquifer simulation)	Anaerobic sludge collected from wastewater treatment plant.	Synthetic groundwater (50 mg N-NO ₃ ⁻ /L) with 50 mM phosphate buffer. NaHCO ₃ (2 g/L) was supplied as an inorganic carbon source.	DGGE primers Eub 27 F and Eub 518 R	<i>Thiobacillus denitrificans</i> , <i>T. thioparus</i> , and <i>Paracoccus denitrificans</i>	[81]
Two-chamber cell with a proton exchange membrane (Nafion 117) Anode and cathode: Graphite felt	Activated sludge	Synthetic nitrate-contaminated water (50 mg N-NO ₃ ⁻ /L) added to cathode chamber with 2.94 g/L of NaHCO ₃ .	DGGE primers Eub 27 F and Eub 518 R	<i>Dyella</i> sp., <i>Nitratireductor</i> sp., <i>Shinella</i> sp., <i>Pseudomonas</i> sp. <i>Aeromonas</i> sp., and <i>Curtobacterium</i> sp.	[87]
Two-chamber cell Anode: Iridium mixed metal oxide-coated titanium-electrode mesh Cathode: Packed granular graphite with a stainless-steel mesh	Microbial cultures enriched from the cathodic effluent of a denitrifying BES	Synthetic nitrate-contaminated groundwater (33 mg N-NO ₃ ⁻ /L) and pH around 7	Illumina 16 S rRNA gene amplicon sequencing, 341 F and 785Rmod	<i>Gallionellaceae</i> (40–70% of the relative abundance)	[88]
Two-chamber cell with a cation exchange membrane (QQ-YLM) Anode and cathode: carbon felt.	Activated sludge from a domestic wastewater treatment plant	Synthetic medium (21 mg N-NO ₃ ⁻ /L) with dissolved oxygen concentration of 0.2–0.5 mg/L	Pyrosequencing V1–V3, 27 F and 533 R	<i>Thauera</i> sp, <i>Ralstonia</i> sp, <i>Hyphomicrobium zavarzinii</i> , <i>Hyphomicrobium aestuarii</i> , <i>Rhodoplanes</i> sp.	[89]
Single-chamber cell Rice husk-intensified cathode driving bioelectrochemical reactor. Anode: Stainless steel rod Cathode: Stainless steel mesh	Active sludge from Wastewater plant for three months in a bottle.	Synthetic nitrate-contaminated groundwater (50 mg mg N- NO ₃ ⁻ /L)	Illumina MiSeq 16 S rRNA, V3-V4 regions, primers 338 F and 806 R	0 mA/m ² : <i>Cloacibacterium</i> , 200 mA/m ² : <i>Flavobacterium</i> , <i>Comamonas</i> , <i>Erysipelothrix</i> , <i>Enterobacter</i> , <i>Novosphingobium</i> , <i>Thermomonas</i> (heterotrophic-hydrogenotrophic synergistic) 400 mA/m ² : <i>Acidovorax</i>	[90]
Single-chamber cell (PVC cylinder) Anode: Graphite rod Cathode: Carbon felt	Activated sludge from wastewater treatment plant	Synthetic groundwater (40 mg N-NO ₃ ⁻ /L) with polycaprolactone as carbon source	Illumina MiSeq V3–V4, primers 338 F and 806 R	<i>Dechloromonas</i> , <i>Thauera</i> , <i>Hydrogenophaga</i> and <i>Acidovorax</i>	[83]
Three-chamber cell Sediment Microbial Fuel Cell and Biofilm Electrode Reactor Electrodes: Carbon felt, and carbon felt modified with graphene oxide and using stainless steel mesh as support	Enriched and domesticated inoculum with indigenous bacteria from groundwater	Synthetic groundwater (35 mg N-NO ₃ ⁻ /L) with 109 mg glucose/L and dissolved oxygen concentration under 0.5 mg/L	High-throughput sequencing was performed on an Illumina HiSeq platform, V3-V4 hypervariable region	<i>Nitrospira</i> , <i>Pseudomonas</i> , <i>Pantanalinema CENA516</i> , <i>Dongia</i> , <i>Bryobacter</i> , <i>Hyphomicrobium</i>	[91]
System heterotrophic denitrification coupled with electro-autotrophic denitrifying packed bed reactor Electrolysis section Anode: Ti/RuO ₂ mesh Cathode: Stainless steel mesh Denitrification section Support material: Haycite	Activated sludge from Sewage Treatment Plant Heterotrophic denitrifying bacteria and H ₂ autotrophic denitrifying bacteria were enriched from an anaerobic seed sludge. Aerobic bacteria were enriched from an aerobic seed sludge	Synthetic groundwater (25–50 mg N-NO ₃ ⁻ /L)	Illumina MiSeq high-throughput sequencing, V4-V5 region, primer 515 F	Denitrifiers bacteria: <i>Thauera</i> , <i>Comamonadaceae</i> unclassified, <i>Rivibacter</i> , <i>Hydrogenophaga</i> , <i>Opiritatus</i> , <i>Zoogloea</i> , <i>Pelomonas</i> , <i>Thiobacillus</i> and <i>Simplicispira</i> were recognized as the major	[92]
Submerged microbial desalination-denitrification cell with the electrodes (carbon paper) separated by polycarbonate plate and to separate the chambers to the outside environment an anion exchange membrane	Electrodes pre-colonized in a two-chamber microbial fuel cell for 5 months. Anode: Active biofilm Cathode: Denitrification biofilm	Synthetic groundwater (20 mg N-NO ₃ ⁻ /L) with a C: N:P ratio of 1:667:1.	DGGE and 16 S rDNA	Deltaproteobacteria, soil bacterium clone CRS5556T-1/GU365999 (84%) <i>Shewanella</i> sp. QRSYY10/EU919225 (82%) <i>Sphingobacterium</i> sp. HPC429/DQ129738 (91%) Alphaproteobacteria, denitrifying bacterium clone 2-9/GQ324227 (93%) <i>Sphingobacteria</i> , <i>Synergistetes bacterium</i> clone 3-5/GQ324231 (88%)	[93]

(continued on next page)

Table 2 (continued)

Reactor architecture	Inocula	Feeding	Method	Dominant associated microbiome on cathode	Ref.
(AMI 7001) in the anodic chamber and cation exchange membrane (CMI 7000) in the cathodic chamber.					

Table 3

Summary of bio-electrochemical denitrification studies in water and wastewater adapted from Ceconet et al. [98].

Type of BES	Separator	Initial Concentration (mg N-NO ₃ ⁻ /L)	Cathodic chamber volume (ml)	Max. nitrate removal rate (mg N-NO ₃ ⁻ /L·d)	Ref.
MFC	PEM	100	156	528	[99]
MFC	CEM	20–110	110	500	[100]
MFC	CEM	28.32	600	51.37	[59]
MFC	CEM	14.42–15.64	600	20.8	[101]
MEC	CEM	11 to 45	675	62.15	[102]
MEC	CEM	33	240	849	[103]
MEC	PEM	50	350	1.7	[81]
MEC	CEM	20–492	1000	17.9	[104]
MEC/MDC	AEM/CEM	20	9	870	[93]

4.2. Key operational parameters in BES

4.2.1. Voltage applied

Nitrate removal efficiency is highly dependent on the applied voltage and must be optimized to achieve the desired efficiency with minimum energy consumption. Electron flow from the cathode to the bacterial cells must be adequate to provide the cells with sufficient energy for growth without compromising process costs by applying too high a voltage. There are some studies in the literature analyzing the effect of this parameter on nitrate removal efficiency. For instance, nitrate degradation from groundwater was studied in the cathodic chamber of a BES using different applied voltages (from +0.597 to −0.703 V vs SHE), reporting that the nitrate removal rate increased from 25.2 to 130.6 mg N-NO₃⁻/L·d when the cathode potential was lowered from + 0.597 to − 0.403 V vs SHE, reaching a nitrate removal efficiency of 86% [111]. In another study, the best nitrate removal conditions were also achieved by applying a cathode voltage of − 0.403 V vs SHE, but only 42.5% of the input nitrate was removed, reaching a removal rate of 0.405 mg N-NO₃⁻/L·h [112]. Following this trend, in another study in a batch system, the nitrate removal increased from 20% to 50% when the total voltage supplied to the BES with abiotic anode increased from 0.7 V to 0.9 V, whereas no higher removal efficiency was obtained at the highest voltage (1.1 V) [113]. Therefore, the application of an electric potential can enhance the denitrification process by decreasing the requirement for an external carbon source. However, the voltage applied must be balanced. Too high voltages can have negative effects on BES performance, such as i) nitrate reduction to ammonium instead of denitrification [114], ii) H₂ production acting as an electron sink due to water electrolysis [115], and iii) a significant decrease in bacterial activity due to a detrimental voltage, evidenced by a drop in bacterial diversity and richness [116]. From the literature, it can be deduced that the optimization of the applied voltage must be performed for each system depending on its configuration and the electrode material, so the selection of the applied voltage must be carefully considered taking into account that among the investigations reviewed, the use of different reactor configurations and materials have an impact on the optimum voltages applied.

4.2.2. Hydraulic retention time

Hydraulic retention time (HRT) is a key parameter in any bioprocess, including autotrophic denitrification systems, as it determines the treatment capacity of the reactor for a specific input flow rate and nitrate concentration. Optimization of this parameter is crucial for up-scaling

the technology since it directly impacts the required volume of the system and, therefore, its capital costs, which will be higher at high HRTs implying higher operational costs. An interesting investigation dealing with denitrifying bioelectrochemical systems reported that when HRT was decreased from 10.89 h to 0.46 h nitrate removal efficiency decreased from 94% to 50%, but nitrate consumption rate increased from 73 mg N-NO₃⁻/L·d to 849 mg N-NO₃⁻/L·d, respectively [103]. These HRT values are comparable with autotrophic and heterotrophic denitrification when applying biomass retention (4–0.5 h) [38]. Another study dealing with the coupling of a multi-cathode biofilm-electrode reactor with a microfiltration unit reported a decrease in nitrate removal efficiency from 90% to 30–40% when decreasing the HRT from 6 h to 20 min [117]. In addition, proper long-term acclimation of autotrophic denitrifying bacteria could also improve nitrate removal performance when high nitrogen loading and short HRTs are imposed [118]. The high removal rates obtained at short HRTs, could be attributed to increased mass transfer to the biofilm, due increased nitrate loading rates and water fluxes [103]. However, changes in HRT also produces changes in the bacterial community [119]. Moreover, it has been reported that endogenous heterotrophic denitrifying bacteria may growth by using dead cells or degradation products as carbon sources [89,120], generating differences in the current demand [103]. This phenomenon could occur at higher HRTs [103] or where chemical–physical heterogeneity is present [120]. Thus, is not clear yet whether the hydrodynamics or the nitrate availability has more relevance for the nitrate removal rate [103]. Nevertheless, the high nitrate removal rates observed at low HRTs provide a promising opportunity for scaling up the autotrophic denitrification in groundwater using series-operated BES as a strategy.

4.3. BES reactor materials

4.3.1. Electrodes

Electrode materials play a decisive role in the overall performance of a BES. In fact represent one of the research topics most frequently addressed by researchers to improve the process. The cathode interacts with the microbial community by transferring electrons for nitrate reduction, which is most efficient if an electroactive biofilm is formed. For this to occur, the material must be biocompatible, electrically conductive, and possess high stability. Carbon-based materials such as graphite felt are commonly used due to their high porosity allowing biomass retention, corrosion resistance, and relatively low costs [121]. Besides, these electrodes could be reinforced with metallic materials

such as stainless steel to optimize electron transfer [122,123].

Another relevant aspect of the reactor configuration is associated with the distance between the electrodes. Ohmic resistance within the BES must be minimized, to avoid energy losses. This can be addressed by establishing a minimum distance between electrodes, as well as a high cathodic surface area. This will allow efficient use of electrons by the microorganisms during nitrate reduction [74]. Selection of electrode materials is an open research niche, and investigations dealing with microbes-materials compatibility should be carried out, to evaluate its effect over nitrate reduction.

4.3.2. Separators

Another important factor that could contribute to the energy loss is the choice of the separator. Energy losses might occur due to the resistance generated by the material itself or by membrane fouling [121]. In case of most BES, the application of ion exchange membranes (IEM) comes with an additional cost, not only related to the materials, but also explicitly as the origin of losses during operation. The most frequently used IEMs are cation exchange membranes (CEM), among which Nafion® (e.g., N 117) perfluorosulfonic acid and Ultrex® CMI-7000 crosslinked polystyrene-divinylbenzene polymer types are the most common. Although these IEMs are excellent in terms of conductivity and selectivity, their applicability in BESs is questionable. The usually complex nature and composition of electrolytes in BESs results in a non-proton assisted electroneutralization process between the electrode chambers, simply due to the significantly higher concentration of other cations. The occupation of sulfonic acid functional groups on the IEM by these cations was shown to be complete [124]. Such phenomenon, together with the high permselectivity may lead to various losses in the system, such as pH-splitting between the electrolytes and consecutive performance loss [125], as well as reduced effective reaction kinetics due to mass transfer limitations [126].

Previous research demonstrated that the ionic conductivity of Nafion significantly decreases with the electrolyte concentration [127]. As mentioned above, since the ionic strength of groundwater is usually very low, the overpotentials related to ion transfer are expected to be a major hindrance to efficient BES operation. Ensuring smaller electrode-membrane distances, and thus, more compact reactor geometry, as well as increasing electrode and membrane surface area to electrolyte volume ratio could be adequate strategies to minimize these effects. However, at the same time, the larger active membrane surface area enhances also those transport processes which are related to membrane imperfections. For instance, O₂ transport through the IEM can have a negative effect on the MFC efficiency by acting as an alternative electron acceptor, as well as by deteriorating the anaerobic electro-active bacteria [128,129]. Considering the low capacity and moderate nitrate removal rates from groundwater in BES presented in the literature, O₂ permeation should be taken into account in process design. Permeability of cathodic H₂ towards the anolyte through – especially swollen – Nafion can also reduce electron recovery efficiency, and can be significant even at mesophilic temperatures [130,131]. These concerns should be addressed in future membrane development for BES applications such as autotrophic denitrification. Novel polymeric membrane materials with sufficient ionic conductivity and potentially higher permselectivity (and faster kinetics) for H⁺-transfer could significantly contribute to minimize ion transfer related losses. For example, Koók et al. [127] demonstrated the improved ionic conductivity of PSEBS-based CEM at low electrolyte (KCl) concentrations compared to Nafion, which not only reduced mass transport losses during MFC operation, but also enhanced the overall electricity production kinetics. In addition, this polymer membrane could be prepared with sufficiently low O₂ cross-over and excellent functional and mechanical stability even in high current density applications ($j = 1\text{--}50\text{ A/m}^2$ relative to the membrane surface area) [127,132]. The PSEBS membranes were furthermore tested in MEC for cathodic H₂ production [133]. It was concluded that they were advantageous for H₂

production due to lower mass transfer limitations and cathodic overpotentials. Besides, other materials, such as anion and cation exchange polymers, ceramics, or polymer-inorganic composites were shown to improve BES operation [134].

5. Capital cost estimation using bes for autotrophic denitrification

Despite research efforts in recent years, BES-based technologies for nitrate removal from groundwater still have significant challenges to overcome before extensive industrial applications. Affordable costs may be one of them. To quantitatively identify factors determining actual costs, an evaluation involving the determination of capital and energy costs was performed. The analysis involved the implementation of a nitrate removal system using BES, considering the drinking water demand equivalent to 5000 habitats (864 m³_{water}/day). Three inlet nitrate concentrations were considered: 14, 23 and 56 mg N-NO₃⁻/L (60, 100 and 250 mg NO₃⁻/L), and systems were conceived in order to provide an effluent with a concentration of 6.8 mg N-NO₃⁻/L. BES reactors were dimensioned applying the nitrate removal kinetics reported by Wang et al. [135] and the modified cassette design reported by Leicester et al. [136] was used for the reactor configuration. Table 4 summarizes the characteristics of the materials considered for cost estimation. The evaluated system was composed of a combination of modules with a maximum useful volume of 100 m³.

As is the case of most biological processes, conversion rates depend on concentration of substrates (electron donor, electron acceptor or nutrients). As a result, the volume of the reactor required to achieve a specific task will depend on substrate concentration within the system, which will be similar to that at the effluent, if good mixing is provided. A way to reduce reactor volume is to organize the system in reactors in series, which will provide higher concentrations in the initial reactors, increasing overall rates and reducing overall system volume. Then, cost analysis involved different scenarios combining in series and in parallel configuration of the required modules, as represented in Fig. 3.

Results of the analysis are included in Figs. 4 and 5, which present the effect of influent concentration and modules organization, on capital and energy-derived costs. Analysis includes only the energy required for BES system operation, without including mixing or pumping. Capital costs are presented as US\$ divided by system capacity (in m³ d⁻¹). On the other hand, energy costs are presented based on the treated water volume (in US\$ per m³). Nitrate concentration in groundwater significantly impacts the capital and energy costs of the proposed system. The capital costs increase significantly as the nitrate concentration in groundwater increases, since removing more nitrate requires an increase in HRT, which implies increasing the number of modules needed to meet drinking water demand (see Fig. 4). On the other hand, energy costs also increase with increasing nitrate concentration, because more electrons are required as inlet concentration increases.

As expected, the inclusion of modules organized in series (Fig. 3) produces a decrease in the required HRT, which has a significant impact on capital costs because the overall reactor size and/or number of modules is reduced. As shown in Fig. 4, the greatest impact is generated

Table 4
Summary of materials considered in capital cost estimation.

Item	Description	Cost US \$/unit
Membranes	Proton exchange membrane	160/m ²
Electrodes	Carbon felt	20/m ²
Current distributor	Stainless steel wire & woven stainless-steel mesh	26/m ²
Cassette structure	PVC Sheet	0.9/kg
Reactor external structure	Fiberglass plastic	40/m ³
Power supply	–	900/kW

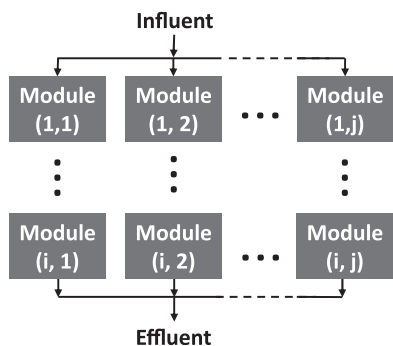


Fig. 3. Organization of modules in series and in parallel, for cost analysis. The number of modules in series is represented by i . The number of modules in parallel, by j . Then, the product of i and j represents the total number of modules.

when considering nitrate contamination of 56 mg N-NO₃⁻/L, where including modules in series can induce an investment reduction of up to 40%. When nitrate concentration is 23 mg N-NO₃⁻/L, the reduction is lower (about 20%), as a result of a lower nitrate load. Specifically, the lowest capital cost that could be achieved (10 modules) is around 9000

US\$/m³.d⁻¹, 4000 US\$/m³.d⁻¹, and 2000 US\$/m³.d⁻¹ for 56, 23 and 14 mg N-NO₃⁻/L of nitrate, respectively.

The capital costs were strongly determined by the price of the membranes, representing 65%, followed by the current distribution system (16%), the electrodes (12%), the power supply (5%), the reactor external structure (2%) and the cassette Structure (below 1%). Estimated capital costs seems very high, and far from commercially competitive. For example, seawater desalination technology by reverse osmosis has a capital cost of 1300 US\$/m³.d⁻¹ [137]. Ionic exchange and reverse osmosis systems for removal of nitrate on groundwater, have an average capital cost of 598 ± 318 US\$/m³.d⁻¹ and 3284 ± 628 US\$/m³.d⁻¹ respectively (data calculated from Jensen et al. [25] equation 21, using the reported annualized capital cost, amortization, and a flow of 0.23 MGD). The cost of a reverse osmosis system could be comparable to the cost of treating groundwater with 23 mg N-NO₃⁻/L of nitrate with a BES technology. If an inlet nitrate concentration of 14 mg N-NO₃⁻/L is considered, the price of the membranes must decrease at least 50%, for BES technology to be competitive. For higher nitrate concentrations, lowering the cost of membranes would definitely not be sufficient.

The energy costs per volume of treated water can also be significantly reduced when system is conceived in modules organized in series. This is associated with a decrease in the number of modules but is also related

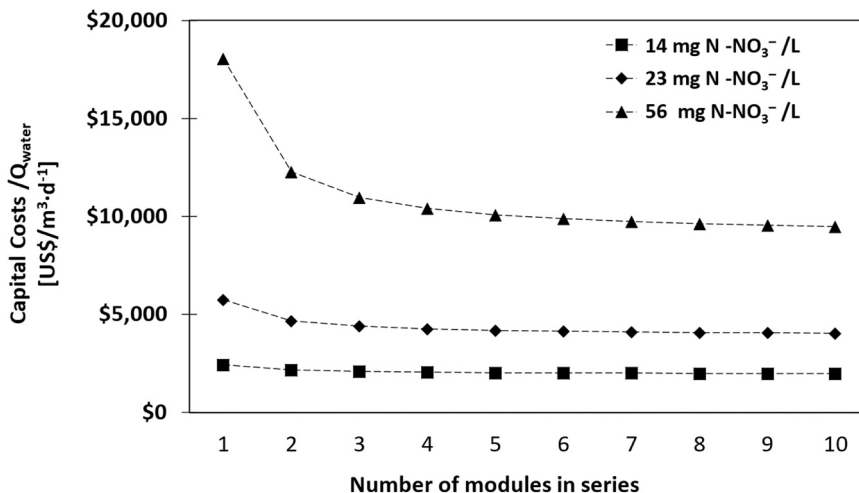


Fig. 4. Capital costs per water flow at different initial nitrate concentrations in groundwater considering reactors operated in series.

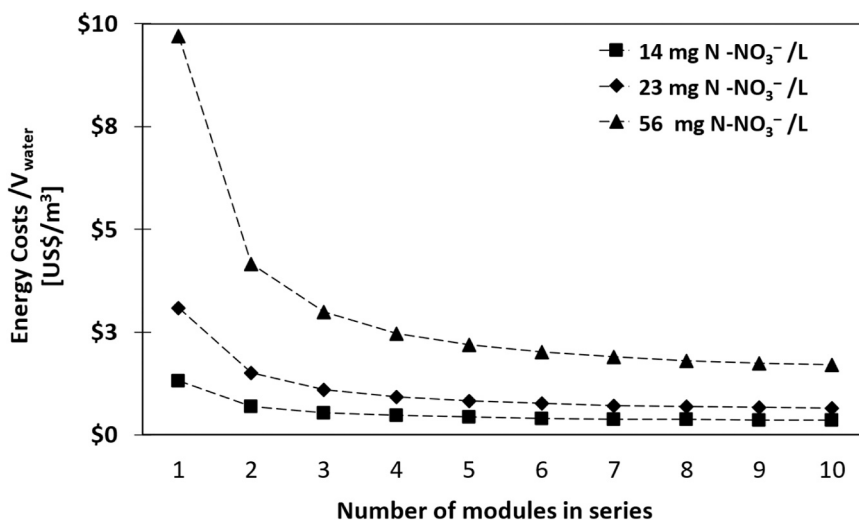


Fig. 5. Energy costs per water volume at different initial nitrate concentrations in groundwater considering reactors operated in series.

to a decrease in energy consumption when the nitrate concentration at the reactor outlet increases. As shown in Fig. 5, energy costs, independent of groundwater nitrate concentration, can be markedly decreased when including a configuration of modules in series. For example, at an inlet concentration of 56 mg N-NO₃⁻/L, changes in the configuration can promote a reduction of close to 85% in the energy requirements. Specifically, the lowest energy cost that could be achieved (10 modules) is around 1.70 US\$/m³ (4.1 kWh/m³), 0.65 US\$/m³ (1.6 kWh/m³), and 0.36 US\$/m³ (0.88 kWh/m³) for 56, 23 and 14 mg N-NO₃⁻/L, respectively. Energy consumption values are comparable with the ones obtained by Ceconet et al. [98] (0.251 kWh/m³). The energy costs estimated here for nitrate removal at groundwater concentrations of 14 and 23 mg N-NO₃⁻/L are comparable to those reported for nitrate removal from groundwater by reverse osmosis, i.e., about 0.42 US\$/m³ (1.03 kWh/m³) [138].

Finally, no economy of scale effect was observed when the analysis was performed for a population of 1000 and 25,000. This implies that treating a larger groundwater flow does not result in lower capital or energy costs, at the conditions of this analysis.

In summary, BES technology capital and energetic costs treating groundwater with 23 and 14 mg N-NO₃⁻/L may be comparable with reverse osmosis removing nitrate from groundwater, with the relevant advantage that nitrate is removed and not merely separated. However, to be competitive, it is imperative to lower the membrane costs, which represent 65% of the capital costs.

6. Conclusions

Autotrophic denitrification is a technically viable and proven alternative to permanently remove nitrate from groundwater. There are a wide variety of well-studied bacterial species involved in denitrification using various electron donors. Of particular interest are electroactive microorganisms, which can reduce nitrate when grown symbiotically with an electrode as an electron donor in BES. BES-based technologies for nitrate removal are relatively new but there is considerable research conducted for other applications of BES, which undoubtedly allows strengthening the fundamental know-how of these systems. However, there are still significant challenges to be addressed for industrial applications. In this context, the development of efficient and low-cost membranes, as well as the improvement of microbial community efficiency by reducing the hydraulic residence time, seems crucial. Future research should concentrate on ensuring systems that can treat industrial-scale volumes at a competitive price, but without compromising system efficiency and avoiding overpotential losses.

CRedit authorship contribution statement

E. Ortega-Martínez, E. Fernandez, R. Cardena, C Etchebehere, A. Cabezas, L. Koók, P. Bakonyi, J. A. Magdalena, E. Trably, N. Bernet: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Validation, Visualization, Writing - original draft, Writing - review & editing. **D. Jeison, J. Toledo-Alarcon, R. Oyarzún, J. L Campos:** Funding acquisition, Project administration, Resources, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: David Jeison reports financial support was provided by ANID Chile. Javiera Toledo reports financial support was provided by ANID Chile. Eduardo Ortega reports financial support was provided by ANID Chile. Jose Luis Campos reports financial support was provided by ANID Chile. Angela Cabezas reports financial support was provided by ANII Uruguay. Claudia Etchebehere reports financial support was provided

by ANII Uruguay. Rene Cardena reports financial support was provided by ANII Uruguay. Jose Antonio Magdalena reports financial support was provided by Complutense University Madrid. Laszlo Kook reports financial support was provided by Hungarian Academy of Sciences.

Data availability

No data was used for the research described in the article.

Acknowledgements

The researchers would like to thank the REDES – CONICYT N°190032 project. Javiera Toledo-Alarcón would like to thank the funding provided by FONDECYT project number 3210456 (ANID, Chile). Eduardo Ortega-Martinez, David Jeison and José Luis Campos were supported by CRHIAM center (ANID/FONDAP/15130015). László Koók was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences. Jose Antonio Magdalena would like to thank Complutense University Madrid for financing his contract at LBE-INRAE (France), with funds from the Ministry of Universities for the requalification of the Spanish University System for 2021–2023 (Modality 1. Margarita Salas), coming from the European Union-Next generation EU funding. Ángela Cabezas and Claudia Etchebehere would like to thank the funding provided by the National System of Researchers in Uruguay (SNI-ANII). René Cardena would like to thank the funding provided by the Postdoctoral fellowship from ANII.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jece.2023.111552.

References

- [1] G. Ferguson, J.C. McIntosh, S. Jasechko, J.-H. Kim, J.S. Famiglietti, J. J. McDonnell, Groundwater deeper than 500 m contributes less than 0.1% of global river discharge, *Commun. Earth Environ.* 4 (2023), 48, <https://doi.org/10.1038/s43247-023-00697-6>.
- [2] W. Qiu, T. Ma, Y. Wang, J. Cheng, C. Su, J. Li, Review on status of groundwater database and application prospect in deep-time digital earth plan, *Geosci. Front.* 13 (2022), 101383, <https://doi.org/10.1016/j.gsf.2022.101383>.
- [3] T. Gleeson, K.M. Befus, S. Jasechko, E. Luijendijk, M.B. Cardenas, The global volume and distribution of modern groundwater, *Nat. Geosci.* 9 (2016) 161–167, <https://doi.org/10.1038/ngeo2590>.
- [4] UN-Water, Groundwater Overview: Making the invisible visible, (2018).
- [5] P. Ravenscroft, Lucy Lytton, Seeing the Invisible: A Strategic Report on Groundwater Quality, 2022.
- [6] R. Damania, S. Desbureaux, A.-S. Rodella, J. Russ, E. Zaveri, Quality Unknown: The Invisible Water Crisis, Washington, DC: World Bank, 2019. <https://doi.org/10.1596/978-1-4648-1459-4>.
- [7] M.H. Ward, R.R. Jones, J.D. Brender, T.M. de Kok, P.J. Weyer, B.T. Nolan, C. M. Villanueva, S.G. van Breda, Drinking water nitrate and human health: an updated review, *Int J. Environ. Res. Public Health* 15 (2018), <https://doi.org/10.3390/ijerph15071557>.
- [8] R. Picetti, M. Deeney, S. Pastorino, M.R. Miller, A. Shah, D.A. Leon, A. D. Dangour, R. Green, Nitrate and nitrite contamination in drinking water and cancer risk: A systematic review with meta-analysis, *Environ. Res* 210 (2022), 112988, <https://doi.org/10.1016/j.envres.2022.112988>.
- [9] J. Schullehner, B. Hansen, M. Thygesen, C.B. Pedersen, T. Sigsgaard, Nitrate in drinking water and colorectal cancer risk: a nationwide population-based cohort study, *Int J. Cancer* 143 (2018) 73–79, <https://doi.org/10.1002/ijc.31306>.
- [10] C. Donat-Vargas, M. Kogevinas, G. Castaño-Vinyals, B. Pérez-Gómez, J. Llorca, M. Vanaclocha-Espí, G. Fernandez-Tardon, L. Costas, N. Aragonés, I. Gómez-Acebo, V. Moreno, M. Pollan, C.M. Villanueva, Long-term exposure to nitrate and trihalomethanes in drinking water and prostate cancer: a Multicase-Control Study in Spain (MCC-Spain), *Environ. Health Perspect.* 131 (2023), <https://doi.org/10.1289/EHP11391>.
- [11] A.S. Jensen, V.R. Coffman, J. Schullehner, B.B. Trabjerg, C.B. Pedersen, B. Hansen, J. Olsen, M. Pedersen, L.T. Stayner, T. Sigsgaard, Prenatal exposure to tap water containing nitrate and the risk of small-for-gestational-age: a nationwide register-based study of Danish births, 1991–2015, *Environ. Int* 174 (2023), 107883, <https://doi.org/10.1016/j.envint.2023.107883>.
- [12] N.H. Ebdrup, J. Schullehner, U.B. Knudsen, Z. Liew, A.M.L. Thomsen, J. Lyngsø, B. Bay, L.H. Arendt, P.J. Clemmensen, T. Sigsgaard, B. Hansen, C.H. Ramlau-Hansen, Drinking water nitrate and risk of pregnancy loss: a nationwide cohort

- study, *Environ. Health* 21 (2022), 87, <https://doi.org/10.1186/s12940-022-00897-1>.
- [13] D.M. Manassaram, L.C. Backer, D.M. Moll, A review of nitrates in drinking water: maternal exposure and adverse reproductive and developmental outcomes, *Environ. Health Perspect.* 114 (2006) 320–327, <https://doi.org/10.1289/ehp.8407>.
- [14] L.A. Croen, K. Todoroff, G.M. Shaw, Maternal exposure to nitrate from drinking water and diet and risk for neural tube defects, *Am. J. Epidemiol.* 153 (2001) 325–331, <https://doi.org/10.1093/aje/153.4.325>.
- [15] E. Abascal, L. Gómez-Coma, I. Ortiz, A. Ortiz, Global diagnosis of nitrate pollution in groundwater and review of removal technologies, *Sci. Total Environ.* 810 (2022), 152233, <https://doi.org/10.1016/j.scitotenv.2021.152233>.
- [16] Washington state department of health, Nitrate Treatment and Remediation for Small Water Systems: Guidance document, 2018.
- [17] J.J. Schoeman, A. Steyn, Nitrate removal with reverse osmosis in a rural area in South Africa, *Desalination* 155 (2003) 15–26, [https://doi.org/10.1016/S0011-9164\(03\)00235-2](https://doi.org/10.1016/S0011-9164(03)00235-2).
- [18] A. Rahman, N.C. Mondal, K.K. Tiwari, Anthropogenic nitrate in groundwater and its health risks in the view of background concentration in a semi arid area of Rajasthan, India, *Sci. Rep.* 11 (2021), <https://doi.org/10.1038/s41598-021-88600-1>.
- [19] M. Thaw, M. GebreEgziabher, J.Y. Villafañe-Pagán, S. Jasechko, Modern groundwater reaches deeper depths in heavily pumped aquifer systems, *Nat. Commun.* 13 (2022), 5263, <https://doi.org/10.1038/s41467-022-32954-1>.
- [20] B.C. Jurgens, K. Faulkner, P.B. McMahon, A.G. Hunt, G. Casile, M.B. Young, K. Beliz, Over a third of groundwater in USA public-supply aquifers is Anthropocene-age and susceptible to surface contamination, *Commun. Earth Environ.* 3 (2022), 153, <https://doi.org/10.1038/s43247-022-00473-y>.
- [21] T. Riedel, C. Kübeck, M. Quirin, Legacy nitrate and trace metal (Mn, Ni, As, Cd, U) pollution in anaerobic groundwater: quantifying potential health risk from “the other nitrate problem, *Appl. Geochem.* 139 (2022), 105254, <https://doi.org/10.1016/j.apgeochem.2022.105254>.
- [22] J. Nolan, K.A. Weber, Natural uranium contamination in major U.S. aquifers linked to nitrate, *Environ. Sci. Technol. Lett.* 2 (2015) 215–220, <https://doi.org/10.1021/acs.estlett.5b00174>.
- [23] R.M. Coyte, R.C. Jain, S.K. Srivastava, K.C. Sharma, A. Khalil, L. Ma, A. Vengosh, Large-scale uranium contamination of groundwater resources in India, *Environ. Sci. Technol. Lett.* 5 (2018) 341–347, <https://doi.org/10.1021/acs.estlett.8b00215>.
- [24] A. Castaño-Sánchez, G.C. Hose, A.S.P.S. Reboleira, Ecotoxicological effects of anthropogenic stressors in subterranean organisms: a review, *Chemosphere* 244 (2020), 125422, <https://doi.org/10.1016/j.chemosphere.2019.125422>.
- [25] V. Jensen, J. Darby, C. Seidel, C. Gorman, Technical Report 6: Drinking Water Treatment for Nitrate, 2012. <http://groundwater.nitrates.ucdavis.edu>.
- [26] V.B. Jensen, J.L. Darby, C. Seidel, C. Gorman, Nitrate in potable water supplies: alternative management strategies, *Crit. Rev. Environ. Sci. Technol.* 44 (2014) 2203–2286, <https://doi.org/10.1080/10643389.2013.828272>.
- [27] S.K. Archana, R.C. Sharma, Sobti, Nitrate removal from ground water: a review, *E-J. Chem.* 9 (2012) 1667–1675, <https://doi.org/10.1155/2012/154616>.
- [28] R.C. Scholes, M.A. Vega, J.O. Sharp, D.L. Sedlak, Nitrate removal from reverse osmosis concentrate in pilot-scale open-water unit process wetlands, *Environ. Sci. Technol.* 7 (2021) 650–661, <https://doi.org/10.1039/D0EW00911C>.
- [29] M. Belkacem, S. Bekhti, K. Bensadok, Groundwater treatment by reverse osmosis, *Desalination* 206 (2007) 100–106, <https://doi.org/10.1016/j.desal.2006.02.062>.
- [30] A. Mengesha, O. Sahu, Sustainability of membrane separation technology on groundwater reverse osmosis process, *Clean. Eng. Technol.* 7 (2022), 100457, <https://doi.org/10.1016/j.clet.2022.100457>.
- [31] I. Ersever, V. Ravindran, H.-H. Tsai, M. Pirbazari, Modeling and design of anaerobic fluidized bed reactor with recycling for denitrification of reverse osmosis concentrates, *Chem. Eng. Sci.* 108 (2014) 111–122, <https://doi.org/10.1016/j.ces.2013.12.036>.
- [32] F. Rezvani, M.-H. Sarrafzadeh, S. Ebrahimi, H.-M. Oh, Nitrate removal from drinking water with a focus on biological methods: a review, *Environ. Sci. Pollut. Res.* 26 (2019) 1124–1141, <https://doi.org/10.1007/s11356-017-9185-0>.
- [33] Y. Shelly, M. Kuk, O. Menashe, G. Zeira, S. Azerrad, E. Kurzbaum, Nitrate removal from a nitrate-rich reverse osmosis concentrate: superior efficiency using the bioaugmentation of an *Acinetobacter* biofilm, *J. Water Process Eng.* 44 (2021), 102425, <https://doi.org/10.1016/j.jwpe.2021.102425>.
- [34] A. Mengesha, O. Sahu, Sustainability of membrane separation technology on groundwater reverse osmosis process, *Clean. Eng. Technol.* 7 (2022), 100457, <https://doi.org/10.1016/j.clet.2022.100457>.
- [35] R.C. Scholes, M.A. Vega, J.O. Sharp, D.L. Sedlak, Nitrate removal from reverse osmosis concentrate in pilot-scale open-water unit process wetlands, *Environ. Sci. Technol.* 7 (2021) 650–661, <https://doi.org/10.1039/D0EW00911C>.
- [36] S.K.M. Huno, E.R. Rene, E.D. Van Hullebusch, A.P. Annachatre, Nitrate removal from groundwater: a review of natural and engineered processes, *J. Water Supply: Res. Technol. - AQUA* 67 (2018) 885–902, <https://doi.org/10.2166/aqua.2018.194>.
- [37] S. Shukla, A. Saxena, Global status of nitrate contamination in groundwater: its occurrence, health impacts, and mitigation measures. *Handbook of Environmental Materials Management*, Springer International Publishing, Cham, 2019, pp. 869–888, https://doi.org/10.1007/978-3-319-73645-7_20.
- [38] Z. Wang, X. Fei, S. He, J. Huang, W. Zhou, Comparison of heterotrophic and autotrophic denitrification processes for treating nitrate-contaminated surface water, *Sci. Total Environ.* 579 (2017) 1706–1714, <https://doi.org/10.1016/j.scitotenv.2016.11.194>.
- [39] Y. Wang, S. Ren, P. Wang, B. Wang, K. Hu, J. Li, Y. Wang, Z. Li, S. Li, W. Li, Y. Peng, Autotrophic denitrification using Fe(II) as an electron donor: a novel prospective denitrification process, *Sci. Total Environ.* 858 (2023), 159721, <https://doi.org/10.1016/j.scitotenv.2022.159721>.
- [40] A. Mohseni-Bandpi, D.J. Elliott, M.A. Zazouli, Biological nitrate removal processes from drinking water supply—a review, *J. Environ. Health Sci. Eng.* 11 (2013), 35, <https://doi.org/10.1186/2052-336X-11-35>.
- [41] S.-S. Wang, H.-Y. Cheng, H. Zhang, S.-G. Su, Y.-L. Sun, H.-C. Wang, J.-L. Han, A.-J. Wang, A. Guadie, Sulfur autotrophic denitrification filter and heterotrophic denitrification filter: comparison on denitrification performance, hydrodynamic characteristics and operating cost, *Environ. Res.* 197 (2021), 111029, <https://doi.org/10.1016/j.envres.2021.111029>.
- [42] F. Hammes, C. Berger, O. Köster, T. Egli, Assessing biological stability of drinking water without disinfectant residuals in a full-scale water supply system, *J. Water Supply Res. Technol. - Aqua* 59 (2010) 31–40, <https://doi.org/10.2166/aqua.2010.052>.
- [43] I.A. Vasiladou, S. Pavlou, D.V. Vayenas, A kinetic study of hydrogenotrophic denitrification, *Process Biochem.* 41 (2006) 1401–1408, <https://doi.org/10.1016/j.procbio.2006.02.002>.
- [44] L. Yu, Y. Yuan, S. Chen, L. Zhuang, S. Zhou, Direct uptake of electrode electrons for autotrophic denitrification by *Thiobacillus* denitrificans, *Electrochem Commun.* 60 (2015) 126–130, <https://doi.org/10.1016/j.elecom.2015.08.025>.
- [45] T.O. Strohm, B. Griffin, W.G. Zumft, B. Schink, Growth yields in bacterial denitrification and nitrate ammonification, *Appl. Environ. Microbiol.* 73 (2007) 1420–1424, <https://doi.org/10.1128/AEM.02508-06>.
- [46] Z. Bi, Q. Zhang, X. Xu, Y. Yuan, N. Ren, D.-J. Lee, C. Chen, Perspective on inorganic electron donor-mediated biological denitrification process for low C/N wastewaters, *Bioresour. Technol.* 363 (2022), 127890, <https://doi.org/10.1016/j.biortech.2022.127890>.
- [47] J. Zhou, L. Ding, M. Shi, R.E.F. Lindeboom, C. Cui, The key factor in high pressure hydrogenotrophic denitrification: hydrogen partial pressure, *J. Water Process Eng.* 49 (2022), 103195, <https://doi.org/10.1016/j.jwpe.2022.103195>.
- [48] K.-C. Lee, B.E. Rittmann, Applying a novel autohydrogenotrophic hollow-fiber membrane biofilm reactor for denitrification of drinking water, *Water Res.* 36 (2002) 2040–2052, [https://doi.org/10.1016/S0043-1354\(01\)00425-0](https://doi.org/10.1016/S0043-1354(01)00425-0).
- [49] K.-C. Lee, B.E. Rittmann, Effects of pH and precipitation on autohydrogenotrophic denitrification using the hollow-fiber membrane-biofilm reactor, *Water Res.* 37 (2003) 1551–1556, [https://doi.org/10.1016/S0043-1354\(02\)00519-5](https://doi.org/10.1016/S0043-1354(02)00519-5).
- [50] T. Tian, H.-Q. Yu, Denitrification with non-organic electron donor for treating low C/N ratio wastewaters, *Bioresour. Technol.* 299 (2020), 122686, <https://doi.org/10.1016/j.biortech.2019.122686>.
- [51] L. Christianson, C. Lepine, S. Tsukuda, K. Saito, S. Summerfelt, Nitrate removal effectiveness of fluidized sulfur-based autotrophic denitrification biofilters for recirculating aquaculture systems, *Aquac. Eng.* 68 (2015) 10–18, <https://doi.org/10.1016/j.aquaeng.2015.07.002>.
- [52] R. Li, M. Guan, W. Wang, Simultaneous arsenite and nitrate removal from simulated groundwater based on pyrrhotite autotrophic denitrification, *Water Res.* 189 (2021), 116662, <https://doi.org/10.1016/j.watres.2020.116662>.
- [53] E.A. Dasi, J.A. Cunningham, E. Talla, S.J. Ergas, Autotrophic denitrification supported by sphalerite and oyster shells: chemical and microbiome analysis, *Bioresour. Technol.* 375 (2023), 128820, <https://doi.org/10.1016/j.biortech.2023.128820>.
- [54] C. Juncher Jørgensen, O.S. Jacobsen, B. Elberling, J. Aamand, Microbial oxidation of pyrite coupled to nitrate reduction in anoxic groundwater sediment, *Environ. Sci. Technol.* 43 (2009) 4851–4857, <https://doi.org/10.1021/es803417s>.
- [55] Y. Hu, G. Wu, R. Li, L. Xiao, X. Zhan, Iron sulphides mediated autotrophic denitrification: an emerging bioprocess for nitrate pollution mitigation and sustainable wastewater treatment, *Water Res.* 179 (2020), 115914, <https://doi.org/10.1016/j.watres.2020.115914>.
- [56] J. suo Lu, T. ting Lian, J. feng Su, Effect of zero-valent iron on biological denitrification in the autotrophic denitrification system, *Res. Chem. Intermed.* 44 (2018) 6011–6022, <https://doi.org/10.1007/s11164-018-3472-3>.
- [57] T. Zheng, J. Li, Y. Ji, W. Zhang, Y. Fang, F. Xin, W. Dong, P. Wei, J. Ma, M. Jiang, Progress and prospects of bioelectrochemical systems: electron transfer and its applications in the microbial metabolism, *Front Bioeng. Biotechnol.* 8 (2020), <https://doi.org/10.3389/fbioe.2020.00010>.
- [58] S.A. Patil, C. Hägerhäll, L. Gorton, Electron transfer mechanisms between microorganisms and electrodes in bioelectrochemical systems, *Bioanal. Rev.* 4 (2012) 159–192, <https://doi.org/10.1007/s12566-012-0033-x>.
- [59] N. Pous, S. Puig, M. Coma, M.D. Balaguer, J. Colprim, Bioremediation of nitrate-polluted groundwater in a microbial fuel cell, *J. Chem. Technol. Biotechnol.* 88 (2013) 1690–1696, <https://doi.org/10.1002/jctb.4020>.
- [60] A. Ceballos-Escalera, N. Pous, M.D. Balaguer, S. Puig, Electrochemical water softening as pretreatment for nitrate electro bioremediation, *Sci. Total Environ.* 806 (2022), 150433, <https://doi.org/10.1016/j.scitotenv.2021.150433>.
- [61] W.G. Zumft, Cell biology and molecular basis of denitrification, *Microbiol. Mol. Biol. Rev.* 61 (1997) 533–616, <https://doi.org/10.1128/mmlbr.61.4.533-616.1997>.
- [62] P. Tavares, A.S. Pereira, J.J.G. Moura, I. Moura, Metalloenzymes of the denitrification pathway, *J. Inorg. Biochem.* 100 (2006) 2087–2100, <https://doi.org/10.1016/j.jinorgbio.2006.09.003>.
- [63] C. Moreno-Vivián, P. Cabello, M. Martínez-Luque, R. Blasco, F. Castillo, Prokaryotic nitrate reduction: molecular properties and functional distinction among bacterial nitrate reductases, *J. Bacteriol.* 181 (1999) 6573–6584, <https://doi.org/10.1128/JB.181.21.6573-6584.1999>.

- [64] M. Winkler, J. Esselborn, T. Happe, Molecular basis of [FeFe]-hydrogenase function, *Biochim. Et. Biophys. Acta (BBA) - Bioenerg.* 1827 (2013) 974–985, <https://doi.org/10.1016/j.bbabi.2013.03.004>.
- [65] P. Albina, N. Durban, A. Bertron, A. Albrecht, J.-C. Robinet, B. Erable, Influence of hydrogen electron donor, alkaline pH, and high nitrate concentrations on microbial denitrification: a review, *Int J. Mol. Sci.* 20 (2019) 5163, <https://doi.org/10.3390/ijms20205163>.
- [66] Y.-X. Cui, B.K. Biswal, G. Guo, Y.-F. Deng, H. Huang, G.-H. Chen, D. Wu, Biological nitrogen removal from wastewater using sulphur-driven autotrophic denitrification, *Appl. Microbiol Biotechnol.* 103 (2019) 6023–6039, <https://doi.org/10.1007/s00253-019-09935-4>.
- [67] O. Franchi, J. Toledo-Alarcón, J.L. Campos, D. Jeison Nuñez, A. Vaksmaa, E. Tapia-Venegas, Diversity of nitrogen-removing microorganisms. Development in Wastewater Treatment Research and Processes, Elsevier, 2022, pp. 133–164, <https://doi.org/10.1016/B978-0-323-91901-2.00009-7>.
- [68] R.B. Cardoso, R. Sierra-Alvarez, P. Rowlette, E.R. Flores, J. Gómez, J.A. Field, Sulfide oxidation under chemolithoautotrophic denitrifying conditions, *Biotechnol. Bioeng.* 95 (2006) 1148–1157, <https://doi.org/10.1002/bit.21084>.
- [69] B.S. Moraes, T.S.O. Souza, E. Foresti, Effect of sulfide concentration on autotrophic denitrification from nitrate and nitrite in vertical fixed-bed reactors, *Process Biochem.* 47 (2012) 1395–1401, <https://doi.org/10.1016/j.procbio.2012.05.008>.
- [70] Q. MAHMOOD, P. ZHENG, J. CAI, D. WU, B. HU, J. LI, Anoxic sulfide biooxidation using nitrite as electron acceptor, *J. Hazard. Mater.* 147 (2007) 249–256, <https://doi.org/10.1016/j.jhazmat.2007.01.002>.
- [71] A. Callegari, S. Bolognesi, D. Ceconet, Operation of a 2-stage bioelectrochemical system for groundwater denitrification, *Water* 11 (2019) 959, <https://doi.org/10.3390/w11050959>.
- [72] S. Dehghani, A. Rezaee, S. Hosseinkhani, Biostimulation of heterotrophic-autotrophic denitrification in a microbial electrochemical system using alternating electrical current, *J. Clean. Prod.* 200 (2018) 1100–1110, <https://doi.org/10.1016/j.jclepro.2018.08.013>.
- [73] F. Zhao, J. Xin, M. Yuan, L. Wang, X. Wang, A critical review of existing mechanisms and strategies to enhance N₂ selectivity in groundwater nitrate reduction, *Water Res.* 209 (2022), 117889, <https://doi.org/10.1016/j.watres.2021.117889>.
- [74] J. Sun, H. Cao, Z. Wang, Progress in nitrogen removal in bioelectrochemical systems, *Processes* 8 (2020) 831, <https://doi.org/10.3390/pr8070831>.
- [75] T. Zhao, B. Xie, Y. Yi, Y. Zang, H. Liu, Two polarity reversal modes lead to different nitrate reduction pathways in bioelectrochemical systems, *Sci. Total Environ.* 856 (2023), 159185, <https://doi.org/10.1016/j.scitotenv.2022.159185>.
- [76] W. Guo, X. Ying, N. Zhao, S. Yu, X. Zhang, H. Feng, Y. Zhang, H. Yu, Interspecies electron transfer between Geobacter and denitrifying bacteria for nitrogen removal in bioelectrochemical system, *Chem. Eng. J.* 455 (2023), 139821, <https://doi.org/10.1016/j.cej.2022.139821>.
- [77] R.C.G. Creasey, A.B. Mostert, T.A.H. Nguyen, B. Virdis, S. Freguia, B. Laycock, Microbial nanowires – Electron transport and the role of synthetic analogues, *Acta Biomater.* 69 (2018) 1–30, <https://doi.org/10.1016/j.actbio.2018.01.007>.
- [78] D.R. Lovley, D.J.F. Walker, Geobacter protein nanowires, *Front Microbiol* 10 (2019), <https://doi.org/10.3389/fmicb.2019.02078>.
- [79] Y. Mao, Y. Xia, Z. Wang, T. Zhang, Reconstructing a Thaueria genome from a hydrogenotrophic-denitrifying consortium using metagenomic sequence data, *Appl. Microbiol Biotechnol.* 98 (2014) 6885–6895, <https://doi.org/10.1007/s00253-014-5756-x>.
- [80] A. Vilar-Sanz, N. Pous, S. Puig, M.D. Balaguer, J. Colprim, L. Bañeras, Denitrifying nirK-containing alphaproteobacteria exhibit different electrode driven nitrite reduction capacities, *Bioelectrochemistry* 121 (2018) 74–83, <https://doi.org/10.1016/j.bioelechem.2018.01.007>.
- [81] V.K. Nguyen, Y. Park, J. Yu, T. Lee, Bioelectrochemical denitrification on biocathode buried in simulated aquifer saturated with nitrate-contaminated groundwater, *Environ. Sci. Pollut. Res.* 23 (2016) 15443–15451, <https://doi.org/10.1007/s11356-016-6709-y>.
- [82] D. Nguyen, S.K. Khanal, A little breath of fresh air into an anaerobic system: how microaeration facilitates anaerobic digestion process, *Biotechnol. Adv.* 36 (2018) 1971–1983, <https://doi.org/10.1016/j.biotechadv.2018.08.007>.
- [83] S. Yao, L. Liu, S. Zhang, X. Tang, Nitrate removal from groundwater by heterotrophic and electro-autotrophic denitrification, *Water* 14 (2022) 1759, <https://doi.org/10.3390/w14111759>.
- [84] N. Pous, S. Puig, M. Dolores Balaguer, J. Colprim, Cathode potential and anode electron donor evaluation for a suitable treatment of nitrate-contaminated groundwater in bioelectrochemical systems, *Chem. Eng. J.* 263 (2015) 151–159, <https://doi.org/10.1016/j.cej.2014.11.002>.
- [85] A. Ceballos-Escalera, N. Pous, P. Chiluiza-Ramos, B. Korth, F. Harnisch, L. Bañeras, M.D. Balaguer, S. Puig, Electro-bioremediation of nitrate and arsenite polluted groundwater, *Water Res* 190 (2021), 116748, <https://doi.org/10.1016/j.watres.2020.116748>.
- [86] R. Liu, X. Zheng, M. Li, L. Han, X. Liu, F. Zhang, X. Hou, A three chamber bioelectrochemical system appropriate for in-situ remediation of nitrate-contaminated groundwater and its reaction mechanisms, *Water Res* 158 (2019) 401–410, <https://doi.org/10.1016/j.watres.2019.04.047>.
- [87] V.K. Nguyen, S. Hong, Y. Park, K. Jo, T. Lee, Autotrophic denitrification performance and bacterial community at biocathodes of bioelectrochemical systems with either abiotic or biotic anodes, *J. Biosci. Bioeng.* 119 (2015) 180–187, <https://doi.org/10.1016/j.jbiosc.2014.06.016>.
- [88] X. Wang, A. PrévotEAU, K. Rabaey, Impact of periodic polarization on groundwater denitrification in bioelectrochemical systems, *Environ. Sci. Technol.* 55 (2021) 15371–15379, <https://doi.org/10.1021/acs.est.1c03586>.
- [89] Y. Xiao, Y. Zheng, S. Wu, Z.-H. Yang, F. Zhao, Bacterial community structure of autotrophic denitrification biocathode by 454 pyrosequencing of the 16 S rRNA gene, *Microb. Ecol.* 69 (2015) 492–499, <https://doi.org/10.1007/s00248-014-0492-4>.
- [90] L. Xue, N. Chen, J. Zhao, C. Yang, C. Feng, Rice husk-intensified cathode driving bioelectrochemical reactor for remediating nitrate-contaminated groundwater, *Sci. Total Environ.* 837 (2022), 155917, <https://doi.org/10.1016/j.scitotenv.2022.155917>.
- [91] Y. Yang, M. Li, R. Liu, X. Ma, J. Li, W. Li, X. Liu, X. Lei, Y. Long, P. Wang, H. Wang, Enhanced self-powered system with graphene oxide modified electrode for simultaneous remediation of nitrate-contaminated groundwater and river sediment, *J. Clean. Prod.* 315 (2021), 128059, <https://doi.org/10.1016/j.jclepro.2021.128059>.
- [92] T. Peng, C. Feng, W. Hu, N. Chen, Q. He, S. Dong, Y. Xu, Y. Gao, M. Li, Treatment of nitrate-contaminated groundwater by heterotrophic denitrification coupled with electro-autotrophic denitrifying packed bed reactor, *Biochem Eng. J.* 134 (2018) 12–21, <https://doi.org/10.1016/j.bej.2018.02.016>.
- [93] Y. Zhang, I. Angelidaki, A new method for in situ nitrate removal from groundwater using submerged microbial desalination-denitrification cell (SMDDC), *Water Res* 47 (2013) 1827–1836, <https://doi.org/10.1016/j.watres.2013.01.005>.
- [94] Y. Sakakibara, M. Kuroda, Electric prompting and control of denitrification, *Biotechnol. Bioeng.* 42 (1993) 535–537, <https://doi.org/10.1002/bit.260420418>.
- [95] E.W. Tow, A.L. Hartman, A. Jaworowski, I. Zucker, S. Kum, M. AzadiAghdam, E. R. Blatchley, A. Achilli, H. Gu, G.M. Urper, D.M. Warsinger, Modeling the energy consumption of potable water reuse schemes, *Water Res X* 13 (2021), 100126, <https://doi.org/10.1016/j.wroa.2021.100126>.
- [96] K.B. Gregory, D.R. Bond, D.R. Lovley, Graphite electrodes as electron donors for anaerobic respiration, *Environ. Microbiol* 6 (2004) 596–604, <https://doi.org/10.1111/j.1462-2920.2004.00593.x>.
- [97] P. Clauwaert, K. Rabaey, P. Aelterman, L. De Schampelaire, T.H. Pham, P. Boeckx, N. Boon, W. Verstraete, Biological denitrification in microbial fuel cells, *Environ. Sci. Technol.* 41 (2007) 3354–3360, <https://doi.org/10.1021/es062580r>.
- [98] D. Ceconet, S. Zou, A.G. Capodaglio, Z. He, Evaluation of energy consumption of treating nitrate-contaminated groundwater by bioelectrochemical systems, *Sci. Total Environ.* 636 (2018) 881–890, <https://doi.org/10.1016/j.scitotenv.2018.04.336>.
- [99] J.-T. Li, S.-H. Zhang, Y.-M. Hua, Performance of denitrifying microbial fuel cell subjected to variation in pH, COD concentration and external resistance, *Water Sci. Technol.* 68 (2013) 250–256, <https://doi.org/10.2166/wst.2013.250>.
- [100] P. Clauwaert, J. Desloover, C. Shea, R. Nerenberg, N. Boon, W. Verstraete, Enhanced nitrogen removal in bio-electrochemical systems by pH control, *Biotechnol. Lett.* 31 (2009) 1537–1543, <https://doi.org/10.1007/s10529-009-0048-8>.
- [101] S. Puig, M. Coma, J. Desloover, N. Boon, J. Colprim, M.D. Balaguer, Autotrophic denitrification in microbial fuel cells treating low ionic strength waters, *Environ. Sci. Technol.* 46 (2012) 2309–2315, <https://doi.org/10.1021/es2030609>.
- [102] D. Ceconet, M. Devecseri, A. Callegari, A.G. Capodaglio, Effects of process operating conditions on the autotrophic denitrification of nitrate-contaminated groundwater using bioelectrochemical systems, *Sci. Total Environ.* 613–614 (2018) 663–671, <https://doi.org/10.1016/j.scitotenv.2017.09.149>.
- [103] N. Pous, S. Puig, M.D. Balaguer, J. Colprim, Effect of hydraulic retention time and substrate availability in denitrifying bioelectrochemical systems, *Environ. Sci.* 3 (2017) 922–929, <https://doi.org/10.1039/C7EW00145B>.
- [104] H.II Park, D. kun Kim, Y.-J. Choi, D. Pak, Nitrate reduction using an electrode as direct electron donor in a biofilm-electrode reactor, *Process Biochem.* 40 (2005) 3383–3388, <https://doi.org/10.1016/j.procbio.2005.03.017>.
- [105] D.C. Goody, K. Hinsby, *Organic Quality of Groundwaters*, in: Natural Groundwater Quality, Blackwell Publishing, Ltd, Oxford, UK, n.d.: pp. 59–70. <https://doi.org/10.1002/9781444300345.ch3>.
- [106] B. Huang, H. Feng, M. Wang, N. Li, Y. Cong, D. Shen, The effect of C/N ratio on nitrogen removal in a bioelectrochemical system, *Bioresour. Technol.* 132 (2013) 91–98, <https://doi.org/10.1016/j.biortech.2012.12.192>.
- [107] R.A. Rozendal, H.V.M. Hamelers, K. Rabaey, J. Keller, C.J.N. Buisman, Towards practical implementation of bioelectrochemical wastewater treatment, *Trends Biotechnol.* 26 (2008) 450–459, <https://doi.org/10.1016/j.tibtech.2008.04.008>.
- [108] B.E. Logan, B. Hamelers, R. Rozendal, U. Schröder, J. Keller, S. Freguia, P. Aelterman, W. Verstraete, K. Rabaey, Microbial fuel cells: methodology and technology, *Environ. Sci. Technol.* 40 (2006) 5181–5192, <https://doi.org/10.1021/es0605016>.
- [109] X. Cao, X. Huang, P. Liang, K. Xiao, Y. Zhou, X. Zhang, B.E. Logan, A new method for water desalination using microbial desalination cells, *Environ. Sci. Technol.* 43 (2009) 7148–7152, <https://doi.org/10.1021/es901950j>.
- [110] T. Hülsen, K. Hsieh, D.J. Batstone, Saline wastewater treatment with purple phototrophic bacteria, *Water Res.* 160 (2019) 259–267, <https://doi.org/10.1016/j.watres.2019.05.060>.
- [111] N. Pous, S. Puig, M. Dolores Balaguer, J. Colprim, Cathode potential and anode electron donor evaluation for a suitable treatment of nitrate-contaminated groundwater in bioelectrochemical systems, *Chem. Eng. J.* 263 (2015) 151–159, <https://doi.org/10.1016/j.cej.2014.11.002>.

- [112] H. Xu, N. Tong, S. Huang, W. Hayat, S. Fazal, J. Li, S. Li, J. Yan, Y. Zhang, Simultaneous autotrophic removal of sulphate and nitrate at different voltages in a bioelectrochemical reactor (BER): evaluation of degradation efficiency and characterization of microbial communities, *Bioresour. Technol.* 265 (2018) 340–348, <https://doi.org/10.1016/j.biortech.2018.06.040>.
- [113] V.K. Nguyen, S. Hong, Y. Park, K. Jo, T. Lee, Autotrophic denitrification performance and bacterial community at biocathodes of bioelectrochemical systems with either abiotic or biotic anodes, *J. Biosci. Bioeng.* 119 (2015) 180–187, <https://doi.org/10.1016/j.jbiosc.2014.06.016>.
- [114] V.K. Nguyen, Y. Park, H. Yang, J. Yu, T. Lee, Effect of the cathode potential and sulfate ions on nitrate reduction in a microbial electrochemical denitrification system, *J. Ind. Microbiol. Biotechnol.* 43 (2016) 783–793, <https://doi.org/10.1007/s10295-016-1762-6>.
- [115] S. Kondaveeti, S.-H. Lee, H.-D. Park, B. Min, Bacterial communities in a bioelectrochemical denitrification system: the effects of supplemental electron acceptors, *Water Res* 51 (2014) 25–36, <https://doi.org/10.1016/j.watres.2013.12.023>.
- [116] H. Xu, N. Tong, S. Huang, W. Hayat, S. Fazal, J. Li, S. Li, J. Yan, Y. Zhang, Simultaneous autotrophic removal of sulphate and nitrate at different voltages in a bioelectrochemical reactor (BER): evaluation of degradation efficiency and characterization of microbial communities, *Bioresour. Technol.* 265 (2018) 340–348, <https://doi.org/10.1016/j.biortech.2018.06.040>.
- [117] M. Prosnansky, Y. Sakakibara, M. Kuroda, High-rate denitrification and SS rejection by biofilm-electrode reactor (BER) combined with microfiltration, *Water Res* 36 (2002) 4801–4810, [https://doi.org/10.1016/S0043-1354\(02\)00206-3](https://doi.org/10.1016/S0043-1354(02)00206-3).
- [118] W. Xing, D. Li, J. Li, Q. Hu, S. Deng, Nitrate removal and microbial analysis by combined micro-electrolysis and autotrophic denitrification, *Bioresour. Technol.* 211 (2016) 240–247, <https://doi.org/10.1016/j.biortech.2016.03.044>.
- [119] Q. Deng, C. Su, Z. Chen, T. Gong, X. Lu, Z. Chen, X. Lin, Effect of hydraulic retention time on the denitrification performance and metabolic mechanism of a multi-chambered bio-electrochemical system, *J. Environ. Manag.* 299 (2021), 113575, <https://doi.org/10.1016/j.jenvman.2021.113575>.
- [120] N. Pous, C. Koch, A. Vilà-Rovira, M.D. Balaguer, J. Colprim, J. Mühlberg, S. Müller, F. Harnisch, S. Puig, Monitoring and engineering reactor microbiomes of denitrifying bioelectrochemical systems, *RSC Adv.* 5 (2015) 68326–68333, <https://doi.org/10.1039/C5RA12113B>.
- [121] D. Su, Y. Chen, Advanced bioelectrochemical system for nitrogen removal in wastewater, *Chemosphere* 292 (2022), 133206, <https://doi.org/10.1016/j.chemosphere.2021.133206>.
- [122] H. Wang, Q. Yang, Q. Yan, Q. Wen, Primary insight into the cathode strengthened electrons transport and nitrous oxide reduction during hydrogenotrophic denitrification in bioelectrochemical system (BES), *J. Environ. Chem. Eng.* 9 (2021), 104723, <https://doi.org/10.1016/j.jece.2020.104723>.
- [123] S.S. Gadegaonkar, Ü. Mander, M. Espenberg, A state-of-the-art review and guidelines for enhancing nitrate removal in bio-electrochemical systems (BES), *J. Water Process Eng.* 53 (2023), <https://doi.org/10.1016/j.jwpe.2023.103788>.
- [124] R.A. Rozendal, H.V.M. Hamelers, C.J.N. Buisman, Effects of membrane cation transport on pH and microbial fuel cell performance, *Environ. Sci. Technol.* 40 (2006) 5206–5211, <https://doi.org/10.1021/es060387r>.
- [125] T.H.J.A. Sleutels, A. terHeijne, P. Kuntke, C.J.N. Buisman, H.V.M. Hamelers, Membrane selectivity determines energetic losses for ion transport in bioelectrochemical systems, *ChemistrySelect* 2 (2017) 3462–3470, <https://doi.org/10.1002/slct.201700064>.
- [126] M. Oliot, S. Galier, H. Roux de Balmann, A. Bergel, Ion transport in microbial fuel cells: key roles, theory and critical review, *Appl. Energy* 183 (2016) 1682–1704, <https://doi.org/10.1016/j.apenergy.2016.09.043>.
- [127] L. Koók, J. Žitka, S. Szakács, T. Rózsenszki, M. Otmar, N. Nemestóthy, K. Bélafi-Bakó, P. Bakonyi, Efficiency, operational stability and biofouling of novel sulfomethylated polystyrene-block-poly(ethylene-ran-butylene)-block-polystyrene cation exchange membrane in microbial fuel cells, *Bioresour. Technol.* 333 (2021), 125153, <https://doi.org/10.1016/j.biortech.2021.125153>.
- [128] F. Zhao, F. Harnisch, U. Schröder, F. Scholz, P. Bogdanoff, I. Herrmann, Challenges and constraints of using oxygen cathodes in microbial fuel cells, *Environ. Sci. Technol.* 40 (2006) 5193–5199, <https://doi.org/10.1021/es060332p>.
- [129] L. Koók, N. Nemestóthy, P. Bakonyi, A. Göllei, T. Rózsenszki, P. Takács, A. Salekovic, G. Kumar, K. Bélafi-Bakó, On the efficiency of dual-chamber biocatalytic electrochemical cells applying membrane separators prepared with imidazolium-type ionic liquids containing [NTf₂] – and [PF₆] – anions, *Chem. Eng. J.* 324 (2017) 296–302, <https://doi.org/10.1016/j.cej.2017.05.022>.
- [130] C. Francia, V.S. Ijeri, S. Specchia, P. Spinelli, Estimation of hydrogen crossover through Nafion® membranes in PEMFCs, *J. Power Sources* 196 (2011) 1833–1839, <https://doi.org/10.1016/j.jpowsour.2010.09.058>.
- [131] A. Selim, G.P. Szijjártó, L. Románszki, A. Tompos, Development of WO₃-nafion based membranes for enabling higher water retention at low humidity and enhancing PEMFC performance at intermediate temperature operation, *Polymers* 14 (2022) 2492, <https://doi.org/10.3390/polym14122492>.
- [132] L. Koók, L.F.M. Rosa, F. Harnisch, J. Žitka, M. Otmar, N. Nemestóthy, P. Bakonyi, J. Kretschmar, Functional stability of novel homogeneous and heterogeneous cation exchange membranes for abiotic and microbial electrochemical technologies, *J. Memb. Sci.* 658 (2022), 120705, <https://doi.org/10.1016/j.memsci.2022.120705>.
- [133] R. Cardena, L. Koók, J. Žitka, P. Bakonyi, B. Galajdová, M. Otmar, N. Nemestóthy, G. Buitrón, Evaluation and ranking of polymeric ion exchange membranes used in microbial electrolysis cells for biohydrogen production, *Bioresour. Technol.* 319 (2021), 124182, <https://doi.org/10.1016/j.biortech.2020.124182>.
- [134] L. Koók, G. Dörög, P. Bakonyi, T. Rózsenszki, N. Nemestóthy, K. Bélafi-Bakó, J. Abonyi, Directions of membrane separator development for microbial fuel cells: a retrospective analysis using frequent itemset mining and descriptive statistical approach, *J. Power Sources* 478 (2020), 229014, <https://doi.org/10.1016/j.jpowsour.2020.229014>.
- [135] C. Wang, J. Dong, W. Hu, Y. Li, Enhanced simultaneous removal of nitrate and perchlorate from groundwater by bioelectrochemical systems (BESs) with cathodic potential regulation, *Biochem. Eng. J.* 173 (2021), <https://doi.org/10.1016/j.bej.2021.108068>.
- [136] D.D. Leicester, J.M. Amezcaga, A. Moore, E.S. Heidrich, Optimising the hydraulic retention time in a pilot-scale microbial electrolysis cell to achieve high volumetric treatment rates using concentrated domestic wastewater, *Molecules* 25 (2020), <https://doi.org/10.3390/molecules25122945>.
- [137] D. Brakemeier, Economic Assessment between Reverse Osmosis and Solar Thermal Desalination, Santiago - Chile, 2015.
- [138] N. Pous, S. Puig, M.D. Balaguer, J. Colprim, Effect of hydraulic retention time and substrate availability in denitrifying bioelectrochemical systems, *Environ. Sci.* 3 (2017) 922–929, <https://doi.org/10.1039/C7EW00145B>.