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Challenges and Applications of Nitrate-Reducing Microbial Biocathodes

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Abstract

Bioelectrochemical systems which employ microbes as electrode catalysts to convert chemical energy into electrical energy (or conversely), have emerged in recent years for water sanitation and energy recovery. Microbial biocathodes, and especially those reducing nitrate are gaining more and more attention. The nitrate-reducing biocathodes can efficiently treat nitrate-polluted wastewater. However, they require specific conditions and they have not yet been applied on a large scale. In this review, the current knowledge on nitrate-reducing biocathodes will be summarized. The fundamentals of microbial biocathodes will be discussed, as well as the progress towards applications for nitrate reduction in the context of water treatment. Nitrate-reducing biocathodes will be compared with other nitrate-removal techniques and the challenges and opportunities of this approach will be identified.

1. Introduction

Due to population growth and development of modern agriculture practices, emission of various pollutants is increasing and contributing to climate change and other negative environmental effects. Among these pollutants, nitrate from fertilized fields can contaminate surface and groundwater. In order to remove or decrease nitrate concentration in water, many physical, chemical and biological approaches have been investigated including denitrification - the microbial nitrate reduction to nitrogen. In the following sections we will introduce the nitrogen cycle and current challenges connected with increasing nitrate concentrations. Common methods for nitrate removal will be briefly described, with focus on biological and in particular bioelectrochemical methods. Microbial Fuel Cells, Microbial Electrolysis Cells and Microbial Electrochemical Snorkels will then be described. In the next part, we will focus on the nitrate-reducing biocathodes (NRB). The principles of biocathodes will be discussed and the best condition for their development will be analyzed: optimal pH, oxygen concentration, polarization or external resistance and electrode material. The cyclic voltammograms of nitrate-reducing biocathodes will be compared next in order to identify nitrate reduction peaks. We will also present the most commonly found bacterial species which are able to reduce nitrate with a cathode as the electron source. Because denitrification can lead to harmful by-products or intermediates before the complete nitrate reduction to dinitrogen, these species will be discussed in the next section. Later, we will explain the effect of changing the role of a bioelectrode (between bioanode and biocathode) by changing polarization or other conditions. Finally, the nitrate-reducing biocathodes coupled with other applications, such as power production, nitrification, anammox, and removal of other pollutants such as perchlorate and sulfur compounds will be discussed.

1.1. Nitrogen cycle

In the natural N cycle, the fixation of gaseous dinitrogen (N_2) requires energy in order to activate and break the stable $N\equiv N$ triple bond. This energy can be physical (lightning), creating nitric (NO) or nitrous oxides (N_2O), or biochemical through catalysis by nitrogen fixing bacteria, producing ammonia (NH_3). The latter can be also produced in the process of ammonification - bacterial transformation of nitrogen organic compounds into ammonia. Ammonia can then be used by nitrifying bacteria in nitrification, which is an aerobic process of biological oxidation of ammonia to nitrogen oxides. Usually, nitrification occurs in two sequential oxidative steps performed by different bacterial genera: ammonia oxidation to nitrite (NO_2^-) and nitrite oxidation to nitrate (NO_3^-) (Fig. 1) [1]. Then, nitrate can be assimilated by

plants or reduced by denitrification: a microbial process of dissimilatory reduction of nitrate or nitrite to gaseous oxides (nitric oxide or nitrous oxide) which can be further reduced to dinitrogen (N₂) [2]. Anammox (anaerobic ammonium oxidation) is another metabolic group of bacteria known to carry out a denitrification decoupled from the oxidation of organic carbon in anoxia, i.e. they directly couple the oxidation of ammonium to the reduction of nitrite or nitrate [3]. These bacteria carry out a denitrification decoupled from the oxidation of organic carbon in anoxia, i.e. they directly couple the oxidation of ammonium to the reduction of nitrite or nitrate. Finally, the anaerobic respiration of chemoorganotrophic microbes in organic matter rich environment, can result in dissimilatory nitrate reduction to ammonium (DNRA) and the nitrogen cycle also includes a complete ammonium oxidation (comammox) in one organism that belongs to *Nitrospira* genus [4]. Generally, reactive nitrogen can exist in the form of ammonium, nitrate, nitrite, nitrous and nitric oxides, nitrous and nitric acids, peroxyacyl nitrates, as well as nitrogen organic compounds such as amino acids etc., which circulate between atmospheric, terrestrial and marine ecosystems [5,6].

Nowadays, half of the global nitrogen fixation is anthropogenic [7], mainly performed using the industrial Haber-Bosch process, but also by legume crops or fossil fuel combustion [8]. Currently, food production for around half of the world population depends on nitrogen fertilizer input [9]. The anthropogenic operations provide around 45% of total fixed nitrogen produced annually. From 1960 to 2000, the use of nitrogen fertilizer increased by 800%. The excess of nitrogen in soil increases the productivity and CO₂ uptake of ecosystems, but decreases the biodiversity. The most abundant nitrogen species in these fertilizers is NH₄⁺, which is easily transformed to NO₃⁻ and leaks to surface and ground waters. It results in eutrophication, toxic algae blooms making the environment harmful for aquatic fauna. Part of these nitrogen species will be incompletely denitrified, creating nitrous oxide, which also contributes to climate change (N₂O is a greenhouse gas with global warming potential 265-300 times higher relative to CO₂) and substantial contribution to acid rain and photochemical smog [6,8,10]. Moreover, the presence of nitrate in drinking water might be toxic for humans, which is mainly attributed to its reduction to nitrite. Nitrite causes oxidation of hemoglobin which becomes unable to transport oxygen to tissues. This condition, called methaemoglobinaemia, is particularly dangerous for infants. High intake of nitrate and/or nitrite could be also carcinogenic [11]. The European Union allows up to 11.3 mg N-NO₃⁻ L⁻¹ (50 mg NO₃⁻ L⁻¹) and 0.14 mg N-NO₂⁻ L⁻¹ (0.5 mg NO₂⁻ L⁻¹) in drinking water, with a condition that [NO₃⁻]/50

+ $[\text{NO}_2^-]/3 \leq 1$ [12], while in the United States the limit is $10 \text{ mg N-NO}_3^- \text{ L}^{-1}$ and $1 \text{ mg N-NO}_2^- \text{ L}^{-1}$ [13].

2. Nitrate removal methods

There are several methods for nitrate removal which can be divided into physical, chemical, electrochemical [14], as well as biological and bioelectrochemical methods.

2.1. Physical methods

Physical methods for nitrate removal are separation-based and include reverse osmosis, ion exchange and adsorption. Reverse osmosis is a process using a partially permeable membrane, in which the applied pressure overcomes the osmotic pressure which allows the separation of unwanted ions or other molecules [15–17]. Activated carbon can be used to adsorb the nitrate from water, however its activation requires high temperature or chemical treatment. [18–20]. Ion exchange is a method in which nitrate-loaded water is flowing through an anion exchange resin bed, where nitrate ions are exchanged for chloride. Once the resin's exchange capacity is exhausted, it can be regenerated using a concentrated chloride solution [21,22]. All in all, most of the separation-based methods are effective but expensive, not always selective for nitrate and require reprocessing of the generated waste brine [23]. This nitrate brine could be purified with electrochemical methods, however the challenge of this approach is to combine the reduction of nitrate with oxidation of released ammonium as well as to avoid the formation of undesired by-products of this reaction [24,25].

2.2. Chemical and photochemical methods

Among chemical methods, some involve nitrate reduction by metals, for example zero valent iron [26] or magnesium [27]. Big scale applications of these methods are limited by the difficulty of preventing the formation of ammonia or other undesired products and by the limitation of reaction rate by slow diffusion of large particles. The use of metal powder raises another challenge for subsequent separation [23]. Another approach is to use the photocatalytic reduction of nitrate over semiconductor materials, such as titanium dioxide. This technique could be used for treating brine containing nitrate from separation-based methods, however, it also requires additional electron donors, for example formic acid [28].

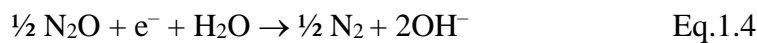
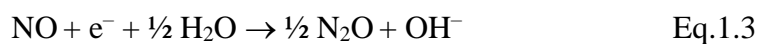
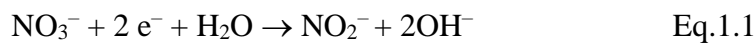
2.3. Electrochemical methods

Electrochemical methods include electrocoagulation and electrodialysis. Electrocoagulation is a process of water treatment that consists of several steps: first, a sacrificial anode is oxidized which leads to the formation of oxides, hydroxides or

oxyhydroxides. These ions cause the destabilization of contaminants resulting in coagulation and formation of floc, which can be more easily separated from clean water [29]. In electro dialysis, the applied electric potential difference influences the transport of ions through ion-exchange membrane. The electro dialysis cell consists of two compartments: diluted feed and concentrated brine; which are formed by anion- and cation-exchange membranes placed between two electrodes. The pollutant ions are migrating to a given electrode through the membrane. Usually, the electro dialysis cell is composed of stacks of membranes and electrodes [30–33].

2.4. Biological methods

The main biological method of nitrate removal is denitrification, which was already briefly defined in Part 1.1. The complete denitrification consists of four reduction steps from nitrate towards dinitrogen and requires the use of 5 moles of electrons per mole of nitrogen atom [34]:



Denitrifying bacteria are mostly facultative anaerobes and denitrification occurs in the absence of oxygen with nitrogen oxides as terminal electron acceptors in the respiratory process. Since the formal potential of $\text{O}_2/\text{H}_2\text{O}$ at pH 7 (0.82 V) is significantly higher than that of NO_3^-/N_2 (0.74 V, Fig. 2), the energy resulting of the oxidation by O_2 of organic matter (e.g. glucose) is higher. Therefore, the respiration of oxygen is preferred over nitrate and, moreover, oxygen acts as a repressor of the gene expression of the nitrate respiratory chain [2]. Indeed, terminal electrons acceptors are not equal in term of energy production and microorganisms regulate their metabolism according to the available electron acceptors present in the environment [35]. In heterotrophic denitrification, organic carbon source such as carbohydrates, organic alcohols, amino acids and fatty acids is required to act as electron donor. Some denitrifying bacteria can, however, utilize inorganic compounds, such as dihydrogen or reduced sulfur compounds as electron donors in lithotrophic denitrification [1,36]. Abundance of accessible electron donors and low oxygen concentration are the two most important factors for the denitrification to occur [37–39]. Other parameters, such as nitrate concentration, hydraulic conditions, pH, temperature, water composition (salinity, inhibitory substances) or microbial

communities have secondary effects [40,41]. For efficient nitrate reduction to dinitrogen, a proper C/N ratio should be kept. For easily accessible compounds, the chemical oxygen demand (COD, mg O₂ L⁻¹)/N-NO₃⁻ w/w ratio ranges from 3.0 to 6.0, depending on the nature of the carbon compound and on the bacterial species. Consequently, the C/N ratio is a factor influencing the final product of heterotrophic denitrification [42]. Denitrification can occur in terrestrial, freshwater and marine systems, where nitrate and nitrite are present [43]. The large scale application of biological denitrification brings several challenges, such as the risk of gaseous nitrous oxide production, the need to dose precise amount of carbon compounds - large enough to ensure complete denitrification and low enough to prevent excessive growth of biomass - redundancy of organic compounds in effluent, and necessity to keep low oxygen concentration [44].

Hydrogenotrophic denitrification (lithotrophic denitrification with the use of H₂ as electron donor) is an interesting process because of the high selectivity for nitrate removal and lack of harmful by-product; the only products are dinitrogen and water therefore no post-treatment is required. However, the main limitation is the risk that dihydrogen can create an explosive atmosphere in treatment plants [45].

Many different strategies can be employed to improve the heterotrophic biological denitrification in water, such as sludge systems or denitrifying bioreactors. In general, the activated sludge process is using biological floc made of bacteria and protozoa to treat wastewaters [46]. Denitrification can occur in an anaerobic digestion process with the use of anaerobic methanogenic sludge [47]. Denitrifying bioreactors include denitrification beds, denitrification walls, denitrification layers or woodchips bioreactors. They have solid carbon substrates in the flow path of nitrate contaminated water. These substrates, usually fragmented wood products, prevent the shortage of electron donors so the reactor can last up to 15 years [48]. Constructed wetlands in free surface flow configuration are simple ecological engineering systems that take advantage of the processes occurring in natural wetlands, such as wetland vegetation, sediments and microbial communities of wetlands to assist in treating waters in controlled environment [49].

The combination of different methods is also studied, for example nitrate removal by ion exchange, where brine is later treated by biological denitrification [50].

2.5. Bioelectrochemical systems for denitrification

Bioelectrochemical systems (BES) can link electrode materials and bioelectrochemistry to biological denitrification. BES include microbial fuel cells (MFC), microbial electrochemical snorkel (MES), microbial electrolysis cells (MEC) and derivatives. In this section we shall discuss successively Bioelectrodes, MFC, MEC and MES.

Bioelectrodes

BES have at least one bioelectrode, i.e. an electrode covered by a biofilm able to perform electron transfer with the electrode surface. Heterotrophic microorganisms oxidize organic matter through catabolism and the electrons released in this process are used for respiration. Respiratory enzymes catalyze the transfer of these electrons from organic compounds to generally soluble terminal electron acceptors, such as oxygen, nitrate or sulfate, which are then reduced. Some bacteria are also able to catalyze an extracellular transfer of electrons to solid terminal electron acceptor such as metal oxides. These bacteria are called exoelectrogens and the process exoelectrogenesis. *Shewanella* and *Geobacter* are the best described genera gathering such exoelectrogen bacteria. These bacteria can form biofilms on solid electrodes and making them a bioanode. Conversely, other bacteria can likewise develop onto cathodes and use them as electron donor [51]. Extracellular solid electron donors in natural environment can be for example sulfur or solid ferrous species like magnetite [52,53].

Extracellular electron transfer can occur by two main pathways: direct electron transfer through close contact or nanowires, or through indirect electron transfer using mediators (Fig. 3) [54]. Direct electron transfer requires a physical contact between the electrode surface and the bacterial cell membrane or nanowires. The membrane should contain small redox proteins such as c-type cytochromes at their external surface. If the microorganisms are not capable of such direct electrons exchange, redox mediators or by-products can be used to carry the electron from the cell to the electrode by indirect - mediated electron transfer. Some bacteria can release outside of cell redox compounds, such as menaquinone, that can be used also as electron shuttles. Manganese and iron oxides can be also used as electron acceptor [55,56]. The extracellular polymeric substances of bacteria in biofilms can contribute to either direct or indirect electron transfer. It helps attaching cells to the solid electrode and therefore shortens the gap between cell membrane and the electrode surface for direct transfer. Non-electroactive proteins such as protamine, can also promote electron transfer, probably by influencing the architecture of the biofilm-like material [57]. In case of indirect electron transfer, the polymeric

substances are beneficial because it accumulates electron shuttles between bacteria and electron donors or acceptors [58].

Microbial Fuel Cells (MFC)

MFC are bioelectrochemical systems capable of energy production from biomass exploiting electrogenic bacteria [59]. A standard MFC is shown on Fig. 4. It consists of two compartments separated by an ion-exchange membrane. In the first compartment, the oxidation of electron donors (organic compounds like acetate) is performed by the biofilm covering the electrode – the electrode here plays the role of electron acceptor making it a microbial bioanode. The transferred electrons are then transported to the cathode in the second compartment, where a reduction reaction occurs. In an MFC at least one electrode should be colonized by an electrogenic biofilm –bioanode or biocathode. Usually, when the MFC is designed to produce power, oxygen is used as the electron acceptor. Many modifications were proposed to increase the cathodic efficiency, including the air-cathode MFC, in which the uptake of oxygen from the air removes the necessity of energy consuming air bubbling of the catholyte [60]. Another possible electron acceptor is nitrate, reduced on NRB [34]. Nitrate reduction rate (NRR) achieved with NRB should be competitive with other methods for nitrate removal, such as constructed wetlands or denitrifying bioreactors. For constructed wetlands, the highest reported values of NRR are in a range of about 3.7 to 4.5 g N-NO₃⁻ m⁻³ day⁻¹ [61,62]. Denitrification walls have a NRR of up to 5.5 g N-NO₃⁻ m⁻³ day⁻¹ [48,63,64] and for denitrification beds, it could be up to 22-23 g N-NO₃⁻ m⁻³ day⁻¹ [48,65]. The articles exploring NRB performances are giving a wide range of values of NRR which are listed in Tables 1 and 2 in the Supplementary Material. Some of the most efficient nitrate reducing BES are detailed next.

In an early study describing MFC including NRB, Viridis et al. reached a NRR of 410 g N-NO₃⁻ m⁻³ day⁻¹. This setup was built to treat one wastewater stream containing acetate and ammonium, which was first fed into an anaerobic anodic chamber in order to remove organic compounds, and then was passed through external aerobic nitrification stage and finally the denitrification occurred in the cathodic chamber. The acetate was removed completely and only small concentrations of nitrate remained in the cathodic effluent, some having been reduced to ammonium. The best denitrification values were achieved for an external resistance value between the anode and the cathode of 5-10 Ω [66]. Clauwaert *et al.* operated their BES in MFC mode (producing electrical energy) and MEC mode (electrical energy is applied to drive the electrochemical reactions). The reported NRR was up to 500 g N-NO₃⁻ m⁻³ day⁻¹ with the pH controlled at 7.2 in the cathodic compartment (220 g N-NO₃⁻ m⁻³ day⁻¹ with no control

of the pH) [67]. Zhang *et al.* also built a denitrifying BES consisting of two chambers, in which the cathode potential was kept at around 0.04 V vs standard hydrogen electrode, SHE (–0.2 V vs saturated calomel electrode, SCE). These authors followed nitrate reduction starting from different initial concentrations and they described the results of reduction rate using the Monod equation, which allowed to estimate that the maximum NRR in this experiment was up to 1330 g N-NO₃⁻ m⁻³ day⁻¹ [68]. Clauwaert *et al.* in the first work describing MFC with NRB obtained 146 g N-NO₃⁻ m⁻³ day⁻¹ [34]. Al-Mamun *et al.* combined MFC with an anaerobic sequencing batch reactor which allowed to treat insoluble, polymeric and complex organic compounds together with nitrate, coupling it with energy harvesting. They reached a NRR up to 125.7 g N-NO₃⁻ m⁻³ day⁻¹ [69]. One of the highest reported NRR values so far was that of Vijay *et al.*, who used soil and cow manure and combined heterotrophic and autotrophic denitrification to remove 6500 g N-NO₃⁻ m⁻³ day⁻¹. The electrode surface area was relatively low and there was cow manure and soil in the cathodic chamber (serving as bacterial and electro donor source), therefore probably the significant part of nitrate was removed by biological denitrification. Nevertheless, closed circuit MFC increased the rate by up to two or three times compared with open circuit, which means that bioelectrochemical denitrification also played a role [70].

These examples are showing that employing bioelectrochemical nitrate reduction can be beneficial to treat nitrate-polluted waters at larger scale. The NRR can be much higher than those obtained with constructed wetlands or denitrifying bioreactors. However, the research focusing on NRB is still in its early stage. NRB are usually prepared in smaller scale and the NRR per 1 m³ is calculated on the basis of laboratory results. Increasing the scale could affect the efficiency. Choosing the most optimal system for application could also be difficult because of very different materials and conditions in different studies. For example, due to the popular choice of electrode materials with unclear defined surface area, such as graphite granules or felt, comparison of efficiencies vs electrode area is problematic. The actual cost of its application is an additional question to consider. Nevertheless, MFC employing NRB have a great potential to be applied to polluted wastewaters treatment.

Microbial Electrolysis Cells (MEC)

MEC are devices similar to MFC, also consisting of two electrodes in anodic and cathodic compartments, separated by a membrane. In contrast to MFCs, MEC does not produce power, but it is connected to a power source. The applied voltage is used to generate chemical compounds such as hydrogen or methane. For nitrate removal application, this hydrogen can

be used as electron donor to reduce nitrate through lithotrophic denitrification [71]. In order to eliminate the need of external power supply, MEC is sometimes paired with MFC, that produces electric energy needed for hydrogen production [72]. Recently, applying three air cathode MFCs connected in series with MEC, resulted in an increase of denitrification rate from 56.5% to 80.6% (control experiment was autotrophic denitrification MFC, starting concentrations were 20-50 mg N-NO₃⁻ L⁻¹) [73].

Microbial Electrochemical Snorkel (MES)

MES is a specific type of MFC composed of two electrodes in short-circuit, which could be even simplified to one piece of electrode immersed in two different environments: sediment and water (Figure 5). In sediment, the anodic biofilm formed on the electrode oxidizes the available organic electron donors. Since the sediment is anaerobic and also poor in nitrates, the access to electron acceptors is limited so the solid electrode can be used for this role. Therefore, the electrons coming from the oxidation are then transported via the electrode to the cathodic biofilm formed on the part located in water, where they are used to reduce the electron acceptor: oxygen, nitrate or other compounds. Since the two bioelectrodes are in short-circuit, no power can be produced in such configuration but it allows reaching the maximum current. In the seminal study that introduced the concept of MES, the device was applied to COD treatment and reached around 55% of removal. For comparison, the traditional MFC with 1000 Ω resistance reached only 35% of COD removal [74]. The MES also accelerated the oxidative reactions in oil-contaminated sediment, the oxygen uptake and the CO₂ evolution in microcosms [75]. Additionally, it increased reduction of sulfate driven by organic contaminant oxidation in the bulk sediment [76]. The system with MES consisting of calcined petroleum coke placed in the columns, through which the contaminated influent was circulating, was introduced as Electroactive Biofilm-Based Constructed Wetlands for improving the COD and phosphate removal efficiency [77]. Moreover, positive electric potentials were measured in the system containing electrodes, which was interpreted as the confirmation of presence of electroactive biofilms. Furthermore, MES was also applied for accelerating copper metal recovery [78].

Little research so far has been dedicated to study the nitrate removal using MES. In 2015, Yang et al. reported a MES consisting of a piece of carbon felt in the sediment and iron rod connecting sediment and water environment [79]. The authors achieved 98% of nitrate removal in MES system in 16 days compared with 29% in 24 days for control experiment with sediment without MES. However, the initial nitrate concentration was only around 2 mg N-

$\text{NO}_3^- \text{ L}^{-1}$ ($8.8 \text{ mg NO}_3^- \text{ L}^{-1}$), while the World Health Organization describes surface water concentrations up to $18 \text{ mg NO}_3^- \text{ L}^{-1}$ as low [11]. The European Union defines the nitrate-contaminated water for nitrate concentration over $50 \text{ mg NO}_3^- \text{ L}^{-1}$ [80], therefore such low concentrations are usually not considered as a pollution. Moreover, a green rust-like coating was found on a cathodic part of MES. Green-rust is a generic name for $\text{Fe}^{\text{II}}\text{-Fe}^{\text{III}}$ layered double hydroxyl mineral, which is a corrosion crust on iron based materials. This can lead to the conclusion that nitrate reduction did not occur biocathodically, but rather by a chemical reaction with Fe species[81–83].

Another publication does not focus exactly on MES but it is worth mentioning because it reports the influence of the external resistor on the efficiency of sediment microbial fuel cell (SMFC) in nitrate treatment [84]. In these SMFC, both anode and cathode were made of carbon felt, acclimated in a lake for two months, linked with a resistor varying from 1000 to 1Ω . It was found that with decreasing resistance, the current density and nitrate removal efficiency were increasing. With very low resistance, such microbial fuel cell was quite similar to a microbial electrochemical snorkel. The SMFC with 1Ω resistance achieved 60% nitrate removal in 115h, with a low starting nitrate concentration of $2.5 \text{ mg N-NO}_3^- \text{ L}^{-1}$ ($11 \text{ mg NO}_3^- \text{ L}^{-1}$), which was the concentration of nitrate in the lake.

Recently, the possibility of applying the MES composed of stainless steel electrodes immersed in sediment and in water for nitrate reduction was analyzed [85]. In this study, the principle of the MES was confirmed by measuring continuously the current flowing in short-circuit from the anodic to the cathodic sides and nitrate addition induced clearly an increase of the biocathodic activity. The development of specialized electroactive biofilms on both anode and cathodes of MES was confirmed by voltammetric and 16s RNA analysis. The results obtained from this laboratory experiment were then compared with the one from the MES developed in a constructed wetland.

3. Nitrate-reducing biocathodes

As it was explained in the previous part, BES coupled with NRB can give promising results in terms of denitrification. However, growing a NRB requires specific environment. This part will describe the concept of biocathodes and define the conditions necessary for development of NRB, such as anaerobic environment, the optimal pH, the optimal polarization or external resistance, the best electrode materials and the influence of water conductivity. The results will also be analyzed, including comparison of cyclic voltammetry and discussion on

bacterial communities. Finally, the products of nitrate reduction will be discussed, as well as the possibility of changing the role of bioelectrode: switch between bioanodic and biocathodic conditions.

3.1. Biocathodes

One of the bottlenecks of the bioelectrochemistry research field is the cathodic performance. Very often, the cathode is made of, doped, or covered with platinum which serves as a catalyst, because it has a lower overpotential for oxygen reduction [86]. However, it has limited availability, high cost and possible negative environmental effects. Non precious metal-based catalysts, such as lead, cobalt or iron based materials were also studied and showed promising improvements in power output, but the concerns are risk of metal leaching from the cathode and sensitivity for conditions occurring in MFC: e.g. decrease of catalytic activity in sulfate-rich, chloride-rich or high pH environment. Another solution is to use a redox mediator in the cathodic chamber, which transfer electrons from the electrode to terminal electron acceptor. They also reduce overpotential and usually are reversible redox species. The most commonly used mediator is ferricyanide, which has fast reduction kinetics, relatively high redox potential, and it is well soluble in water. However, it is not a sustainable choice for application for electricity generation, because it is sensitive to light, due to the low rate of regeneration by oxygen it should be regularly replenished, and in long-term operation it can diffuse through the membrane [87].

The idea of using microbial catalyst on a cathode came after the first proof that the electrode can serve directly as the electron donor for bacteria, which was described in 2004 [88]. In this study, the electrode poised at -500 mV vs Ag/AgCl was the only possible electron donor in nitrate-rich anaerobic environment. The source of bacteria was sediment inoculum. In such setup, nitrate was reduced to nitrite, which was not observed in control experiment without polarization or with dead bacteria; moreover, cathodic current was observed when nitrate was added to the cathodic chamber. Microbial analysis and further tests showed that the bacteria species responsible for this reduction was *Geobacter* sp. In another study, published soon after, a current (200 mA) was applied to the bioreactor with the sludge from sewage treatment plant used as bacteria source. In that case, the complete denitrification on biocathode (nitrate to dinitrogen) was observed for the first time. Moreover, the decrease of nitrate concentration in the experimental environment resulted to the cathodic potential decrease from -80 to -260 mV vs Ag/AgCl [89].

In 2007, the first MFC in which microorganisms performed the complete denitrification in cathodic chamber was described, at a rate up to $0.146 \text{ kg NO}_3^- \text{N m}^{-3} \text{ net cathodic compartment (NCC) d}^{-1}$ or $0.080 \text{ kg NO}_3^- \text{N m}^{-3} \text{ total cathodic compartment (TCC) d}^{-1}$, using only electrons supplied by microorganisms from the anodic chamber, oxidizing organic material [34]. The authors tested different external resistance or applied voltage between anode and cathode. They found that with fixed external resistance, the produced current was proportional to the denitrification rate. Moreover, the higher voltage between electrodes was mainly due to higher cathodic potential. Besides, no further denitrification and current production was observed when the cathodic potential was higher than 0 V vs SHE. The maximum power production in this setup was $8 \text{ W m}^{-3} \text{ NCC}$ with an external resistance of 25Ω . Generally, power production when nitrate is the terminal electron acceptor is not competitive with MFC equipped with an oxygen-reducing biocathode. For example in the same MFC operating either with oxygen or with nitrate, the produced power was 10.27 and 1.92 W m^{-2} , respectively [90]. However, the ability of water treatment from nitrate makes it interesting for further studies.

3.2. Conditions for best performance of NRB

While analyzing the different publications on NRB, one can notice a number of conditions applied in order to successfully grow an efficient biocathode. Usually, the NRB is grown in anaerobic environment, neutral pH, on a carbon or metal electrode with a specific applied potential, and with a mixed bacterial community. Each of these conditions was carefully verified by at least a few groups. This section will focus on summarizing the results of these studies focusing successively on anaerobic environment, pH, salinity, biocathode polarization, external resistance and electrode material.

Anaerobic environment

The nitrate-reducing biocathodes are usually kept in anaerobic environment, in order to prevent the competitive reaction with oxygen. The low dissolved oxygen (DO) concentration, together with electron donor availability, are considered as the two most important factors for the effective work of denitrifying bacteria [39,40]. The oxidation of organic matter is preferably performed with dioxygen, however, in water with low oxygen concentration, facultative anaerobic bacteria will use nitrate for their metabolism instead. In case of low oxygen and nitrate concentration, the next preferable electron acceptors will be manganese oxide, iron oxide, sulfate and carbon dioxide. Table 1 summarizes the influence of oxygen concentration on nitrate reduction.

There are several studies investigating the exact influence of oxygen on biocathodic nitrate reduction. Guo et al. tested three different concentrations of dissolved oxygen in the catholyte of MFC: 1.5, 3.4 and 4.4 mg O₂ L⁻¹, while the concentrations of nitrate and ammonium were 473 and 104 mg respectively [91]. With the increase of the concentrations of oxygen, the conversion of nitrate and ammonium, as well as the power produced, were lower. Moreover, in oxygen-rich environment, more aerobic nitrifying and less anaerobic denitrifying bacteria were observed. Sima et al. tested five different concentrations of DO in water of a SMFC for nitrogen removal (nitrification and denitrification), which were 0, 1, 3, 5 and 8 mg L⁻¹ [92] (Table 1). There was no nitrate accumulation in sediment and water in these anoxic conditions, while the concentrations above 5 mg L⁻¹ favored such accumulations. The authors concluded that the oxygen concentration of 3 mg L⁻¹ is an optimal compromise between nitrification, which requires oxygen and denitrification, in which there is a competition with oxygen, and allows to achieve the best total nitrogen (TN) removal.

Zhang and Angelidaki studied the effect of DO concentration on the nitrate and nitrite reduction and power production in SMFC [93]. As expected, the nitrate and nitrite removal was less and less effective with increasing levels of DO, however the effect was different regarding the power density. High power density was observed initially with low DO concentration (close to 0 mg L⁻¹), it decreased when the DO was 3.2 mg L⁻¹, and then increased again with DO of 8 mg L⁻¹. The authors explain this by initial inhibition of the denitrification process on the electrode, and with increasing DO concentration, the oxygen took the role of the electron acceptor. Based on these examples, one can conclude that DO concentration around 3 mg L⁻¹ can be considered as a limit above which the electromicrobial denitrification process is inhibited.

However, in case of simultaneous nitrification/denitrification processes, some manuscripts report denitrification above oxygen concentration of 3 mg L⁻¹. Virdis et al. observed successful denitrification up to 4.4 mg L⁻¹ of DO, above which it was inhibited [94]. Furthermore, one year later the same group increased this limit and reported a BES for simultaneous nitrification and denitrification, which achieved the maximum TN removal efficiency at 5.7 mg O₂ L⁻¹ [95] (Table 1). Zhu et al. also performed nitrification and denitrification in cathodic chamber and they found that the oxygen concentration of 5 mg L⁻¹ was optimal for nitrogen removal, but higher concentrations were inhibiting denitrification [96]. There are few explanations for this remarkable efficiency of nitrate reduction in the presence of oxygen. Its excess could be consumed during the nitrification process which can occur in

bulk water, but in the deeper layers of the biofilm on the bioelectrode the conditions can be anoxic and the development of denitrifying microorganism is possible [66,97]. Zhu et al. showed this phenomenon by measuring DO in the depths of biofilm, showing variations from anoxic conditions up to values similar as in the bulk solution (5 mg L^{-1}) [96]. Another explanation is the aerobic denitrification - a process in which bacteria can simultaneously use dioxygen and nitrate as terminal electron acceptors through respiration [98,99]. For example, *Pseudomonas aeruginosa* was found to use both these electron acceptors with DO concentrations up to 2.2 mg L^{-1} [100]. Recently, a few studies described aerobic denitrification in MFCs [101–103], yet they are mostly focused on coupling this process with nitrification or other reactions like phenol removal. Furthermore, Sun et al. studied the algal-bacterial biocathode which was operating in two stages alternately: during daytime with light and oxygen used as electron acceptor and at night, in a dark environment where nitrate replaced oxygen as electron acceptor [104]. The power produced by this BES was higher during daytime (110 mW m^{-2}) than at night (40 mW m^{-2}), however, the complete ammonium and high nitrate removal of 86% in 192h was achieved (high starting concentrations of 314 and 330 mg N-NH_4^+ and $\text{N-NO}_3^- \text{ L}^{-1}$ were used respectively). Therefore, there could be cases when denitrification can occur in oxygen concentrations higher than 3 mg L^{-1} .

Influence of pH

Protons are necessary in each step of denitrification (Eq. 1.1 to 1.4), therefore it is important to keep a proper pH in a cathodic chamber. Table 2 summarizes the influence of pH on denitrification rate in few selected studies. In most of the definitions of the MFC, the protons are said to be released at the bioanode, cross the proton-exchange membrane and be used in the reduction reactions occurring at the biocathode. In reality, this diffusion is not sufficient enough and local acidification happens in the anodic compartment coupled to local alkalization in the cathodic one, which may cause electrode deactivation [105]. Indeed, lower pH of anodic effluent and higher pH of cathodic effluent as a function of electrode potential, was measured by Pous et al. [106]. Keeping the correct pH at the biocathode is important for achieving the best performance. This is why in many publications the authors use phosphate buffer as catholyte [70,104,115–122,107–114]. Rezaee et al. made a composite carbon felt/multiwall carbon nanotube biocathode and they were testing it in different conditions, including pH values ranging from 6 to 9 [123]. They found that nitrate reduction was more efficient at pH 7 and 8 (over 80%) than at pH 6 (less than 60%) or 9 (less than 50%). Vijay et al. studied the influence of cathodic pH on the nitrate reduction efficiency and they tested very wide range of pH: 2, 4,

6, 7 and 9 [70]. They had a similar conclusion that pH 7 was the most optimal for this kind of experiment. Clauwaert et al. increased the nitrate removal from 0.22 to 0.5 kg NO₃⁻N m⁻³ NCC d⁻¹ by keeping the constant pH value of 7.2 (in the control experiment without pH control, the pH of catholyte was increasing due to cathodic reactions) [67]. Cheng et al. proposed to change the role of bioelectrode by switching the potential and providing either electron donors or acceptors [124]. In this way, the biofilm that was first cathodic and alkalized the environment, could later benefit from this higher pH as a bioanode. In summary, pH in a range of 7-8 seems to be the optimal value for NRB performance.

Influence of salinity/conductivity

Conductivity and salinity have a direct influence on the efficiency of bioelectrochemical treatments. In case of low conductivity, the treatment efficiency and energy production of MFCs are limited. For example, when treating nitrogen in water ($\sigma < 1600 \mu\text{S cm}^{-1}$) the low conductivity induces an incomplete nitrate reduction and nitrous oxide production [125]. However, despite a low conductivity ($955 \mu\text{S cm}^{-1}$), Pous et al. were able to treat groundwater with 57% nitrate removal without nitrite accumulation [126].

In the case of waters with high salinity, the conditions can be toxic for the microbial communities. A selection of the population is then observed in favour of halophilic microorganisms [127]. With a salinity of 20 g L⁻¹, Chaudhary et al. were able to achieve 49.5% nitrate removal while reaching a current of -43.5 mA cm^{-2} thank to an haloalkaliphilic enriched biofilm [128].

Biocathode polarization

In many of the studies about nitrate reducing biocathodes, an external potential is applied to the electrode, and found to be crucial to ensure good performance. This potential should be generally low enough to drive the growth of bacteria and high enough to prevent the electrolysis of water [129]. Above 0 V vs SHE the efficiency of cathodic denitrifying bacteria is severely limited [34,130]. In most of the articles, one can find that the applied potential was from around -0.3 V [90,107,108,131–135] or -0.25 [110,129,136–138], up to around -0.1 V vs SHE [111,131,139–142] (See Table S1&S2, Supplementary Material). In some studies, applying potentials of -0.7 V vs SHE or lower was found to increase NRR but the mechanism included electrolysis of water and hydrogenotrophic denitrification using hydrogen generated at the cathode [112,116,143].

Several studies focused on applying different potentials in order to determine the optimal potential value, these are summarized in Table 3 and discussed in this section. Viridis et al. studied the influence of four tested potential values (100, 0, -100 and -200 mV vs SHE) applied on biocathode on cathodic nitrate reduction and on N₂O accumulation [144]. They found that the highest current and nitrate removal was found for an applied potential of -100 mV vs SHE. At lower potential values (-200 mV), higher residual current as well as higher N₂O reduction rates were observed. In the same study, different currents were applied to the cathode, and the highest nitrate reduction, however together with highest N₂O accumulation, was observed for the highest current (15 mA). Pous et al. applied a wide range of potentials from +0.597 to -0.703 V vs SHE to the cathode while varying anodic electron donors [106]. In this study, the optimal nitrate removal rate and the lowest accumulation of undesired intermediates was achieved with applied potential of -0.123 V vs SHE. Yu et al. used *Thiobacillus denitrificans* pure culture to grow biocathodes applying the following potentials: -0.5, -0.3, -0.1 and +0.25 V vs SHE, and they found that the NRR is highest at the most negative applied potential (-0.5 V vs SHE) [145]. Gregoire et al. developed a biocathode by applying the relatively high potential of -0.055 V vs SHE (-0.250 V vs Ag/AgCl) and they observed a maximum current density of -905 mA m⁻² [113]. The potential was then shifted to more negative value of -0.153 V vs SHE (-0.350 V vs Ag/AgCl) and a large increase of reduction current up to -3200 mA m⁻² was noticed. Yet, when the higher potential of +0.197 V vs SHE was applied to grow another biocathode (0 V vs Ag/AgCl), no current was flowing between the bioelectrodes, which is an indication that the biocathode was not operating at this potential. Nguyen et al. wanted to develop nitrate-reducing biocathodes operating at high potential by applying from +0.97 V to +1.197 V vs SHE (-0.1 to +1 V vs Ag/AgCl) [146]. In the systems with biotic anodes and cathodes, they did not observe any significant difference in nitrate removal while changing these potentials. In addition, nitrate removal was lower than the control without applied potentials. In conclusion, the potential in the range from -0.3 to -0.1 V vs SHE turns out to be the most suitable for cathodic biological nitrate reduction.

External resistance

Cathodic potential can be applied, but the potential difference between anode and cathode can also be established with a proper resistor. According to Ohm's Law ($I = U/R$), the current is increasing with decreasing resistance, and therefore nitrate removal increases too. However, when the resistance decreases and the current increases, the voltage must also decrease, and this will cause the decrease of power ($P = I*U$). This simple reasoning gave the

bases for the idea of the microbial electrochemical snorkel, but was also proven in several articles about MFC. Jia et al. studied nitrate removal in MFC and they observed total denitrification in 25h and average current $I = 0.41$ mA when $R = 10 \Omega$ was applied. At the same time, with $R = 1000 \Omega$, only 38% of nitrate was consumed and $I = 0.12$ mA [147]. Similarly, in the study of Viridis et al., for a reactor with $R = 5 \Omega$ the nominal current production was 24.3 mA and the nitrate concentration dropped almost completely (from 55 to 1.6 mg $\text{N-NO}_3^- \text{L}^{-1}$); for $R = 100 \Omega$ the current was 4.8 mA and nitrate removal was very limited (from 66.7 to 54 mg $\text{N-NO}_3^- \text{L}^{-1}$). However, the optimal power for this study was achieved for $R = 20 \Omega$ [66]. Pous et al. noticed that with the lower external resistance, more nitrate can be introduced with achieving always complete denitrification (three times more nitrate with 5 Ω comparing to 50 Ω) and the maximum power was produced for $R = 25 \Omega$. In the study of Wang et al., the nitrate reduction was 60% with $R = 1 \Omega$ and only 12% with $R = 510 \Omega$ [84], but the power density was decreasing with decreasing external resistance. More information about applied resistances can be found in Table S1&S2 in the Supplementary Material. In summary, there is a trade-off between optimum power and optimum nitrate reduction.

Electrode material

The base materials for electrodes should be conductive, chemically stable, have good mechanical strength and low cost. For bioelectrodes, the additional requirements are high surface roughness and good biocompatibility [148]. Various carbon and metal materials are used as the electrode material in MFC with NRB, and they are listed in Tables S1&S2 in the Supplementary Material. Zhang et al. compared the current and power density while using graphite felt, carbon paper or stainless steel as electrode material for oxygen-reducing biocathode [149]. They found the best performance using graphite felt (350 mA m^{-2} and 109.5 mW m^{-2}) and very poor results when using stainless steel ((18 mA m^{-2} and 3.1 mW m^{-2}) (Fig. 6).

Indeed, in the great majority of studies about NRB, different carbon materials are used as electrode, for example carbon paper [93,114,115,133,135], carbon cloth [134,137,143,145,150,151], graphite felt [84,112,155,116,118,119,122,146,152–154], carbon felt [68,117,130,156–161], graphite rods, bars and plates [70,107,113,131,138,162,163], graphite or carbon brushes [69,110,164–169] and graphite granules [34,66,139,142,144,170–172,67,90,108,109,121,125,126,136]. Composites materials were also used to increase the performance, for example carbon felt/multiwall carbon nanotube composite [123], coated carbon materials [140,141,173,174] or layered air-cathode [175]. Different carbon materials

can favor development of different microbial communities [176]. However, another research was comparing granular graphite and stainless steel mesh as the NRB material in MFC for swine manure treatment [177]. The authors found similar results for nitrate reduction for both of these materials and they concluded that in a longer perspective, stainless steel mesh would be more promising because of the clogging effect in graphite granules compartment. Erable et al. immersed graphite and stainless steel electrodes into marine sediments and they found that in the same medium, these two electrodes were colonized by different microbial community which leads to development of anodic and cathodic properties for graphite and steel respectively [178]. Yet, despite these results, the number of studies involving stainless steel as cathodic material remains very limited. Metals like iron and nickel were also tested as cathode materials. A microbial electrochemical snorkel with iron gave good results for treating water of low nitrate concentrations [79], however some rust-like coating were observed on the cathodic part of iron rod, therefore the mechanism could involve chemical reactions with iron rather than a bioelectrochemical process [81]. Nickel foam was used to create an algal-bacterial biocathode which could use oxygen and nitrate as electron acceptor during day and night mode respectively [104]. Such biocathode resulted in good nitrate removal efficiency (86% in 192h) starting from the concentration of 330 mg N-NO₃⁻ L⁻¹. To conclude, carbon-based materials are the most popular choice for NRB, however there are also studies with metal electrodes.

3.2.1. Voltammetric properties of nitrate-reducing biocathodes

Cyclic voltammetry is widely used for studying nitrate reducing biocathodes and several groups tried to identify the nitrate reduction peak potential. To the best of our knowledge, the highest reduction peak potential for nitrate was observed so far by Rogińska et al., with biocathodes developed in a microbial electrochemical snorkel [84]. The biocathode positioned on the surface water of a laboratory wetland was short-circuited with a bioanode in sediments. In the absence of nitrate, a biocathodic response is already observed, that could be due to residual nitrogen species or oxygen species (curve a, Figure 7A). After addition of nitrate in the laboratory wetland, the cathodic response was increasing very clearly with an onset potential near 250 mV versus SHE and a half-wave potential close to 50 mV vs SHE (curve b of Figure 7A). Cheng et al. observed also relatively high redox potential with photoelectrotrophic denitrification system, where a denitrification biocathode was coupled with a TiO₂ photoanode [179]. In their study, they found the onset potential and peak potential for nitrate reduction to be 0.15 V and 0 V vs SHE, respectively, while no or little signals were present for an abiotic cathode with nitrate or for a biocathode without nitrate. A little bit more negative onset potential

was found in the study of Yi et al. who prepared a bioelectrochemical system aiming at the detection of biochemical oxygen demand (BOD) and nitrate [180]. The NRB developed in this experiment had the onset potential of below -0.1 V vs Ag/AgCl which should correspond to around 0.09 V vs SHE. Gregoire et al. in their study focused on enrichment of NRB (for example by keeping anaerobic conditions and providing additional carbon source) in order to achieve a higher current. They also developed a biocathode by applying two different potentials on the electrode [113]. When the potential applied to the biocathode was lower (-0.25 V vs Ag/AgCl), the cyclic voltammogram (CV) showed clearer reduction peak with an onset potential of -0.125 V vs Ag/AgCl (0.07 V vs SHE). The reduction signal on the CV was less clear in case of the NRB developed at the more positive applied potential of 0 V vs Ag/AgCl.

A number of publications show a nitrate reduction wave starting around 0 V vs SHE (-0.2 V vs Ag/AgCl). Pous et al. performed several CVs of the microcosms sampled from denitrifying biocathodes in different media, such as NO_3^- , $\text{NO}_3^- + \text{NO}_2^-$, NO_2^- or buffer. Figure 7B reports an example of CV from this study. The authors found that the peak with the onset potential at -0.25 V vs Ag/AgCl (-0.055 V vs SHE), with an inflection point at -0.3 V vs Ag/AgCl (-0.105 V vs SHE), obtained in media with NO_3^- or $\text{NO}_3^- + \text{NO}_2^-$, was due the reduction of nitrate to nitrite. Another signal around -0.7 V vs Ag/AgCl (-0.505 V vs SHE), obtained in media with $\text{NO}_3^- + \text{NO}_2^-$ or only NO_2^- , was assigned to further nitrite reduction. No clear signals were observed for the blank electrode [131]. The same group created one more bioelectrochemical system, which was aiming at reducing nitrate and arsenite [139]. While performing the CV, they found the potential for nitrate reduction to be -0.02 V vs SHE. In other study, they developed a bioelectrode which, depending on conditions, was oxidizing acetate or reducing nitrate. For this bioelectrode, the reduction wave was starting at around -0.05 V vs SHE and the potential of the nitrate reducing signal was -0.175 V vs SHE [107]. Kondaveeti and Min compared a biocathode on abiotic carbon and a platinum cathode [114]. For the biocathode, the observed reduction peak was at -0.2 V vs Ag/AgCl (around 0 V vs SHE).

More negative potentials were also attributed to nitrate reduction in some publications. Huang et al. tested the denitrifying properties of modified carbon felt cathodes [156]. They attributed different nitrate reduction peaks: -0.45 V vs SCE for bare electrode and -0.332 V vs SCE for a modified one. Yu et al. studied nitrate removal with biocathode colonized by *Thiobacillus denitrificans* [145]. They observed the peak of nitrate reduction at $-$

0.31 V vs SHE. Furthermore, Vijay et al. prepared a MFC for simultaneous removal of nitrate and uranium. In this study, the authors assigned a signal at -0.552 V vs Ag/AgCl (-0.335 V vs SHE) to nitrate reduction.

In general, in most of the studies on nitrate reduction started in a region from 0 to -0.1 V vs SHE, but lower potential values were also attributed to nitrate reduction. In order to clearly identify the peak for nitrate reduction, it is necessary to compare it with adequate control experiments without nitrate. The diversity observed between biocathodes is likely due to the different microbial communities present on these electrodes, as discussed next.

3.2.2. Bacteria

Fundamental understanding of microbial communities present on NRB is required to have a complete overview of the BES and to maximize the efficiency of the bioelectrochemical device. Most of the published studies focusing on NRB are based on the selective pressure process to achieve electroactive nitrate removal. Hence, most of the biological inocula used consist in biologically uncontrolled medium such as sediments, wastewater and others which leads to the need to identify the bacterial species that developed during the incubation phase. Several methods of analysis are employed, which can be divided into techniques for studying electroactive microbes, microbial community and extracellular electron transfer [181]. One of the most common methods is using 16S rRNA-gene sequencing to identify the bacterial communities.

Over the years, because of the decrease of the 16S rRNA-gene sequencing cost and the rise of interest in MFC technologies, a more precise knowledge developed on which bacterial groups are involved in electroactive biofilms.

Proteobacteria is the most known phylum for electroactive properties and it is found on both anodes and cathodes of MFCs. Gregory et al., who first observed biological nitrate reduction with electrode being the only electron donor, also studied the differences between the microbial community on the electrode poised at -0.5 V vs Ag/AgCl and the control electrode without current flow [88]. They found the increase of *Geobacteraceae* family (δ -*Proteobacteria*) on polarized electrodes, most of the sequences strongly related to *Geobacter grbiciae*. There was also more γ -*Proteobacteria* on the poised electrodes, and more β -*Proteobacteria* on the controls.

When it comes to electroactive denitrification, two main bacterial groups can be noted, both belonging to the β -*Proteobacteria* class. The first group is the *Thiobacillus* genus included

in the *Hydrogenophilales* order. Among this genus, the two main cited species are undeniably *Thiobacillus* spp and *Thiobacillus denitrificans* which were confirmed to be able to reduce nitrate to nitrite with the use of electrode as the sole electron donor [111,145]. It was also observed that an electrode poised between -0.12 V vs SHE up to -0.5 V vs SHE favored their development. The second most important group is taxonomically larger, with two bacterial genera involved: *Thauera* and *Azoarcus*. Those two genera are phylogenetically close, both belonging to the *Zoogloeaceae* family and show high level of similarity in term of 16S rRNA gene sequences [182].

The *Thauera* genus is the most cited in publications reporting biological electroactive denitrification [110,157,159,183]. This genus has become such common in the bio-electrochemistry research field that some new characterized species were directly isolated from biofilms developed from Microbial Fuel Cells [184].

As for the *Azoarcus* genus, a large part of its species are noted for their ability to reduce nitrate, nitrite [185] but also the ability of some of them to perform N_2 fixation [186]. Its presence in cathodic biofilms has been noted in many studies but mostly in the case of oxygen reduction bio-cathodes [187–189] and also in some bio-electrochemical systems involved in nitrate removal applications [136,190,191].

In addition, some of the bacterial species known for their anodic electroactivity such as *Geobacter metallireducens* or *Shewanella oneidensis* are now studied for their potential ability to perform cathodic electron uptake in order to perform potential nitrate reduction [192,193].

Despite of the results reported in the references above, it is fundamental to keep in mind that the knowledge about cathodic denitrifying species remains highly limited and many things stay undiscovered, both in term of species and in term of metabolism. The fact that most of the identified electroactive nitrate reductive species belong to the β -*Proteobacteria* class does not mean that no other potential undiscovered species exist. One confirmation of this hypothesis comes from the results from Chen et al. who found the highest relative abundance of *Proteobacteria* (in total 66.2%), in particular β -*Proteobacteria* (50%) in the cathodic chamber after 80 days of operating of MFC for nitrate reduction. Other present *phyla* were *Bacteroidetes* (21.6%), *Chlorobi* (8.1) and *Actinobacteria* (4.1) [194]. However, the authors continued the experiment and analyzed the bacterial community after 350 days of operation of MFC. In this longer perspective, the total percentage of *Proteobacteria* was relatively stable, but the number of γ -*Proteobacteria* increased significantly and replaced β -*Proteobacteria* which became

scarcely represented. The amount of *Bacteroidetes* and *Actinobacteria* were also rather unchanged, however *Chlorobi* disappeared and *Planctomycetes* and *Firmicutes* became present [90].

The specific conditions applied to biocathodes can influence not only the different bacteria species, but also their relative number. Specific cathode conditions and voltage caused the low abundance of bacteria on closed circuit cathode in comparison to the electrode let at open circuit in the work of Guo et al. [91].

Moreover, extremophilic microorganisms (i.e. evolving in extreme environments such as very high or very low temperatures, pH or salinity conditions) are underused in the case of nitrate removal [128] even if they may overcome the some of the current bottlenecks of BES, particularly in the case of denitrification [195].

Chaudhary et al. reported the case of electrochemical enrichment of haloalkaliphilic nitrate-reducing microbial biofilm at the cathode of bioelectrochemical systems. In the case, the biofilm, evolving at high salinity such as 20 g NaCl L⁻¹ were mainly made of unreported *Pseudomonas*, *Natronococcus*, and *Pseudoalteromonas spp.* [128].

Some thermophilic bacteria have also been reported as nitrate-reducing in BES. Fu et al. used either a thermophilic anaerobic digester sludge and a pure culture of a thermophilic nitrate-reducing bacterium *Calditerrivibrio nitroreducens* to inoculate an MFC operated at 55°C [196]. The *Thauera* genus, discussed above, has also been isolated from MFC operating at different temperatures (up to 45°C) [183]. Furthermore, Zhao et al. showed that raising the temperature from 36 to 48°C lead to a switch in the microbial communities. The communities went from thermophilic autotrophic nitrifiers to thermophilic aerobic denitrification bacterium (dominated by *Aquamicrobium*, *Brachymonas* or *Comamonas*, and *Alishewanella* [101].

3.3. Reaction products

The final product of a complete denitrification is dinitrogen, however, side products such as nitrite or nitrous oxide can be accumulated. This is highly undesirable because these compounds are more harmful pollutants than nitrate itself. Nitrite is toxic for aquatic fauna and flora as well as for humans (especially infant) by oral exposure [197,198] and nitrous oxide is significantly contributing to the greenhouse gas footprint [199].

Pous et al. built an MFC which was operating for three months [126]. They reported accumulation of different products during different operation periods: between days 31 and 45, around 43% of accumulated nitrate was reduced only to nitrite. Later, nitrite was almost

completely removed, but during days 84-97 the authors estimated that 60% of the initial nitrate was in the form of nitrous oxide (based on coulombic efficiency, electron transfer and Tafel plot). It can be concluded that the biofilm was maturing and growth of bacteria catalyzing different reactions was promoted depending on availability of the compounds in the biofilm environment. In a different study, these researchers again observed that between 34.4 and 54.1% of reduced nitrate accumulated in the form of nitrite indicating that nitrite reduction rate is lower than nitrate reduction rate. By analyzing the biocathode with CV, they also found that nitrite reduction happens at more negative potential than nitrate [131].

Doan et al. saw that increased current applied on a biocathode increases nitrate removal, but also the accumulation of nitrous oxide [200]. Up to 70% of reduced nitrate was accumulated as nitrous oxide when the applied current density was 20 A m^{-2} .

Virdis et al. observed that the nitrous oxide production was changing depending on the potential values applied on the biocathode. $0.209 \text{ mM N h}^{-1}$ was produced when 0 V vs SHE was applied and when the potential decreased to -0.25 V vs SHE , the nitrous oxide emission dropped to $0.072 \text{ mM N h}^{-1}$. However, N_2O could be also reduced on the biocathode, however with much slower rate [144]. Rather different effects were observed by Yu et al., who tested biocathodes with applied potentials from -0.5 to $+0.25 \text{ V vs SHE}$. They reported that more nitrite was accumulated with lower potential applied, for example for $+0.25 \text{ V}$ there was 0.19 mM nitrite and for -0.5 V there was 1.39 mM of nitrite. However, no clear influence of potential on the nitrous oxide accumulation was observed in this study [145].

Srinivasan et al. tried to determine the specific factor influencing the nitrite concentration by changing the electron donor concentrations, DO or pH. However, none of these factors influenced the nitrite accumulation (about 66% of the initial nitrogen-nitrate) in this study [201]. Therefore, it seems that the cathodic potential could have a major influence on the nitrite and nitrous oxide accumulations but the exact nature of this influence is still to be studied.

3.4. Changing the polarity of the bioelectrode

A bioelectrode does not have to necessarily keep the same role: in some studies, its function was modified by switching polarization value and providing different substrates. Such switching role has the important advantage of preventing excessive acidification or alkalization around the electrode. Usually, anodic oxidation reactions lead to a decrease of pH and reduction reactions require protons. The proton-exchange membrane does not provide an efficient

exchange of H^+ ions [105]. Regularly changing the role of the bioelectrode can in principle solve the problem: the protons released during the oxidation phase being consumed during the reduction phase. This operation was applied for oxygen [202] and nitrate reduction [124]. In case of nitrate, the first study was described by Cheng et al. in 2012, who created the anocathophilic biofilm. They observed the inhibition of the anodic reaction due to acidification - when this effect was neutralized by adding sodium hydroxide, anodic current production resumed. The same biofilm was also used as a biocathode when sodium acetate was replaced by sodium nitrate, which resulted in current reversal (Fig 8). Such change of behavior was repeated several times, with 85 and 87% of anodic oxidation and cathodic reduction efficiency, respectively [124]. Pous et al. also developed a switchable bioelectrode which operated as a bioanode in the presence of acetate and with an applied potential of +0.397 V vs SHE, yet it could be switched to cathodic conditions by applying -0.303 V vs SHE and nitrate. The switch also caused the current inversion and this inversion could be repeated several times. They obtained a nitrate removal rate of $0.532 \text{ g N-NO}_3^- \text{ m}^{-2} \text{ d}^{-1}$. They found that both acetate oxidation and nitrate reduction were taking place at the same formal potential of -0.175 V vs SHE, which could suggest that a similar electron transfer mechanism is used in both processes [107].

Yun et al. developed a biocathode from a bioanode by changing the potential from +0.15 to -0.4 V vs SHE; this biocathode successfully treated water polluted not only by nitrate, but also by two other model pollutants: nitrobenzene (nitroaromatic) and Acid Orange 7 (an azo-dye) [203]. Similarly, Liang et al. switched the applied potential from +0.44 to -0.26 V vs SHE to switch from an anodic biofilm to a cathodic one which allowed to reach up to 98% efficiency of nitrate reduction. However, in this case the anodic bacteria was lost from the electrode and it was impossible to recover a bioanodic behavior afterward [110]. Lin et al., with the use of this method, obtained a very high reduction rate of $2.74 \text{ g N-NO}_3^- \text{ m}^{-2} \text{ d}^{-1}$ [204]. Yi et al. tested another application - they used the bidirectional extracellular electron transfer of *Shewanella loihica* for detection of BOD and nitrate [180].

More recently, several studies have focused on these bidirectional electrodes for denitrification. Liang et al. achieved 93% nitrate removal by constructing bidirectional electron transfer biofilms via periodic polarity reversal [138]. The study showed that the exoelectrogens were dominated by *Geobacter pickeringii* while denitrifiers were *Chryseobacterium sp.*, *Acidovorax sp.* and *Stenotrophomonas sp.*.

Other studies have shown that polarization conditions have a direct impact on the BES formed. Guo et al. studied the interspecies electron transfer between *Geobacter* and a denitrifying bacterium in the case of biofilms formed with polarity inversion at various frequencies (between 12 and 48h) [135]. The highest current density, 2.19 A m^{-2} was obtained with polarity inversion every 12 hours. This study showed symbiotic interactions in which *Geobacter* transferred electrons from the electrodes to the denitrifying bacteria for denitrification via c-type cytochromes. Zhao et al, showed that a single polarity inversion led to a biofilm dominated by *Afipia* allowing a slow nitrate removal while a periodic polarity inversion led to a biofilm dominated by *Geobacter* allowing a stable nitrate removal by dissimilatory nitrate reduction to ammonia [134].

With the same approach, Wang et al developed a BES where, instead of alternating 2 types of polarization, they alternated periods with polarization at $-0.4 \text{ V vs Ag/AgCl}$ and periods at OCP. The current was then close to 0 during the OCP period and reached -24 mA during polarization (compared to -16 mA with constant polarization). However, since nitrate removal was less efficient during OCP periods, the removal rate of nitrate was less efficient with the periodically polarized system (86% vs 98% under constant polarization), with a higher NO_2 production.

4. NRB coupled with other applications

Very often, the studies about NRB are coupled with other applications. Articles about MFC are usually also describing the power production of the device. Other research try to pair nitrate reduction with the removal of other pollutants, such as ammonium, perchlorate, sulfur compounds, etc. This part will describe the BES used for these purposes and report significant results from different approaches.

4.1. Power production

BES equipped with NRB are very often coupling water treatment with energy recovery. The maximum power of MFC can be found by analyzing its bell-shaped power-current curve (Fig. 9, situation (a)). However, the maximal efficiency for water treatment is achieved with the maximal current, and therefore with a low or zero voltage (Fig. 9, situation (b)) [81]. According to Ohm's law ($P = U \cdot I$), there is no power produced with $U \approx 0$. Therefore, the highest power production is not necessarily equal to the highest efficiency in term of denitrification. Clauwaert et al. found that the highest denitrification rate was achieved for current equal to $58 \text{ A m}^{-3} \text{ NCC}$ and voltage equal to 0.075 V . Yet, the highest power of 8 W m^{-3}

³ NCC was for $I = 35 \text{ A m}^{-3} \text{ NCC}$ and $U = 0.214 \text{ V}$ [34]. Viridis et al. observed the highest NRR of $410 \text{ g N-NO}_3^- \text{ m}^{-3} \text{ day}^{-1}$ when the resistance was 5Ω . The current for this resistance was $133 \text{ A m}^{-3} \text{ NCC}$ and the power was $13 \text{ W m}^{-3} \text{ NCC}$. When the resistance was 20Ω , the reduction rate decreased to $345 \text{ g N-NO}_3^- \text{ m}^{-3} \text{ day}^{-1}$ and the current was $99 \text{ A m}^{-3} \text{ NCC}$ but the power increased to the maximum which was $34 \text{ W m}^{-3} \text{ NCC}$ [66].

Several attempts were carried out in order to increase either the current or the power density of NRB. By changing the potential of biocathode from -0.055 to -0.155 V vs SHE (-0.25 to $-0.35 \text{ V vs Ag/AgCl}$) Gregoire et al. increased the current from -905 to -3200 mA m^{-2} [113]. Samrat et al. compared phosphate and carbonate buffered solutions as catholyte in MFC with seawater bacteria. They found the increase of power and current density from 0.6 W m^{-3} and 7.5 A m^{-3} to 2.1 W m^{-3} and 26.6 A m^{-3} after replacement of the phosphate solution by a carbonate one. This could be explained by the fact that when the phosphorus content decreased, the bacteria went to resource acquisition mode and increased the nitrate reduction and therefore energy production [160]. Al-Mamun et al. studied the influence of initial nitrate loading on current and power. By gradually increasing the influent nitrate concentration from 41 to $140 \text{ g N-NO}_3^- \text{ m}^{-3} \text{ day}^{-1}$, the specific current production increased from 16.2 to $27.4 \text{ A m}^{-3} \text{ NCC}$ and specific power production increased from 1.77 to $5.2 \text{ W m}^{-3} \text{ NCC}$ [121]. In a different study, this group changed the initial nitrate concentrations from 25 to $200 \text{ g N-NO}_3^- \text{ m}^{-3} \text{ day}^{-1}$ and estimated $150 \text{ g N-NO}_3^- \text{ m}^{-3} \text{ day}^{-1}$ to be the optimal in terms of current and power production, which for this experiment were $45.88 \text{ A m}^{-3} \text{ NCC}$ and $7.1 \text{ W m}^{-3} \text{ NCC}$ [69]. Chen et al. observed a very big difference of current peak depending on the modification of carbon-based electrodes in the beginning phase of the study. However, after this initial phase, all electrodes were going towards similar values of current [170].

MFC with oxygen-reducing biocathodes are generally more efficient for energy production. For example, Xie et al. built a three chamber MFC with one anodic and two cathodic compartments. One of the cathodic compartments was aerobic and dedicated to nitrification coupled with oxygen reduction while the other compartment was anaerobic and performing nitrate reduction. The power produced by the MFC with the oxic and anoxic chambers was 14 and $7.2 \text{ W m}^{-3} \text{ NCC}$, respectively [205]. Zhang et al. proposed a similar MFC but in the shape of a tube in which the outer chamber was the aerobic cathodic compartment, the middle chamber was anodic and the inner one was the anaerobic cathode devoted for nitrate reduction. They found that the anaerobic chamber was producing $0.001 \text{ kWh m}^{-3} \text{ NCC}$ and the aerobic one, even after subtracting the energy required for its operation (pump), produced

0.031 kWh m⁻³ NCC [206]. Sun et al. prepared a photo-bioelectrochemical system equipped with algal-bacterial biocathode, which was reducing oxygen during the day and nitrate during the night. The maximum power was 110 mW m⁻² and 40 mW m⁻² in day and night mode, respectively [104]. This is why perhaps the main focus for NRB should be their efficiency in nitrate reduction. However, the studies of NRB are still recent and an increase of power production efficiency can be still achieved. More results of power production reached up to date in BES with NRB can be found in Table S1&S2, in the Supplementary Material.

4.2. Nitrification-denitrification MFC

Bioelectrochemical systems are often used to combine several processes of water treatment and for example denitrification can be coupled with nitrification. Nitrification is a process of ammonium oxidation to nitrate that can be later reduced to dinitrogen through denitrification. Simultaneous nitrification and denitrification (SND) requires careful choice of conditions in order to keep both reactions happening at similar rate to avoid nitrate or nitrite accumulation. Other approaches for removing pollutants like excess organic carbon will also be discussed next.

Biological nitrification (in solution) and bioelectrochemical denitrification (at the biocathode) can be integrated into one cathodic compartment (Fig. 10) [94–96]. One of the challenges of this solution is to find the optimal DO concentration in the catholyte, as oxygen is required for nitrification, but is inhibiting denitrification. However, as discussed in part 1.3.2 several studies proved that oxygen concentrations between 4 and 5 mg L⁻¹ do not noticeably inhibit denitrification, the deeper layers of biofilm remaining in anoxic conditions [96]. Therefore, these DO concentrations are a tradeoff for reaching the optimal nitrogen removal rate. Viridis et al. found that the optimal DO concentration in their experiment was 4.35 mg L⁻¹, allowing 94% efficiency of TN removal (starting ammonium concentration was 0.111 kg N m⁻³ d⁻¹) [66]. Moreover, the protons released during nitrification help the balance of the optimal pH in the catholyte which can be alkalized due to cathodic reactions. Another challenge is the necessity of providing carbon and electron source for the nitrification step. These carbon compounds are electron donors to denitrifiers species and hence are competitor with the cathode that would then perform non exo-electrogenic denitrification [96].

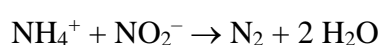
SMFC and similar constructed wetlands-MFCs were also used as incubation medium for simultaneous nitrification and denitrification. Constructed wetlands-MFC are MFCs integrated into constructed wetlands. By applying constructed wetlands-MFC with three biocathodes, Xu et al. increased both nitrification and denitrification rates by 1.8 times in

comparison to the control experiment [207]. Qiu et al. coupled SMFC with oxygen-producing submerged plants. The presence of these plants increased nitrification rate in both BES and control experiments, however, nitrate was present in the effluents. On the other hand, the setups without plants were less efficient in terms of nitrification, and less nitrate was found in the effluent. One can conclude that in this experiment the submerged plants and aerobic environment had bigger impact than the presence of BES [208].

Another approach is to perform nitrification and denitrification in separated chambers. San-Martín et al. prepared a stack BES with 5 pairs of anodes and cathodes, with separated nitrification chamber. The wastewater polluted with COD and nitrogen was first fed to the anodic chamber in order to oxidize organic compounds. Later, this water was transferred to the external nitrification chamber where ammonium was oxidized and the newly produced nitrate was finally removed in the cathodic chamber. This study was an interesting step towards application in urban wastewater treatment plant, which reached a TN removal rate of 70% [209]. Liang et al. created two-stage MFC system with an oxic biocathodic compartment where oxygen was reduced and nitrification occurred, and an anoxic biocathodic compartment for denitrification. Owing to the oxic part and to increased scale (50 L), the MFC was producing power and removing COD and ammonium from water (95 and 97% respectively, with starting concentrations of 70 mg N-NH₄ L⁻¹). However, even with the longest hydraulic retention time (HRT) (18h), effluent still contained 11 mg N-NO₃⁻ L⁻¹ which resulted in 84% of efficiency of TN removal [210]. A similar setup with two cathodic chambers, aerobic and anaerobic, was studied by Li et al. In the aerobic chamber, simultaneous reduction of oxygen and short-cut nitrification occurred (short-cut nitrification is the process of oxidizing ammonium to nitrite instead of nitrate). Nitrite was then reduced in the second anaerobic cathodic chamber. This setup was also producing power and removing nitrogen with a 99.9% efficiency (with starting concentration of 50 mg N-NH₄ L⁻¹, in 4 days) [211].

4.3. NRB coupled with anammox

Another strategy to remove nitrogen from water is to couple denitrification with anaerobic ammonium oxidation (anammox) - an anaerobic ammonium oxidation process coupled with nitrite reduction, as shown in the following reaction [212]:



One of the bottlenecks of anammox process is the by-product nitrate accumulation. In order to overcome it, denitrification was proposed to be coupled with this process. Biological

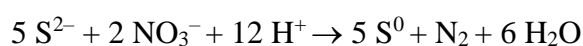
denitrification requires the supply of organic compounds as electron and carbon source for bacteria. However, these compounds can restrain the anammox bacteria. By using the electrode as electron source, bioelectrochemical denitrification solves this problem and therefore coupling denitrification with anammox is an interesting idea to be studied. Li et al. proposed to introduce ammonium and nitrite to the cathodic chamber of MFC, inoculated with anammox biomass. They observed an improvement in TN removal in closed circuit system in comparison to open circuit [213]. In other experiment performed by Qiao et al., the optimal conditions for coupling anammox and autotrophic denitrification was achieved with MEC. For a voltage of 1.5 V and the $\text{NO}_2^- \text{-N}/\text{NH}_4^+ \text{-N}$ ratio of 1.28, the efficiency of TN removal was 99% ($1.38 \text{ kg N m}^{-3} \text{ d}^{-1}$) [214].

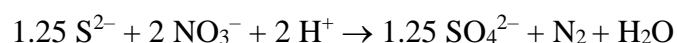
4.4. NRB coupled with perchlorate removal

Salts of perchlorate (ClO_4^-) are often used as oxidizers in rocket fuels, fireworks, missiles etc. They can be also present in fertilizers and therefore later contaminate soil and water [215]. Perchlorate is toxic for human health even in small amount and in the European Union, its concentration in food should not exceed $10 \mu\text{g kg}^{-1}$ [216]. Perchlorate can be reduced in MFCs and NRB can be adapted to reduce perchlorate by gradually changing the influent concentrations of both electron acceptors (namely nitrate and perchlorate) [217]. Both electron acceptors can be also reduced simultaneously, however Xie et al. noticed that increasing nitrate concentrations can gradually decrease the efficiency of perchlorate reduction [218]. This effect was also confirmed in other studies [219] which indicates a possible competition between the two compounds as terminal electron acceptor. Nevertheless, Jiang et al. obtained an efficiency of the simultaneous perchlorate and nitrate removal (in 1:1 ratio) of 40.97% and 86.03%, respectively, while the efficiency for perchlorate only was 53.14% and 87.05% for nitrate only [159]. Wang et al. observed that the decrease of perchlorate concentration was very much accelerated after nitrate was reduced: therefore, both salts could have been completely removed in 120h. These authors also found that optimal biocathodic potential for this reaction was -0.2 V vs SHE [220].

4.5. NRB coupled with removal of sulfur compounds

Sulfide can be released in wastewater from petrochemical plants or some manufactures; it can be also generated by biological breakdown of sulfates in anaerobic environment [221]. Sulfide can be used as electron donors in autotrophic denitrification as shown in the following equations [222]:





Usually, recovered S^0 is the main purpose of the specific biotechnological process. In MFC, sulfide, instead of, or together with, organic matter, can be used as an electron donor in the anodic chamber [223]. Its oxidation can be coupled with nitrate reduction in the cathodic chamber [224]. Zhong et al. obtained a stable removal of sulfide and nitrate in MFC, combined with power recovery [225]. Cai et al. built an efficient MFC for simultaneous sulfide and nitrate treatment, obtaining 99.4% and 96.5% of removal respectively, regardless of the initial concentration of sulfide (between 60 and 540 mg L^{-1}) and ratio between pollutants [226].

4.6. NRB coupled with removal of other pollutants

The compounds such as sulfate, phosphate and uranium were also coupled with nitrate reduction in bioelectrochemical systems. Tetteth et al. created a macrophyte cathode SMFC, which was able to remove COD, nitrate and sulfate, reaching up to 99% removal NO_3^- and SO_4^{2-} (with starting concentrations of 125.6 $\text{mg N-NO}_3^- \text{L}^{-1}$ and 75 $\text{mg S-SO}_4^{2-} \text{L}^{-1}$) [164]. Ge et al. constructed pyrite-based constructed wetlands-MFC which simultaneously removed COD, nitrate and total phosphorus [227]. Vijay et al. were searching the solution for treatment of nuclear wastewater, which contains nitrate and uranium. They added glycerol-3-phosphate, which by employing the phosphatase enzyme, released phosphate which with uranium creates insoluble uranyl phosphate. At the same time, nitrate reduction and power production were also achieved in this study [152]. Nitrate reduction has then in principle a potential to be coupled with several other processes.

5. Conclusions

This review summarized the current research about NRB. In order to grow a NRB and based on the literature review, one should apply potential in the range -0.3 to -0.1 V vs SHE at a cathode made of carbon or of an alloy like stainless steel, keep the DO level below 3 mg L^{-1} and the pH in the range 7-8. Chosen single-strain cultures as well as mixed communities from wastewater treatment plants or sediments can be used as a source of bacteria. Using these guidelines should allow optimal denitrification rate, energy production and limiting side products. Efforts towards coupling nitrate reduction with other reactions were also reviewed. However, the specific conditions which are easily applicable at the laboratory scale, such as anaerobic atmosphere, optimal pH and C/N ratio, could be difficult to reach and maintain in larger scale application. Indeed, the chemical and physical environment can be challenging to

control in wastewater treatment plants or in constructed wetland for example. We reckon that this is the biggest challenge of further research on NRB.

Nevertheless, taking into consideration the novelty of this research, the ever increasing interest of researchers from diverse field, and the growing number of published studies on this topic, one can predict that the optimal, low-cost and efficient nitrate reducing system employing NRB will be designed and applied in the near future. Part of the solution may come from a bioelectrochemical system in snorkel configuration that does not require any external power and has shown promising performance so far.

Acknowledgement

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Figures

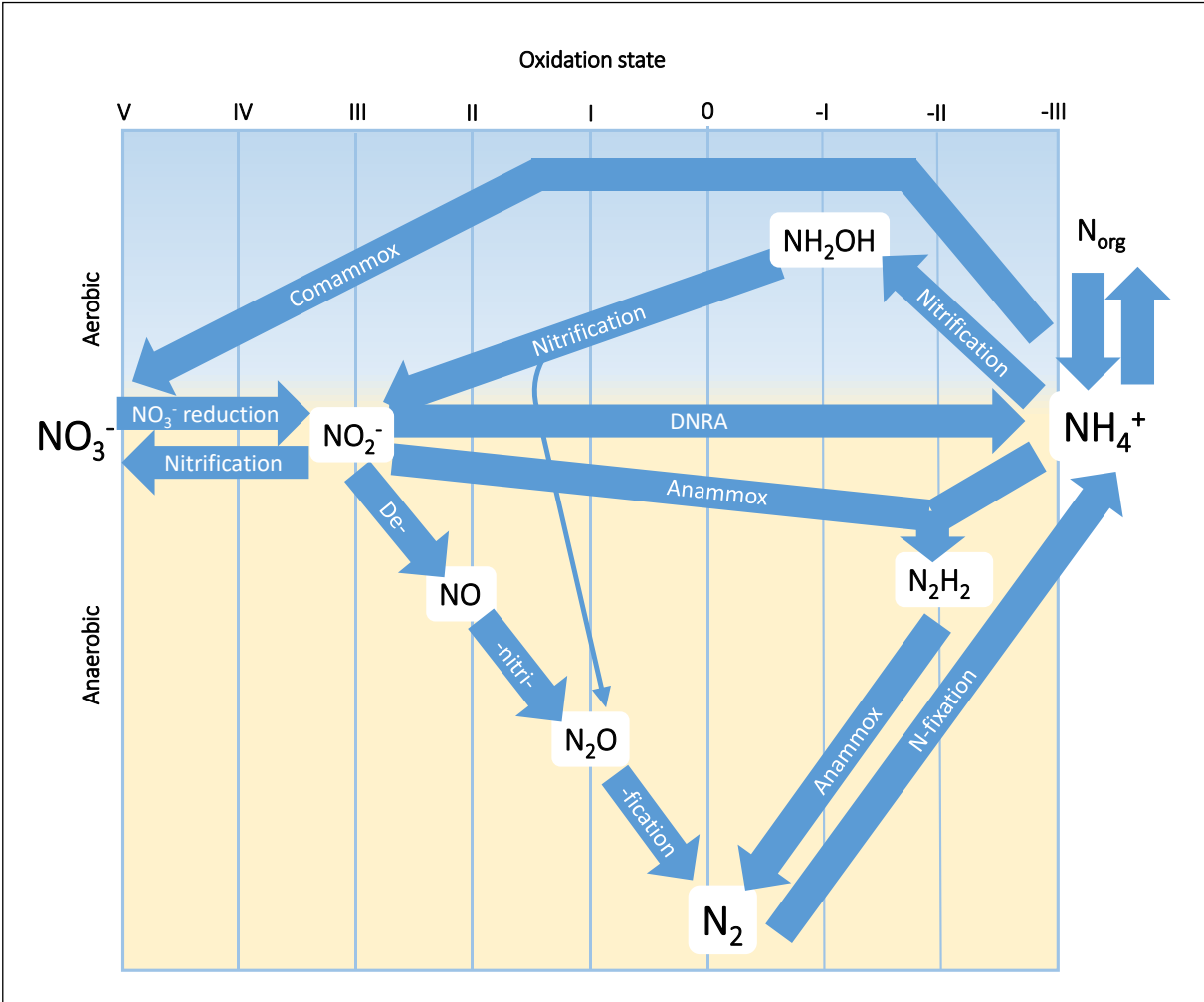


Figure 1. Major transformations in the nitrogen cycle. DNRA: dissimilatory nitrate reduction to ammonium. Adapted from Canfield et al. [6].

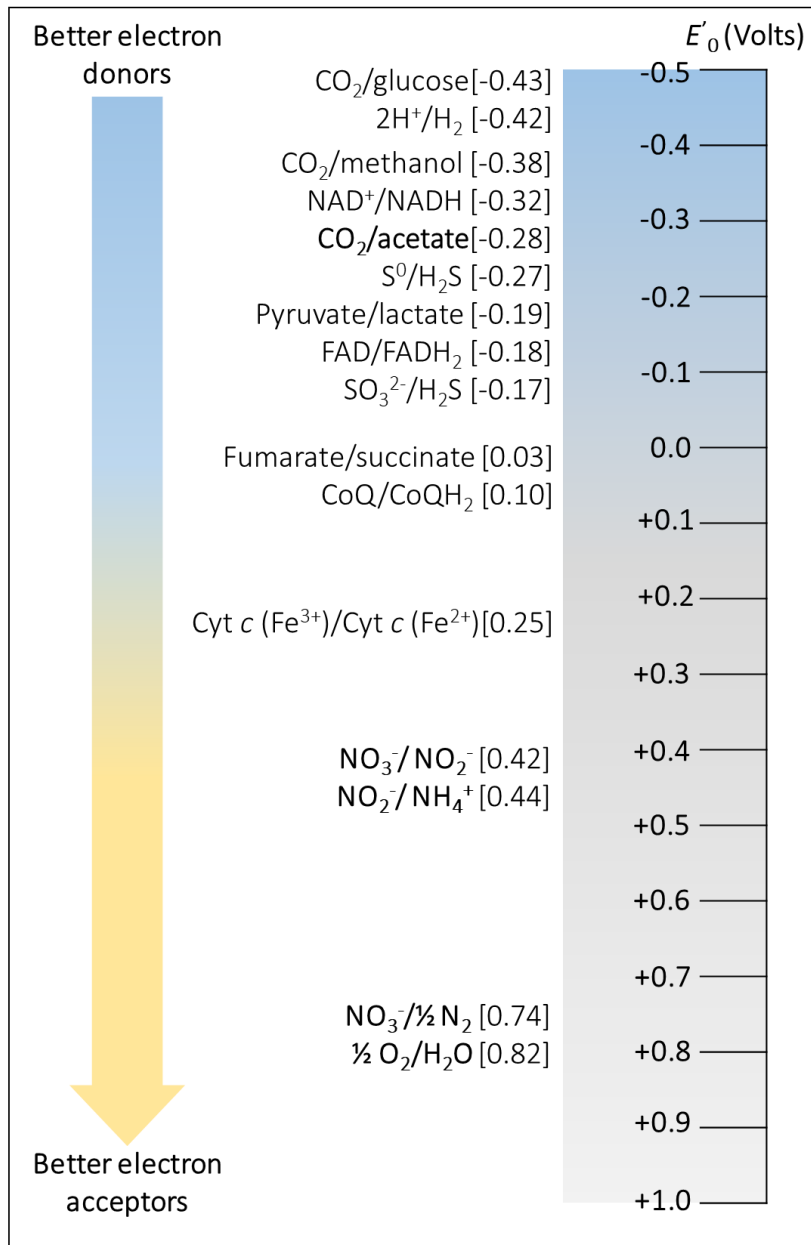


Figure 2. The electron tower. Electrons move spontaneously from donors with more negative reduction potentials (E'_0 vs SHE at pH 7) to acceptors with more positive reduction potentials. Adapted from Willey et al. [228].

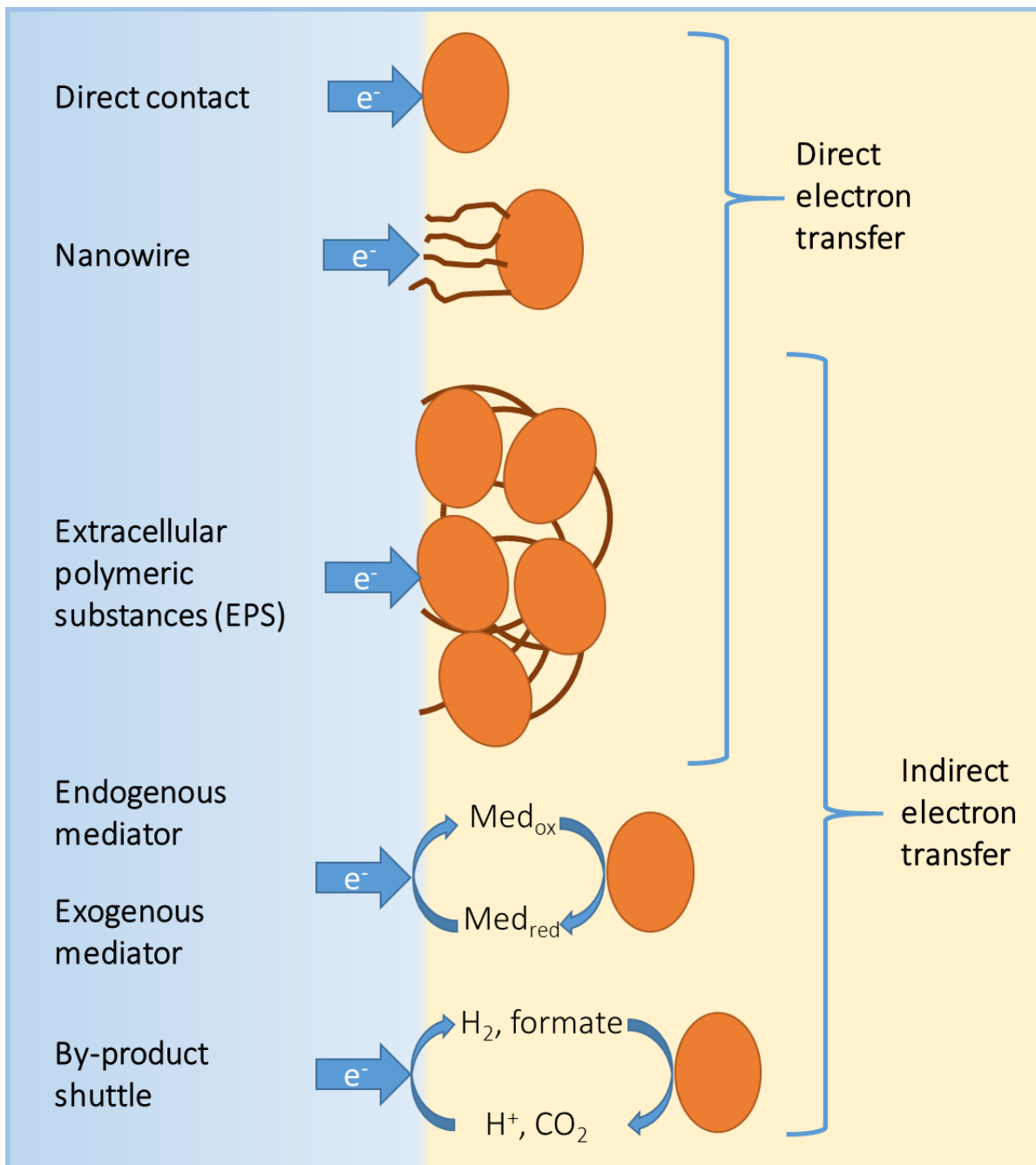


Figure 3. Different pathways of cathodic electron transfer. Adapted from Choi et al. [54].

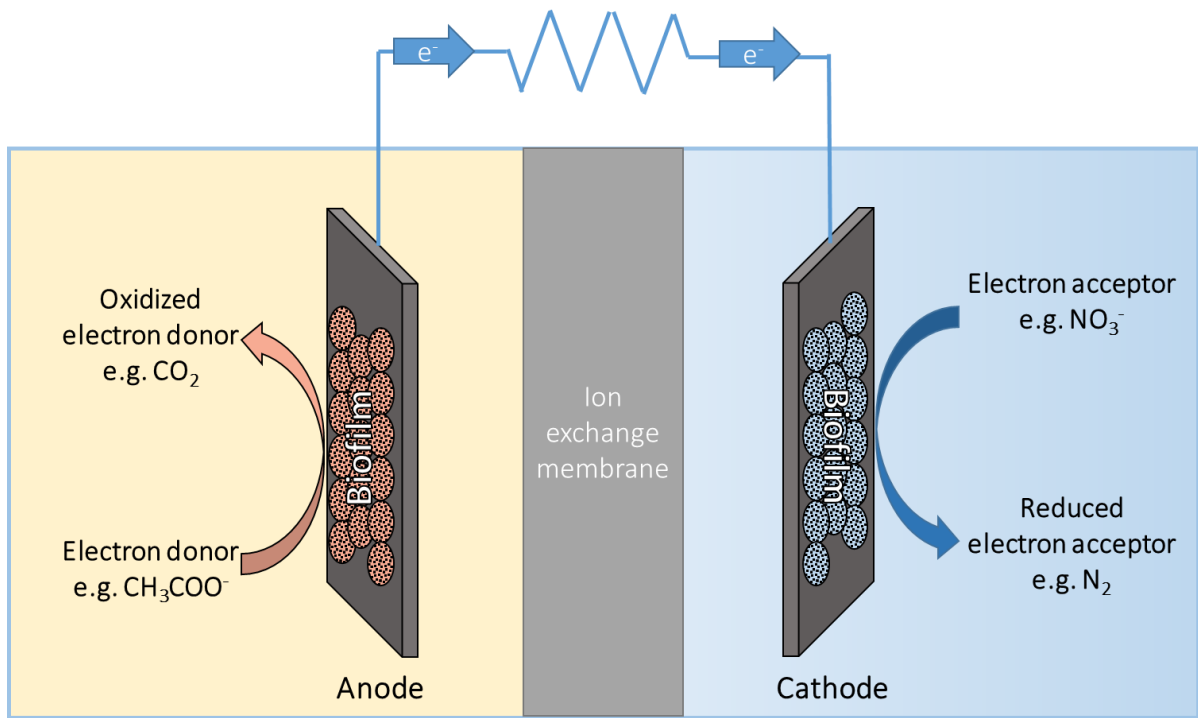


Figure 4. A schematic diagram of a two-chamber microbial fuel cell.

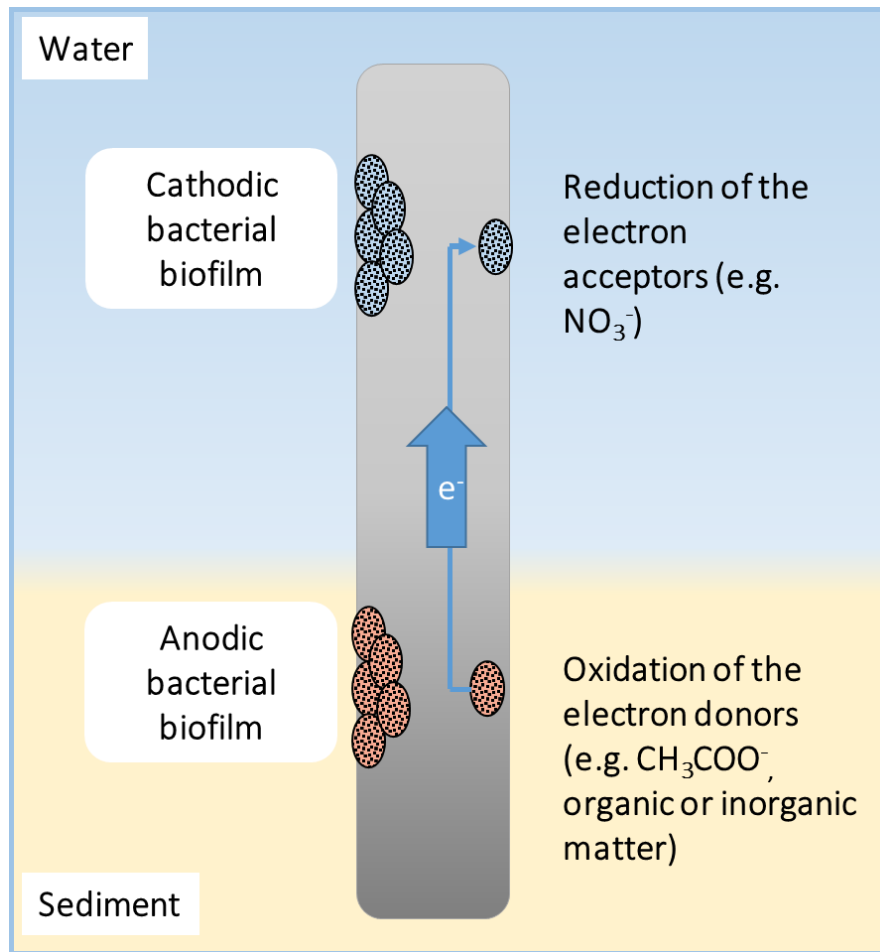


Figure 5. Schematic diagram of microbial electrochemical snorkel. Adapted from Rogińska et al. [85].

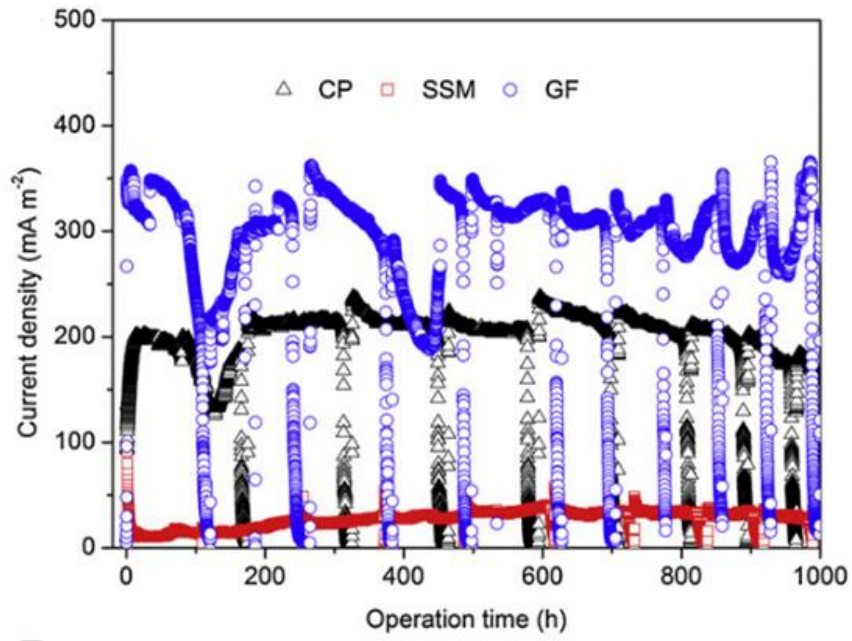


Figure 6. Current density for different materials used in microbial fuel cells. CP - carbon paper, SSM - stainless steel mesh, GF - graphite felt [149].

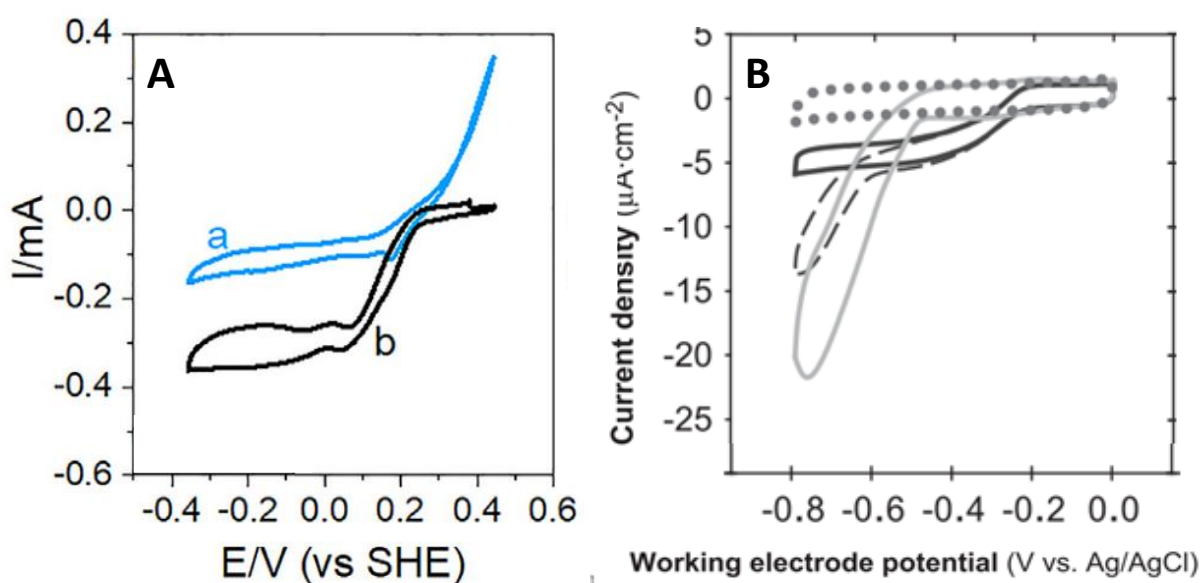


Figure 7. Cyclic voltammograms of nitrate-reducing biocathodes: (A) Response of a biocathode developed in a microbial electrochemical snorkel, (a) in the absence and (b) in the presence of nitrate (max concentration of $44 \text{ mg N-NO}_3^- \text{ L}^{-1}$). Scan rate: 1 mV s^{-1} [85]. (B) Response of a biocathode developed at $-0.32 \text{ V vs Ag/AgCl}$ in the presence of only nitrate (dark gray plain line), nitrate and nitrite (dashed line), only nitrite (light gray plain line) and blank electrode (dotted line). Scan rate: 0.5 mV s^{-1} [131].

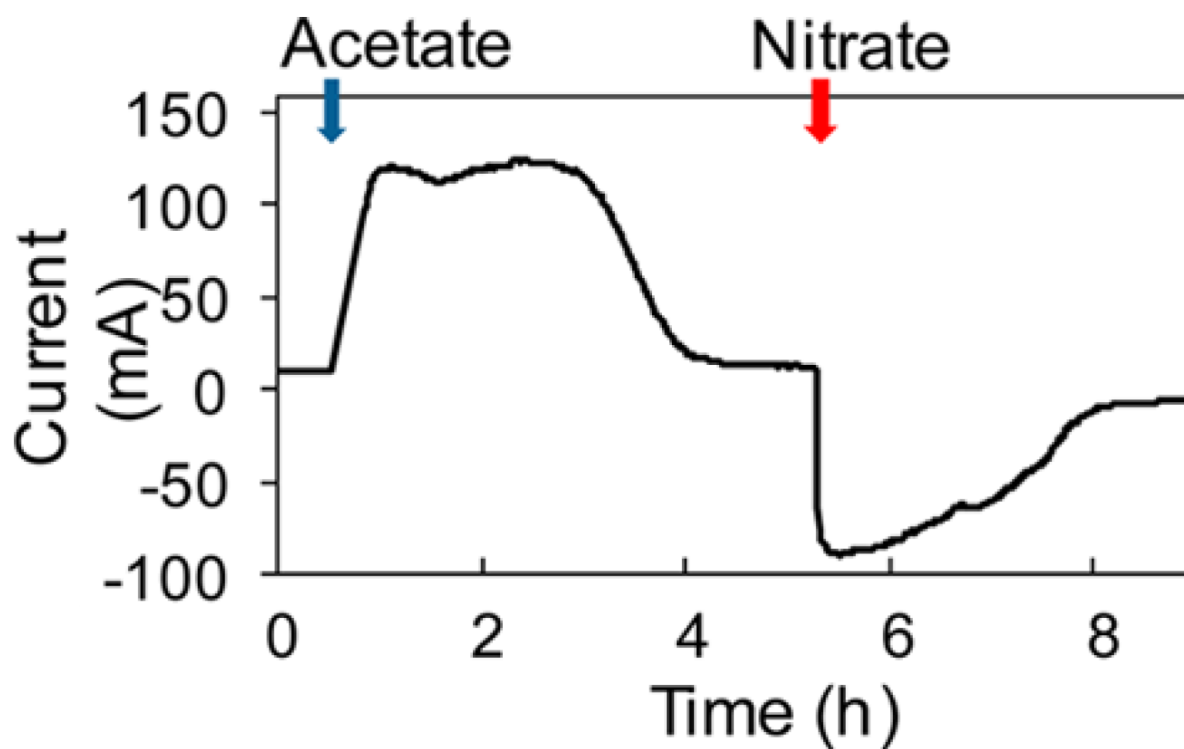


Figure 8. Examples of changes in current after "switching" electrode conditions from bioanodic to biocathodic. A. The electrode potential was -0.203 V vs SHE (-0.4 V vs Ag/AgCl). The pH was controlled on values 6.8–7.5. The electrode was fed either with acetate or with nitrate [124].

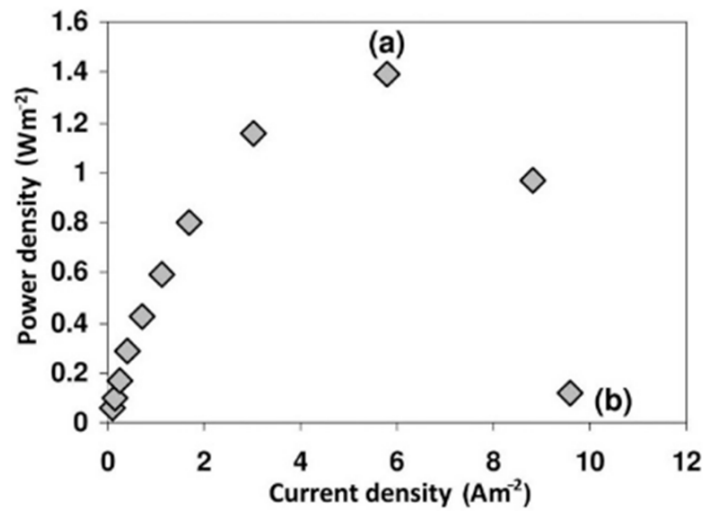


Figure 9. Power-current curve of a microbial fuel cell [81].

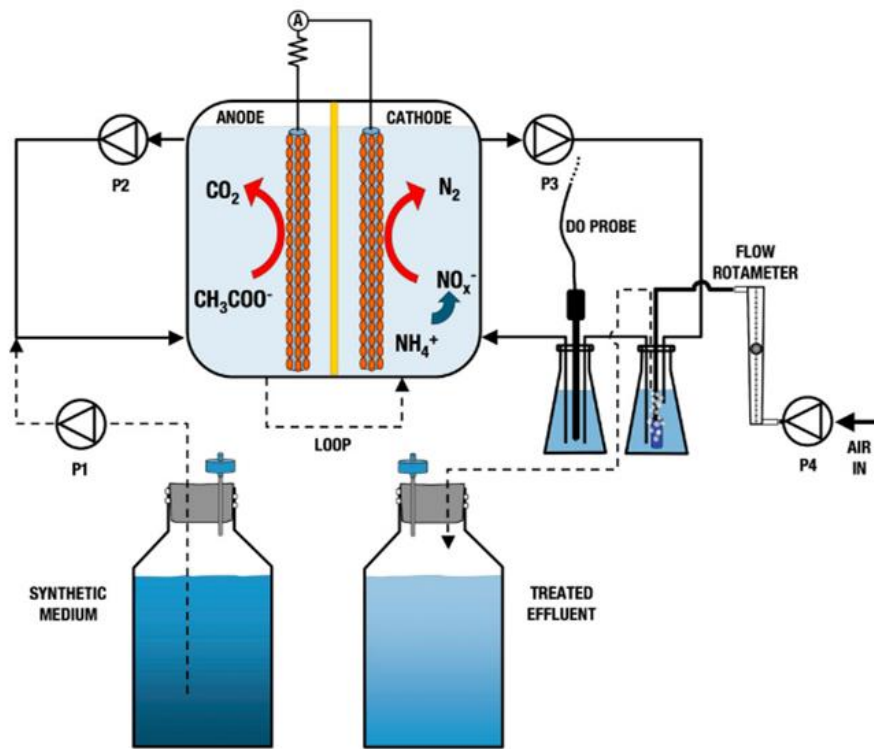


Figure 10. An example of nitrification-denitrification bioelectrochemical system, proposed by Viridis et al. [66].

Tables

Table 1. Summary of chosen studies of influence of oxygen concentration on nitrate reduction.

Type of BES	Dissolved oxygen mg L ⁻¹	Starting nitrogen concentration	Nitrate reduction rate	Energy production	Reference
MFC	1.5	0.722 g L ⁻¹ KNO ₃	1.565 mg (L·h) ⁻¹	1776 mW m ⁻³	[91]
	3.4		1.382 mg (L·h) ⁻¹	1764 mW m ⁻³	
	4.4		1.174 mg (L·h) ⁻¹	1021 mW m ⁻³	
SMFC	0	14.16 and 864.43 mg of TN in water and sediment, respectively	42.11% of TN (after 49 days)	7.24 mW m ⁻²	[92]
	1		55.22% of TN (after 49 days)	9.69 mW m ⁻²	
	3		60.55% of TN (after 49 days)	34.77 mW m ⁻²	
	5		56% of TN (after 49 days)	34.96 mW m ⁻²	
	8		38.35% of TN (after 49 days)	67.21 mW m ⁻²	
SMFC	0	10 mg N L ⁻¹	~62%	41.3 mW m ⁻²	[93]
	1.1		~55%	~37 mW m ⁻²	
	3.2		~27%	24.8 mW m ⁻²	
	7.8		7.8%	55.5 mW m ⁻²	
MFC Flow: 0.7 L d ⁻¹ , HRT 6.86h	1.97	53 mg N-NH ₃ L ⁻¹	66.9% of TN	0.19 mW	[94]
	4.35		94.1% of TN	0.89 mW	
	5.02		80.3% of TN	1.79 mW	
	7.24		29.0% of TN	2.74 mW	
BES for nitrification and denitrification	3.2	13 mg N L ⁻¹ (NH ₄ ⁺)	3.12 mg TN L ⁻¹ h ⁻¹	[95]	
	4.7		3.18 mg TN L ⁻¹ h ⁻¹		
	5.7		3.39 mg TN L ⁻¹ h ⁻¹		
	7		2.95 mg TN L ⁻¹ h ⁻¹		
MFC	4.2	~77 mg N L ⁻¹	0.029 kg TN m ⁻³ d ⁻¹	[96]	
	5		0.064 kg TN m ⁻³ d ⁻¹		
	6		Inhibition of denitrification process		

Table 2. Summary of chosen studies of influence of pH on nitrate reduction.

Type of BES	pH	Starting nitrogen concentration	Nitrate reduction rate	Reference
BES	6	50 mg NO ₃ ⁻ -N L ⁻¹ 6h	55.3%	[123]
	7		85.5%	
	8		84.2%	
	9		43.4%	
MFC	2	345 mg NO ₃ ⁻ -N L ⁻¹	50%	[70]
	4		55%	
	6		76%	
	7		97%	
	9		91%	

Table 3. Summary of chosen studies of influence of applied potential on nitrate reduction or measured current.

Type of BES	Applied potential	Starting nitrogen concentration	Nitrate reduction rate	Current	Reference
MFC	100 mV vs SHE	0.652 g NaNO ₃ L ⁻¹ 200 mL min ⁻¹	-0.327 mM-N h ⁻¹	7.8 mA	[144]
	0 mV vs SHE		-0.432 mM-N h ⁻¹	10.4 mA	
	-100 mV vs SHE		-0.484 mM-N h ⁻¹	12.4 mA	
	-200 mV vs SHE		-0.424 mM-N h ⁻¹	11.3 mA	
BES	-500 mV vs SHE	2 mM of nitrate	75.6% after 15 days	160.8 μA	[145]
	-300 mV vs SHE		58.5% after 15 days	76.5 μA	
	-100 mV vs SHE		48.4% after 15 days	29.5 μA	
	250 mV vs SHE		26.8% after 15 days	11.4 μA	
MFC	197 mV vs SHE	0.606 g KNO ₃ L ⁻¹		No catalytic current	[113]
	-53 mV vs SHE			-905 mA m ⁻²	

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