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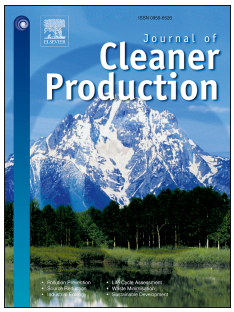

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Denitrification of water in a microbial fuel cell (MFC) using seawater bacteria

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Abstract

The sea contains various microbes which have an ability to reduce and oxidize substances like iron, sulphur, and nitrate. Most of these processes happen in the seawater, but can also be applied for purification of wastewater. In the present work, a consortium of seawater bacteria has been used for the first time in a microbial fuel cell to reduce nitrate in synthetic water samples and produce electricity by oxidizing organic matter. The concentrations of nitrate and nitrite were reduced to well below their permissible limits. Moreover, the growth of the bacterial consortium in cathode causes an increased electricity production in the cell because of the increased bacterial activity. The performance of the cell with a bicarbonate buffered solution in the cathode was superior to that obtained with the commonly used phosphate buffered solution. As bicarbonate is the natural buffering agent found in the sea, the use of bicarbonate buffered solutions is eco-friendly. The same seawater bacterial consortium was used in both the anode and the cathode, confirming their adaptability to different environments. Unfortunately, denitrification was accompanied by the generation of high concentrations of ammonium in the anode and the cathode, probably because of the use of nitrogen gas for

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sparging the anolyte. This aspect merits further investigation.

Keywords: Bicarbonate buffer, biological cathode, denitrification, microbial fuel cell, phosphate buffer, seawater bacteria

1. Introduction

Compounds containing nitrogen (N) are abundant, with an annual production of about 4.13 x 10^{14} g as N by fixation processes (Zhang and Zindler, 1993). Among them, nitrate (NO_3^-) is one of the predominant compounds produced which is a highly mobile and stable species. Also, most of the compounds are converted to NO_3^- after their use in biological systems (Galloway, 1998). The released NO_3^- enters the groundwater sources in the form of domestic and industrial wastewater, and fertilizers that are added to the soil for higher crop yields. This leads to widespread environmental contamination (Lasagna et al., 2016).

When nitrate is ingested by people, it is converted to nitrite and then to nitrosamines, which may cause gastric cancer (Bogárdi et al., 2013). Moreover, nitrate intake causes methaemoglobinaemia in infants, even when exposed for a very short duration (Fewtrell, 2004). The World Health Organization (WHO) has prescribed guideline values (GV) of 50 mg NO_3^- L^{-1} and 10 mg nitrite (NO_2^-) L^{-1} (World Health Organization, 2011). To obtain concentrations less than the GV, methods such as adsorption, reverse osmosis, ion exchange, electro-dialysis (Bhatnagar and Sillanpää, 2011), catalytic denitrification (Hao and Zhang, 2017), and biological denitrification (Ghafari et al., 2008) have been used.

Because of the disadvantages of many of the processes, it is preferable to use the biological route, wherein bacteria can reduce NO_3^- to nitrogen (N_2) gas by donating the electrons which are released in their metabolic pathways (Munn, 2011). An additional disinfection step such as UV treatment or passing the water through a bed of silver nanoparticles (Swathy et al., 2014) is needed before the water can be used for drinking.

The reduction and oxidation steps can be made to occur in the anodic and cathodic compartments of a microbial fuel cell. This is similar to a conventional fuel cell, where the oxidation of substrates occurs in the anode, and reduction occurs in the cathode. When oxidation-reduction and transfer of electrons occur in the presence of, or are mediated by microorganisms, then it is called a microbial fuel cell (MFC). The current obtained is produced by

the oxidation of organic substrates and the produced electrons (e^-) are used for the reduction of the nitrate in the cathode (Fig. 1). It is also observed that

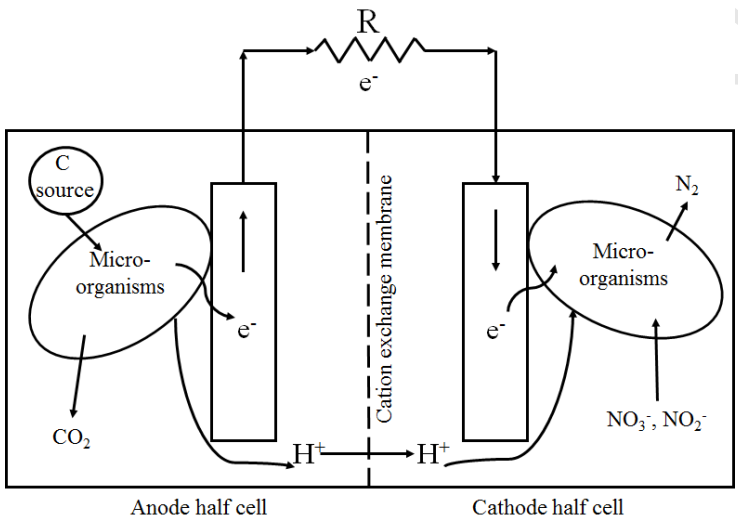


Figure 1: Schematic of a microbial fuel cell. Here R represents a resistor.

the energy yield from the treatment of wastewaters is higher in an MFC than compared to alternate pathways like the combustion and the use of a fuel cell (Gude, 2016). Recently, there have been developments in the enhancement of the power output from MFC by changing the genes related to electron transfer and biofilm production in microorganisms using transcriptional and translational regulation techniques (Cao et al., 2017).

The overall reaction is (Zumft, 1997; Clauwaert et al., 2007)

$$NO_3^- + 5e^- + 6H^+ \rightleftharpoons \frac{1}{2}N_2(g) + 3H_2O \quad (1)$$

The reduction of NO_3^- mostly occurs in the absence of oxygen (O_2), as the enzymes involved in the denitrification process are repressed by O_2. Therefore an anaerobic condition is maintained in the cell (Munn, 2011). The MFC produces nitrate-free water and also generates electricity. The first application of this method for nitrate removal was by Clauwaert et al. (2007), who observed complete denitrification with power and current densities of about 4 W/m^3 TCC (total cathodic compartment volume) and 19 A/m^3 TCC, and

a cell voltage of 0.214 V. Similar experiments, but with simultaneous carbon, nitrate, or phenol removal were performed with different COD/N (chemical oxygen demand/nitrogen) ratios (Virdis et al., 2010; Feng et al., 2015).

Attempts were made to reduce the resistances at the cathode side by adjusting the pH, using transient operation, and by design modifications (Clauwaert et al., 2009; Behera et al., 2010; Liang et al., 2013; Zhu et al., 2013; Yang et al., 2015). In most of these systems, phosphate buffered solutions (PBS) were used as the media to control the pH and also to provide sufficient conductivity for the flow of ions. However, the increased use of phosphate and its subsequent discharge into water sources causes eutrophication, which is a serious environmental problem (Morse et al., 1998; Loganathan et al., 2014). Therefore to limit the use of PBS, buffers such as bicarbonate, and boric acid-borate were used (Fan et al., 2007; Chen et al., 2015). The power density at the anode with a bicarbonate buffered solution (BBS) was about 39% higher compared to PBS for a pH = 9.0 (Fan et al., 2007).

There are also reports on the direct use of low-conductivity waters like groundwater sources without buffer addition, and with a high nitrate content, and their subsequent denitrification. Pous et al. (2013) observed a 64% removal efficiency of nitrate for a 15-day period of operation using water with a conductivity less than 1000 μS/cm. For water with higher conductivities in the range 1000 - 4000 μS/cm, Puig et al. (2012) observed about 45 - 90% removal when the system was operated for 3 days. Groundwater sources were also treated using PBS, but with a modified design of the fuel cell. Anion exchange membranes were used to permit the movement of anions from the surrounding groundwater to the buffered anolyte and catholyte solutions, which results in desalination and denitrification (Tong and He, 2013; Zhang and Angelidaki, 2013). As bicarbonate is a naturally occurring buffering compound (Weber and Stumm, 1963), its use instead of phosphate can help in reducing the load of phosphate contamination on the environment.

The methods discussed above for denitrification either used a single strain of bacteria, or a consortium taken from a waste sludge or a bio-reactor used in wastewater treatment plants. Denitrifying and nitrifying bacteria are also present to a greater extent in seawater. It is estimated that annually about 1.4 x 10^{14} g N is fixed by marine ecosystems and about 1.0 x 10^{14} - 2.8 x 10^{14} g N is denitrified to N_2 gas in the oceans (Fowler et al., 2013). Therefore, the use of seawater bacteria as an inoculum may increase the rates of denitrification. In the present work, seawater bacteria taken from the Adriatic sea at

the shores of Italy have been used. There are reports on the use of seawater bacteria for oxidizing substrates in the anode of an MFC (Tommasi et al., 2016), but there are no reports on their use for denitrification. For the first time, seawater bacteria have been used for denitrification in the present work.

The nitrate removal capability of these bacteria was examined for one month under external resistance using PBS. Then BBS was used instead of PBS for another month. Electrochemical measurements were made to confirm the superiority of BBS compared to PBS with the seawater bacteria. The present work is mainly confined to the results obtained with BBS.

2. Materials and methods

2.1. Experimental setup and materials

Two different microbial fuel cells (MFC) designs were used in the experiments. Both designs consist of two chambers, an anode and a cathode, and were made of poly(methyl methacrylate). The dimensions of a single square chamber was 8 x 8 x 2 cm^3 (volume ≈ 128 mL), and that of circular chamber was 7 cm (diameter) and 15 mm (width) (volume ≈ 57.7 mL). All the experiments were conducted in duplicate under the same operating conditions (Supplementary Fig. S1). The anode and cathode chambers were separated by a cation exchange membrane (CEM) (CMI-7000, Membranes International, USA) to facilitate the movement of cations from the cathode to the anode and vice versa. As cations (mainly H^+) are lost at the cathode, other cations must move from the cathode to the anode to maintain electroneutrality. For the movement of electrons from the anode to the cathode through the external circuit, graphite rods were stitched to a commercial carbon felt (C-FELT) (soft felt SIGRATHERM GFA 5, SGL Carbon, Germany) using a conductive carbon thread and the rods were connected to a potentiostat using copper wires with crocodile clips. The carbon felt was used to increase the effective area exposed, thereby increasing the transfer of electrons.

The square cell was operated in a semi-batch mode, wherein an initial amount of substrate was added to the chamber. The total volume of the solution taken was about 110 mL. Sampling and addition of new substrate were done periodically.

The circular MFC was operated in a semi-continuous mode, wherein the solution was re-circulated from the chamber to reservoir (bottle A) using a peristaltic pump (ISMATEC-ISM404B, Germany) at a flow rate of 36 mL/min (Fig. 2). During oxidation and reduction, carbon dioxide (CO_2)

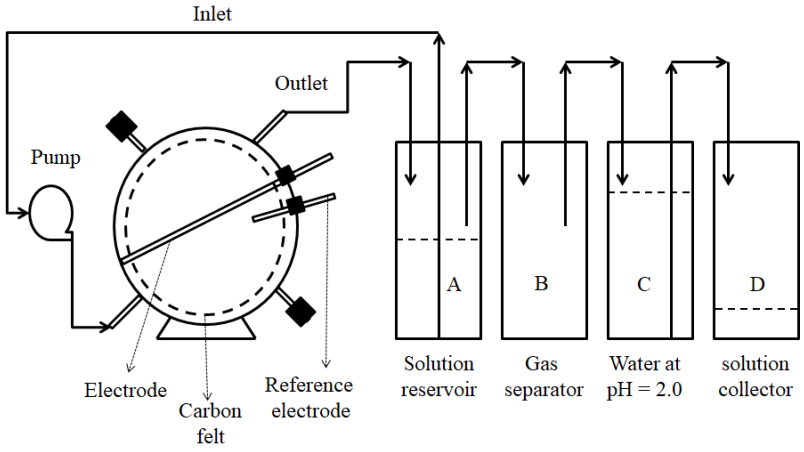


Figure 2: A side view of the circular MFC setup used in the experiments (A photograph of the setup is shown in Supplementary Fig. S3).

is released at the anode and N_2 gas at the cathode. From the outlet of each chamber, both the liquid and the gas fractions enter the bottle A, where the gaseous fraction is separated from the liquid and moves to the bottle B. The water vapour associated with the humid gas is condensed in B, and the relatively dried gas enters the bottle C, which contains water at a pH = 2.0. The low pH prevents the dissolution of CO_2. Hence the gas displaces the water into the bottle D (Fig. 2). The volume of water collected in D is equal to the volume of gas released from the chamber. The total volume of the solution taken was about 200 mL in the circular chamber and the bottle A.

The chemicals sodium acetate (AR) (CH_3COONa), peptone (bacteriological for microbiology), sodium phosphate dibasic dihydrate (AR) ($Na_2HPO_4 \cdot 2\,H_2O$), sodium phosphate monobasic monohydrate (AR) ($NaH_2PO_4 \cdot H_2O$), potassium nitrate (AR) (KNO_3), sodium bicarbonate (AR) ($NaHCO_3$) were purchased from Sigma Aldrich and used to prepare the anolyte and catholyte without further purification. Here AR represents analytical reagent grade chemical. Ultrapure Millipore water was used to prepare the anolyte solution. For the catholyte, commercially and locally available mineral bottled water was used as the solvent.

2.2. MFC startup and operation

The MFCs used in the experiments were inoculated with the effluent from an active MFC that was previously enriched. The enrichment procedure is given in Tommasi et al. (2016), who also present a detailed discussion of the different types of bacteria in the seawater consortia. The consortium consisted of species such as Proteobacteria phylum (*Shewanella* and *Geobacter*), Firmicutes (*Clostridium*) and Ascomycota (*Saccharomyces*). Phosphate and bicarbonate buffers of approximately 80 mM concentration were prepared. Phosphate buffer was prepared by the addition of 8.2 g/L $Na_2HPO_4 \cdot 2\,H_2O$ and 5.9 g/L $NaH_2PO_4 \cdot H_2O$ to millipore water. Bicarbonate buffer was prepared by the addition of 5.46 g/L $NaHCO_3$, 48.1 mg/L $Na_2HPO_4 \cdot 2\,H_2O$ and 35.9 mg/L $NaH_2PO_4 \cdot H_2O$ to mineral water. The anolyte used consisted of PBS with 10 mL inoculum, 8 g/L CH_3COONa, 10 g/L peptone, 12 g/L glucose ($C_6H_{12}O_6$). As we are interested in denitrification of water, a catholyte containing 0.16 g/L KNO_3 (100 mg NO_3/L) was used. In our experiments we have not considered the use of natural water, as this could lead to complications arising from the interactions of seawater bacteria with other contaminants present in the natural source of water. To avoid substrate limitations at the anode, the anolyte had a higher concentration of the carbon source than that required by stoichiometry. For the production of 1 mole of N_2, 10 moles of e^- are required and these are produced at the anode by oxidation of glucose and acetate which release 24 and 8 moles of e^-, respectively, per mole of substrate oxidized (2 - 3) (Logan and Rabaey, 2012).

$$C_6H_{12}O_6 + 6H_2O \rightleftharpoons 6CO_2 + 24H^+ + 24e^- \quad (2)$$

$$CH_3COO^- + 2H_2O \rightleftharpoons 2CO_2 + 7H^+ + 8e^- \quad (3)$$

All the solutions were adjusted to a pH between 7 - 7.5 before their injection into the MFC using 2 N sodium hydroxide (NaOH) or hydrochloric acid (HCl) solution. Phosphate buffered solution and BBS were purged with nitrogen and argon gas, respectively for about 5 minutes, to maintain the cells in an anaerobic condition. The solution volumes in the anode and the cathode chambers of the cells were kept constant. The samples from the chambers were collected periodically and an equal amount of fresh solution was added. This procedure corresponds to the working of the MFC in multiple operational cycles.

A chamber containing a cathode, an electrolyte and bacteria will be referred to as a biotic cathode (biocathode), and one without bacteria will be

referred to as an abiotic cathode. Similar remarks apply to the anode. In the present work, a biotic anode was used along with abiotic and biotic cathodes.

The solution containing bacteria was prepared by adding 1 mL of the enriched inoculum to 10 mL of the solution. Initial studies were done using square cell with an abiotic cathode. To confirm the dual nature of seawater bacteria, the bacteria enriched in the anodic compartment during the experiments with an abiotic cathode were used in the cathodic compartment for denitrification. The biotic experiments at the cathode were first performed with square setup and then the circular setup with BBS was used. The circular setup was used to decrease the limitations that can arise because of the slow diffusion of species and nutrients in the square setup.

All the cells used were initially connected under open circuit voltage to attain a steady voltage. Then the cells were connected to a resistance of 1.5 kΩ. Periodic linear sweep voltammetry (LSV) measurements was conducted using a potentiostat.

2.3. Electrochemical measurements on MFCs

The electrochemical analysis were conducted using a multi-channel VSP potentiostat/galvanostat (BioLogic, France). The data from these experiments were acquired using a EC-Lab software version 10.1x. Polarization curves i.e. the variation of the cell potential with current density, were obtained using LSV by imposing a linear decrease of the electric potential from the open circuit voltage (OCV) (current $I = 0$) to the short circuit voltage (SCV) ($I = I_{max}$) of the cell, at a scan rate of 1 mV/s. From these $I - V$ curves, the power density and the current density were calculated using the equations $I_d = I/V_c$ and $P_d = (IV_o)/V_c$, where I_d and P_d are current and power density, and V_o and V_c represent the voltage output and the volume of the cathodic chamber, respectively. For a full cell, a two-electrode configuration was used, where the working electrode (WE) was connected to the anode and both the counter (CE) and the reference (RE) electrodes were connected to the cathode.

2.4. Chemical analysis of samples

Samples collected at certain time intervals were analysed using a Metrohm ion chromatograph for anions (Cl^-, SO_4^{2-}, NO_3^-, PO_4^{3-}, NO_2^-, CH_3COO^-) and cations (Na^+, K^+, NH_4^+). When PBS was used, the eluent for the anions contained 2.5 mM Na_2CO_3, 1.0 mM $NaHCO_3$, and 2.0 mM NaOH and the samples were diluted about 200 times before injection into the column. When

BBS was used, the eluent for the anions contained 1.0 mM $NaHCO_3$ and 3.2 mM Na_2CO_3, and the samples were diluted 25 times. The separation of ions was achieved using a Metrosep A Supp 5 - 250/4.0 anion column with a Metrosep Guard column. The cation analysis was done using a Metrosep C4 - 150/4.0 column. The eluent for the cations was a mixture of 1.7 mM HNO_3 and 0.7 mM dipicolinic acid, and the samples were diluted 200 times.

The composition of the gaseous samples was determined using an offline gas chromatograph (VARIAN CP4900), where H_2, N_2, CO_2, and O_2 were detected.

3. Results and discussion

3.1. Ability of seawater bacteria to denitrify water

Denitrification occurred at a faster rate with a biotic cathode (i.e. one having seawater bacteria) than an abiotic cathode (Fig. 3). In Figs. 3 - 5,

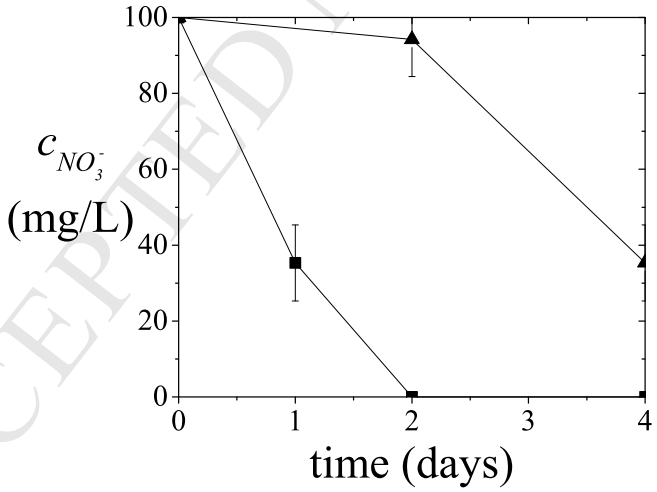


Figure 3: Variation of the concentration of nitrate in the catholyte with time: ▲, abiotic cathode; ■, biotic cathode. A PBS was used in the cathode of the square cell.

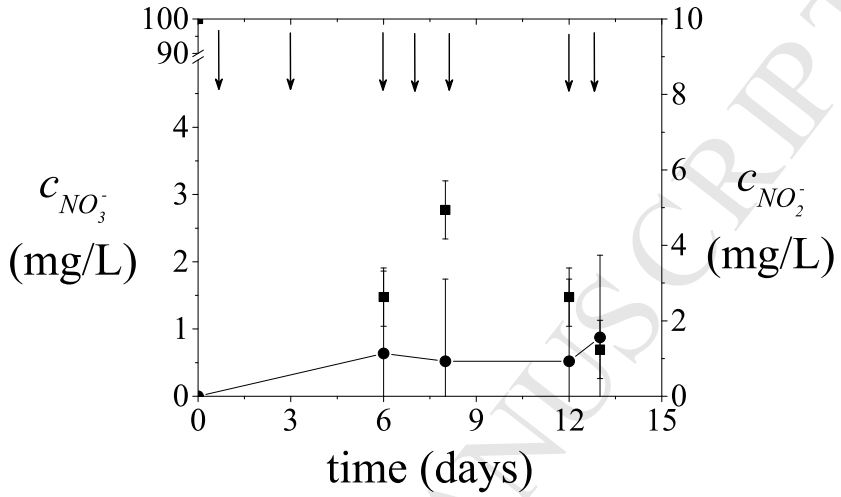
and 8, the error bars represent the 95% confidence limits associated with the estimation of the concentration from the calibration curve (see Appendix A).

At the end of two days, the removal of nitrate was about 100% with the biocathode and 6% with the abiotic cathode. Also, nitrite was not detected in the system. Thus there was a total conversion of nitrate to N_2. This was also confirmed by the gas analysis of the samples collected, which contained about 99.8% N_2 gas. Thus, a consortium of bacteria taken from the free surface of sea, where most of the nitrogen-fixing bacteria are present (Zhang and Zindler, 1993), significantly enhanced the rate of denitrification.

The use of PBS to examine the denitrification capacity of bacteria is good on a lab scale. However, it is not feasible at the field level, as the presence of high concentrations of phosphates in water causes algal growth. In the case of seawater bacteria, the use of bicarbonate is a sustainable solution for denitrification, as it is a natural buffering agent present in the sea. Also, it mimics the environmental conditions to which the seawater bacteria are acclimatized and provides a suitable means to maintain the pH in the desirable range of 7 - 8.2. Probably because of this, there is a faster adaptation and bacterial growth. Also, as there is no separate organic carbon in the cathode, the bacteria may use the bicarbonate present as a carbon source. This increases the bacterial metabolism rates compared to the phosphate buffer solution. It has been observed that denitrifying bacteria can adapt to an environment with supply of carbon source as CO_2 or HCO_3^- (Ghafari et al., 2009). For seawater bacteria, if there is high N and low P, which is the case with BBS, the bacteria may go into a survivalist mode and the resource acquisition (NO_3^- reduction) machinery may dominate the growth machinery (Arrigo, 2005). Thus the bacteria can survive the changes in the environmental conditions when the buffer is changed from PBS to BBS. The nitrate and nitrite levels obtained were very much lower than GV values prescribed by WHO (50 mg/L for NO_3^- and 10 mg/L for NO_2^-) were obtained when BBS was used in the cathode (Fig. 4a).

The nitrate reduction in the biotic cathode is mostly dependent on the availability of the electrons in the cathode and also on the mass transfer of the nitrate from the bulk solution to the cathode surface. In order to reduce this resistance, the catholyte was continuously recirculated. Moreover, to permit free movement of the ions, the conductivity of the catholyte was increased by doubling its concentration to 160 mM (conductivity $\approx$ 9.8 mS/cm). When the concentration of the bicarbonate was increased, there was a 9-fold increase in the concentration of NO_2^-, and a decrease in the amount of NO_3^- reduced (Fig. 4b). Such a decrease of the denitrification capability of bacteria with an increase of conductivity has not been reported

(a)



(b)

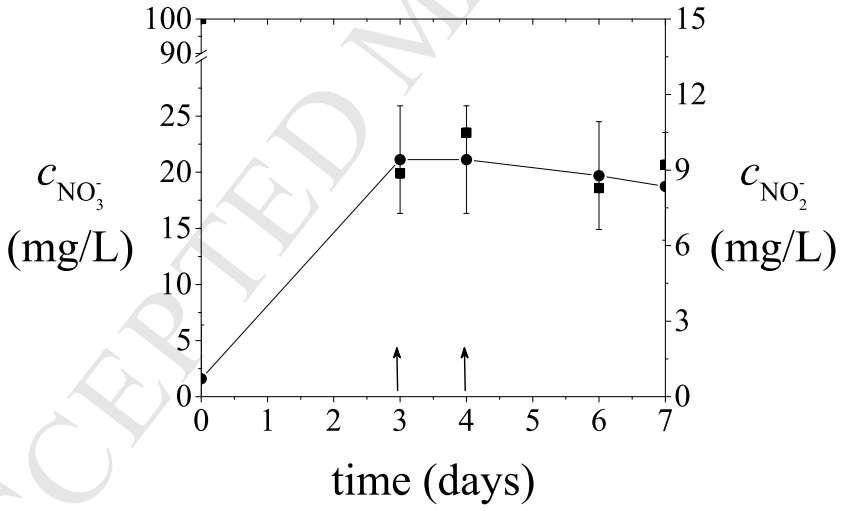


Figure 4: Variation of the concentration of nitrate, ■ and nitrite, ● in the biotic cathode with time: concentration of BBS in the cathode = 80 mM (a), 160 mM (b). The arrows represent an injection of 10 mL of fresh catholyte. The circular cell was used.

earlier. However, there are reports of the decrease in performance as the conductivity was increased for experiments with a bioanode and a biocathode using an aerobic process (De Schamphelaire et al., 2010; Lefebvre et al.,

2012; Karthikeyan et al., 2016). It has been reported that the presence of higher salt concentrations can adversely affect the physiology of bacteria, as the salt tolerance for different organisms is different. It is important to find out the different kinds of bacteria in seawater which affect denitrification. However, this is beyond the scope of the present work and merits further investigation.

The electrons needed for the reduction of nitrate are liberated by the oxidation of glucose and acetate at the anode. The concentration of acetate in the anode compartment decreases with time, except for a sudden increase when fresh anolyte is added (Fig. 5). Moreover, there was a steady decrease

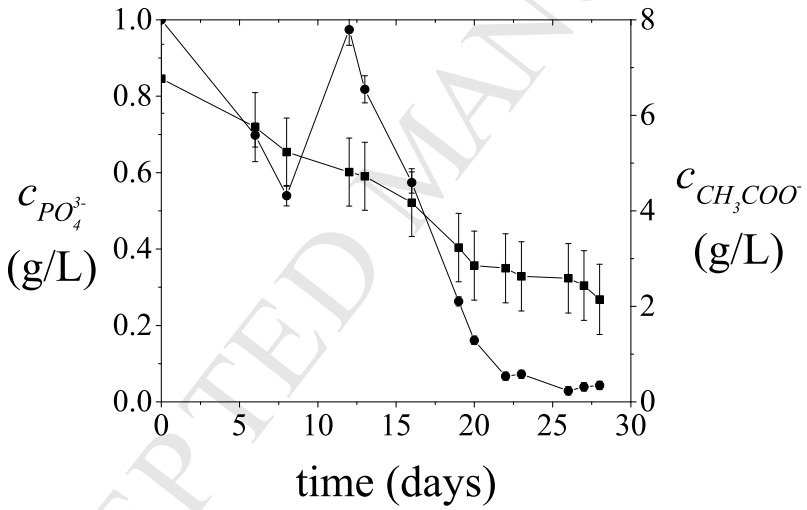


Figure 5: Variation of the concentration of phosphate in the biotic catholyte, ■ and acetate in the biotic anolyte, ● with time for the circular cell. Here the cathode and the anode contain BBS and PBS, respectively. For reasons discussed in the text, the BBS contains some phosphate.

in the concentration of phosphate in the cathode compartment (Fig. 5), probably because of the use of phosphate as a nutrient source by the bacteria. Here the presence of phosphate in BBS is mainly because of the addition of inoculum from a previous MFC which was operated with PBS. An increase of the optical density of the catholyte confirms the growth of bacteria (Supplementary Fig. S2). This indicates that the bacteria used nitrate, phosphate,

and the electrons from the anode for their growth. Hence, there was a stable removal of nitrate from the solution (Fig. 4).

The performance obtained with the present MFC that use seawater bacteria in both the anode and cathode is difficult to compare with that of other devices. This is mainly because of the use of wide variety of microorganisms, materials and cell architectures by different groups.

3.2. Higher power generation with BBS at the cathode

The reduction of the nitrate in the cathode is possible due to the availability of the electrons produced at the anode. Open circuit voltage (OCV) conditions were initially used for 1 day and 5 days for the square and circular cells to get the bacterial population acclimatized to the conditions in the cell. When PBS was used in the biotic cathode, the open circuit voltage (V_{OCV}) was about 0.37 V. When the BBS was used as the catholyte in the circular cell, there was an increase in V_{OCV} to 0.45 V (Fig. 6). There was

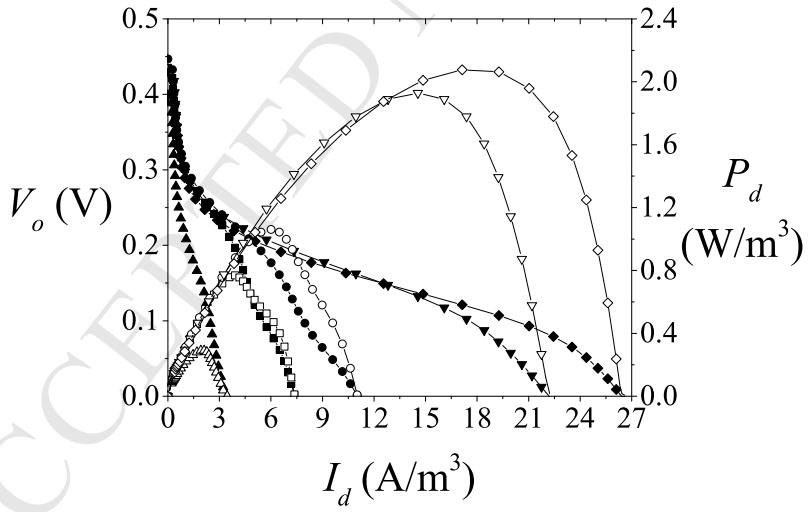


Figure 6: Polarization (filled symbols) and power density (open symbols) curves for the circular cell with BBS at the cathode and measured at different times during the course of the test: ■,□, 6 days; ●,○, 6 days, after replenishment; ▲,△, 8 days; ▼,▽, 12 days, after replenishment; ◆,◇, 14 days.

also a significant increase in the maximum power and current densities from 0.6 W/m^3 and 7.5 A/m^3 to 2.1 W/m^3 and 26.6 A/m^3, respectively, as the buffer solution was changed from PBS to BBS (Fig. 6). As mentioned in the previous section, the decrease in the P content when the buffer is changed from PBS to BBS may cause the bacteria to go into a resource acquisition mode. This may lead to a higher reduction of NO_3^-, thereby producing a higher peak values of the current and the power densities.

For BBS, the OCV was almost constant during the course of experiments, but there was a considerable increase in the peak power and current densities after 8 days (Fig. 6). This is mainly because of the availability of enough substrate to permit the oxidation and reduction reactions to take place and generate the required electrons. There was a sudden decrease in the voltage as the current density was increased near OCV condition. This could be because of activation resistance, also called charge-transfer resistance. It derives from the slowness and irreversibility of the reactions taking place at the surface of the electrodes (Zhao et al., 2009; Hidalgo et al., 2015).

The anode was buffered using PBS having a conductivity of ≈ 10 mS/cm, which is 40% higher than that of BBS. Therefore, to check the performance of the cell with a higher BBS conductivity at the cathode, the concentration of bicarbonate was doubled. There was an almost two-fold increase in the current and the power density, even though there was very less change in the OCV (Fig. 7). As mentioned earlier, with an increase in the bicarbonate concentration, there was a decrease in the reduction of NO_3^- and NO_2^- to N_2 (Fig. 4). However, based on the measurement of the current density, there is an extra flow of electrons from the anode to the cathode because of the increased conductivity. This should cause an increased reduction of NO_3^-, contrary to observation. It is possible that the N_2 gas produced may further reduced to ammonium (NH_4^+) by the reaction (Kim et al., 2005).

$$\frac{1}{2}N_2 + 3e^- + 4H^+ \rightleftharpoons NH_4^+ \tag{4}$$

The competition for e^- by the reactions (1) and (4) may cause a decrease in the reduction of NO_3^-.

3.3. Biological denitrification and a possible chemical nitrification in the cell

The reduction of nitrate occurs as shown by the reaction (1). With an excess availability of electrons it is possible to reduce the nitrate and nitrite

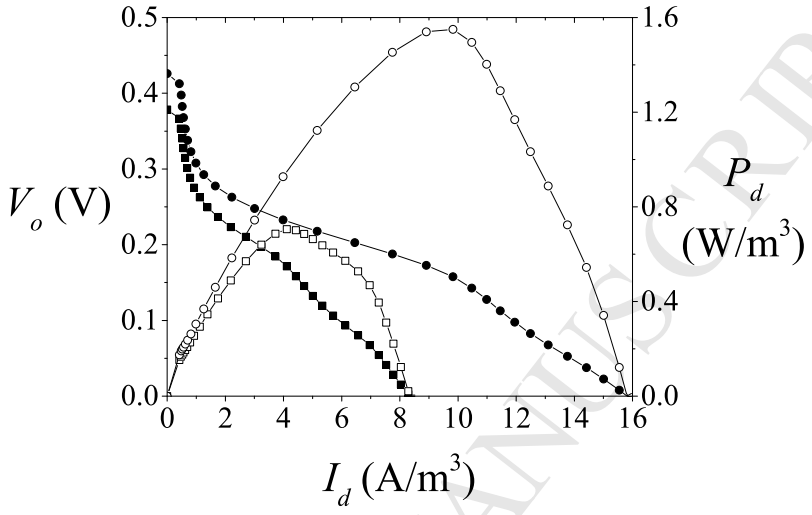


Figure 7: Polarization (filled symbols) and power density (open symbols) curves for the full cell with BBS at the cathode, measured before (■, □) and after (●,○) addition of the concentrated bicarbonate solution.

to NH_4^+ by the reactions (4) and (5) (Kim et al., 2005)

$$NO_2^- + 6e^- + 8H^+ \rightleftharpoons NH_4^+ + 2H_2O \quad (5)$$

In our experiments, NH_4^+ was observed in both the cathode and the anode (Fig. 8). The concentration of NH_4^+ in the anode was very high compared to its value in the cathode. Thus NH_4^+ appears to diffuse against its concentration gradient if it is produced in the cathode. The phenomenon of reverse diffusion has been observed in multicomponent systems (Krishna, 2016). However, in view of the large difference in concentrations, further investigation is needed. As the solution in the anode was initially sparged with N_2 gas, dissolved N_2 can diffuse from the anode to the cathode. With the availability of excess e^- and H^+, N_2 may be reduced to NH_4^+ in the cathode and diffuse to the anode.

If a bacterial population has to achieve nitrification in the anode using a species such as *Planctomycetes* (anammox bacteria), it requires NO_2^- along

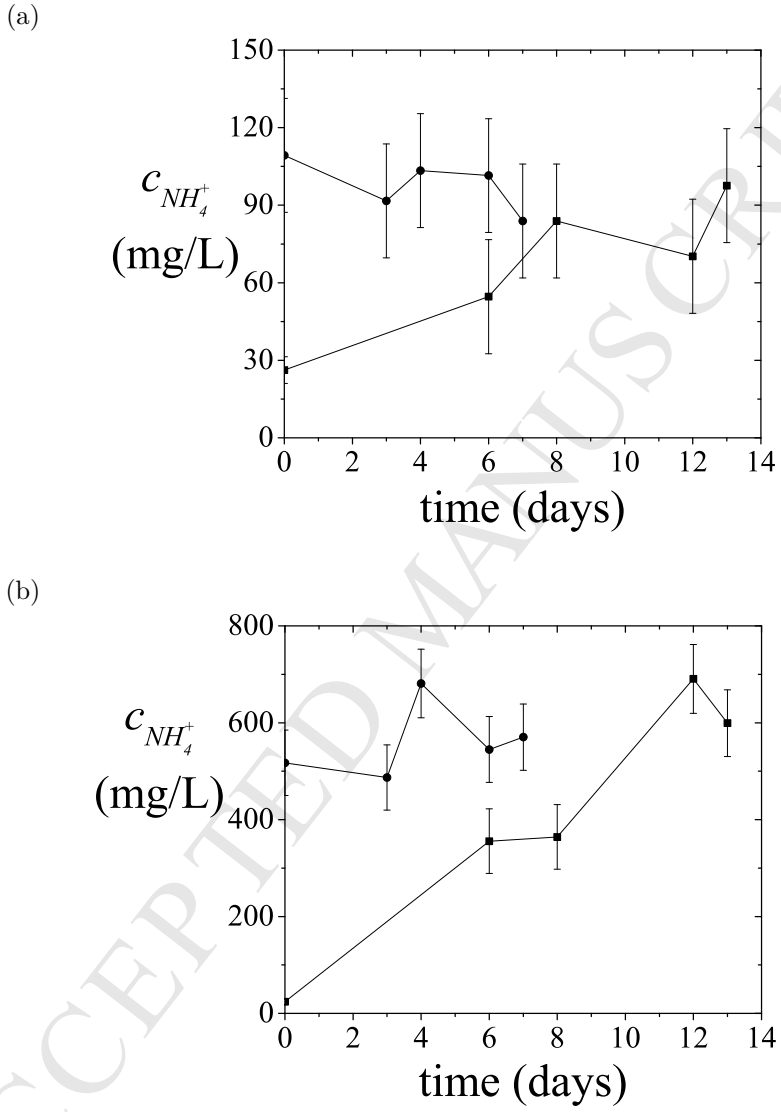


Figure 8: Variation of the concentration of NH_4^+ with time in the (a) catholyte and (b) anolyte: ■, 80 mM HCO_3^-; ●, 160 mM HCO_3^- at the cathode.

with NH_4^+ for the oxidation reaction (Kuenen, 2008)

$$NH_4^+ + NO_2^- \rightleftharpoons N_2 + 2H_2O \tag{6}$$

As there is no nitrite present in the anode, a reaction that may occur is

(Kim et al., 2005, 2006)

$$NH_4^+ + 4OH^- \rightleftharpoons \frac{1}{2}N_2 + 4H_2O + 3e^- \quad (7)$$

Thus chemical nitrification may occur in the anode and biological denitrification in the cathode.

4. Conclusions

The role of seawater bacteria in global nutrient cycling, which includes the nitrogen cycle, is extremely important for ecological equilibria. It has been shown for the first time that seawater bacteria can be used in a MFC to reduce NO_3^- to N_2. The levels of NO_3^- and NO_2^- were well below the guideline values prescribed by the World Health Organization. The bacteria were able to function in a phosphate buffered solution (PBS) in the anode and bicarbonate buffered solution (BBS) in the cathode. The BBS is environmentally friendly and can denitrify water with higher power and current densities than PBS in the presence of seawater bacteria. However, a high concentration of HCO_3^- (160 mM) decreased the denitrification capacity of the cell. Hence, seawater bacteria can be used for the denitrification of water with low to medium concentrations of bicarbonate. The high concentrations of NH_4^+ in the cathode and the anode may result from sparging the anolyte with N_2 or because of the presence of higher amounts of substrate in anode which results in the production of a higher amount of e^-. It appears that chemical nitrification may occur in the anode but more detailed studies are required. Therefore, a proper selection of the sparging gas and the use of a stoichiometric amount of substrate is necessary.

A. Calibration curve and confidence limits

The quantitative analysis of the ions in the solution was mostly done using ion chromatography. In a typical chromatogram the area under the peak corresponding to NO_3^- is proportional to its concentration $c_{NO_3^-}$. The construction of the calibration curve and confidence intervals is done as described in Snedecor and Cochran (1968, pg. 162). The procedure is discussed below.

The calibration curve is constructed as follows. Considering samples having known concentrations of the ions, let c_i denote the concentration of NO_3^-

in a sample i, a_i the area under the chromatogram, and n the total number of samples. The deviation from the mean values is given by

$$c_{d,i} \equiv c_i - \bar{c}, \quad a_{d,i} \equiv a_i - \bar{a} \tag{A-1}$$

where

$$\bar{c} = \frac{1}{n}\sum_{i=1}^{n} c_i, \quad \bar{a} = \frac{1}{n}\sum_{i=1}^{n} a_i$$

denote the mean values of the concentration and the area. The slope of the regression line is given by

$$b = \frac{\sum_{i=1}^{n} c_{d,i}\, a_{d,i}}{\sum_{i=1}^{n} c_{d,i}^2} \tag{A-2}$$

Consider an experiment with m independent measurements of the area under the chromatogram for a single unknown sample. If the mean values of these measurements is a, the estimated value for the concentration of ions in this unknown sample, c is given by

$$c = \bar{c} + \frac{a - \bar{a}}{b} \pm \frac{t_{0.05,n-2} S}{b} \sqrt{\frac{1}{m} + \frac{1}{n} + \frac{(a-\bar{a})^2}{b^2 \sum_{i=1}^{n} c_{d,i}^2}} \tag{A-3}$$

where

$$S = \sqrt{\frac{\sum_{i=1}^{n} a_{d,i}^2 - b^2 \sum_{i=1}^{n} c_{d,i}^2}{n-2}}$$

$t_{0.05,n-2}$ denote tabulated value of the quantity t, which follows the Student's t-distribution with $n-2$ degrees of freedom, and S is the sample standard deviation from the regression line. The subscript 0.05 denotes the 95% confidence limits, i.e. the interval within which there is a 95% chance of finding the actual concentration of the unknown sample. In (A-3), the term after the ± sign gives the confidence limits. For a set of known concentrations, an example of a calibration curve is shown in Fig. 9.

A similar procedure is used for the other ions.

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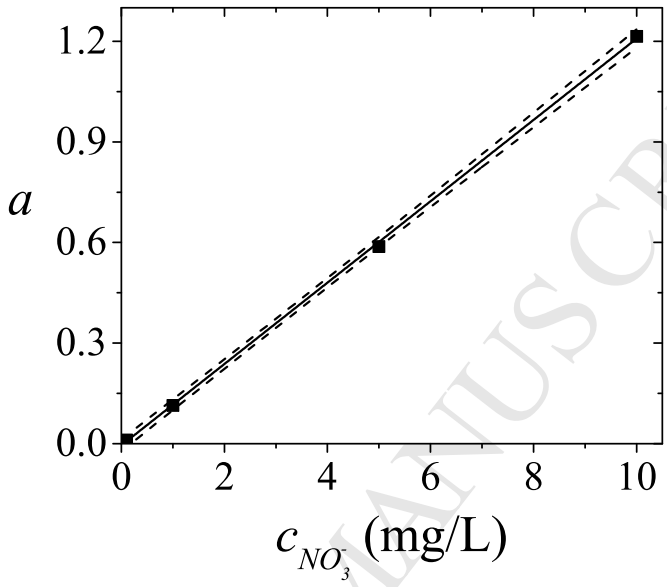

Figure 9: Variation of the area under the chromatogram, a with the concentration of NO_3^-, $c_{NO_3^-}$ obtained using an ion chromatograph: ■, data; ——, linear regression line; - - - - - - -, 95% confidence limits for c_F.

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Supplementary Information

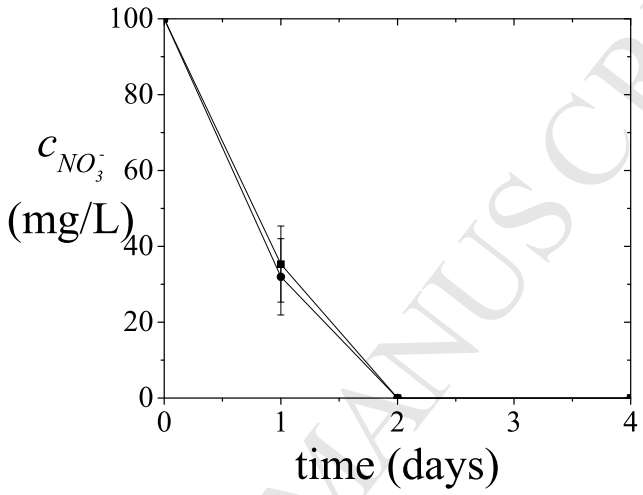

Figure S1: Variation of the concentration of nitrate in the catholyte with time of two different experiments with same operating conditions.

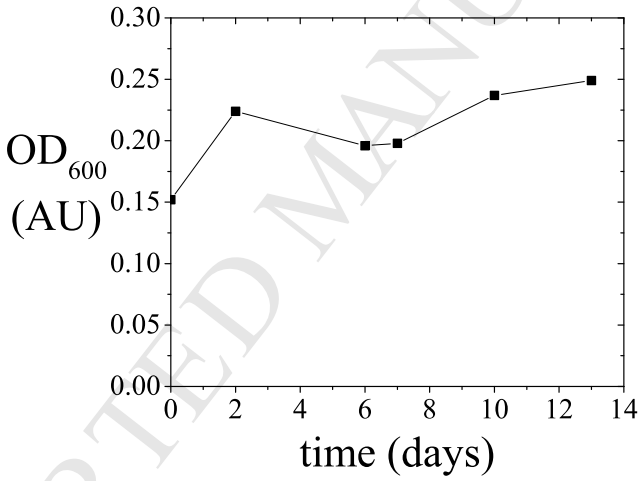

Figure S2: Variation of the optical density (OD_{600}) of the catholyte with time. The OD was measured at 600 nm using a spectrophotometer.

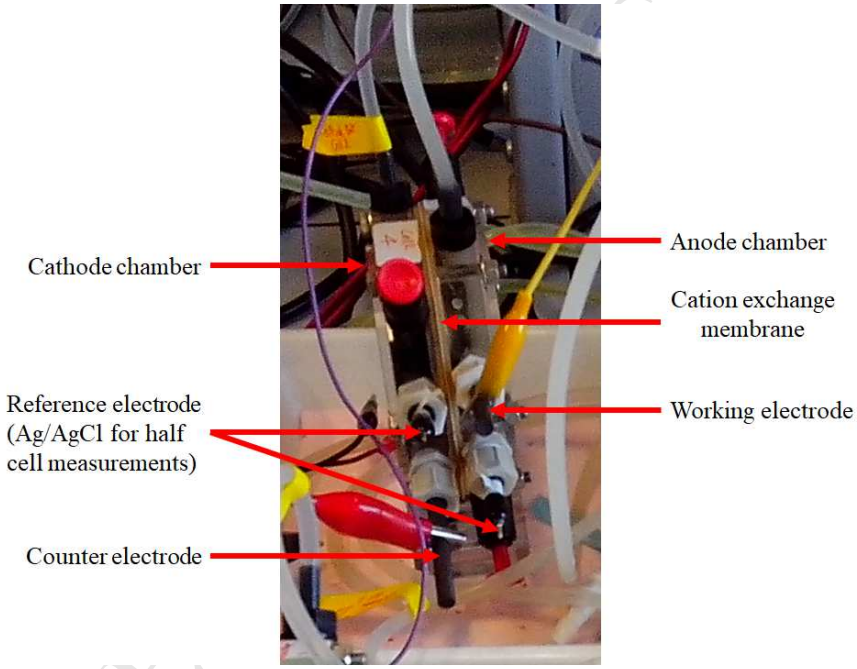

Figure S3: Picture of the Microbial Fuel Cell with the cathodic and anodic chambers connected by a cation exchange membrane. The cell is connected to a potentiostat using the working and counter electrodes.

Highlights

- Seawater bacteria can perform denitrification at the cathode of an MFC.
- Use of bicarbonate (HCO_3^-) buffer makes the process environmentally sustainable.
- Seawater bacteria in HCO_3^- buffer increases the power and current densities.