Hindawi Publishing Corporation
Mathematical Problems in Engineering
Volume 2013, Article ID 410916, 9 pages
http://dx.doi.org/10.1155/2013/410916

Research Article

A Microbial Fuel Cell Modified with Carbon Nanomaterials for Organic Removal and Denitrification

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Received 26 July 2013; Accepted 23 September 2013

Academic Editor: Bashir Ahmad

This paper investigated microbial denitrification using electrochemical sources to replace organic matter as reductant. The work also involved developing a system that could be optimised for nitrate removal in applied situations such as water processing in fish farming or drinking water, where high nitrate levels represent a potential health problem. Consequently, the study examined a range of developments for the removal of nitrate from water based on the development of electrochemical biotransformation systems for nitrate removal. This also offers considerable scope for the potential application of these systems in broader bionanotechnology based processes. Furthermore, the work discussed the context of improved microbial fuel cell (MFC) performance, potential analytic applications, and further innovations using a bionanotechnology approach to analyse cell-electrode interactions. High nitrate removal rate of more than 95% was successfully achieved by using a MFC system modified with carbon nanomaterials.

1. Introduction

A biological nitrate removal using microbial fuel cells (MFCs) has attracted great attention due to its ability to directly generate electricity, while accomplishing water and wastewater treatment. MFCs are bioelectrochemical systems (BESs) that use electrochemically active microorganisms. The microorganisms act as catalyst for the electrochemical oxidation of the organic material, and the electrode is therefore referred to as a microbial bioanode [1]. This oxidation occurs in an anaerobic environment, resulting in producing electrons, protons, and CO_2. The protons, which are created at the anode to maintain a charge balance, typically migrate through the solution to the cathode. In contrast, the electrons flow through an external electrical circuit with a load resistance to the cathode and in turn combine with protons and an oxidant to generate electricity. In a conventional MFC, the cathode is abiotic, due to the use of expensive and sustainable catalysts. Microbial biocathodes have been shown as greatly promising alternative, as inexpensive and sustainable electrode materials can be used [2]. In addition, anaerobic biocathodes can offer the advantages of having a MFC system with both anaerobic anode and cathode chambers. This helps minimise the risk of oxygen leaking in the anode chamber, thus increasing the efficiency of its reaction, and also helps reduce the cost of the catalyst used. The first development of a biocathode was achieved by Clauwaert et al. in [3], demonstrating that a complete denitrification can be performed using microorganisms in the cathode with electrons supplied by microorganisms oxidising acetate in the anode. Simultaneous organic removal, power production, and full denitrification were achieved without relying on H_2-formation or external power. It was also reported that a bacterial culture enriched in *Geobacter* species could reduce nitrate to nitrite by using the cathode as the terminal electron donor [4]. In 2008, Lefebvre and coworkers proposed a novel type of two-chambered MFC, where the costly catalyst on the cathode surface was replaced by an autoheterotrophic denitrifying biofilm [5]. Denitrification was performed by microorganisms using electrons supplied by bacteria oxidising domestic wastewater and with acetate as the substrate in the anode chamber.

The MFC performance can be improved through a proper design of the MFC reactor and the selection of

appropriate materials. A wide variety of carbon-based materials, including carbon paper, carbon cloth, carbon felt, and graphite granules (GGs), have been examined as electrode in MFC [6–9]. Carbon based materials such as graphite felt (GF), carbon fibres, and carbon cloth have been widely used as electrode materials for electrochemical properties due to their stability, high surface area, and availability at a reasonable cost. GF electrodes have been shown to be attractive materials due to their interesting characteristics in applications, such as electrosynthesis and metal recovery. However, GF electrodes have low electrochemical activity due to poor kinetics and reversibility that limit their use as active electrode materials. Therefore, great attention has been paid to the modification of such electrode materials in order to improve their electrochemical properties. The modification of the electrode surface area may also improve such aspects as bacterial adhesion, hence increasing the electron transfer from bacteria to the electrode surface. *Geobacter* sp. can make an electrical connection with graphite electrodes and can accept electrons from an electrode when the electrode is poised at a negative potential [4]. It was reported that the electrochemical activity of the GF can be improved by treating the GF with concentrated sulphuric acid [10]. Alternative novel modification techniques were also developed to enhance the GF electrochemical activity material, as shown in [11]. Furthermore, it has been demonstrated that carbon nanomaterials have the ability to facilitate the electron transfer process during the electroreduction and electrooxidation of electroactive species such as NADH and hydrogen peroxide [12, 13] and during the enzyme-substrate interaction [14]. Additionally, the porous structure of the carbon nanomaterials can give the electrode better wetting properties [15]. This can allow the analyte to diffuse into the carbon nanomaterial bundles with lower friction, as mentioned in [16].

In this study, a mediatorless H-shaped MFC is constructed using bacteria both in the anode and cathode in order to perform biological nitrate removal. A mixed bacterial culture in the cathode can perform denitrification through the use of electrons supplied by a mixed bacterial culture oxidising acetate in the anode. This configuration represents an anodic (oxidative) reaction in water heavily contaminated with organics, while the reduction for nitrate is carried in relative clean environment, so avoiding the addition of organic matter required for conventional denitrification systems. Furthermore, in an attempt to improve the MFC, modifications to the electrochemical properties of the electrode were investigated through the use of a cyclic voltammetry using carbon nanomaterials to coat the graphite felts electrodes. Among all the nanomaterials used in this study, graphitised carbon nanofibers (GCNFs) were selected for further investigation, as they offered the best electrochemical performance and were thought to provide the largest active surface area. The performance of the MFC system coupled with the GCNFs modified electrodes was evaluated and significant improvements were observed. The highest voltage output achieved was about 41 mV with over 95% nitrate removal.

2. Materials and Methods

2.1. MFC Design and Operation

2.1.1. MFC Construction. An H-shape MFC, which consisted of two separated chambers joined with a glass tube containing a 4.7 cm × 4.5 cm diameter proton exchange membrane (PEM), was constructed. The volume of each chamber was approximately 200 mL with a 50 mL headspace. Anodic and cathodic chambers were operated in anaerobic conditions, where the top of each chamber was sealed with a rubber stopper. A platinum wire was introduced from the top of each chamber through the rubber stopper to solder one end of a rectangular prism shaped graphite felt electrode (GFE), having a surface area of 40 cm^2 and a weight of 0.719 g. Given that the rectangular prism-shaped electrode (SA) had a length (L) of 4 cm, a width (W) of 0.5 cm, and a height (H) of 4 cm, the outer surface area was calculated as

$$\begin{aligned} \mathrm{SA} &= 2LW + (2L + 2W)\,H \\ \mathrm{SA} &= 2 \times 4 \times 0.5 + (2 \times 4 + 2 \times 0.5) \times 4 = 40\ \mathrm{cm}^2. \end{aligned} \tag{1}$$

Each chamber has two side ports to allow the provision of fresh substrate, as well as the purge of nitrogen, and to allow the removal of the treated one.

2.1.2. MFC Inoculation and Operation. A soil sample was collected from a depth of 3 cm using a clean spatula and bag and was then delivered to a laboratory within 5 min. An inoculum solution was prepared by adding 10 g soil to a 250 mL flask containing 70 mL of an autoclaved solution. The latter was prepared with 1 g CH_3-COONa-$3H_2O$ and 3 g KNO_3 in 500 mL distilled water and then autoclaved at 121°C for 15 min. The inoculum solution was shaken and kept for 20 min before being used. The mixed culture of soil inoculum was simultaneously inoculated into each chamber during the MFC start-up. Both anode and cathode chambers were similarly filled with an artificial wastewater medium. This artificial wastewater medium, which was prepared according to [17], contained inorganic salts dissolved in 990 mL of 5 mM phosphate buffer and 10 mL of a trace mineral solution. The phosphate buffer solution used to form the basis of the salt solution was prepared as a mixture of KH_2PO_4 and K_2HPO_4. The salt solution was autoclaved at 121°C for 15 min and left to cool. Nitrogen gas was then purged for 30 min to remove oxygen. The solution's pH was checked and adjusted to 7 in each chamber through the addition of 1 M HCl or 1 M NaOH. The former (HCl) was used to reduce pH, while the latter (NaOH) was used to increase pH.

In order to maximise the growth of the biofilm-forming organisms, enrichment was conducted in three different modes for three months, following the procedure given in [18]. The MFC reactor was first operated in a fed-batch mode under a closed circuit condition through 10 KΩ in the first month. This essential growth mode allowed the microorganisms to grow at a constant load using acetate as a carbon source in the anodic chamber and nitrate as an electron acceptor in the cathodic chamber. This was facilitated through the weekly addition of 2 mM (169.5 mg/L) sodium

acetate CH_3-COONa-$3H_2O$ and 8.4 mM (847.9 mg/L) potassium nitrate (KNO_3) into the anodic and cathodic chambers, respectively. The second mode was performed as starvation mode for a month, where the addition of a carbon source was stopped and the nitrate fed was carried out weekly. The main goal of this mode was to enable consumption of carbon source added in the first mode. The mode was also given a selection of organisms that were capable of either using residual acetate or internally storing carbon to be subsequently used for cellular maintenance needs. This purpose was achieved by replacing the medium solution in both chambers after two weeks of the starvation mode period, which helped avoid sodium and nitrite accumulations in the anode and cathode chambers, respectively. Furthermore, the weekly addition of the carbon source was restarted and the external load was reduced from 10 KΩ to 500 Ω to allow a higher current to flow between the electrodes for the rest of the enrichment period. This resulted in higher availability of an electron acceptor in the cathode and offered the opportunity of the growth of exoelectrogenic organisms. During the enrichment process, both chambers were purged with gaseous nitrogen for 10 min after each fuel addition in order to obtain anaerobic conditions. Moreover, the solution in each chamber was continuously mixed using a magnetic stirrer to enhance mass transfer. The experiment was conducted at room temperature.

The enrichment process was completed and the MFC operation was then started, where the power generated is computed as the production of cell voltage across an external resistance due to the current flow through the resistor. The MFC system is operated at a steady state when the power generated equals the power consumed for an extended time. In steady state MFC systems, sustainable power can be generated as the product of a steady current passing through a fixed load and a constant voltage drop across this load. Due to the possibility of many steady conditions in a MFC system, it is important to define the condition in which the MFC produces the maximum sustainable current, as well as computing the maximum sustainable power. In order to obtain a steady state condition, the MFC system was initially conducted through an external resistance of 500 Ω in several batch modes using acetate as the carbon source and nitrate as the electron acceptor under anaerobic conditions. The cell voltage was measured every hour using a digital multimeter connected to a personal computer through a data acquisition system (34405A, Agilent). The nutrient medium was completely replaced at the operation start-up and when the voltage dropped to less than 5 mV as an end of batch. Previous study [19] found that a stable MFC system was achieved when the voltage output was reproducible after replacing the medium at least twice. In this study, a stable voltage generation (sustainable voltage) of 30 mV approximately was produced after three batches. Furthermore, in order to define the steady state that provides the maximum power output, a polarization curve was obtained by measuring the stable voltage generated at various external resistances. In addition, a series of batch-mode MFC tests were performed to investigate the effect of nitrate and acetate concentrations on the MFC's performance and its denitrification activities. The operation of these tests was carried out under closed circuit with an external resistance of 500 Ω. The effect of external resistance on the denitrification process was studied by operating the MFC under three different loads. Nitrate reduction and nitrite accumulation rates were studied and evaluated throughout the tests. Nitrate and nitrite concentrations were measured using an ultraviolet spectrophotometric screening method proposed in [20] and a development spectrophotometric method proposed in [21], respectively.

2.2. MFC Developments and Modifications

2.2.1. Investigation of the Electrochemical Properties of the Electrode. This section aims to investigate the electrochemical properties of a GFE using cyclic voltammetry experiments. The cyclic voltammetry is an active technique that involves the application of potential to an electrode and monitoring of the current response through the electrochemical cell over a period of time. The applied potential induces a change in the concentration of the electroactive element at the electrode surface through electrochemical oxidation or reduction. This technique can either be used for organic or inorganic substances including studies of adsorption processes on surfaces, electron transfer and reaction mechanisms, kinetics of electron transfer processes, and transport of species in solution. Furthermore, the electrochemical experiments were conducted using Epsilon electrochemistry and a three-electrode arrangement. The three electrodes were comprised of a working electrode at which the redox reaction takes place, a reference electrode through which no current flows, and a counter electrode (an auxiliary electrode) which completes the circuit. The working electrode was graphite felt, the auxiliary electrode was platinum (Pt), and the reference electrode was a silver/silver chloride electrode (Ag/AgCl). These electrodes are connected to a potentiostat that provides the desired potential. The potentiostat was linked up with a personal computer controlled electrochemical application that was used for collecting and calculating the data. The electrochemical experiments were carried out in a one compartment electrochemical cell with a volume of about 25 mL at room temperature and in an oxygen free environment (by bubbling nitrogen through the solution). The electrolyte used in the electrochemical experiments was 1 mM methyl viologen (MV^{+2}) in a 0.1 M phosphate buffer solution (PBS), which was composed of a mixture of KH_2PO_4 and K_2HPO_4 and with a pH of 7. The methyl viologen (MV^{+2}) has been considered as the simplest redox system in which MV^{+2} reduced to the relatively stable cation radical MV^+ [22, 23]. The MV^+ is relatively stable in oxygen free solutions, resulting in a deep blue colour, which is evidence of the formation of MV^+ in the solution, in contrast to the colourless MV^{+2} [24, 25]. The methyl viologen can act as a good electron transfer mediator for a biological system [23] and can also be electroreduced on a surface of various electrodes [26, 27]. Additionally, the effect of the pH value on electrochemical behaviour was investigated through the use of a range of pH values from 4 to 8, in the steps of 1. The interaction between the concentration and the peak current obtained

was also studied, where methyl viologen concentrations of 0.25 mM, 0.5 mM, 1 mM, 2.5 mM, and 5 mM were used. Moreover, the effect of the PBS concentration was studied, and PBS concentrations of 0.2 M, 0.1 M, 0.05 M, 0.01 M, 0.005 M, and 0.001 M were used. In addition, in order to study the correlation between the redox mechanism and the surface area used, the graphite (carbon) felt electrode was cut into multiple pieces with different surface areas. The electrochemical experiments were performed as follows.

(1) A solution of 0.1 M phosphate buffer in 25 mL distilled water of pH 7 was prepared, and a 1 mM methyl viologen (MV^{+2}) concentration was then made in the 0.1 M phosphate buffer solution (i.e., using the phosphate buffer solution as diluent). The resultant solution was poured into an electrochemical cell with a volume of about 25 mL.

(2) The three electrodes were carefully connected to an external cell box in the faraday cage and were then fitted into the electrochemical cell, by making sure that all electrodes were submerged but not touching the cell bottom.

(3) The computer programme was set to the following conditions: an initial potential (IP) of −200 mV, a final potential (FP) of −200 mV, a switching potential (SP) of −900 mV, and a scan rate (SR) of 20, 50, 100, 200, or 250 mVs^{-1} at room temperature.

(4) The solution in the cell was purged with nitrogen for 2 min, while stirring through the use of a small magnetic stirrer to achieve anoxic conditions.

(5) The experiment was run and a voltammogram was taken.

(6) Steps 1 to 5 were repeated with different pH values of 4, 5, 6, and 8.

(7) Steps 1 to 5 were repeated with different methyl viologen concentrations of 0.25 mM, 0.5 mM, 2.5 mM, and 5 mM, while using a pH value of 7.

(8) Steps 1 to 5 were repeated with different phosphate buffer concentrations of 0.2 M, 0.05 M, 0.01 M, 0.005 M, and 0.001 M, while using a 1 mM methyl viologen concentration and adjusting the pH value to 7 in all the phosphate buffer concentrations.

(9) Steps 1 to 5 were repeated with different surface areas of the graphite (carbon) felt electrode. All the pieces of the graphite (carbon) felt electrodes were washed with distilled water before being used.

2.2.2. Electrode Modifications Based on Carbon Nanomaterials. Graphite felt has a large specific surface area and good stability [28]. However, it has a lower electrochemical activity that leads to a limited voltage efficiency and a lower power density, compared to the other materials used in the MFC system. This work aims to improve the electrochemical activity of the GF electrode through the use of carbon nanomaterials. The carbon electrode surface area can be enlarged by dispersing the carbon nanomaterials on the surface of the electrode to form a randomly dispersed array of high surface area. Four types of carbon nanomaterials were used in this study to modify the GF electrode. These carbon nanomaterials include single-walled carbon nanotubes (SWCNTs), graphitised carbon black (GCB), carbon nanofibres (CNFs), and graphitised carbon nanofibres (GCNFs). The electrochemical performance of the modified GF electrodes was investigated and evaluated through cyclic voltammetry. The experiments were performed as follows.

(1) The tests were performed in 1 mM methyl viologen (MV^{+2}) in a 0.1 M phosphate buffer solution of pH 7 at a room temperature.

(2) Four graphite felt electrodes were cut into a similar size of 4 mm × 4 mm × 2 mm and washed with distilled water before treating.

(3) Suspensions of carbon nanomaterials were prepared by mixing 0.7 mg of each nanomaterial with 700 μL of N,N.Dimethylformamide (DMF) as the dispersing agent and agitating the mixture using a sonicating tip (Ultrasonic Processor, Sonics Vibra Cell) for 1 h. Close to 10 μL of the resultant solution (i.e., the nanomaterial and DMF solution) was dropped directly onto the GF electrode surface and was allowed to dry at 40°C for 45 min to evaporate the solvent.

(4) The electrodes were tested following steps 1 to 5 described in the procedure given in Section 2.2.1.

3. Results and Discussions

3.1. Electrochemical Properties. The maximum power density was evaluated through the examination of a polarization curve, which characterises voltage as a function of current. The power production over a range of current densities was obtained by changing the external resistance R_{ext} using a resistor box, when the voltage production became stable. The MFC reactor was initially operated under an open circuit condition. Once the reactor achieved a stable voltage output of 0.349 V, the resistor box was switched on and the external resistance was varied from 10 Ω to 10 kΩ in steps of 250 Ω every 10 min and the cell voltage was measured at each resistance. Current and power levels were calculated with the voltage and resistance based on Ohm's law. Current and power densities were calculated by normalising the current and voltage through an electrode surface area. Polarization and power density curves are displayed in Figure 1(a). The polarization curves illustrating the three characteristic regions of voltage drop in the MFC are shown in Figure 1(b). These regions include a rapid voltage decrease due to the flow of current through high external resistance, an almost constant decrease in voltage, and a second significant voltage drop at high current densities. The decrease in the cell voltage is a consequence of electrode overpotentials (activation, bacterial metabolic, and mass transfer losses) and ohmic losses. The maximum power density obtained was 1.26 mW/m^2 at a current density of 10.23 mA/m^2. This is slightly lower than that reported in [29], where the highest power density achieved was 1.7 mW/m^2 at a current density of 15 mA/m^2.

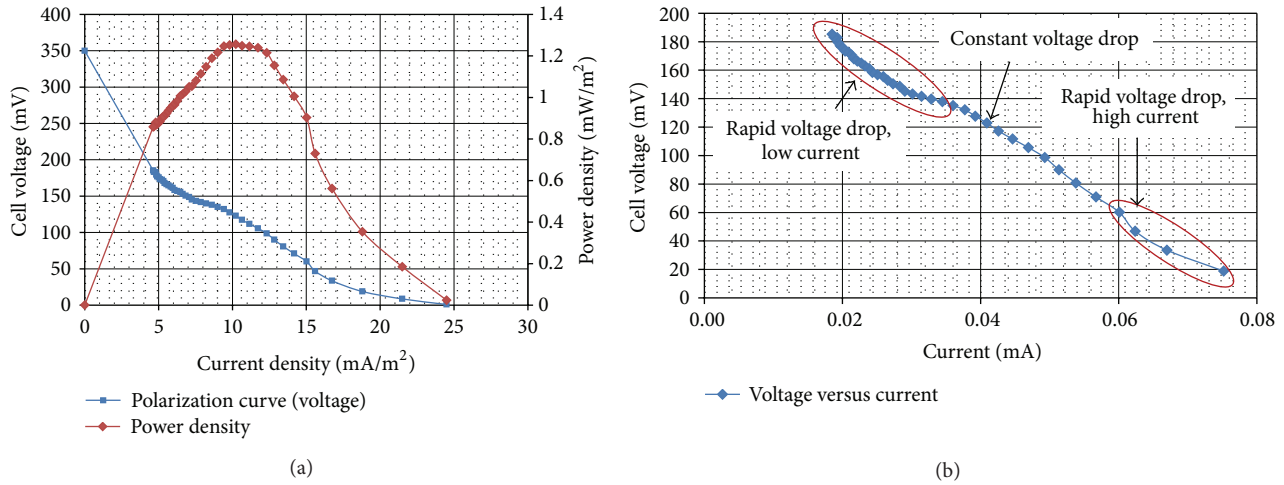

FIGURE 1: (a) Polarization curves. (b) The three characteristic regions of voltage drop.

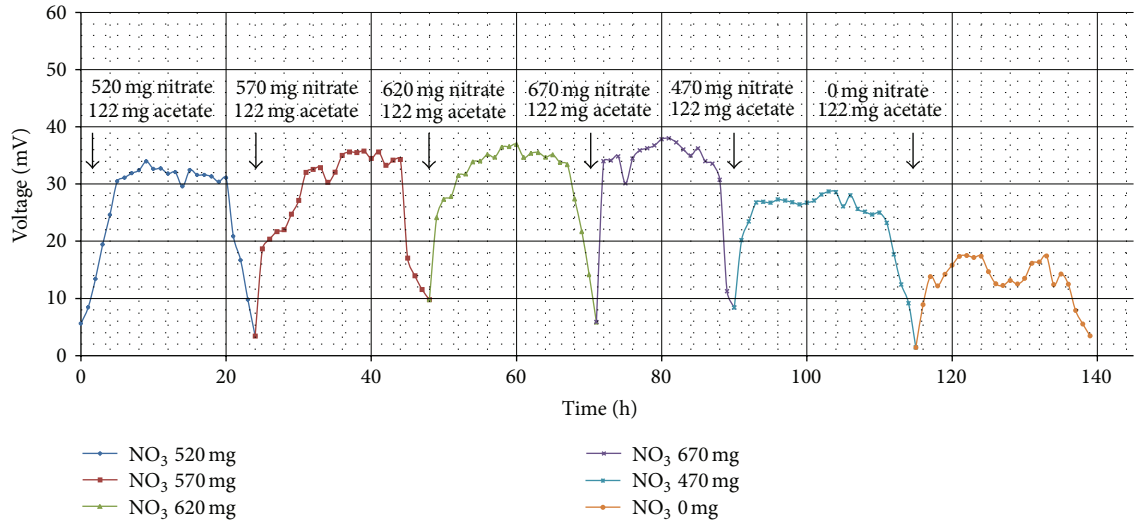

FIGURE 2: Effect of nitrate concentrations on voltage generation.

This is a result of two factors: firstly, [29] used a graphite-Mn(IV) and a graphite-Fe(III) electrode as an anode and a cathode, respectively; however, a pure GF electrode is used here in both chambers. Modification of GF electrodes using nanomaterials and the improvements observed are discussed in Section 3.5. Furthermore, the results achieved in this study were based on the enrichment of soil inoculum only; however, anaerobic digester sludge was used in [29]. Based on the slope of the linear region of the polarization curve, an internal resistance R_{int} of 2893 Ω could be determined. It is also shown in Figure 1(a) that the power output was maximal when $R_{int} = R_{ext} = 3000\ \Omega$.

3.2. Effects of Nitrate Concentration on MFC Performance. The effects of different nitrate concentrations on denitrification activity and voltage output were investigated at a fixed external resistance of 500 Ω. The MFC system was operated with a number of batches at a range of nitrate concentrations between 470 mg/L and 670 mg/L in steps of 50 mg/L in the cathodic chamber, while a fixed acetate concentration of 122 mg/L was added in the anodic chamber at the beginning of each batch. An illustration of typical profiles of cell voltages produced is shown in Figure 2. The denitrification activity was examined through the measurement of nitrate and nitrite concentrations at the end of each batch. The denitrification activity (including nitrate reduction and nitrite accumulation rates) and current generation achieved are outlined in Table 1.

The results showed that both nitrate removal and nitrite accumulation rates exhibited an increase with the increase of nitrate concentration. Increasing the nitrate concentration from 470 mg/L to 670 mg/L improved the denitrification activity by almost 2% (from 87.32% to 89.3%). However, an increase in nitrite accumulation by a factor of approximately

Table 1: Nitrate removal and nitrite accumulation of several closed batches fed with 122 mg/L acetate and different nitrate concentrations.

Nitrate concentration (mg/L)	Nitrate removal		Nitrite accumulation mg/L	Average current (μA)
	mg/L	%		
470	410.39	87.32	5.153	46
520	455.12	87.524	6.626	52.4
570	500.3	87.8	10.31	54
620	547.26	88.27	12.52	61
670	598.21	89.3	16.93	65

3 (from 5.15 mg/L to 16.93 mg/L) was incurred. Previous work [29] suggested that the current production was dependent on glucose and nitrate. The experimental results obtained here confirmed this suggestion and also demonstrated that the denitrification rate was supported by the current production (which was dependent on nitrate). However, the system was also operated with the absence of nitrate as an electron acceptor in the cathodic chamber, and a low electrical current was observed.

3.3. Effects of Acetate Concentration on Denitrification Activity. To investigate the effect of acetate on the MFC performance, the fuel cell was fed with several batches at different acetate concentrations in the anodic chamber. Acetate concentrations of 72, 122, 172, 222, and 272 mg/L were used. Each batch was performed with an external resistance of 500 Ω at a nitrate concentration of 520 mg/L in the cathodic chamber. Cell voltage profiles produced are shown in Figure 3. The denitrification rate and current production obtained are given in Table 2. The addition of sodium acetate can allow the bacteria to provide more electrons and to increase the voltage output. This was observed as an increase in the cell voltage when the concentration of acetate was increased. Meanwhile, increasing acetate concentration resulted in an increase in the current generation. In the cathodic chamber, an increase in the nitrate removal from 85.6% to 92.23%, together with a decrease in the nitrite accumulation from 7.4 mg/L to 5.2 mg/L, was observed when the acetate concentration increased from 72 mg/L to 272 mg/L. This indicates that when acetate concentration of 272 mg/L was used, 1.08% of the nitrate removed was turned into nitrite. The remaining 91.15% (474.97 mg/L) of the nitrate removed was possibly converted into nitrogen gas. The experimental results proved that the sodium acetate highly supports the denitrification activity. However, the MFC performance was evaluated in the absence of sodium acetate and a denitrification rate of 71.7% was achieved while producing low cell current. This is due to the bacteria making use of the carbon stored internally by the cells from the previous batches, where carbon sources are used. It has to be noted that operating the MFC system in carbon starvation mode (i.e., without using a carbon source) during the enrichment forced the bacteria to store carbon internally.

3.4. Effects of External Resistance on Denitrification Activity. To investigate the effect of electricity generation on the denitrification process, further experiments were conducted with an MFC under different external resistances of 500, 5000, and 10000 Ω. Sodium acetate and nitrate concentrations of 122 mg/L and 520 mg/L, respectively, were used. Average currents of 52 μA (500 Ω), 22 μA (5 KΩ), and 14 μA (10 KΩ) were produced, as shown in Table 3. The results confirmed that the denitrification rate is strongly dependent on the cell current produced, which was varied here by external resistance. It was observed that using higher resistance led to low cell current, resulting in lower nitrate removal rates and higher accumulation rates. This was possibly due to insufficient electron donors being available on the cathode.

3.5. MFC Performance with Modified GF Electrodes. The interaction between the bacterial biofilm and the electrode surface area can affect the MFC's performance. The reactants transport, including substrate, electrons, and electron accepting species from the bulk to the electrode surface, and the reaction kinetics on the electrodes surfaces can also influence the MFC's performance. The reaction kinetics on the electrode surface and the mass transfer are affected by the electrode materials [30], surface chemical properties of the electrodes [31], size and shape of the electrodes [32], and biofilm condition [33]. The electrode should provide a good environment for the bacteria to attach to and transport electrons, a large surface area, and a high conductivity [30]. The internal resistance of an MFC consists of two parts: nonohmic and ohmic resistances [34]. The former comprises a charge transfer resistance and a diffusion resistance [35], and these can be reduced by increasing the electrode surface area as well as selecting electrodes with good catalytic abilities. Ohmic resistance can be decreased by arranging the electrodes closely, using solutions with high conductivity and using a membrane with low resistivity.

Modification of the GF electrode surface to enhance its reaction kinetics and mass transfer is a good way to improve the MFCs performance. The GF electrode was modified with carbon nanomaterials, including SWCNTs, GCB, CNFs, and GCNFs, which are promising materials that can provide great stability and high conductivity. The electrochemical behaviour of the modified GF electrodes was investigated by using cyclic voltammetry. Cyclic voltammograms of methyl viologen redox reactions on different modified GF electrodes were recorded and compared. Comparisons of the kinetic methyl viologen redox reactions on the unmodified GF electrode were also considered. The results indicated an improvement in the electrochemical activity of the methyl viologen on the modified electrodes compared to the unmodified electrode. The GCNFs modified electrode exhibited the best electrochemical activity among the other modified electrodes, due to its having the largest surface area which greatly increased the rate of electron transfer. Therefore it was chosen to enhance the MFC's performance. In an

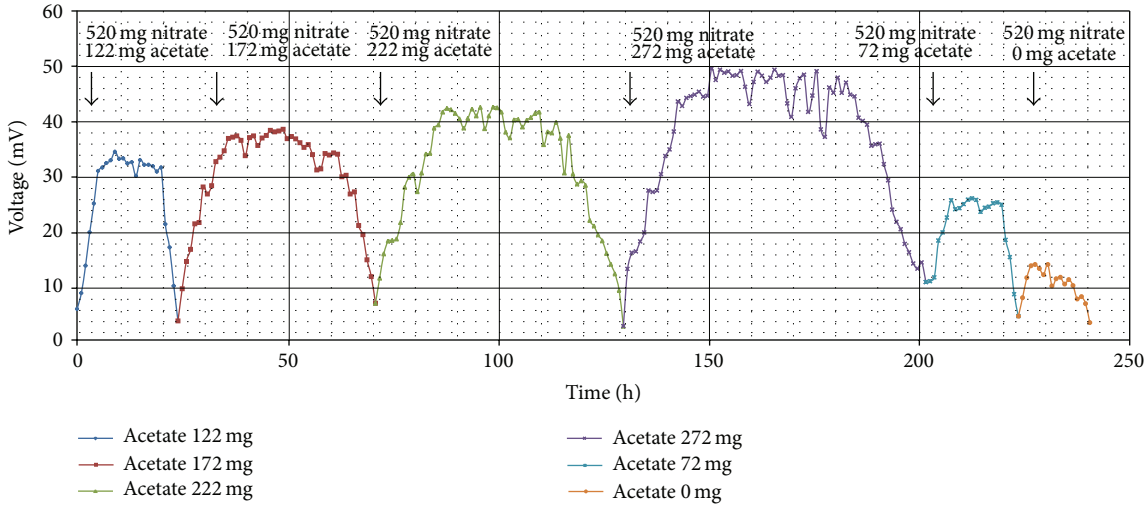

Figure 3: Voltage generation using acetate at different concentrations.

Table 2: Nitrate removal and nitrite accumulation of several closed batches fed with 122 mg/L acetate and different nitrate concentrations.

Acetate concentration (mg/L)	Nitrate removal		Nitrite accumulation mg/L	Average current (μA)
	mg/L	%		
72	445.01	85.6	7.4	41
122	455.12	87.5	6.6	52.4
172	466.8	89.8	6.4	59
222	472.3	90.83	5.89	63
272	480.17	92.2	5.2	74

Table 3: Nitrate removal, nitrite accumulation, average current, and CE of several closed batches fed with 520 mg/L nitrate and 122 mg/L acetate concentrations under different external resistances: 500, 5000, and 10000 Ω.

External resistance (Ω)	Nitrate removal		Nitrite accumulation mg/L	Average current (μA)
	mg/L	%		
500	455.12	87.5	6.6	52.4
5,000	434.98	83.65	7.61	22.2
10,000	412.5	79.33	8.34	14

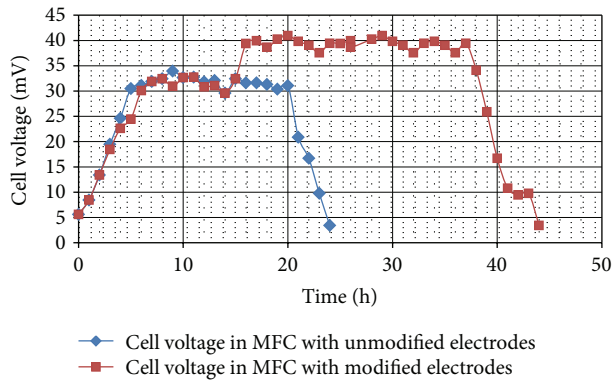

Figure 4: Voltage generation in MFC with modified and unmodified electrodes.

MFC reactor, both anode and cathode were provided by GCNFs modified electrodes. A modified MFC system was enriched following the procedures described in Section 2.1.2 for three months, and its performance was evaluated in terms of power generation and nitrate removal. The results demonstrated that the GCNFs modified MFC system offered about 8% nitrate reduction rate higher than that achieved using unmodified electrodes (the unmodified MFC system removed 87.5% of the nitrate). This is due to the long term stability provided by the GCNFs modified MFC system, where an average of 35 mV was obtained over a period of 44 h; see Figure 4.

4. Conclusions

Nitrogen and nitrate have important environmental impact and there are many problems associated with their release into the environment. Nitrate can be removed from water through the use of physicochemical methods. However, these processes are not viable and their use is problematic. An alternative promising and versatile approach that can be used for nitrate removal is biological denitrification. However a major drawback is the potential bacterial contamination of treated water; thus additional filtration and disinfection are potentially needed to meet current drinking water standards. The stated aims and objectives of this study were to investigate the electrochemical removal of nitrate from water and associated technical problems. The results of the work showed that these aims were successfully achieved setting a good platform for future work in this area.

In order to perform biological nitrate removal, a mediatorless H-shaped MFC was constructed using bacteria both in the anode and cathode. A mixed bacterial culture in

the cathode performed denitrification through the use of electrons supplied by a mixed bacterial culture oxidising acetate in the anode. The effects of acetate/nitrate on current generation and nitrate removal and the denitrification activity as a function of external resistance were also studied. The results demonstrated that the denitrification rate is highly dependent on current production, which is influenced by external resistance and acetate and nitrate concentrations. An Increase in the nitrate reduction rate was observed when the nitrate concentration increased. However, an increase in the nitrite accumulation rate was also induced which can be problematic due to its toxicity. In contrast, increasing the acetate concentration improved nitrate removal rates and reduced nitrite accumulation, with these results indicating the importance of the feed ratios for the two compartments. Higher external resistances were shown to inhibit the denitrification activity due to lower electrical currents produced. In addition, a polarization curve was obtained by changing the external resistance using a resistor box, and a maximum power density of 1.26 mW/m^2 was achieved at a current density of 10.23 mA/m^2. This was also compared well with previous findings.

The electrochemical behaviours of a graphite felt electrode were investigated through the use of the cyclic voltammetry technique. Enhanced redox behaviours were achieved by modifying the GF electrode surface using carbon nanomaterials, such as SWCNTs, GCB, CNFs, and GCNFs. The electrochemical properties associated with the modified GF electrodes were studied, evaluated, and compared with those of the unmodified GF electrode. The GCNFs modified electrode offered the best electrochemical activity compared to the other modified electrodes, due to the large surface area provided and the improved electron transfer rate. Using these types of electrodes should improve the interaction with the microbes as these particles are much smaller than the felt surfaces giving the microbe more surface to directly interact with. Enhancing the reaction kinetics and mass transfer of the GF electrode through the GCNFs material helped improve the MFC's performance. The maximum voltage obtained was 40.94 mV and more than 95% of nitrate was removed, as compared with the unmodified electrode (33.95 mV maximum voltage, 87.5% nitrate removal). In this study only a few conditions of electrode modification were investigated. There are many possibilities that need to be investigated to see if the electrode's performance can be improved further. The optimisation study could have a strong impact on potential development of MFC technology.

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