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Full Length Research Paper

Application of multi-walled carbon nanotubes to enhance anodic performance of an *Enterobacter cloacae*-based fuel cell

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The effect of multi-walled carbon nanotube (MWCNT) modification of anodes and the optimisation of relevant parameters thereof for application in an *Enterobacter cloacae* microbial fuel cell were examined. The H – type microbial fuel cells were used for the fundamental studies, with a carbon sheet as a control anode and platinum coated carbon sheets as the cathode. Anodes were correspondingly modified with MWCNTs dispersed in either 0.1% chitosan or 1% Nafion®. Maximum power output was observed four hours after inoculation of the anode chamber with the microorganism. A 252.6% increase in power output of the fuel cell was observed at an anode modified with 10 mg MWCNTs/ml dispersed in 0.1% chitosan compared to unmodified anodes (13.8 µW). MWCNTs dispersed in chitosan yielded nearly 50% greater power outputs than when dispersed in Nafion®; attributed to increased aggregation in the latter as evidenced by scanning electron microscopy imaging. When Nafion™ 117 membrane was used as a proton exchanger it generally resulted in higher power outputs than the CMI 7000S membrane. These studies also showed that the time-consuming carboxylic acid functionalisation of MWCNT for such applications is not a necessary requirement for enhancing power outputs. The studies thus illustrate the utility of a MWCNT modified anode as a support matrix for *E. cloacae* in a microbial fuel cell and provide clarity on parameters which can be applied to other such studies in the emerging area of nanostructured material utilisation in alternative energy generation.

Key words: Multi-walled carbon nanotubes, microbial fuel cell, *Enterobacter cloacae*, functionalise, anode.

INTRODUCTION

In the enduring search for high efficiency alternative energy solutions, fuel cells have received considerable attention. Microbial fuel cells (MFCs) are considered to be relatively cheap, environmentally friendly and can utilise waste material as a carbon source (Shukla et al., 2004; Bullen et al., 2006; Du et al., 2007). The dual promise of both bioremediation and sustainable energy generation, has warranted sufficient attention in the literature and coupled with recent advances suggest that microbial fuel cells are closer to practical implementation (Watanabe, 2008). However, MFCs' drawback is the low power output (Katz et al., 2003) often attributed to surface area limitations (as a result of cost and practicality) and resistance to electron and proton transfer (Min et al., 2005; Rismani-Yazdi et al., 2008).

Carbon nanotubes exhibit excellent electron transfer characteristics with a high surface area to volume ratio. In addition, multi-walled carbon nanotubes (MWCNTs) provide a viable support for biofilm growth. These properties have been exploited in recent studies utilising *Escherichia coli* for enhancement in power output using MWCNTs (Sharma et al., 2008) combined with polymers such as polyaniline (Qiao et al., 2007) and polypyrrole (Zou et al., 2008) as supporting materials at the anode. These studies have however been limited to *E. coli* as biocatalyst for biohydrogen generation as the fuel for the anode.

The hydrogen generation capacity of *Enterobacter cloacae* has been reported in the literature (Kumar and

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Das, 2001) and recently too its application in microbial fuel cells (Mohan et al., 2008). Tremendous scope exists for improvements in power output, cost and time for such applications requiring continuous research efforts to be directed towards optimisation of such systems.

In this work we constructed a fuel cell utilising *E. cloacae* and examined the effect of MWCNTs in enhancing the power outputs in an H-type microbial fuel cell. In addition, we examined the effects of functionalisation of the MWCNTs, the dispersing agents used to immobilise the MWCNTs and the nature of the membrane used in this setup.

MATERIALS AND METHODS

Microbial growth

E. cloacae strain 16657 and *Clostridium butyricum* strain 2477 both from DMSZ (Germany) were screened for hydrogen production in reinforced clostridal media (Merck, South Africa). *E. cloacae* generated the highest amount of hydrogen (data not shown) and was selected for use in subsequent experiments.

Multi-walled carbon nanotubes (MWCNTs) preparation

The protocol for purification and carboxylic acid functionalisation of MWNCTs was adapted from Tkac and Ruzgas (2006). MWCNTs (≈ 0.04 g; external diameter: 10 - 15 nm; internal diameter: 2 - 6 nm; length: 0.1 - 10 µm; purity > 90%), purchased from Sigma Aldrich (South Africa), were added to a mixture of 10 ml of 55% nitric acid and 30 ml of 95% sulphuric acid (both from Merck) and sonicated in an Elmasonic S10H water bath sonicator (Elma Ultrasonic Technology) for 6 h to achieve acid functionalisation of the nanotubes. NaOH (0.1 M) and Milli-Q water (Millipore, USA) was added to the sediments until the pH was approximately 7, rinsed with water and then dried for 24 h at 70°C.

A solution of chitosan (0.1%; w/v) (Sigma-Aldrich, South Africa) was prepared in 1% acetic acid (Merck) while 1% Nafion® (Sigma-Aldrich) was prepared in absolute ethanol (Hu et al., 2006; Tkac and Ruzgas, 2006). Solutions of functionalised MWCNTs between 2 and 15 mg/ml were prepared separately in 0.1% chitosan and 1% Nafion® solutions. Non-functionalised multi-walled carbon nanotube solutions were prepared to a concentration of 10 mg/ml. These solutions were sonicated for 30 min prior to use.

Electrode preparations

Anodes (2 x 2 cm) were prepared from carbon paper, supplied by Johnson Matthey Technology Centre (UK). The anodes were modified with the respective multi-walled carbon nanotube solutions by pipetting 200 µl of the modifier (MWCNTs dispersed in either 0.1% chitosan or 1% Nafion®; 2-15 mg MWCNTs/ml) and evenly distributing the respective solutions onto each side of the carbon paper and drying at 70°C for 15 min. In all cases the cathode comprised of 2 x 2 cm platinum impregnated carbon sheets (Johnson Matthey Technology Centre).

Proton exchange membrane preparation

Nafion™ 117 membranes (Johnson Matthey Technology Centre) were rinsed by boiling in Milli-Q water for 1 h, to remove excess hydrogen peroxide, followed by boiling in 0.5 M H_2SO_4 (Merck) for 1 h, to replace the sulphonic acid groups and then rinsing for 1 h to remove excess sulphuric acid (Zawodzinski et al., 1993). The cleaned membranes were cut into 25 mm diameter disks before use. The CMI 7000S membrane (Membranes International, USA) was pre-conditioned as per manufacturer's instructions by immersing the disks in a solution of 0.1 M sodium phosphate buffer (Merck) at pH 6.8 for 24 h, to allow for membrane expansion before use.

Fuel cell construction and power determination

The MFC design was adapted from Logan et al. (2005) and Mohan et al. (2008). The microbial fuel cell consisted of two 250 ml Pyrex bottles (Schott Duran) connected by glass tubes with the proton exchange membrane clamped in the centre, as shown in Figure 1. The anode chamber contained 200 ml of deaerated (using nitrogen gas, 99% pure (Afrox)) reinforced clostridial media inoculated with 20 ml of a 16-h old *E. cloacae* culture. The cathode contained 200 ml of 0.02 M potassium ferricyanide (Merck) in 0.1 M sodium phosphate buffer (pH 6.8). Copper wire was wrapped around the electrodes and crocodile clips were used for attachment to a 680 Ω resistor that completed the external circuit. The pre-cleaned Nafion™ 117 membrane was clamped in the middle (B; Figure 1). Voltage measurements were performed at 2-h intervals for 72 h at room temperature (20 ± 2°C), with a digital multimeter. The current at each time interval was calculated using the equation: $I = V/R$, where I is the current (in amperes), V: voltage (V) and R: resistance (Ω). Power (µW) was subsequently calculated using the equation: $P = VI$, where P is power output (W) (Min et al., 2005; Mohan et al., 2008).

Scanning electron microscopy

Anodes modified with MWCNTs-chitosan and MWCNTs-Nafion® were removed after a 72 – h incubation period in the anode chamber culture, fixed with 2.5% glutaraldehyde (Sigma-Aldrich) for 4 h at 4°C and dehydrated by successive immersion (10 min) in ethanol (30, 50, 70, 80, 90% and then absolute ethanol). The specimens were oven-dried, mounted onto specimen stubs using graphite paste, coated with gold and imaged using a Vega© Tescan scanning electron microscope (Anaspec, South Africa).

RESULTS AND DISCUSSION

Figure 2 shows the power output monitored over time for the *E. cloacae* MFC. The maximum power output (17.26 µW) was reached at 4 h. Similar trends were observed in other MFC set ups in during the study, hence subsequent power output data refer to readings at 4 h unless otherwise stated. The maximum power output at 4 h could be due to rapid adaptation of the *E. cloacae* to the operating conditions and rapid fermentation of glucose readily available in the defined media.

Proton exchange membrane selection

A MFC utilising a Nafion™ 117 membrane yielded a maximum power output of 17.7 ± 0.7 µW while that using CMI-7000S membrane yielded 13.7 ± 0.5 µW. The former

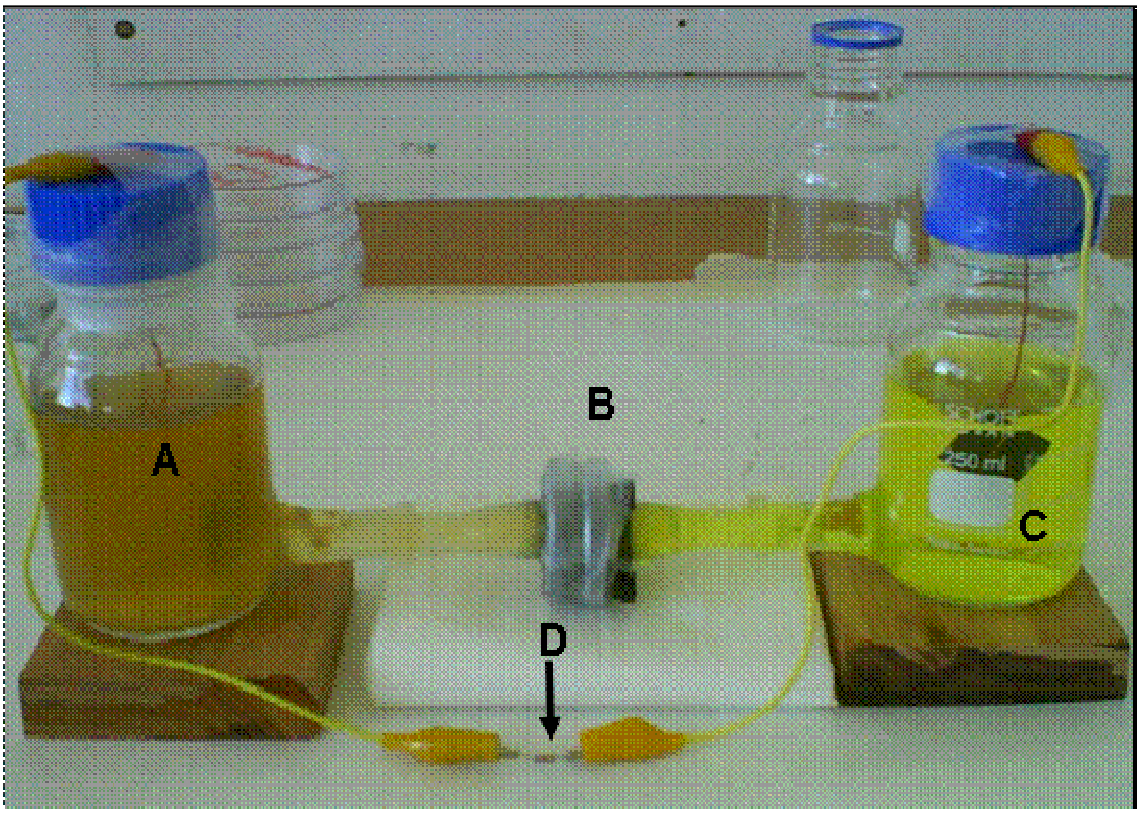

Figure 1. The setup of the H-type microbial fuel cell system used. A: anode chamber; B: proton exchange membrane junction; C: cathode chamber; D: resistor on the external circuit; length of the anode-cathode chambers connector: 200 mm; inner diameter of the connector tube: 14 mm.

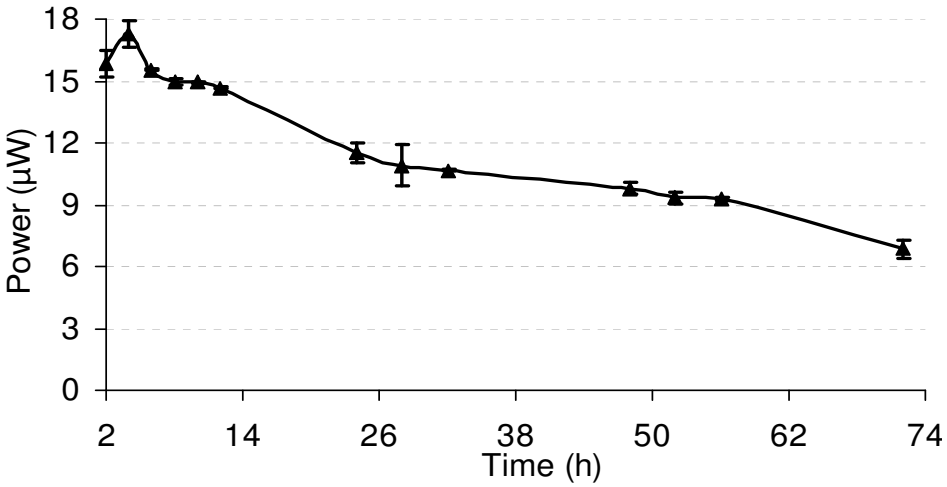

Figure 2. Time-dependent power generation in an *E. cloacae* microbial fuel cell. External resistance: 680 Ω; Electron acceptor: 0.02 M potassium ferricyanide (pH 6.8). Data represent the mean ± sd (n = 3).

gave consistently higher power output for the first 24 h after which there was no significant ($P > 0.05$) difference in the power output from the MFC with the two membranes (Figure 3). This suggests that under the conditions utilised in this study the CMI-7000S membrane required a conditioning period to match the performance of the Nafion membrane. The CMI-7000S membrane is thicker than the Nafion^{TM} 117 which could have led to an

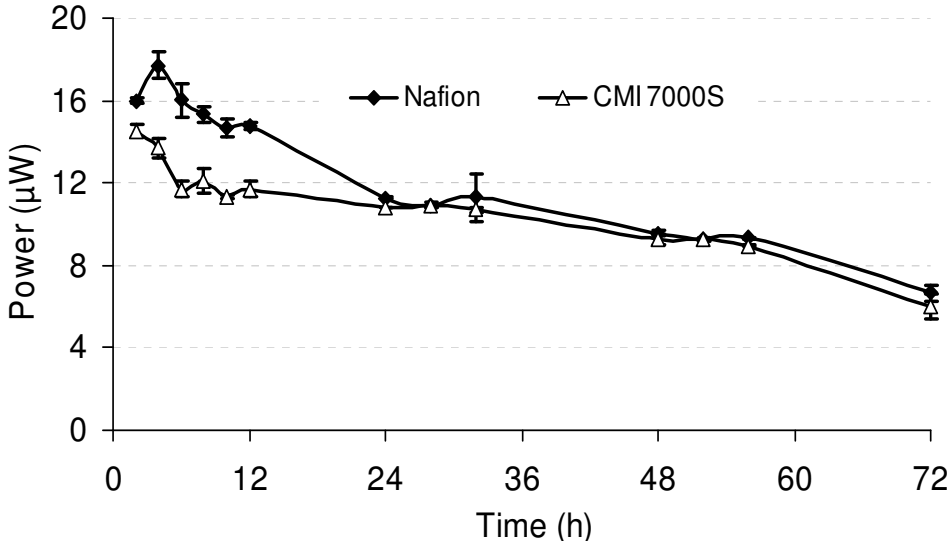

Figure 3. Comparison of the power outputs utilising Nafion™ and CMI 7000S proton exchange membranes at 4 h at an unmodified carbon paper anode (2 cm x 2 cm). External resistance: 680 Ω; Electron acceptor: 0.02 M potassium ferricyanide (pH 6.8). Data represent the mean ± sd (n = 3).

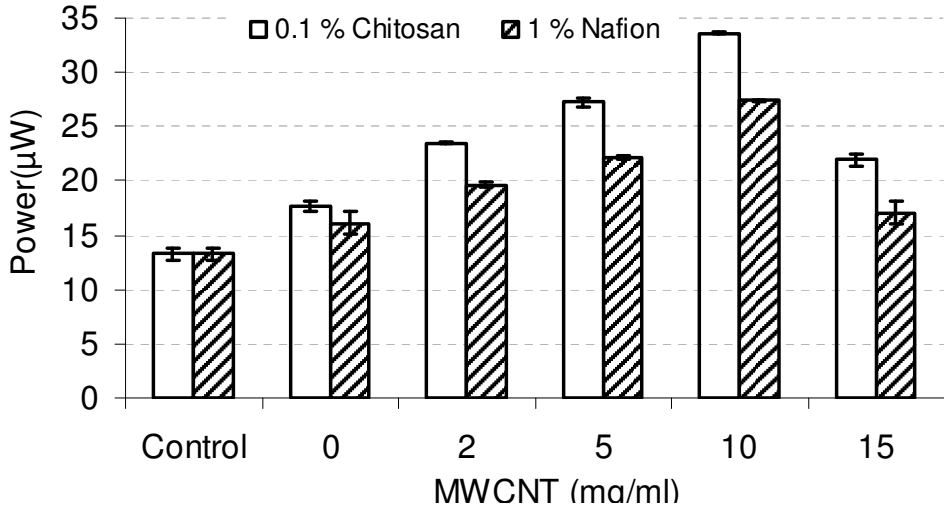

Figure 4. The effect of increasing concentrations of MWCNT dispersed in either 0.1% chitosan or 1% Nafion and immobilised on carbon paper anodes on the power output of an *E. cloacae* MFC at 4 h. Control: plain unmodified anode and 0 refers to the anode coated with respective dispersing agents only. Data represent the mean ± sd (n = 3).

initial low flow of hydrogen ions and the subsequent power output. The CMI membrane can therefore be useful for prolonged life span in a microbial fuel cell especially when waste material is used as a carbon source.

Effects of MWCNTs

Figure 4 shows an increase in power output of the MFC in the presence of anodes modified with MWCNTs compared to the controls (studies conducted in the absence of MWCNTs and dispersing agents and those at anodes coated with the dispersing agents only). This is in keeping with similar studies (Wu et al., 2008). There was a direct relationship between carbon nanotube concentration and power output from 2 to 10 mg MWCNTs/ml dispersed in both 0.1% chitosan and 1% Nafion®. The chitosan-MWCNTs mixture yielded consistently higher

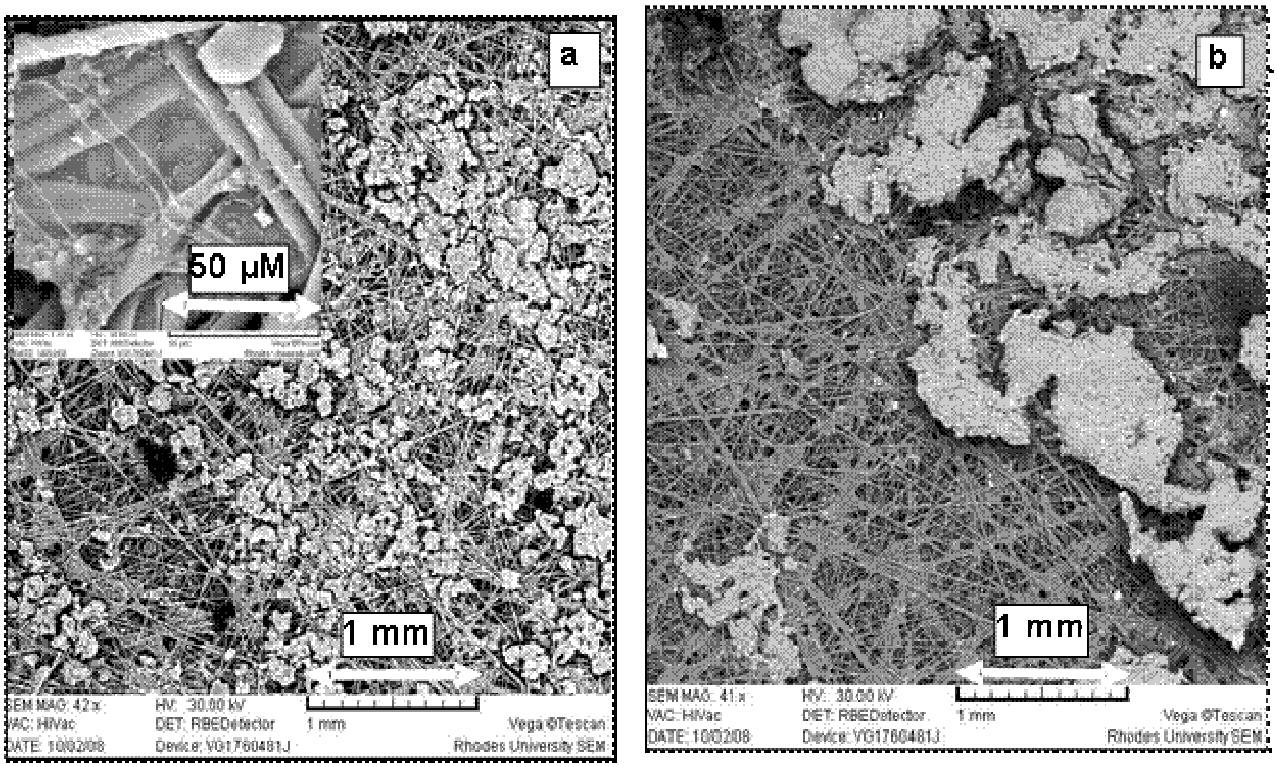

Figure 5. SEM imaging indicating the comparative dispersion of non-functionalised MWCNT in 0.1% chitosan (a) and 1% Nafion® (b) on the modified carbon paper electrodes after 72 h incubation in an anode chamber with *E. cloacae* culture.

power output than the Nafion®-MWCNTs modified anode. The highest power for the former was 33.6 ± 0.1 µW and the latter was 27.4 ± 0.1 µW. The minimum (13.3 µW) and maximum (33.6 µW) power output recorded in this study can be converted to power densities of 16.6 and 42.0 mW/m^2, respectively. These densities are comparable to those (19-33 mW/m^2) reported by Logan et al., (2005) using a similar fuel cell setup with marine sediment organisms cultured in cysteine.

The observed increase in the power output with an increase in MWCNTs concentration was due to the formation of a disperse layer that increased the surface area (for biofilm formation) and conductivity of the anode. However, the proportionality of the enhanced power output and MWCNTs concentration is true up to a certain concentration (in this case 10 mg/ml) after which the power decreased due to changes in the nanotube properties. Thus the decrease in power at 15 mg MWCNTs/ml can be attributed to the formation of an insulating layer that reduced conductivity at the anode.

Higher power outputs observed at chitosan modified anodes than at the Nafion® modified surface concur with earlier reports by Tkzac and Ruzgas (2006) and are accoun-ted for by the enhanced dispersing properties of the polymer, as evidenced by the micrographs in Figure 5, allowing uniform dispersion of the nanotubes. The modification of the surface with chitosan and Nafion® in the absence of MWCNTs (0; Figure 4) shows only a small increase in power output compared to the control.

A comparison of the effect of acid functionalised and non-functionalised MWCNTs showed that the latter yielded slightly more power than the former in the MFC (Figure 6). This can be attributed to metallic impurities in the non-functionalised nanotubes which may impart catalytic properties to this layer. However, the relatively small difference suggests that the time-consuming and resource intensive functionalisation step as utilised by previous authors in MFC may indeed not be a requirement, nor present distinct advantages in enhancing the role of these in MFC production.

Conclusions

Anode modification of the MFC with 10 mg/ml MWCNT increased the output of an *E. cloacae* fuel cell by 252.6%. In this setup there was minimal difference observed between the use of Nafion® and CMI 7000S as proton exchange membranes beyond 24 h. Dispersion of MWCNT in 0.1% chitosan resulted in increased power outputs compared to dispersion in 0.1% Nafion and was attributed to lowered aggregation in the former. This research also suggests that the carboxylic acid functionalisation offers no observed benefit to fuel cell performance when the MWCNTs are entrapped on the electrode surface via the methods examined. These studies thus suggest specific

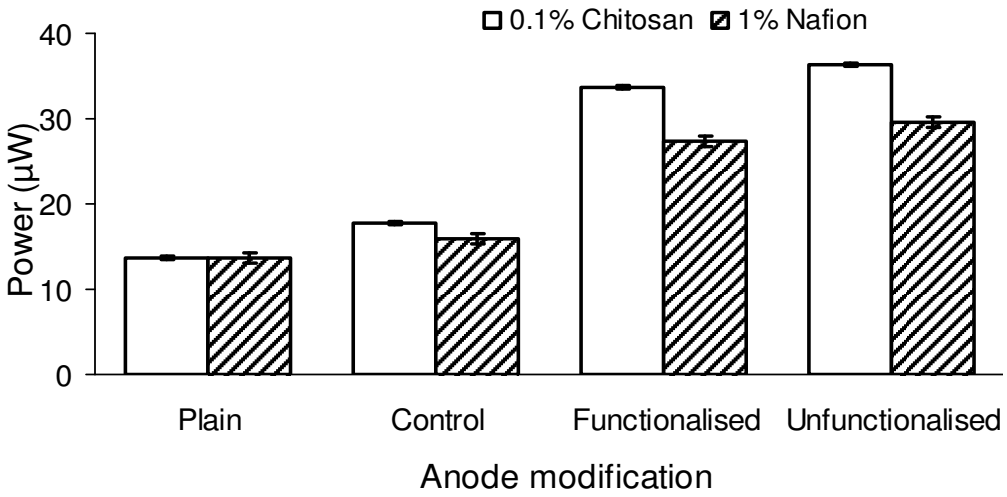

Figure 6. Effects of acid functionalisation of the MWCNT on the power output of *E. cloacae* fuel cells. Control: plain unmodified anode and 0 refers to the anode coated with respective dispersing agents only. Data represent the mean ± sd (n = 3).

improvements in power outputs with particular reference to the emergent application of nanostructured materials in microbial fuel cells.

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