Different types of carbon nanotube-based anodes to improve microbial fuel cell performance

N. Thepsuparungsikul, T. C. Ng, O. Lefebvre and H. Y. Ng

ABSTRACT

The microbial fuel cell (MFC) is an innovative technology for producing electricity directly from biodegradable organic matter using bacteria. Among all the influenceable factors, anode materials play a crucial role in electricity generation. Recently, carbon nanotubes (CNTs) have exhibited promising properties as electrode material due to their unique structural, and physical and chemical properties. In this study, the impacts of CNT types in CNT-based anodes were investigated to determine their effect on both efficiency of wastewater treatment and power generation. The CNTs, namely single-walled CNT with carboxyl group (SWCNT), multi-walled CNT with carboxyl group (MWCNT-COOH) and multi-walled CNT with hydroxyl group (MWCNT-OH) were used to fabricate CNT-based anodes by a filtration method. Overall, MWCNTs provided better results than SWCNTs, especially in the presence of the -OH groups. The highest power and treatment efficiencies in MFC were achieved with an anode made of MWCNT-OH filtered on Poreflon membrane; the open circuit voltage attained was 0.75 V and the maximum power density averaged 167 mW/m^2, which was 130% higher than that obtained with plain carbon cloth. In addition, MWCNT-OH is more cost-effective, further suggesting its potential to replace plain carbon cloth generally used for the MFC anode.

Key words | anode, carbon nanotube, microbial fuel cell, power generation, wastewater treatment

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INTRODUCTION

Energy issues and security have received a lot of attention in recent years. For continued advances, clean, efficient and affordable energy services must be available. Approximately 87% of the world's energy production comes from fossil fuel, but it is fast being exhausted. This may lead to a global energy crisis in the near future. To date, one of the most readily available sources of waste biomass is municipal wastewater. Generally, low strength wastewater is treated using an aerobic process. However, energy requirement in such processes is high due to the need for aeration. Wastewater should therefore be considered not as a waste to dispose of, but as a source of energy that may be harvested. The microbial fuel cell (MFC) is one such ecologically friendly method, with potential for treatment of wastewater and the simultaneous recovery of electrical energy. MFCs use bacteria to directly convert organic matter into energy, carbon dioxide and water. MFCs divert and harvest energy used by bacteria to promote cell growth in the form of electricity. The MFC technology is still in its infancy and suffers from considerable limitations, but practical generation of sustainable bioenergy might be achievable with future research and development (Liu *et al.* 2005; Logan *et al.* 2007).

Among the factors that influence MFC performance, the anode is a crucial parameter, regulating electricity generation and organic removal efficiency (Logan *et al.* 2007; Wei *et al.* 2011). The specific requirements for an MFC anode include high electrical conductivity, high specific surface area, high surface roughness, high porosity, chemical stability, easiness of fabrication, appropriate mechanical strength and toughness, strong biocompatibility and cost-effectiveness (Wei *et al.* 2011; Zhou *et al.* 2011). It is known that different anode materials have impact on microbial adhesion, electron transfer, electrode resistance and electrode surface reaction (Wei *et al.* 2011; Zhou *et al.* 2011). As such, improved microbial colonization and high electron transfer capability can enhance electricity generation and organic removal efficiency (Zhou *et al.* 2011). Carbon cloth and carbon paper are commonly used anode materials due to their high electrical conductivity and good chemical

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stability in microbial culture (Logan *et al.* 2006; Qiao *et al.* 2007); however, both materials display low specific surface area and low porosity (Rabaey & Verstraete 2005; Qiao *et al.* 2007). In addition, carbon paper is fragile and may not be suitable for long-term MFC operation (Kim *et al.* 2007). Therefore, it is necessary to develop new types of anode materials for MFCs, and carbon nanotubes (CNTs) are promising candidates due to their unique structural, electrical, physical and chemical properties (Qiao *et al.* 2007; Sharma *et al.* 2008).

An extensive review of studies making use of CNTs as anode material for MFCs is summarized in Table 1. Multi-walled CNTs (MWCNTs), made of several graphene layers rolled in on themselves to form a tube, are the preferred material (Qiao *et al.* 2007; Sharma *et al.* 2008; Zou *et al.* 2008; Tsai *et al.* 2009; Sun *et al.* 2010; Higgins *et al.* 2011; Thepsuparungsikul *et al.* 2012), but single-walled CNTs (SWCNTs), made of a single graphene layer, have also been used (Xie *et al.* 2011). However, in the absence of comparison, it remains unclear which type of CNTs makes the most suitable MFC anode. Additionally, the role of the functional groups on the CNT surface has yet to be investigated as all research so far has been conducted with carboxyl (-COOH) functional groups (Table 1). Alternatively, hydroxyl (-OH) functional groups may improve CNT characteristics in terms of bacterial adhesion due to strong adhesion forces of hydrogen bonds between the terminal -OH groups on CNTs and the hydrogen bond acceptors or donors present on the bacterial cell walls (Xu & Logan 2006; Parreira *et al.* 2011).

Clearly, CNTs show promise as the anode of an MFC; however, there is a need for a systematic study to assess the optimal CNT material to be used. In this study, novel types of CNT-based anodes were fabricated using an innovative filtration method. The impact of the CNT nature (single- or multi-wall) and functional groups (-COOH or -OH) of CNTs were further evaluated based on morphological and physical characteristics, biocompatibility assessment and performance in an actual MFC system. The main goal of this study is to establish the CNT-based anode as a suitable alternative to conventional MFC anodes, namely carbon cloth.

MATERIALS AND METHODS

Anode preparation

SWCNTs with -COOH functional groups (SWCNT-COOH, diameter 5 nm, length 0.5–1.5 μm, purity >90%) were obtained from Carbon Solutions Inc., USA. MWCNTs with -COOH (MWCNT-COOH) and -OH (MWCNT-OH) functional groups (diameter 20 nm, length 10–30 μm, purity >95%) were obtained from the Chengdu Organic Chemicals Co. Ltd, China. SWCNT-COOH, MWCNT-COOH and MWCNT-OH were coated on a Poreflon

Table 1 | Literature review of MFCs equipped with CNT-based anodes

Anode material	Cathode material	Anode preparation	Maximum power density (mW/m^2)	Open circuit voltage (V)	References
Carbon felt with chitosan/ MWCNT	Carbon felt with Pt	Dipping and drying	Not reported	0.60	Higgins *et al.* (2011)
Ni foam with polyaniline/ MWCNT	Not reported	Not reported	42	0.45	Qiao *et al.* (2007)
Carbon paper with MWCNT-COOH/Sn-Pt	Carbon paper with MWCNT-COOH/Sn-Pt	Not reported	2470	0.91	Sharma *et al.* (2008)
Carbon cloth with MWCNT	Carbon cloth with MWCNT	Dipping and drying	65	0.20	Tsai *et al.* (2009)
Carbon paper with MWCNT-COOH	Carbon paper with Pt	Dipping and drying	290	0.51	Sun *et al.* (2010)
Carbon paper with polypyrrole/MWCNT	Carbon paper	Spraying	228	0.18	Zou *et al.* (2008)
Polyester fabric with SWCNT	Carbon cloth with Pt	Dipping and drying	1098	Not reported	Xie *et al.* (2011)
Poreflon membrane/ MWCNT-COOH	Carbon cloth with Pt	Filtration	120	0.73	Thepsuparungsikul *et al.* (2012)

membrane filter (0.1 μm pore-sized, Sumitomo Electric Fiine Polymer Inc., USA) by filtration. For this purpose, 60 mg of CNTs were dispersed in 40 mL of adequate dispersion solvent by ultrasonication: SWCNT-COOH was dispersed in distilled water for 60 min, MWCNT-COOH was dispersed in 0.5% (v/v) Triton X-100 (Sigma-Aldrich, USA) for 30 min, and MWCNT-OH was dispersed in 1.0% (v/v) sodium dodecyl sulfate (Merck Chemicals, Germany) for 60 min. The homogenized CNT suspension was then vacuum-filtered through a Poreflon membrane, followed by drying at room temperature. The thickness of the CNT film on the Poreflon membrane measured by a digital micrometer (Coolant Proof IP65, Mitutoyo, USA) averaged 0.1 mm. In addition, carbon cloth (non-wet-proofed, type B, E-TEK, USA) without any coating was used for comparison to CNT-based anodes.

To facilitate the identification of the various anodes used in this study, SWCNT-COOH, MWCNT-COOH and MWCNT-OH filtered on a Poreflon membrane will be referred to as SWCNT-COOH/Poreflon, MWCNT-COOH/Poreflon and MWCNT-OH/Poreflon, respectively, in the rest of this paper.

Construction and operation of MFCs

Membrane-less single-chambered air-cathode MFC reactors, each having a total volume of 40.50 cm^3, were constructed with a 2-cm-deep serpentine path separating the anode from the cathode. MFCs using four different types of anode: (i) SWCNT-COOH/Poreflon, (ii) MWCNT-COOH/Poreflon, (iii) MWCNT-OH/Poreflon, and (iv) plain carbon cloth were constructed in duplicate. In all cases, carbon cloth coated with platinum (Pt) catalyst (0.5 mg/cm^2, 30% wet proofed, BASF Fuel Cell Inc., USA) was used as the cathode material. Polytetrafluoroethylene (Gashub, Singapore) layers were applied on the air-side of the cathode following the method of Cheng *et al.* (2006). The apparent anode and cathode surface areas were similar at 20.25 cm^2 each. All reactors were operated at a continuous flow rate of 90 μL/min (hydraulic retention time (HRT) of 7.5 h) and at room temperature (30 ± 2 °C).

Domestic wastewater collected from the effluent of the primary clarifiers of the Ulu Pandan Water Reclamation Plant, Singapore, with a chemical oxygen demand (COD) ranging from 210 to 280 mg/L and a pH ranging from 7.4 to 7.8, was used for inoculation. The inoculation was considered completed when the maximum power output was stable (after about 3 weeks of operation). Afterwards, synthetic acetate wastewater (2 g/L) prepared in a nutrient solution following the method of Oh *et al.* (2004) was continuously fed into the MFCs.

Analytical methods and calculations

The morphology and structure micrographs of the anodes were determined by a field emission electron microscope (FESEM, JSM-6700F, JEOL, Japan) and a scanning electron microscope (SEM, JSM-5600LV, JEOL, Japan). The samples were mounted onto an aluminum stub and coated with Pt. The room temperature electrical conductivity was measured according to the standard van der Pauw dc four probe method based on four-point resistivity systems (Signatone, USA) (Van der Pauw 1958). The specific surface area, pore volume and pore diameter of the anode materials were analyzed using a surface area analyzer (NOVA 4200e, Quantachrome Instrument, USA). For this purpose, 0.05–0.20 g of samples were heated at 150 °C for at least 12 h prior to N_2 adsorption/desorption measurement. The specific surface area and pore volume were calculated using the Brunauer–Emmett–Teller method (Brunauer *et al.* 1938) and the pore size distribution was determined by the Barrett–Joyner–Halenda method (Barrett *et al.* 1951).

The MFC reactors were evaluated in terms of their electrical performance and organic removal efficiency. The voltage (V) across an external resistance of 5 Ω was monitored with a 10-min interval using a multimeter and a computerized data acquisition system (GL800, Graphtec, Taiwan). The power density (P, mW/m^2) and Coulombic efficiency (C_E, %) were calculated according to Logan *et al.* (2006). Polarization curves were drawn by varying the external resistance from 50,000 to 400 Ω and were then used to determine the internal resistance (R_{int}, Ω.m^2) and the maximum power density (P_{max}, mW/m^2). In this study, both P_{max} and R_{int} were normalized to the anode surface area. The COD and suspended solids (SS) concentrations of the feed and effluents were measured following APHA's *Standard Methods* (APHA 2005). The acetate concentration was determined using a gas chromatograph equipped with a flame ionization detector (GC-2010, Shimadzu, Japan).

Biocompatibility studies

The inhibition effect of SWCNT-COOH, MWCNT-COOH and MWCNT-OH on the growth of model bacterium *Escherichia coli* K12 (*E. coli* K12, ATTC 29181) was assessed (i) in nutrient broth (NB, Difco Laboratories, USA) solution and (ii) directly on NB agar plates. Prior to

experimentation, stock culture of *E. coli* was aerobically cultivated in autoclaved NB solution under shaking (150 rpm) at 37 °C for 20 h until the stationary phase was reached. For the first method, 10 mL of this *E. coli* stock culture was then mixed with CNTs at a concentration ranging from 50 to 2,000 mg/L under shaking (150 rpm) at 37 °C for 5 h, and then spread on NB agar plates. For the second method, 100 μL of the *E. coli* stock culture was directly spread on NB agar plates prepared with CNTs at a concentration ranging from 10 to 400 mg/L. In all cases, the plates were left to incubate at 37 °C for 20 h, followed by enumeration. All experiments were conducted in triplicate and control plates were prepared in the absence of CNTs.

In addition to microbiological methods, CNT biocompatibility was evaluated by microscopic observations. For this purpose, fresh samples of SWCNT-COOH/Poreflon, MWCNT-COOH/Poreflon and MWCNT-OH/Poreflon were soaked in the *E. coli* stock culture under shaking (150 rpm) at 37 °C for 20 h and subsequently rinsed with 0.1 M phosphate buffer (pH 7.4) and fixed with 2.5% (v/v) glutaraldehyde (Sigma-Aldrich, USA). The samples were then rinsed with distilled water, dehydrated with ethanol series and finally dried using a critical point dryer. The growth and morphology of *E. coli* on the samples were then assessed by FESEM. Finally, after 7 months of MFC operation, bacterial growth on the MFC anodes was also observed by FESEM.

RESULTS AND DISCUSSION

Anode morphology

The morphology and structure of CNTs filtered on Poreflon membrane and plain carbon cloth, as observed by SEM and FESEM, are shown in Figure 1. SWCNT-COOH/Poreflon, MWCNT-COOH/Poreflon and MWCNT-OH/Poreflon showed a uniform dispersion of highly entangled CNT networks (Figures 1(a)–1(c), respectively), which might support the growth of microbes and serve as nanowires to facilitate electron transfer between the microbes and the anode (Wang *et al.* 2011). During filtration, CNTs formed continuous ropes caused by tube self-assembly induced by the van der Waals force (Pham *et al.* 2008). In the process, SWCNTs formed densely packed and extremely thin fibres (Figure 1(a)), whereas MWCNT fibres were thicker and formed a porous structure (Figures 1(b) and 1(c)) that is believed to enhance substrate diffusion and microbial colonization (Rabaey & Verstraete 2005).

Anode characterization

The anode electrical conductivity, specific surface area, pore volume and pore diameter are shown in Table 2. The control anode (plain carbon cloth) displayed the lowest conductivity, specific surface area, pore volume and pore diameter. In terms of conductivity, the anodes could be ranked as follows: SWCNT-COOH/Poreflon (136 ± 12 S/cm) > MWCNT-OH/Poreflon (103 ± 8 S/cm) > MWCNT-COOH/Poreflon (91 ± 13 S/cm). High conductivity of CNTs filtered on Poreflon membrane may result in enhanced electron transfer (Higgins *et al.* 2011) and the higher conductivity of SWCNTs as compared to MWCNTs can be directly related to their higher degree of entanglement, resulting in a more compact structure (Figure 1(a)). In terms of specific surface area, however, MWCNT-OH (188 ± 15 m^2/g) and MWCNT-COOH (148 ± 11 m^2/g) were superior to SWCNT-COOH (124 ± 14 m^2/g) and the same trend could be seen for the pore volume and pore diameter, as already suggested by the micrographs (Figure 1). Increased specific surface area is expected to facilitate microbial colonization on the anode (Higgins *et al.* 2011; Xie *et al.* 2011), while high porosity may favor substrate penetration into the inner parts of the anode (Rabaey & Verstraete 2005). High specific surface area and porosity are also known to provide more active sites for bacterial catalytic substrate oxidation (Cheng *et al.* 2006; Higgins *et al.* 2011). Finally, pore size in all cases belonged to the mesoporous (2–50 nm) range (Table 2), a size generally considered optimal for microbial colonization deep inside the anode (Rabaey & Verstraete 2005).

MFC performance

The performance of MFCs equipped with various anodes is summarized in Table 3. In comparison with CNTs filtered on Poreflon membrane, control anodes (carbon cloth) performed poorly and this can be directly related to the improved structure with more uniform CNT dispersion on Poreflon membrane (Figure 1).

Polarization and power density curves obtained during steady power production stages are shown in Figure 2. The lowest internal resistance was achieved from SWCNT-COOH/Poreflon at 0.7 ± 0.1 Ω·m^2, followed by MWCNT-OH/Poreflon (0.8 ± 0.1 Ω·m^2) and MWCNT-COOH/Poreflon (1.0 ± 0.1 Ω·m^2) with plain carbon cloth achieving the highest internal resistance at 1.2 ± 0.2 Ω·m^2. Thus, the internal resistances followed the trend of anode conductivities (Table 2). Overall, these values of internal resistance were quite high but could be lowered with an improved MFC design, which is beyond the scope of this study. However, the improved structure of CNT

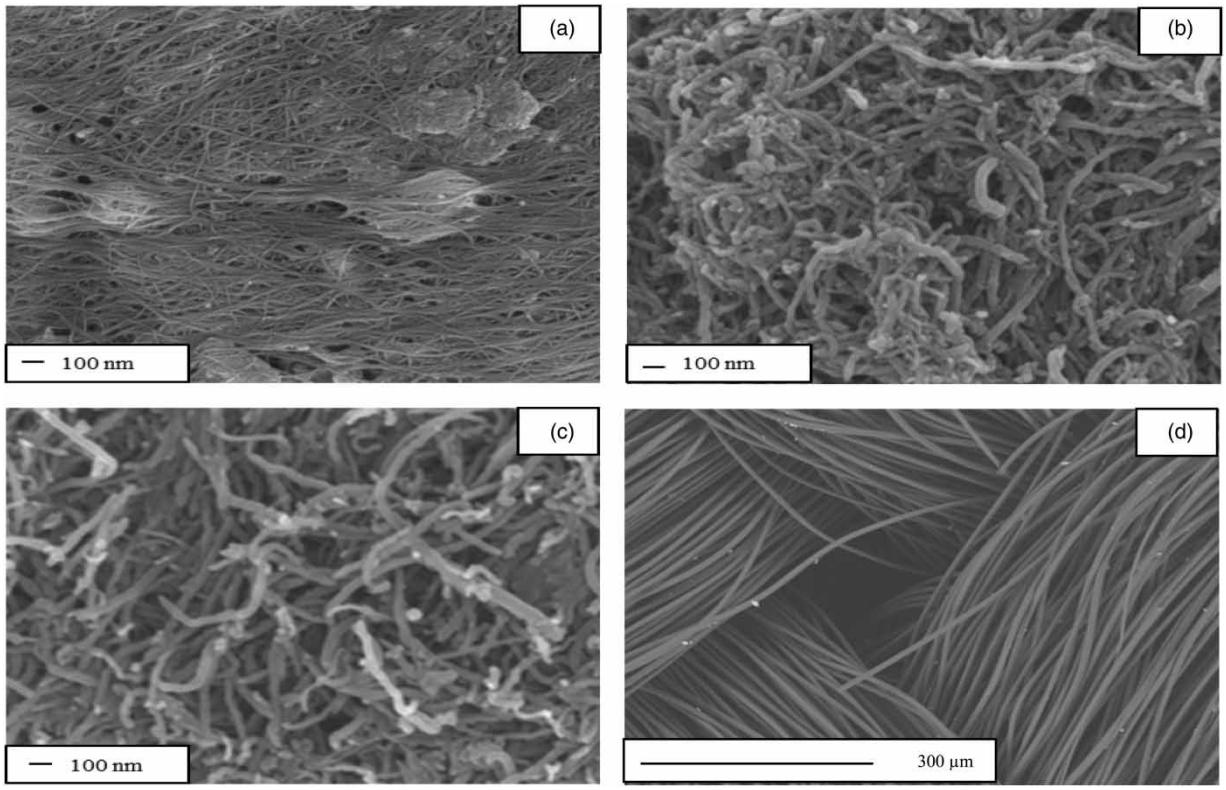

Figure 1 | FESEM micrographs of CNT-based anodes (×50,000 magnification): (a) SWCNT-COOH/Poreflon; (b) MWCNT-COOH/Poreflon; (c) MWCNT-OH/Poreflon; and (d) SEM micrograph (×250 magnification) of carbon cloth.

Table 2 | Electrical conductivity, specific surface area, pore volume and pore diameter of various CNT-based anodes and carbon cloth-based anodes

Types of anodes	Electrical conductivity (S/cm)	Specific surface area[a] (m^2/g)	Pore volume[a] (cm^3/g)	Pore diameter[a] (nm)
SWCNT-COOH/Poreflon	136 ± 12	124 ± 14	0.15 ± 0.04	3.3 ± 0.5
MWCNT-COOH/Poreflon	91 ± 13	148 ± 11	0.22 ± 0.03	3.6 ± 0.4
MWCNT-OH/Poreflon	103 ± 8	188 ± 15	0.28 ± 0.06	3.7 ± 0.4
Carbon cloth	44 ± 7	60 ± 6	0.05 ± 0.03	2.1 ± 0.3

[a]Measured from the anode material.

Table 3 | MFC performance using various CNT-based anodes and operated with synthetic acetate wastewater

				Domestic wastewater			Synthetic wastewater		
MFC	Internal resistance ($\Omega.m^2$)	OCV (V)	Maximum power density (mW/m^2)	COD removal (%)	SS removal (%)	C_E (%)	COD removal (%)	Acetate removal (%)	C_E (%)
SWCNT-COOH/ Poreflon	0.7 ± 0.1	0.46 ± 0.04	67 ± 10	68 ± 5	66 ± 4	10 ± 2	79 ± 4	97 ± 1	24 ± 4
MWCNT-COOH/ Poreflon	1.0 ± 0.1	0.78 ± 0.04	146 ± 6	69 ± 3	69 ± 4	8 ± 1	86 ± 4	97 ± 2	18 ± 3
MWCNT-OH/ Poreflon	0.8 ± 0.1	0.75 ± 0.02	167 ± 7	78 ± 3	72 ± 4	18 ± 2	89 ± 4	98 ± 1	32 ± 4
Carbon cloth	1.2 ± 0.2	0.58 ±0.06	73 ± 16	56 ± 8	58 ± 6	2 ± 1	65 ± 7	95 ± 4	6 ± 2

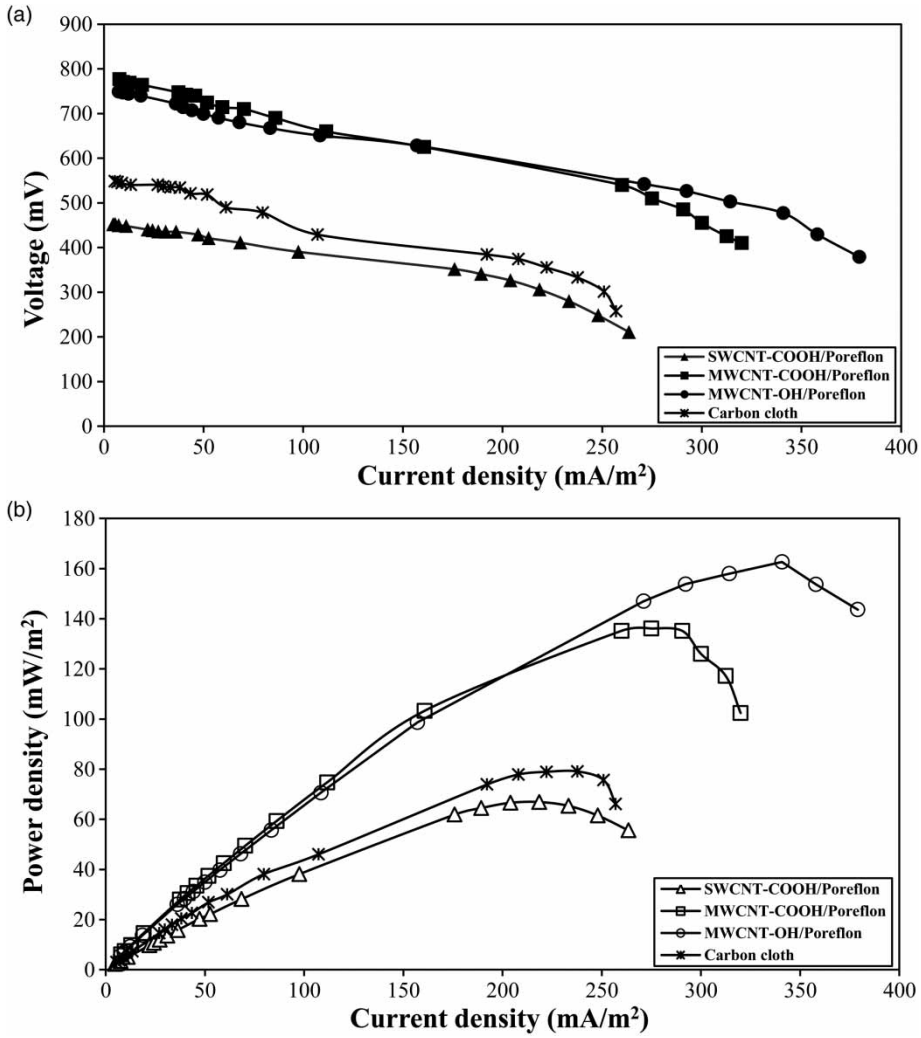

Figure 2 | (a) Polarization curves and (b) power density curves obtained with MFCs equipped with CNT-based anodes and operated with synthetic acetate wastewater.

anodes resulted in considerably increased open circuit voltage (OCV); OCV was higher with MWCNTs than with SWCNTs and the values obtained with MWCNT-COOH/Poreflon (0.78 ± 0.04 V) and MWCNT-OH/Poreflon (0.75 ± 0.02 V) were generally higher than those reported in the literature, except when a hexacyanoferrate catholyte was used (Table 1). Due to high OCV, MWCNTs generated higher maximum power density than SWCNTS and the highest value of 167 ± 7 mW/m^2 was generated from MWCNT-OH/Poreflon anode, followed by MWCNT-COOH/Poreflon (146 ± 6 mW/m^2) and SWCNT-COOH/Poreflon (67 ± 10 mW/m^2).

The dramatic depletion of power density using SWCNT-COOH-based anodes can be explained by the morphology and density of the CNT networks dispersed on the Poreflon membrane. As the diameter and length of SWCNTs were lower than those of MWCNTs (5 nm vs 20 nm diameter and 0.5–1.5 μm vs 10–30 μm length, respectively), their distribution was denser and formed smoother surfaces than MWCNTs (Figure 1). In contrast, the looser network dispersion and rougher surface of MWCNT-based anodes allowed bacteria to penetrate into the inner layers of MWCNTs. In conclusion, this shows that the size and density of CNT networks coated on Poreflon membrane played a more important role than their conductivity.

In comparison to MWCNT-COOH, MFCs equipped with MWCNT-OH anodes exhibited almost 15% higher maximum power density and this can be related to the higher specific surface area and pore diameter of MWCNT-OH (Table 2), therefore facilitating bacterial growth. Moreover, the -OH functional groups on MWCNT surfaces may have enhanced the adhesion forces between the bacterial cell walls and the surfaces of MWCNTs and improved the substrate oxidation rate.

A similar trend was reported by Xu & Logan (2006) who mentioned that OH-functionalized colloids produced higher adhesion forces to proteins (bovine serum albumin, lysozyme and poly-D-lysine) than COOH-functionalized colloids.

The organic removal efficiency is another important factor to evaluate MFC performance as a treatment system. All MFC reactors were operated continuously with a HRT of 7.5 h at an initial COD concentration of 240 and 340 mg/L for domestic and synthetic acetate wastewater, respectively, and the COD, SS and acetate removal efficiencies, and corresponding C_E are summarized in Table 3. The best performance was achieved with MWCNT-OH/Poreflon anode, with 78 ± 3% COD removal efficiency and 72 ± 4% SS removal efficiency with domestic wastewater, and 89 ± 4% COD removal efficiency and 98 ± 1% acetate removal efficiency with synthetic acetate wastewater. The improved organic removal efficiency of MWCNT-OH over other anodes can again be explained by its high specific surface area, porosity and roughness, allowing enhanced colonization of bacteria on the anode and deep penetration of the substrate. On the other hand, the carbon cloth anodes were prone to clogging, which could hinder substrate diffusion (Xie *et al.* 2011). Better performance using acetate as compared to domestic wastewater, making it a suitable substrate for electricity generation, has already been reported elsewhere (Min & Logan 2004; Liu *et al.* 2005; Rabaey & Verstraete 2005). For the same reason, use of acetate resulted in higher C_E than domestic wastewater and the highest C_E was achieved with MWCNT-OH/Poreflon (32 ± 4%), more than five times higher than when using plain carbon cloth (6 ± 2%). This can again be explained by the improved

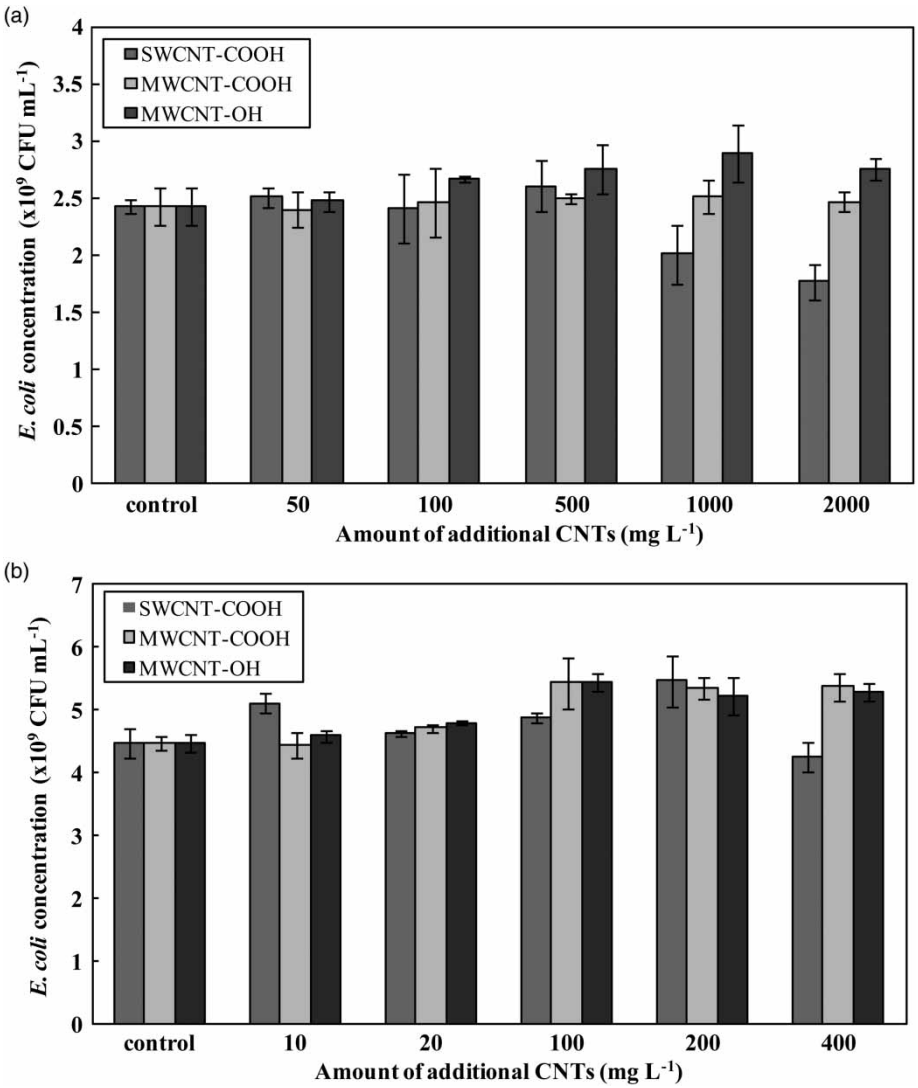

Figure 3 | *E. coli* concentration (CFU/mL) after an incubation period of 20 h at 37 °C in the presence of CNTs in NB solution (a) and on NB agar plates (b).

bacterial attachment and electron transfer on MWCNT-OH/Poreflon. However, the low C_E from all the MFCs indicates that the majority of the substrate was not utilized for power generation, as the main electron acceptor was not the anode but oxygen diffusing via the air-cathode as demonstrated widely by others (Min & Logan 2004; Fan *et al.* 2007).

In summary, MWCNT-OH/Poreflon as an anode exhibited the best MFC performance in terms of power generation and organic removal efficiency. Compared to the literature (Table 1), the OCV from MWCNT-OH anode obtained in this study (0.75 V) was higher than that obtained with MWCNT-COOH anodes (Qiao *et al.* 2007; Zou *et al.* 2008; Tsai *et al.* 2009; Sun *et al.* 2010; Higgins *et al.* 2011). This can be related to the higher specific surface area, porosity, electrical conductivity and stronger bonding between bacteria and the anode of MWCNT-OH. The cost of anode materials used in this study was US$400/g for SWCNT-COOH, US$5.2/g for MWCNT-COOH and MWCNT-OH, US$270/m^2 for Poreflon membrane and US$1,000/m^2 for plain carbon cloth. To fabricate each reactor, 60 mg of CNTs and 20 cm^2 of carbon cloth or Poreflon membrane were utilized for anodes in this study. Therefore, MWCNT/Poreflon was found to be the most cost-effective anode at US$1/anode, twice lower than plain carbon cloth (US$2/anode) and 25 times lower than SWCNT/Poreflon (US$25/anode).

Biocompatibility

For MWCNT-COOH and MWCNT-OH, the *E. coli* concentration determined after an incubation period of 20 h at

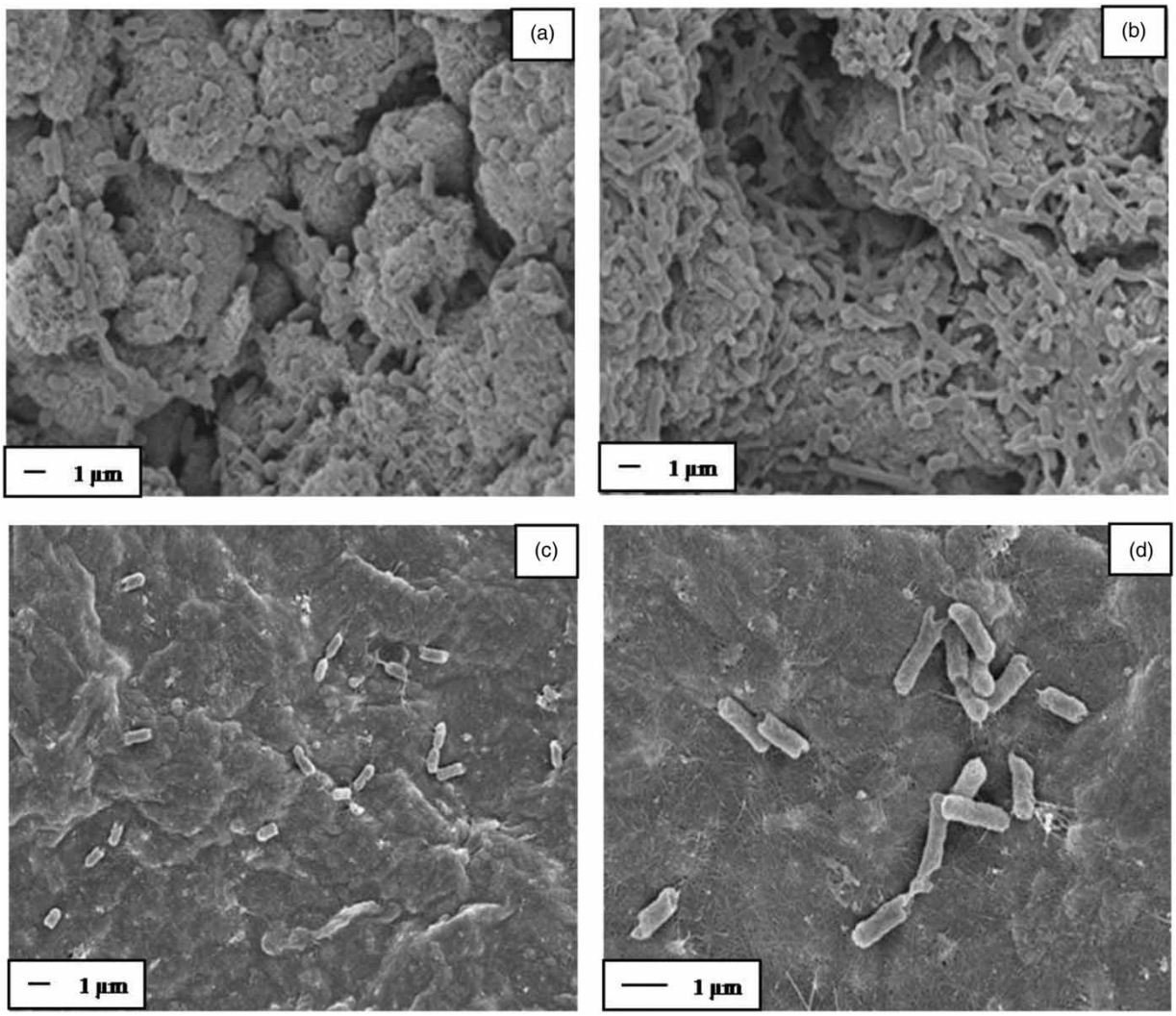

Figure 4 | FESEM micrographs of *E. coli* biofilm on CNT-based anodes after incubation at 37 °C for 20 h: (a) MWCNT-COOH (×5,000 magnification); (b) MWCNT-OH (×5,000 magnification); (c) SWCNT-COOH (×5,000 magnification); and (d) SWCNT-COOH (×10,000 magnification).

37 °C in NB solution (Figure 3(a)) and NB agar plates (Figure 3(b)) showed no significant deviation from the control up to 2,000 mg/L of MWCNTs in NB solution and 400 mg/L of MWCNTs on NB agar plates, indicating an absence of toxicity of MWCNTs on *E. coli*. However, SWCNT-COOH ≥1,000 mg/L in NB solution and ≥200 mg/L on agar plates inhibited the growth of *E. coli* with a cell concentration 32 and 11% lower than the control at 2,000 mg/L of SWCNTs in NB solution and at 500 mg/L of SWCNTs on NB agar plates, respectively. This could be explained by the increase of the degree of carboxyl groups in SWCNTs, which might have a direct impact on bacterial toxicity, as reported by others (Magrez *et al.* 2006; Kang *et al.* 2008). Particularly, Kang *et al.* (2008) explained that the shorter length of SWCNTs (0.5–1.5 μm vs 10–30 μm of MWCNTs) may allow them to penetrate through the cell membrane of *E. coli*, disrupting its activity and subsequently destroying the cell.

These conclusions were supported by FESEM observations of CNTs filtered on Poreflon membrane and incubated with *E. coli* stock culture at 37 °C for 20 h (Figure 4), which showed denser *E. coli* colonization with MWCNTs (Figures 4(a) and 4(b)) than with SWCNTs (Figure 4(c)). Moreover, at higher magnification (Figure 4(d)), SWCNT-COOH/Poreflon showed damaged cell membranes and mis-shapen *E. coli* cells as already reported by Kang *et al.* (2008).

These observations were corroborated with FESEM micrographs of the anodes sampled from the MFCs after 7 months of operation (Figure 5). Less biofilm was found developing on the SWCNT/Poreflon (Figure 5(a)) than on

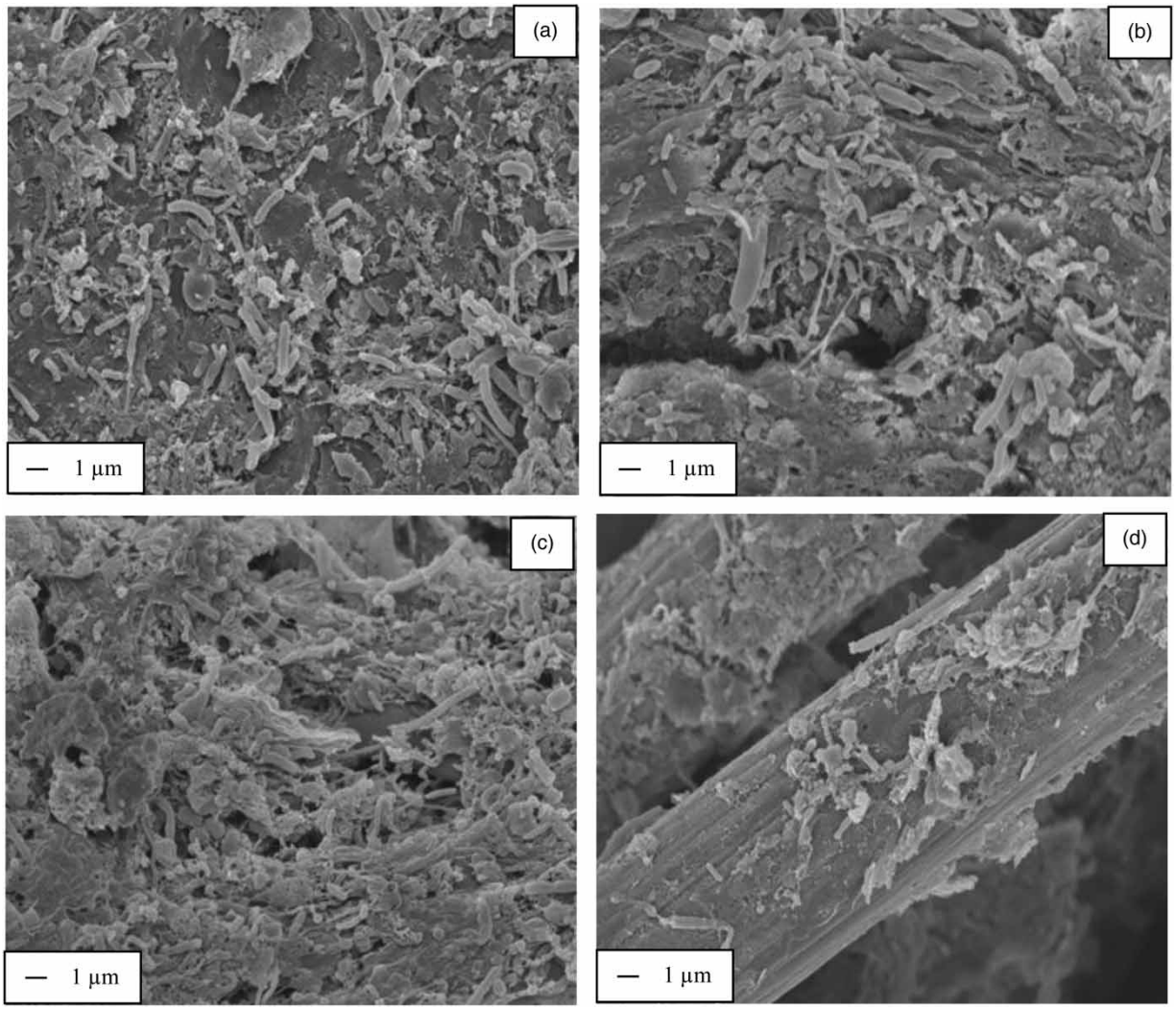

Figure 5 | FESEM micrographs of CNT-based anodes after 7 months of operation in an MFC (×5,000 magnification): (a) SWCNT-COOH/Poreflon; (b) MWCNT-COOH/Poreflon; (c) MWCNT-OH/Poreflon; and (d) carbon cloth.

the MWCNT/Poreflon (Figures 5(b) and 5(c)). From Figures 5(a)–5(c), it also appears that the biofilm covered the totality of the anode surface with an abundance of rod-shaped, spherical-shaped and spiral-shaped microorganisms growing individually or in chains; however, biofilm was scarce on carbon cloth-based anodes (Figure 5(d)), and this can be related to the improved physical properties of the anodes obtained by filtration. Again, the high porosity obtained using MWCNTs is very apparent in these micrographs (Figures 5(b) and 5(c)).

CONCLUSION

In this study, CNTs were identified as possibly good alternatives to conventional carbon cloth as MFC anodes. The advantages of CNTs include high specific surface area, conductivity, pore volume, and pore diameter, along with a unique network structure and strong biocompatibility. In addition, the preparation procedure of CNTs on Poreflon membrane was simple, time-saving and cost-effective. MWCNTs further performed better than SWCNTs, and the presence of the -OH functional groups resulted in greatly improved performance in terms of electron transfer capability, microbial attachment, substrate diffusion and substrate oxidation. MWCNT-OH/Poreflon as the anode of an MFC greatly enhanced the OCV (0.75 V) of the system and generated a maximum power density 130% higher than the plain carbon cloth. In addition, the Coulombic efficiency was satisfactory using both the domestic (18%) and synthetic acetate (32%) wastewater.

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