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Direct electron transfer in *E. coli* catalyzed MFC with a magnetite/MWCNT modified anode

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Although microbial fuel cells (MFCs) are not yet commercialized, they have drawn great attention as a low cost and environmentally friendly alternative energy source. There are still several challenges to overcome for MFCs to appear in industry. Currently, real-world applications of MFCs are limited because of their low power density due to slow electron transfer between the bacterial cells and the electrode. To solve this problem, a batch type MFC with modified anode surface has been investigated in this article. The carbon anode surface was coated with magnetite/MWCNT nanocomposite by directly applying a magnetic field and the *Escherichia coli* (*E. coli*) catalyzed MFC was assembled for polarization studies. This conductive biocatalytic layer coated on the anode helps the electron transfer between the bacteria and the anode, by forming micro channels of MWCNT between them. The magnetite in the anode creates a magnetic field and attracts the bacteria towards the anode layer, speeding up the electron transfer and enhancing the MFC performance even without any mediator. The mediator-less MFC with magnetite/MWCNT nanocomposite generates high power density of 1050 mW m^{-2}, almost 30% more than our previous report created by *E. coli* K12 in mediated MFC. Conductivity and resistivity of the conductive biocatalytic electrode was also investigated with cyclic voltammograms and electrochemical impedance spectroscopy, respectively.

Introduction

Renewable and clean forms of energy are one of the world's greatest and present needs. Microbial fuel cells (MFC) represent a new method of renewable energy recovery though the concept dates back to 1970s.[1] MFC converts chemical energy into electricity through catalytic activity of microorganisms mostly bacteria to oxidize organic and inorganic matter; in simple words using bacterial catalysis MFC directly generate electricity from wastes.[2] Unlike typical fuel cells, MFCs are attractive with low-cost and environmentally friendly technology using bio-materials without the use of inorganic matter such as expensive or precious metals. MFCs also have operational and functional advantages over the current technologies like high efficiency, operating in ambient or even low temperatures, no requirement of gas treatment and potential for wide spread applications.[3–5] Although MFCs have the advantages of clean power generation and simultaneous waste utilization, their commercialization has been ceased due to their low power output. The recent developments of MFC with enhanced power output have rekindled the research trends in MFC providing possible opportunities for their practical applications.[5–8]

In an MFC, microbes in the anode act as bio-catalyst for the decomposition of organic substrate to generate electron and proton. Electrons produced by the microbes are transferred to the anode and flow to the cathode linked by a conductive material containing a resistor, or operated under a load. The current is generated by diverting the catabolic electrons to the anode. Meanwhile, the proton migrates in electrolyte and reaches the cathode through the membrane or separator and combines with oxygen with the help of electron to produce water.[7] There are various factors affecting the performance of MFC during this process resulting in a lowering of the attainable voltage and power. Among the whole processes, oxygen reduction in cathode, concentration or mass transfer losses *etc.*, possibly reduce the power of MFC (Logan *et al.*, 2012).[9] The slow kinetics of the electron transfer between the bacterial cells and the fuel cell anode is one of the main reasons for the low power capability of MFC. In order to start the transfer of electrons from the electrochemical active microbes towards the electrode or to transfer electrons towards a final electron acceptor, an energy barrier needs to be overcome, which results in a voltage loss or activation over potential.

Generally, the transfer of electrons from microbes to anodes are assisted by mediators such as thionine, methylene

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blue, 2-hydroxyl 1, 4-naphthoquinone (HNQ) *etc.*, which are used as electron shuttle to facilitate the transfer of electron between the bacteria and the working electrode/anode in the system.[10] This type of MFC is called mediated fuel cells where the mediators have to be continuously replenished in order to avoid any poisonous effect and improve the efficiency of the fuel cell. Recently, there has been mediator-less (ML) MFCs reported by Kim *et al.* and others.[4,6,11–14] Their practicality is increased by production of electrical energy through various organic wastes processing without any mediators. ML MFC employ metal reduction microbes including *Shewanella*,[11,15,16] *Geobacter sulfurreducens*,[8,17] *Rhodoferax ferrireducens*[4] *etc.*, to enhance the electron transfer from microbe to anodic electrode. These microbes use various strategies to transfer electrons directly or indirectly towards the working electrodes like, shuttling them using self produced mediators like membrane proteins or cell metabolites or through electrically conductive self produced nanowire.[18,19] ML MFC with coulombic efficiency as high as 80% was reported by Chaudhuri *et al.*,[4] but the power density of the MFCs remained low since the high coulombic efficiency was obtained under very high over potentials for glucose oxidation. *E. coli* being one of the readily available and easily grown bacterial strains can transfer electrons from electrochemical active microorganisms to electrode through mediators.[16,17] Zhang *et al.*,[6] shows that an electrochemically evolved *Escherichia coli* (*E. coli*)-catalyzed MFC with a carbon based anode exhibits a maximum power density greater than 600 mW m^{-2} and 760 mW m^{-2} in ref. 19 and 20 in the absence of any artificial electron mediators, which is much higher than the previously reported value of 91 mW m^{-2} achieved in an *E. coli*-based MFC with efficient mediators used in both the anode and the cathode by Park *et al.*[10]

In this work, instead of using artificial mediators, we modified the anode surface by coating magnetite/MWCNT-*E. coli*, creating network channel between the microbes and anode. The magnetite/MWCNT nanocomposite was prepared and introduced into *E. coli* containing solution to form a nanocomposite of magnetite/MWCNT-*E. coli*. MWCNT with high surface area and high conductivity is used to improve the electron transfer; magnetite is employed to make a nanocomposite of conductive biocatalytic layer of magnetite-MWCNT-*E. coli* by creating a strong magnetic field. This magnetic field helps to improve the interconnection and interaction between magnetite coated anode and microbes. The modification effect of anode surface on the performance of MFC is evaluated.

Experimental

Synthesis of magnetite/CNT nanocomposite

Magnetite/CNT nanocomposite was prepared using CNT (diameter 5–10 nm, nanosolution) and magnetite particles (particle size 20–40 nm, Aldrich) by a simple adsorption process. The CNTs were purified by acid treatment to eliminate any residual growth catalysts and impurities. 200 mg CNT was dispersed in a 1 L beaker of 60% HNO_3 by ultrasonication for 1 h. The content was stirred at a constant temperature of 80 °C for 24 h. The mixture was then filtered while flushing continuously with distilled water until it reached a pH 7.0. Thus purified CNTs were collected after drying in air/vacuum oven at 120 °C. 200 mL of organic solvent cyclohexane (MW: 84.16, −99.5%, Dae Jung) was taken in a 500 mL beaker and magnetite particles were added to it. The mixture was dispersed for 30 min by ultrasonication and then purified CNTs were added into the dispersed magnetite suspension. The content was stirred for more than 4 h for the adsorption of the two nanoparticles. To eliminate the organic solvent cyclohexane, ethyl alcohol anhydrous 99.9% (MW: 46.07, Dae Jung) was added to this mixture and centrifuged 3–4 times. For a strong adsorption, nanocomposite was heat treated in nitrogen atmosphere (300 °C) for 1 h and the magnetite/MWCNT nanocomposite was collected after cooling.[21–23] After the synthesis, a magnet was placed near the nanocomposite to confirm its magnetic property. The particles were strongly attracted by magnetic force in both solid state and dispersed suspension. The photographs of magnetite/CNT nanocomposite both particles and in ethanol suspension in the presence and absence of an externally placed magnet is shown in figure (Fig. 1) supporting the magnetic property of magnetite in magnetite/MWCNT nanocomposite. Morphological property of the synthesized Magnetite/CNT nanocomposite was examined by field emission scanning electron microscope (FESEM) (S-4700, HITACHI) and X-ray diffraction using high-resolution X-ray diffractometer (X'PERT-MRD, Philips) in order to investigate the structure of the nanocomposite with different magnetite contents.

Microbe cultivation

Escherichia coli (*E. coli*) K12 used in this experiment is a gram-negative bacteria of 2 μm size and the aerobic culture was done in Luria-Bertani (LB) medium. The *E. coli* K12 colony in LB agar medium was extracted into a falcon tube containing 5 mL LB medium. Seed culture was carried out for 12 h at 160 rpm in a shaking incubator (37 °C). 1 mL of the seed culture medium was mixed with 20 mL of LB medium in a falcon tube followed by the main culture for 12 h at 160 rpm in a shaking incubator (37 °C).[11]

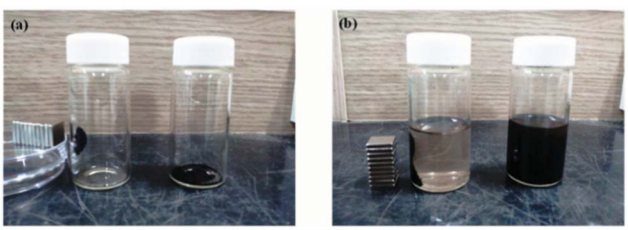

Fig. 1 The photographs of Magnetite/CNT nanocomposite (a) particles in the presence (left image) and in the absence of a magnet and (b) suspended in ethanol in the presence (left image) and absence of an externally placed magnet.

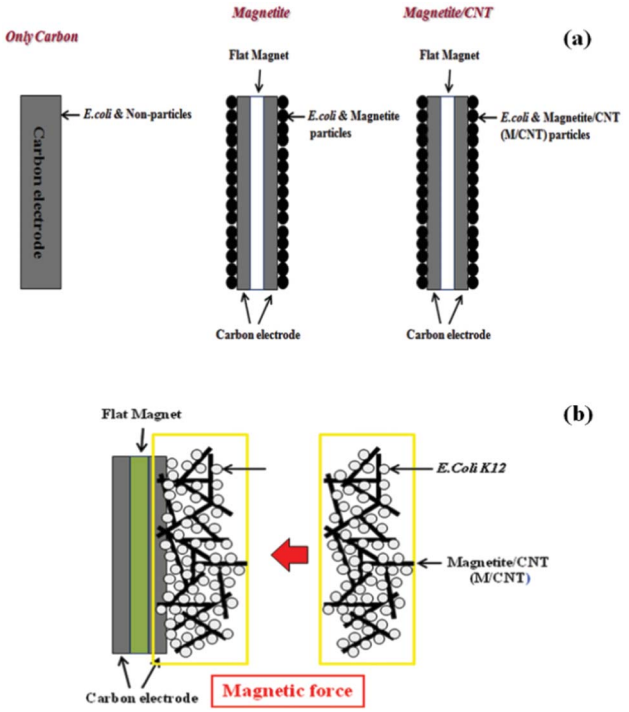

Fig. 2 (a) Assignments of the anode electrode (only carbon, magnetite, magnetite/CNT). (b) Concept of magnetite/CNT electrode modification.

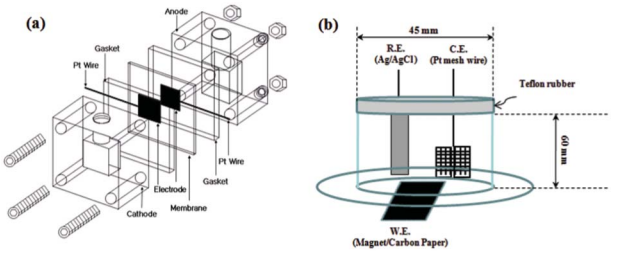

Fig. 3 Schematic diagram of fuel cell assembly for (a) Polarization curve & Impedance and (b) Cyclic Voltammetry.

Assembly of anode

For anode preparation, 0.05 g of magnetite/MWCNT nanocomposite was added to *E. coli* culture medium in main culture, vortexed for uniform dispersion of particles and then cultivated continuously by the culturing process for 2–3 h. A magnetic carbon electrode made of a magnet (10 × 10 × 2 mm) incorporated with carbon paper (TGPH-090, Toray International Inc.) was placed close to the culture medium. A sort of network is formed between *E. coli* and magnetite/CNT nanocomposite and they get adsorbed onto the magnetic carbon electrode resulting in a conductive biocatalytic electrode (magnetite/CNT). The difference between conventional anode and modified electrode is clearly demonstrated in Fig. 2a. A schematic diagram of electrode modification through magnetic force is depicted in Fig. 2b. In order to investigate the effect of magnetite and MWCNT particles on fuel cell performance, a conventional carbon electrode, a magnetite electrode and a conductive biocatalytic electrode were used separately with the designated names only carbon, magnetite and magnetite/MWCNT respectively and their fuel cell performance and electrochemical characterization were evaluated. Also, fuel cell performance of synthesized magnetite/MWCNT electrode with different magnetite content (10, 30, 50 and 80 wt%) was comparatively analyzed.

Fabrication and evaluation of MFC

A small two-chamber MFC consisting of anode (10 mL) and cathode (10 mL) was used to evaluate the performance of fuel cell (Fig. 3a). The two electrodes were separated using Dupont's cation exchange membrane (Nafion 117). To minimize the internal resistance, the distance between the electrodes and cation exchange membrane was set below 0.5 mm. Platinum wire was used as external wire for counter electrode. An 8 mL *E. coli* suspension mixed with 100 mM of phosphate buffer solution (pH 7.0) was injected into the anode; a mixture (1 : 1) of 50 mM ferricyanide and 100 mM phosphate buffer solution (pH 7.0) was injected into the cathode. 1 mL of 0.5 M glucose ($C_6H_{12}O_6$) was injected into anode as electron donor and was purged with N_2 for 30 min to make anaerobic atmosphere. Dissolved oxygen was supplied to cathode as electron acceptor by a continuous flow of oxygen with air pump. Cathode was applied with carbon cloth (BASF) with 20% platinum content, and all evaluation of fuel cell performance was done in an incubator at 37 °C.

Electrochemical characterization

The MFC reactor was operated in batch mode. The open circuit potential (OCP) and voltage output with external resistance were acquired at 1 min intervals by an automatic data acquisition system connected to a computer. The power output curve was plot by measuring the external potential at different external resistances (ranging from 100 KΩ to 100 Ω) using a variable load box and a digital multimeter (Model 2000, Keithley). Current (I) was calculated using Ohm's law (V = I × R) at each given resistance (R) and the power density was obtained by current and voltage (P = V × I) normalized with the anode geometrical surface area. MFC performance was evaluated using polarization and power density curve.

Electrochemical impedance spectroscopy (EIS), (Gamry Reference 600 TM potentiostat installed with Gamry EIS 300 software) was measured for the anode using a three point method to investigate the complex resistance generated from unit cell of anode when fuel cell is working. Anode impedance spectra were recorded using the anodes (only carbon, magnetite and magnetite/MWCNT) as working electrode, Ag/AgCl as reference electrode and cathode as counter electrode. All EIS evaluations were conducted at the open circuit potential in a frequency range of 100 kHz–0.1 Hz and the amplitude of the applied AC signal was 10 mV.

Cyclic voltammograms (CV) was measured for current *versus* potential curve in a Gamry Reference 600 TM potentiostat installed with Gamry PHE200 software. In order to evaluate electrochemical activity of each anode (only carbon, magnetite and magnetite/CNT), a half cell of 100 mL volume was made and current-potential curve was measured using three-point

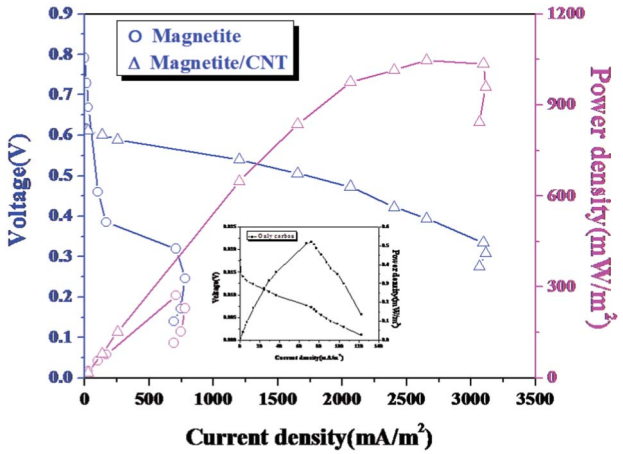

Fig. 4 Polarization curves and power output of ML MFC with only carbon, magnetite and magnetite/CNT modified anodes.

method (Fig. 3b). Each anode was placed on top of a round shaped glass plate covered with a cylindrical stopper (height: 60 mm, diameter: 45 mm) fixed with reference electrode (Ag/AgCl) and counter electrode (Pt mesh wire). A 50 mL *E. coli* suspension mixed with 100 mM phosphate buffer solution (pH 7.0) and 1 mL 0.5 M glucose ($C_6H_{12}O_6$) was injected and nitrogen purged for 30 min to make an anaerobic atmosphere. More than 3 cycles of CV curve was obtained in the potential range of −600 mV–+600 mV at a scan rate of 25 mV s^{-1}.

Result and discussion

The performance of MFC fabricated with magnetite/MWCNT modified anode

Electrochemical characterization of the fuel cell with a conductive biocatalytic anode (magnetite/MWCNT-*E. coli*) was evaluated and the polarization curves and power density curves are shown in Fig. 4. From the figure, it is noted that the power generation in magnetite/MWCNT-*E. coli* coated conductive biocatalytic anode MFC is much greater than only carbon and magnetite anode MFCs. To be specific, magnetite/MWCNT-*E. coli* coated conductive biocatalytic anode delivers a maximum power density of 1050 mW m^{-2} at a current density of 2650 mA m^{-2} and a cell voltage of 390 mV. This maximum power density obtained is 2000 times larger than the only carbon anode and more than 4 times larger than the magnetite anode MFCs respectively. It is seen that the modified anode has a great influence in MFC performance. The magnetite in the conductive bio-catalytic anode seems to enrich the *E. coli* population on carbon anode surface by the attractive force created by the magnetic field. Similar studies by Kim and B. E. Logan *et al.* (2005)[24] have proved to increase the effect of enrichment of bacteria by using a ferric medium (ferric iron-coated carbon electrode) as the anode on initial power production. They observed that ferric-reducing bacteria were selectively attached to the ferric oxide-coated electrode and suggest that the power generation in the MFC was primarily due to direct electron transfer by bacteria attached to the electrode and not through any suspended bacteria or bacteria producing mediators in the biofilm. It has been reported that the startup was most successful with the ferric iron-coated anode, resulting in increased power generation of MFC. Kim *et al.*[24] also mentioned that these iron oxide-coated anodes improved biocompatibility, and thus produced higher power densities and coulombic efficiency, compared to plain anodes. Lowy *et al.* (2006)[25] found that graphite modified with a graphite paste containing Fe_3O_4 or Fe_3O_4 and Ni^{2+} possesses 1.5–2.2 times greater kinetic activity than plain graphite. Sun *et al.*[23] also confirmed the magnetic properties and behavior of magnetite coated carbon nanotubes. The bacterial adhesion to metal oxide surfaces and magnetite has been investigated earlier. Li *et al.* (2004)[26] and Williams *et al.* (2006)[27] found out that the adhesion between magnetite and microbes arose by the attractive force between magnetite's positive charge and microbial surface's negative charge. Hence it is confirmed that the magnetite in magnetite/MWCNT's magnetic field has increased the efficiency of the initial contact of microbes on the anode surface or microbe population. This magnetic field helps to improve the interconnection and interaction between magnetite coated anode and microbes by speeding up electron transfer rate and enhancing the MFC performance even without any mediator.

This high performance of conductive biocatalytic anode can also be attributed to very little loss of activation polarization compared to a rapid loss of activation polarization of the other electrodes. Generally to transfer the electrons from the electrochemically active microbes towards a final electron acceptor, an energy barrier needs to be overcome, which results in a voltage loss or activation over potential.[4,28] In this case it can be explained that the loss of activation which occurs during the transfer of electron decreases significantly due to the formation of a dense conductive network of MWCNT-magnetite-*E. coli* between the microbes and the electrode. With rich *E. coli* population on the anode surface the energy barrier becomes very less and the electrons migrate towards the anode easily without any external mediator. This dense conductive network formed by modified anode also has better electrical contact between *E. coli* and anode surface resulting in improved electron migration speed.

To confirm the effect of modified anode, electrochemical characterization of fuel cells with different anodes such as conventional plain carbon electrode (Only Carbon) and a magnetite electrode (only magnetite) were performed and the polarization curves and power density curves are included in Fig. 4. The maximum obtained power densities of only carbon and magnetite electrodes were 0.52 and 270 mW m^{-2} respectively. Only carbon anode produced very low power density. In order to achieve a better performance of MFCs, the anode material should have a larger surface area with relatively fewer activation losses.[4] So the with very low surface area and high activation loss without any mediator carbon electrode in MFC renders low power density. Magnetite anode produced a maximum power density of 220 mW m^{-2} at 320 mV cell potential and current density of 710 mA m^{-2}. Magnetite employs a strong magnetic field and creates the *E. coli* population on the anode surface. Magnetite electrode has

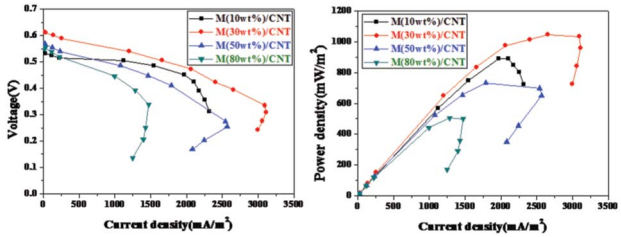

Fig. 5 Polarization curves of ML MFCs using conductive biocatalytic electrode with different content of magnetite (a) polarization curve; (b) power output (resistance : 100 KΩ–100 Ω).

increased power up to ~220 mW m^{-2} compared to power levels of carbon electrode increasing the ratio from 0.5% to 200%. This is far greater than the only carbon electrode but, the conductive biocatalytic electrode (magnetite/MWCNT) produced a maximum power density of 1050 mW m^{-2}. MWCNT with high surface area and high conductivity coupled with magnetic properties of magnetite has greatly enhanced power production with improved electron transfer. These results were verified by the impedance spectra explained later.

To optimize the magnetite content in magnetite/MWCNT nanocomposite, the performance of ML MFC with magnetite/MWCNT anodes of different magnetite contents (10, 30, 50 and 80 wt%) were evaluated. The polarization and power density curves are shown in Fig. 5 (a) and (b) and the open circuit voltage, maximum current density and power density of magnetite/MWCNT electrode with different magnetite contents are derived in Table 1 for a clear understanding. The maximum power densities obtained with 10, 30, 50 and 80 wt% magnetite in magnetite/MWCNT nanocomposite electrodes are 890, 1050, 730, and 500 mW m^{-2} respectively. The maximum power density was generated from the MFC using a conductive biocatalytic electrode with 30 wt% of magnetite in magnetite/MWCNT nanocomposite. It is interesting to see that the power density increases from 10% to 30%, and decreases again from 30% to 50% and further decreases to 80%. This results suggest that 30 wt% of magnetite is the optimum ratio for CNT-magnetite-*E. coli* conductive biocatalytic electrode. As the magnetic field of the magnetite in magnetite/MWCNT increased up to 30% the anode provides feasibility for the higher amount of bacterial adhesion resulting in continuous and fast electron transportation. Auffan *et al.* (2008) investigated the relation between redox state of Iron based nanoparticles including magnetite and *E. Coli*, and the toxicity and key factors responsible for the oxidative stress induced by the nanoparticles.[29] The survival tests for *E. Coli* in the presence of magnetite nanoparticles suggest that, the microbe can withstand up to a certain amount of magnetite content and certainly shows a decrease in bacterial survival after that. This was due to the toxic effect of nanoparticles itself which was confirmed by various experiments. They have shown that exposure to increasing concentrations of iron based nanoparticles like magnetite results in a dose dependent decrease in the survival of *E. Coli*. The main source of toxicity is found to be an oxidative stress induced by oxygenation of reduced magnetite either within nanocomposite lattice or on the release of magnetite to the electrolyte solution. Auffan *et al.* also show that 30–40% of the iron atoms present are localized at the surface of the nanocomposite and in our case magnetite/MWCNT; it is likely that the entire surface and subsurface region of the magnetite is fully oxidized.[29] So when magnetite content increases from 10% to 30%, power density increases significantly due to enrichment of *E. coli* population due to the presence of magnetite (magnetic field) and when it further increases to 50% and 80% the efficiency of the MFC is damped due to bacteria's struggle to survive and complete oxidation. This explains why 30 wt% of magnetite is the optimum ratio for MWCNT-magnetite-*E. coli* conductive biocatalytic electrode. The maximum power density (1050 mW m^{-2}) produced from the MFC using a conductive biocatalytic electrode without any mediator is 30% higher than the maximum power density 700 mW m^{-2} generated from *E. coli*-catalyzed (HNQ) mediated microbial fuel cell from our previous report.[30] Generally *E. coli* and *yeast* are known to produce electricity in the presence of mediators and a variety of chemicals have been used to facilitate the shuttling of electrons from inside the cell to electrodes outside the cell by their redox properties. From the above results, possibility of creating mediator-less microbial fuel cell with *E. coli* by improving the contact between *E. coli* and carbon electrode using magnetite leads to new direction towards applications.

Table 1 OCV, maximum current density and power density of Magnetite/CNT electrode with different magnetite content

Magnetite (wt%)	10	30	50	80
OCV (V)	0.53	0.61	0.57	0.56
Maximum current density (mA m^{-2})	2300	3100	2500	1500
Maximum Power density (mW m^{-2})	890	1050	730	500

Electrochemical characterization of modified anode

In order to identify the complex resistance generated in anode, electrochemical impedance was measured by applying only carbon, magnetite and magnetite/MWCNT electrodes to the unit cells. Nyquist plots of impedance spectra for the anode of ML MFC with and without conductive biocatalytic magnetite/MWCNT electrodes are shown in Fig. 6 (b) in frequency range

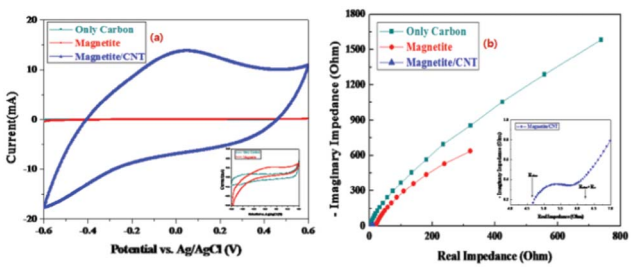

Fig. 6 (a) Cyclic voltammograms for the anode of ML MFCs in N_2-saturated 0.1 M PBS (scan rate 25 mVs^{-1}) with only carbon, magnetite and magnetite/CNT modified anodes. (b) Nyquist plots for the anode of ML MFC with only carbon and magnetite anodes; inset shows magnetite/CNT modified anode in high frequency (Frequency range of 100 kHz–0.1 Hz).

100 kHz–1 mHz. Impedance spectrum of magnetite/MWCNT at high frequency is also shown. In Nyquist plot, in the high frequency region, the x-intercept gives the electrolyte resistance (R_s) and the diameter of semicircle represents the charge transfer resistance (R_{ct}). The smaller the R_{ct} the faster is the charge transfer rate. In each anode, the observed electrolyte resistance (R_s) showed no significant difference. However, the diameter of the semicircle or the charge transfer resistance (R_{ct}) observed in each anode was very low; 30 wt% magnetite/MWCNT presents a small semicircle of 1.6 Ω which explains only a small amount of resistance exists during electron transfer. The other two electrodes like only carbon and magnetite have no identified charge transfer resistance but Warburg impedance (Fig. 6 (b)). This confirms that the electron transfer between *E. coli* and electrode surfaces that form a network with magnetite/MWCNT particles has been facilitated.

In order to investigate the electrocatalytic activity of anodes; only Carbon, magnetite and magnetite/MWCNT anodes were applied in a half cell and analyzed with cyclic voltammetry. Cyclic voltammograms of the anodes in ML MFC with and without conductive biocatalytic magnetite/CNT electrode in N_2 saturated 0.1 M phosphate buffer solution with scan rate 25 mVs^{-1} is shown in Fig. 6 (a). There is a distinct oxidation peak at around 50 mV against reference electrode (Ag/AgCl) in the current-potential curve of a conductive biocatalytic magnetite/CNT electrode. This means there is increased electron transfer efficiency of *E. coli* which forms a network with magnetite/CNT particles. On the other hand, no significant oxidation or reduction peak was observed with only carbon and magnetite electrodes as seen in Fig. 6 (a) inset. The featureless and rectangular shaped CV is the typical carbon double layer behavior owing to its structural variations. Magnetite/MWCNT shows larger current responses compared to *E. coli*-catalyzed mediated MFC previously reported by us.[30]

Morphological analysis of modified anode surface

FESEM was used to investigate the morphological property of synthesized materials and anode surfaces attached with *E. coli*. Fig. 7 shows the FESEM images of (a) CNT, (b) magnetite and (c) magnetite/MWCNT nanocomposite after synthesis respectively. The images show that the magnetite and MWCNT are uniformly synthesized. The composite also appear visually uniform, and clearly indicate the presence of magnetite on the surface of the MWCNTs as well as the composite matrix. FESEM images of the anode electrode surfaces after cell test are given in Fig. 8 with (a) only carbon electrode; (b) magnetite electrode and (c) magnetite/MWCNT composite electrode. The

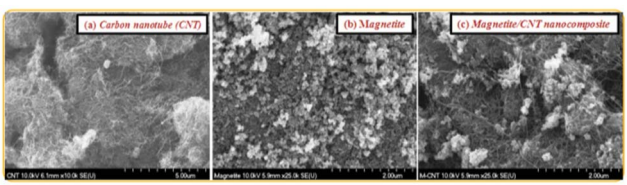

Fig. 7 SEM images of (a) CNT; (b) Magnetite and (c) Magnetite/CNT nanocomposite.

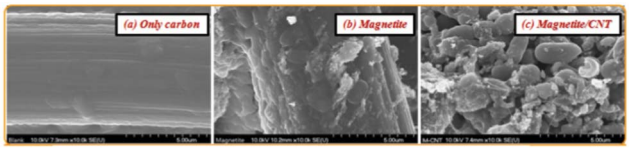

Fig. 8 SEM image of the anode electrode surfaces after cell test.

E. coli is scarcely adsorbed onto the surface of CNT on the only carbon electrode and magnetite electrode was covered with *E. coli* like a bio-film. The magnetite/CNT particles on the composite electrode and *E. coli* formed a sort of network at the surface of conductive biocatalytic electrode (magnetite/CNT). A strong adsorption of *E. coli* on to the surface of the magnetite/CNT electrode can be clearly observed from the FESEM image. A conductive biocatalytic anode was made by a formation of network from magnetite/CNT nanoparticles and *E. coli*, which then strongly adsorbed to the electrode surface due to the magnetic force of magnetite. Consequently, the interaction of magnetite particles and the formation of complex network with conductive CNT were found to be important factors for improving electrical contact of *E. coli*, and improving fuel cell performance.

In order to investigate the crystal structure of synthesized magnetite/MWCNT nanocomposite with different magnetite content, X-ray diffraction was conducted using high-resolution X-ray diffractometer (X'PERT-MRD, Philips). Fig. 9 clearly shows that, the diffraction peak at $2\theta = 26.5^\circ$ can be well indexed to the (002) reflection of graphite, while the other diffraction peaks in the range of $15^\circ < 2\theta < 90^\circ$ correspond to the (220), (311), (400), (422), (511) and (440) reflections of magnetite (Fe_3O_4), suggesting the presence of magnetite as magnetic phase in the composites [JCPDS 01-1111]. As magnetite content increases, the diffraction peak become broader and weaker at the $2\theta = 26.5^\circ$. It confirms the variation of magnetite in magnetite/MWCNT nanocomposite with different proportions *i.e.* 10, 30, 50 and 80 wt% (Fig. 9).[23]

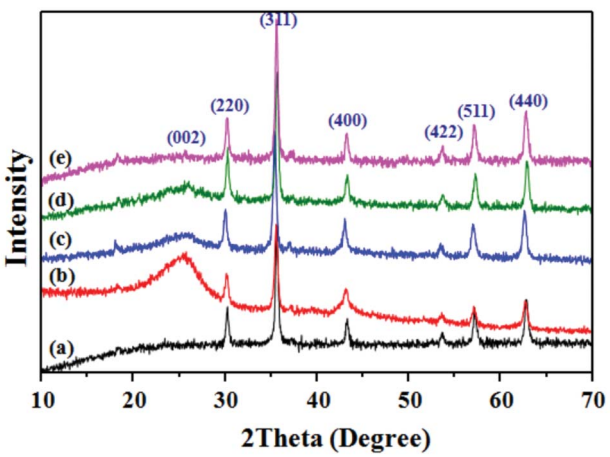

Fig. 9 XRD patterns of Magnetite/CNT nanostructure fabricated with different amount of magnetite; (a) magnetite (b) 10 wt% (c) 30 wt% (d) 50 wt% (e) 80 wt%.

Considering the batch type MFC of small scale used to investigate the modified anode in this article, the long term efficiency of the modified anode is not included to be compared with other systems. We have focused only on the performance improvement and with proportional expansion of the MFC system, enhanced performance can be assured.

Conclusion

We have developed a mediator-less microbial fuel cell with *E. coli* as catalyst and modified magnetite/MWCNT coated anode. This anode coating forms a complex micro channel network interface between the microbes and the anode. The magnetite in the anode coating attracts the microbes onto the anode surface with an attractive magnetic force and enriches the *E. coli* population on the same. This facilitates the transfer of electrons from the microbes towards the anode without any mediator. The conductive biocatalytic magnetite/MWCNT anode is highly electrochemically active and the electron transfer migration speed between *E. coli* and anode interface is improved due to this formation of complex network. The loss of activation polarization was greatly reduced by applying this conductive biocatalytic anode and high performance and high power density is obtained. Also ML MFC with magnetite/MWCNT modified anode produce maximum power with 30 wt% of magnetite producing maximum power density of 1050 mW m^{-2}. Even though currently complex, it is feasible that our understanding of the process increases new possibilities to microbial limits in power production.

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