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Research Article

Effect of electrode surface properties on enhanced electron transfer activity in microbial fuel cells

The influence of electrode surface chemistry over biofilm growth was evaluated for photo-bioelectrocatalytic fuel cell. A consortium of photosynthetic bacteria was grown onto different electrodes designed with polyethylenimine (PEI) and multi-wall carbon nanotubes as hydrophilic and hydrophobic modifier, respectively. The designed electrodes were loaded with 0.08, 0.17, and 0.33 $\mu\text{g}/\text{cm}^2$ of PEI to change the hydrophilicity. However, 0.56, 0.72, and 0.83 mg/cm^2 of multiwall carbon nanotubes were used to alter the hydrophobicity of the electrodes. The surface chemistry of electrode and bio-interaction was evaluated as a function of contact angle and biofilm formation. The results were compared with those obtained with a carbon paper electrode. The contact angle on the untreated electrode (carbon paper) was 118° , whereas for hydrophobic and hydrophilic electrodes, the maximum and minimum contact angles were 170° and 0° , respectively. Interestingly, the maximum biofilm growth (0.2275 g, wet basis) was observed on highly hydrophobic surface; however, the maximum electrochemical performance (246 mV) was shown by the most hydrophilic electrode surface. PEI-based electrode with good biofilm formation showed comparatively higher electrogenic activity.

Keywords: Biofilm / Carbon paper / Cyclic voltammetry / Multiwall carbon nanotubes / Polyethylenimine

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1 Introduction

Research on microbial fuel cell (MFC) is thriving and there is tremendous potential for improvement through electrochemical, microbiological, and systems engineering [1–4]. The anode of a MFC plays a crucial role in its overall performance. The anode significantly affects the bioelectrical properties of a MFC

as it is one of the key factors for electrons transfer from microbes to anode [5, 6]. It is reported that an increase in anode surface area for biofilm formation combined with optimized porosity and fluid flow facilitation throughout the anode material enhances MFC performance [6–8]. Affinity of the electrogenic bacteria to the anode material also has an impact on the rate of biofilm formation, and subsequently on the MFC performance [9, 10]. Recently, it is reported that positively charged and hydrophilic surfaces are easily colonized by electroactive microbes like *Geobacter* and are more suitable for electroactive biofilm formation. Thus, modification of carbon surfaces with functional groups like $-\text{N}(\text{CH}_3)_2$, $-\text{NH}_2$, and $-\text{CH}_2\text{PPh}_3$ enhance MFC performance [11]. Goncalves and Govind [12] observed increased metabolic activity, whereas Kramer et al. [8] reported the contrary results stating that biofilm formation was

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Abbreviations: CP, carbon paper; CV, cyclic voltammetry; MFC, microbial fuel cell; MWCNT, multiwall carbon nanotubes; PEI, polyethylenimine; PNB, Bibel and Pfening medium; SEM, scanning electron microscope

retarded by (polyethylenimine) PEI as it prevented electron transfer to the anode. However, PEI, a cationic polymer, has been reported to be an effective bacterial flocculating agent and has been used for artificially constructed microbial consortia using dielectrophoresis [13]. Branched PEI is a polycationic organic polymer that has a high density of primary, secondary, and tertiary amino groups and has also been used as an attachment factor for culturing both eukaryotic and prokaryotic cell lines [8]. There have been recent reports where PEI has been used for encapsulation of bio-electrodes for biosensor and bio-fuel cell applications [14, 15]. These reports showed that the introduction of PEI helped to reduce the electron transfer resistance and facilitated the diffusion of negative charge on the electrode. This work attempts to find the effect of electrode surface properties on the growth of biomass and its interaction with the surface for the effective electron transport activity in photo-bioelectrocatalytic fuel cell. Hence, PEI was selected to harness its attributes by using a novel method for electrode modification in the research area of MFC. PEI is mixed with carbon powder for electron facilitation and electrode modification to retain porosity and make the electrode hydrophilic. Three different concentrations of PEI have been used to vary the degree of hydrophilicity of the electrode. Similarly, three different concentrations of multiwall carbon nanotubes (MWCNT) were used to modify the electrodes with varying degree of hydrophobicity. Finally, the effect of electrode modification on an indigenous photosynthetic consortium was evaluated by micrographs, biomass growth, contact angle of electrode surface, open circuit potential (OCP), etc.

2 Materials and methods

2.1 Chemicals and reagents

MWCNT (size OD 10–15 nm, ID 2–6 nm, and length 0.1–10 μm) and PEI (50% w/v in water) were procured from Sigma–Aldrich (USA). Toray carbon paper (CP) and carbon powder were purchased from ElectroChem Inc. USA. All other chemicals were of analytical grade and were used as received without further purification.

2.2 Biocatalyst

Hydrogen producing photosynthetic bacterial consortium was enriched from the samples collected from brackish water lake, the Chilika lagoon, Chilika, Odisha, ($19^{\circ} 50' \text{ N}$, $85^{\circ} 30' \text{ E}$, in the eastern part of India). The microbes were assessed based on their photo-fermentative hydrogen production from short chain organic acids. The enriched photohydrogen-producing bacterial consortium was grown in sterile Bibel and Pfening medium (PNB medium). PNB medium contained KH_2PO_4 , 0.5 g; CaCl_2 , 0.05 g; MgCl_2 , 0.2 g; yeast extract, 1.0 g; NaCl , 0.4 g; sodium pyruvate, 3.0 g; and trace solution, 1 mL (1 L of trace solution contained boric acid [0.3 mg], cobalt chloride [0.2 mg], zinc sulfate [0.1 mg], sodium molybdate [0.03 mg], manganese chloride [0.03 mg], cupric chloride [0.01 mg], EDTA [0.005 mg], and ferric citrate [1%], 0.5 mL; per liter of distilled water). The

initial pH of the medium was set at 7.0. The medium in each bottle was initially flushed with argon till it became completely anaerobic (Resazurin was used as an indicator in a control reactor). The bottles were then sealed with rubber septum stoppers and aluminum caps. The inoculated serum bottles were then incubated at 30°C in an incubator under illumination intensity of 5000 lux and grown for 5 days. All chemicals used were of analytical grade.

2.3 Bioanode fabrication

CP was used as the support material for the bioanode fabrication. Surface area of the CP (both sides) was 18 cm^2 . The hydrophobic and hydrophilic electrodes were prepared by using varying amounts of MWCNT and PEI solution. Further, to prepare the bioanode, the fabricated electrodes were placed in PNB medium inoculated with photosynthetic hydrogen-producing bacterial culture. The temperature was kept at 30°C with luminous intensity of 5000 lux for the growth of photo hydrogen-producing bacterial consortium.

2.3.1 Hydrophobic electrode using MWCNT

MWCNT were dispersed in 8 mL of isopropyl alcohol (IPA) with a fixed minimal amount (0.6 μg PEI) and sonicated for 90 min to obtain stable homogeneous suspension. The homogeneous suspension was layered on both sides of the CP and allowed to dry to obtain a uniform coating having MWCNT loading of 0.56, 0.72, and 0.83 mg/cm^2 . The electrodes, henceforth referred to as CP/MWCNT, were then stored at 45°C to keep them moisture-free until use.

2.3.2 Hydrophilic electrode using PEI

The aqueous PEI solution was mixed with a fixed minimal amount of carbon black powder (12 mg) and dispersed in 6 mL of isopropyl alcohol. The carbon powder was used to keep the layer electrically conductive. The mixture was then sonicated for 90 min to obtain stable homogeneous suspension. Similar to the fabrication of hydrophobic electrode, the hydrophilic electrode was fabricated by putting the coating of the obtained suspension on the CP, which was then dried to get PEI loading of 0.08, 0.17, and 0.33 $\mu\text{g}/\text{cm}^2$. The electrodes henceforth referred to as CP/PEI.

2.4 Measurement of biomass loading

The electrodes (untreated or treated CP) were immersed in PNB medium inoculated with photo hydrogen-producing bacteria consortium for 2 wk for biomass growth on the electrode. Santoro et al. [17] biomass loading method has been used for the analysis. The change in electrode weight after the microbial growth was used as an indirect method to represent the amount of bacteria attached to the electrode surface over time. The “wet” mass and “dry” mass of the bacterial growth on the electrode were measured using a high precision balance. The electrode was taken out from the photo hydrogen-producing bacterial

Figure 1. Schematic of the bio-electrochemical system (half-cell).

culture, and the biomass was dried for 24 h in at $28 \pm 2^\circ\text{C}$ and measured to get the “dry” mass of the bacterial growth over the electrode, whereas the ‘wet’ mass was the weight measured after 20 min of drying. The other experimental errors were within $\pm 5\%$.

2.5 Contact angle

Contact angles of the hydrophobic and hydrophilic electrodes were measured using the sessile drop technique, where a droplet was gently placed on the electrode surface without any vibrations or other disturbances and without a biofilm over it. The images were captured using a sophisticated camera [16].

2.6 Scanning electron microscope analysis

The images of the electrode were obtained using a scanning electron microscope (SEM) (1430 vp; Leo, Germany). A small portion of the electrode was cut and mounted using a carbon tape on an aluminum stub followed by sputtering with double-layer gold coating to increase the conductivity. However, the bioelectrode was first dried at $28 \pm 2^\circ\text{C}$ temperature for 3 h before gold coating.

2.7 Half-cell setup

A three-electrode electrochemical cell as shown in Fig. 1 was used for the electrochemical studies. Ag/AgCl (saturated KCl) was used as the reference electrode, a platinum rod as the counter electrode, and the fabricated electrode was used as the working electrode. OCP measurement and cyclic voltammetry (CV) studies were performed using a potentiostat (CH Instruments, USA) [18]. The electrochemical cell was operated in batch mode with addition of fresh media, and the cell was autoclaved at 121°C , 15 bar pressure for 20 min prior to use in each experiment.

Figure 2. SEM image of (A) CP, (B) CP/PEI (0.17 mg/cm^2), and (C) CP/MWCNT (0.56 mg/cm^2).

3 Results and discussion

3.1 Morphology of electrode

Figure 2 shows the SEM image of the fabricated electrodes without biomass growth. Fig. 2A displays the SEM image of bare CP, which shows carbon fibers with complex porous net kind of

Figure 3. Contact angles measurement of (A) CP, (B) CP/PEI ($0.17 \mu\text{g}/\text{cm}^2$), and (C) CP/MWCNT ($0.56 \text{ mg}/\text{cm}^2$).

structure. Figs. 2B and 2C show the SEM images of representative CP/PEI ($0.17 \text{ mg}/\text{cm}^2$) and CP/MWCNT ($0.56 \text{ mg}/\text{cm}^2$) modified electrodes. In Fig. 2B, the PEI polymer can clearly be seen on the carbon fibers and it partially covers the pores between the carbon fibers of the CP. It can be seen in Fig. 3B that the PEI

percolated to the inner layers of the CP and formed a polymer layer up to some depth of the CP. Fig. 3C shows the SEM image of CP/MWCNT showing a highly porous and uniform coating over the CP.

3.2 Contact angle

The contact angle is a measurement of the wetting properties of a solid surface by a liquid. A small contact angle indicates that the liquid will spread over or wet the surface, while a high contact angle indicates poor wetting property of the surface by the liquid [19]. Thus, the wetting ability of liquid solutions on the surface of electrodes could be qualitatively characterized by the contact angles to a certain extent. The representative contact angle measurements of water droplet on electrode surfaces are shown in Fig. 3.

The contact angles on all the studied electrodes are shown in Table 1. The bare CP exhibits a contact angle of 118° . The contact angle increases on addition of MWCNT to the electrode, indicating that the wetting ability of the anode backing layer decreases or hydrophobicity increases. The contact angles of the CP modified with PEI (CP/PEI) are smaller than those of CP. As the loading of PEI in the CP increases, the contact angle decreases and reaches to 0° for $0.33 \mu\text{g}/\text{cm}^2$ of PEI loading. It shows that the wetting ability or hydrophilicity of the electrodes improves by addition of the hydrophilic PEI.

3.3 Biofilm growth

Analysis of Fig. 4, which shows the SEM micrographs of CP, CP/PEI ($0.17 \mu\text{g}/\text{cm}^2$), and CP/MWCNT ($0.56 \text{ mg}/\text{cm}^2$), confirmed the presence of biofilms on all the three electrodes (Fig. 4A, C, E). Figure 4B, D, F shows the morphology of bioelectrodes at selected areas. In case of CP bioelectrode (Fig. 4A, B), the outline of carbon fibers is clearly visible. The biofilm growth appears to be on the surface enveloping almost most of the pores. Broken biofilms or cracks are visible at a few places. It can be seen that the biofilm was more cohesively bonded with the hydrophobic surface having a contact angle of 170° . In Fig. 4E, F, the cohesiveness of the biofilm can be observed on the electrode but the carbon fiber strands were not well enveloped. However, the biofilm grown on to the CP/PEI ($0.17 \mu\text{g}/\text{cm}^2$) is attached adhesively on to the carbon fiber strands (Fig. 4C, D), which is having a low contact angle and high hydrophilicity.

Table 1. Properties of various electrodes

Type of electrode	Content	Contact angle ($^\circ$)	Loading ("wet" mass, g)	Loading ("dry" mass, g)	OCP with reference to Ag/AgCl (mV)
CP	–	118	0.2464	0.0230	155
CP/PEI	$0.08 \mu\text{g}/\text{cm}^2$	20	0.1447	0.0142	224
	$0.17 \mu\text{g}/\text{cm}^2$	15	0.1648	0.0165	237
	$0.33 \mu\text{g}/\text{cm}^2$	0	0.2275	0.0224	246
CP/MWCNT	$0.56 \text{ mg}/\text{cm}^2$	165	0.2498	0.0240	219
	$0.72 \text{ mg}/\text{cm}^2$	167	0.2545	0.0248	208
	$0.83 \text{ mg}/\text{cm}^2$	170	0.2645	0.0257	195

Figure 4. SEM images of biofilm formation on (A, B) CP, (C, D) CP/PEI ($0.17 \mu\text{g}/\text{cm}^2$), and (E, F) CP/MWCNT ($0.56 \text{ mg}/\text{cm}^2$).

Table 1 shows that the biofilm growth ('wet' and 'dry' mass) increases with an increase in the contact angle for hydrophobic electrode (CP/MWCNT). However, it is interesting to note that in case of hydrophilic electrode (CP/PEI), the biomass growth ('wet' and 'dry' mass) increases with a decrease in the contact angle. These results can be correlated with the SEM results in which the growth is in the form of a separate layer for hydrophobic surface, whereas for hydrophilic surface, the growth is on the CP

strands too. Moreover, with the help of OCP, it can be seen that the maximum potential is generated by the most hydrophilic electrode (CP/PEI— $0.33 \mu\text{g}/\text{cm}^2$) layer. It is to be noted that the biofilm growth is mostly adhesive in nature for CP/PEI electrodes and has less biomass compared to other electrodes. However, the CP/PEI treated electrodes show high OCP as compared to bare CP or CP/MWCNT electrodes. Thus, it can be inferred that the biofilm attached adhesively to the surface is more important as

Figure 5. CV of bioelectrodes (A) CP/PEI, and (B) CP/MWCNT; scan rate: 50 mV/s.

compared to the cohesive biofilm formed on the electrode with less adhesion.

3.4 Cyclic voltammetry

CV experiments were performed between -1 V and 1 V with a sensitivity of 10 mA/V and scan rate of 50 mV/s. Figure 5A–B shows the cyclic voltammogram of CP/PEI and CP/MWCNT compared with the CP electrodes. The CV indicated that the electrochemical properties of CP were quite different from those of CP/PEI and CP/MWCNT electrodes. Peak current increase was observed for both CP/PEI and CP/MWCNT electrodes compared to that of CP electrode, indicating increased electron transfer rate on these surfaces [20]. Nanostructures impregnated anodes employed to increase the surface area of the CP electrode using MWCNT and carbon powder as surface impregnations de-

picted higher power output than plain electrode, which might be due to the effective colonization of microbial consortia [21].

The increase in peak height is more (Fig. 5A) for CP/PEI electrode attributed to the polycationic property of PEI, which facilitates the diffusion of negative charge on the electrode. Figure 5B shows the CV of the CP/MWCNT electrodes with increasing concentration of MWCNT. It is observed that the peak height increases with an increase in MWCNT concentration up to 0.72 mg/cm², beyond which the peak height decreases and attributed to reduced current flow. However, for CP/PEI electrodes, the peak height increased with increased PEI content (Fig. 5A), though these findings are contrary to the results reported by Kramer et al. [8]. The probable reason may be the inclusion of carbon power for layering the PEI, which helped to maintain the conductivity of the electrodes and facilitated both biofilm growth and electron flow from the microbes to the electrode surface. Moreover, the highest electrocatalytic activity is shown by CP/PEI (0.33 μ g/cm²) and CP/MWCNT (0.72 mg/cm²) in Fig. 5A and 5B, respectively. These results are in agreement with the OCP results shown in Table 1.

4 Concluding remarks

The effect of hydrophilic and hydrophobic electrode surface was studied on the biofilm growth and bioelectrogenesis. The growth of biomass on CP/PEI electrodes was lower as compared to any of the hydrophobic CP/MWCNT electrodes. The maximum biomass growth was obtained for CP/MWCNT (0.83 mg/cm²), whereas the electrochemical activity of the CP/MWCNT (0.83 mg/cm²) hydrophobic electrode was lowest as compared to any of the CP/PEI hydrophilic electrodes. The maximum OCV of 246 mV was obtained by the electrode with most hydrophilic nature (CP/PEI: 0.33 μ g/cm²). The higher performance of the CP/PEI: 0.33 μ g/cm² was confirmed with the CV analysis in spite of higher growth on hydrophobic electrode.

Practical application

The study documented the influence of electrode surface chemistry on the functioning of a photo-bioelectrocatalytic fuel cell. This communication provides a practical method to design electrodes with hydrophobic and hydrophilic nature. Systematic evaluation of the data illustrated the specific function of hydrophobic nature of electrode surface on the biofilm formation as well as electrogenic activity.

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5 References

- [1] Logan, B. E., Hamelers, B., Rozendal, R., Schroder, U. et al., Microbial fuel cells: Methodology and technology. *Environ. Sci. Technol.* 2006, 40, 5181–5192.
- [2] Navaneeth, B., Hari Prasad, R., Chiranjeevi, P., Chandra, R. et al., Implication of composite electrode on the functioning of photo-bioelectrocatalytic fuel cell operated with heterotrophic-anoxygenic condition. *Bioresour. Technol.* 2015, 185, 331–340.
- [3] Zabihallahpoor, A., Rahimnejad M., Talebnia, F., Sediment microbial fuel cells as a new source of renewable and sustainable energy: Present status and future prospects. *RSC Adv.* 2015, 5, 94171–94183.
- [4] Wan, L. L., Li, X. J., Zang, G. L., Wang, X. et al., A solar assisted microbial electrolysis cell for hydrogen production driven by a microbial fuel cell. *RSC Adv.* 2015, 5, 82276–82281.
- [5] Logan, B. E., Exoelectrogenic bacteria that power microbial fuel cells. *Nat. Rev. Microbiol.* 2009, 7, 375–381.
- [6] Mohan, S. V., Raghuvulu, S. V., Sarma, P. N., Influence of anodic biofilm growth on bioelectricity production in single chambered mediatorless microbial fuel cell using mixed anaerobic consortia. *Biosens. Bioelectron.* 2008, 24, 41–47.
- [7] Choi S., Chae J., Optimal biofilm formation and power generation in a micro-sized microbial fuel cell (MFC). *Sens. Actuators A* 2013, 195, 206–212.
- [8] Kramer, S., Mahoney, S. S., Garner, J. H., Microbial fuel cell biofilm characterization with thermogravimetric analysis on bare and polyethyleneimine surface modified carbon foam anodes. *J. Power Sources* 2012, 210, 122–128.
- [9] Guo, K., Freguia, S., Dennis, P. G., Chen, X. et al., Effects of surface charge and hydrophobicity on anodic biofilm formation, community composition, and current generation in bioelectrochemical systems. *Environ. Sci. Technol.* 2013, 47, 7563–7570.
- [10] Srikanth, S., Pavani, T., Sarma P. N., Mohan, S. V., Synergistic interaction of biocatalyst with bio-anode as a function of electrode materials. *Int. J. Hydrogen Energy* 2011, 36, 2271–2280.
- [11] Picot, M., Lapinsonniere, L., Rothballer, M., Barriere, E., Graphite anode surface modification with controlled reduction of specific aryl diazonium salts for improved microbial fuel cells power output. *Biosens. Bioelectron.* 2011, 28, 181–188.
- [12] Goncalves, J. J., Govind, R., Rapid evaluation of biofilm attachment promoters and biofilm growth orientation using a mini-impedimetric device. *Sens. Actuators B* 2009, 143, 341–348.
- [13] Andrews, J. S., Mason, V. P., Thompson, I. P., Stephens, G. M. et al., Construction of artificially structured microbial consortia (ASMC) using dielectrophoresis: Examining bacterial interactions via metabolic intermediates within environmental biofilms. *J. Microbiol. Methods* 2006, 64, 96–106.
- [14] Das, M., Goswami, P., Direct electrochemistry of alcohol oxidase using multiwalled carbon nanotube as electroactive matrix for biosensor application. *Bioelectrochemistry* 2013, 89, 19–25.
- [15] Das, M., Barbora, L., Das, P., Goswami, P., Biofuel cell for generating power from methanol substrate using alcohol oxidase bioanode and air-breathed laccase biocathode. *Biosens. Bioelectron.* 2014, 59, 184–191.
- [16] Tadmor, R., Yadav, P. S., As-placed contact angles for sessile drops. *J. Colloid Interface Sci.* 2008, 317, 241–246.
- [17] Santoro, C., Guilizzone, M., Baen, J. P. C., Pasaogullari, U. et al., The effects of carbon electrode surface properties on bacteria attachment and start up time of microbial fuel cells. *Carbon* 2014, 67, 128–139.
- [18] Ghosh, A., Basu, S., Verma, A., Graphene and functionalized graphene supported platinum catalyst for PEMFC. *Fuel Cells* 2013, 13, 355–363.
- [19] Zhang, J., Yin, G. P., Lai, Q. Z., Wang, Z. B. et al., The influence of anode gas diffusion layer on the performance of low-temperature DMFC. *J. Power Sources* 2007, 168, 453–458.
- [20] Guo, K., Freguia, S., Dennis, P. G., Chen, X. et al., Effects of surface charge and hydrophobicity on anodic biofilm formation, community composition, and current generation in bioelectrochemical systems. *Environ. Sci. Technol.* 2013, 47, 7563–7570.
- [21] Mohanakrishna, G., Mohan, S. K., Mohan, S. V., Carbon based nano tubes and nano powder as impregnated electrode structures for enhanced power generation: Evaluation with real field wastewater. *Appl. Energy* 2012, 95, 31–37.