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# Studies on Microbial Fuel Cells with Modified MWNTs Anode Materials

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The marine exoelectrons *S. marisflavi* EP1 were intertidal sediments that taken from a marine surface of Xiamen Baicheng Sea. This study examined the electricity production performance of three different anode materials in microbial fuel cells *i.e.*, graphite, multi-walled carbon nanotubes and the esterified multi-walled carbon nanotubes. The results showed that the produced maximum voltage of the above three anode materials were 0.5282 V, 0.4329 V and 0.3123 V, respectively, along with the maximum power densities were 341.6, 241.1 and 151.4 mW/cm$^2$. The maximum power density to use the esterified multi-walled carbon nanotubes as the microbial fuel cell anode was 1.25 times as that of graphite. The growth of bacteria on the surface of the esterified multi-walled carbon nanotubes is faster than that on the surface of the graphite anode. It indicates that esterified multi-walled carbon nanotubes modified anode can significantly increased the electricity production of extracellular electronic metabolic capacity, so as to improve producing electrical properties of the microbial fuel cell.

**Keywords: Marine exoelectrogen, Microbial fuel cells, Esterification, Multi-walled carbon nanotubes, Anode modification.**

## INTRODUCTION

At present, energy shortage and waste treatment are two challenges that people are facing. Microbial fuel cell can not only solve the problem of energy shortage, but also for waste treatment at the same time. Microbial fuel cell is a kind of special fuel cell. It has microorganisms as catalyst in the anode chamber, organic compound such as waste biomass and organic waste as fuel. It generate electricity by biological oxidation process[1]. If microbial fuel cell is used in organic waste treatment, it can not only dispose the waste but also recover part of energy of organic matter. In addition, it can reduce the cost and achieve the double effect of pollution control and resource utilization. According to the statistics[2], in the United States, there are more than 1.26 tons of waste water need to be processed per day. The funds for wastewater treatment are more than 25 billion dollars every year and most of the funds are used for the treatment of wastewater aeration process. This high energy consumption of wastewater treatment technology is not applicable to every country in the world, especially in developing countries.

As a new technology for wastewater treatment, microbial fuel cell can produce energy; moreover, can reduce the energy consumption by agitation and aeration. So it has good prospects for development. In order to make this kind of wastewater treatment technology more economic, the electrogenesis capacity of microbial fuel cell needs to be further improved.

From the microbial fuel cell composition[3,4], the anode, where electrogenic bacteria attached, as carrier, it not only affects the amount of electricigens attachment, but also influences the rate of electron transfer. So the anode is the key factors to improve the electrical properties of microbial fuel cell[5].

Therefore, from the point of improving the production capacity of microbial fuel cell, the best choice of anode materials is very important for improving the power generation performance of microbial fuel cell. There are a lot of anode materials have been widely used, such as graphite[6], carbon paper[7], carbon cloth[8], carbon particle[9,10] and reticulated vitreous carbon[11,12], because they are low-cost and have large surface area, good biocompatibility, except the catalytic activity of the anode of microbial reactions[13,14]. Accordingly, the development of modification of carbon materials and novel anode materials[15] is one of the key to improve the electric capacity of microbial fuel cell. Researches[16,17] shows that use of carbon material to increase the production of electrical properties of microbial fuel cell modification has been proved to be successful. There is great progress in the use of novel anode materials. Carbon nanotubes as a kind of new carbon material is gradually being applied to the microbial fuel cell, because of its specific pore structure, good biocompatibility, large specific surface area, high thermal stability and high conductivity[18]. Qiao *et al*.[19] loaded the carbon nanotubes on polyaniline, using *E. coli* as the electricigens. The maximum power it density reached 42 mW/m$^2$. Liang[20] pressed carbon nanotubes into sheet electrode as the anode of microbial fuel cell. Compare with the anode which made of carbon powder, the electricity production capacity increased by 20 % or so. Sharma *et al*.[21], made an anode through diffusing adsorption of metallic carbon nanotubes into the carbon paper. Compare with the flat of the graphite electrode, their power increased by 6.4 times.

The technology of electrogenic bacteria is one of the key technologies in microbial fuel cell area. It is a kind of new microbial resources discovered in recent years. This kind of microorganisms have a unique extracellular electron transfer pathway[1]. It can transfer electronic to the final electron acceptor located outside of the cells and coupling the microbial metabolism and the growth. So far, there are some electricigens have been obtained. Most of them are proteobacteria, belonging to β, γ, δ subclasses of the modified bacteria. Many electrogenic bacteria are belonging to subclass of Geobacter and Shewanella and most of these electrogenic bacteria are obtained from land-based sources. For 71 % of the earth's surface is covered by oceans, there are many features of the marine environment, including high-salt, high-pressure, low temperature and poor nutrition. Because of the special living environment, marine microorganism has the diversity and specificity on the species, the genome, *etc*. Therefore, marine electrogenic bacteria will become a hot spot in the research of microbial fuel cell.

In this study, we choose marine electrogenic bacteria *Shewanella marisflavi* EP1, which is taken from Baicheng sea waters situated at Xiamen, as the anode microorganisms and multi-walled carbon nanotubes (MWNTs) as the fundamental materials. Esterification modification is prepared by carboxylation and chloridization reaction. The modified materials were characterized by Infrared spectroscopy and raman spectroscopy and dispersion test. Compared the electricity production of graphite anode, modified MWNTs graphite anode and esterification of MWNTs modified are applied to microbial fuel cell.

## EXPERIMENTAL

The sediment, which was collected from coastal sediments in Xiamen, was inoculated into microbial fuel cell anode chamber as catalyst. *Shewanella marisflavi* EP1 was isolated from one microbial fuel cell fed with lactate operated for 2 months. Multi-walled carbon nanotubes (MWNTs) were purchased from Shenzhen Nanotech Port Co. Ltd. Concentrated sulfuric acid, concentrated nitric acid, toluene, *tert*-butyl alcohol, ethyl acetate, thionyl chloride, *etc*. were analytical grade and were purchased from Sinopharm Chemical Reagent Co. Ltd.

### Preparation of esterification multi-walled carbon nanotube

**Carboxylation of multi-walled carbon nanotube:** Added MWNTs and concentrated sulfuric acid into spherical flask (200 mL) successively. Use high power digital ultrasonic cleaner dispersed them for 0.5 h and then adding an appropriate amount of concentrated nitric acid and mix thoroughly. Heated to reflux for 5 h to make it functional; after the solution was cooled to room temperature, pour the excess acid, the lower black liquid was diluted with ice-water, washed until pH value is about 7; standing, filtering, water washing, drying at 100 °C, then carboxyl MWNTs were made.

**Synthesis of esterification of multi walled carbon nanotubes:** Put the carboxylated MWNTs and thionyl choride into spherical flask, then ultrasonic dispersed for 1 h stirring and then acyl chlorination for 2 h at 80 °C. Drying by distillation, adding an appropriate amount of toluene, ultrasonic dispersion after 0.5 h, obtained a black liquid. Potassium carbonate, *tert*-butyl alcohol and the amount of toluene reaction after 6 h with stirring, then dripped it into the black liquid to react after 4 h non-shock chilling, tilting the upper toluene, washing with distilled water, ethanol, acetone respectively. After standing, filtering, water washing, drying, the esterified MWNTs were made.

**Characterization of esterified MWNTs:** Added the MWNTs, carboxylated MWNTs, esterified MWNTs into water, chloroform, acetone, ethyl acetate and toluene solution respectively, ultrasonic dispersed for 0.5 h. Standing, then test its dispersion. Nicolet Magna-550 Fourier transforms infrared spectrometer, KBr powder, scanning range: 400-4000 cm$^{-1}$, after $CO_2$ peak. The Raman spectra of LubRam-IB Raman spectroscopy, the excitation wavelength is 635 nm.

### Application of esterified MWNTs modified anode in microbial fuel cell

**Pretreatment of the graphite electrode:** Sand the graphite electrode surface with the emery paper. Make the ultrasonic cleaning with 1:1 $HNO_3$ and absolute ethyl alcohol, redistilled water in turn. Then put the graphite electrode into 0.5 mol/L $H_2SO_4$ solution for 1 h, removed the electrode, dried under an infrared lamp, spare.

**Preparation of a MWNTs-nafion modified electrode:** The 10 mg esterified MWNTs and unmodified MWNTs material were added to 10 mL absolute ethyl alcohol, which containing 0.5 % Nafion membrane (United States DuPont Co.) solution, ultrasonic dispread after 0.5 h, formed a uniform black solution. Utilizing the coating method to put the black liquor modified graphite electrode in the pretreatment of the infrared lamp, placed in baking to let the solvent volatilizes completely. Then coat the black solution to the pretreated graphite electrode. Baked under an infrared lamp to the solvent volatilizes completely.

**Electricity production experiment of microbial fuel cell modified anode with MWNTs:** The device of production of electric experiment used the two-chambered microbial fuel cell (MFC). The volume of anode chamber and cathode chamber was 14 cm$^3$. Anode and cathode chamber separated by a pretreated proton exchange membrane (PEM, Nafion117, DuPont) apart, the effective area is 7 cm$^2$. The anode materials of microbial fuel cells are coarse graphite electrode (CE), MWNTs/CE, Esterified MWNTs/CE and the cathode material is coarse graphite electrode. The cathode and anode were connected by wires, the external resistance of 500 Ω.

Three microbial fuel cell anode chambers connected with pump in parallel were filled with substrate: 2 g $NH_4Cl$, 20 mM sodium lactate, 10 mL L$^{-1}$ trace element solution, added M6 solution to the volume of culture medium solution reached

1 L, then adjusted the pH value to 7. The composition of M6 is: 19.89 NaCl, 0.745 KCl, 0.35 $NaH_2PO_4$, 0.44 $Na_2HPO_4$ and 0.188 $MgSO_4$. The electrogenic bacteria were inoculated to the anode chamber. Took the logarithmic growth phase (OD600 = 1) aerobic culture of *S. marisflavi* EP1 bacteria liquid 6 mL, centrifugation 5 min in the 7000 rpm speed, the culture medium used to cultured the electrogenic bacteria suspended in the anode chamber, the initial OD600 is 0.27, nitrogen 3 min. Let the multiple channel peristaltic pump continuous operation (BT100-1L, Baoding lange constant flow pump Co. Ltd.). Rotate speed is 25 rpm, the anode chamber system is completely mixed model. Injected M6 to the cathode chamber, the three cathode chambers connected in series. Peristaltic pump (BT00-100 W, Baoding Lange constant flowing pump Co. Ltd.) have anaerobic anode, cathode aeration. Multi channel universal digital meter (Keithley 2000, the United States of America Keithley instruments) collected the voltage data.

## RESULTS AND DISCUSSION

**Dispersion test of modified MWNTs:** The modified MWNTs materials were tested by FT-IR in order to analyze the surface functional groups of these three materials of MWNTs, carboxylated MWNTs and esterified MWNTs. The results of the three Infrared spectra are shown in Fig. 1.

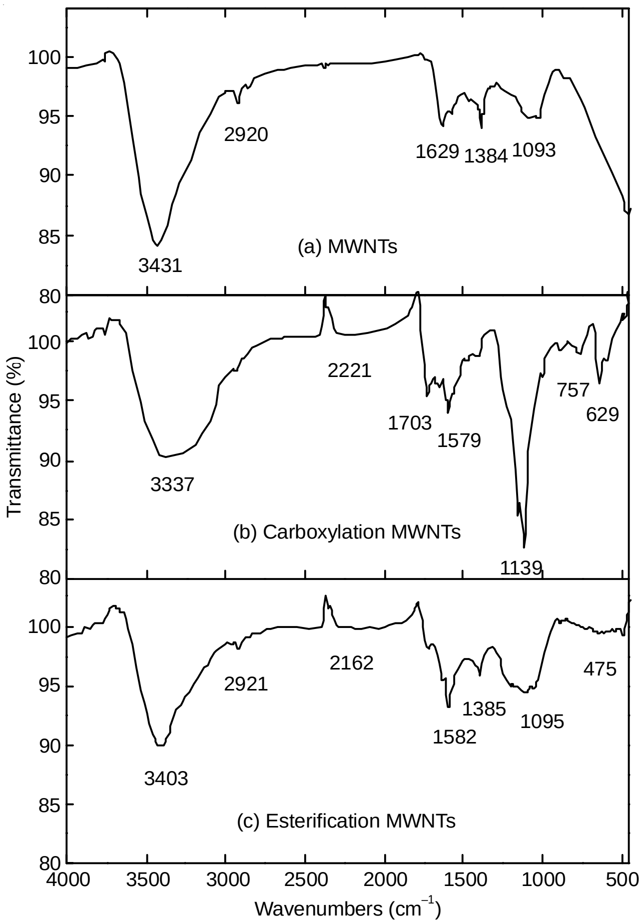

Fig. 1. FT-IR spectra of the modified MWNTs materials

Compared with the absorption peak of MWNTs (Fig. 1a), the infrared spectra of the MWNTs after acid treated (Fig. 1b) shows that, the characteristic absorption disappear when the wave number is 2920 cm$^{-1}$. There are three characteristic absorption peaks of carboxyl, when the wave numbers are 1703, 1579 and 1139 cm$^{-1}$. There is a stretching vibration peak of carbonyl, when the wave number is from 1740 to 1690 cm$^{-1}$. There is an antisymmetric stretching vibration peak of the carboxyl, when the wave number is in the vicinity of 1600 cm$^{-1}$. There is strong absorption peak caused by the in-plane bending vibration of hydroxyl, when the wave number is near 1150 cm$^{-1}$. It can be preliminarily that, carboxyl has been introduced to the surface of MWNTs. Fig. 1c shows that the alkyl is introduced, because the C-H characteristic absorption peak is reproduced when the wave number is near 2921 cm$^{-1}$. The absorption peaks at 1700 and 1582 cm$^{-1}$ caused by the stretching vibration of C=O and the antisymmetric stretching vibration of COO-, respectively.

Compared with Fig. 1b, the absorption peaks caused by the in-plane bending vibration of O-H decreased, when the wave number is near 1400 cm$^{-1}$. There are asymmetric stretching vibration peak and symmetric stretching vibration peak of C-O-C, when the wave number is respectively near 1160 and 1050 cm$^{-1}$. The absorption peak is very obvious, when the wave number is 1095 cm$^{-1}$. This shows that the ester function has been introduced to the surface of MWNTs.

**FT-IR test of modified MWNTs materials:** MWNTs, carboxylated MWNTs and esterification MWNTs were dissolved in deionized water, chloroform, acetone, ethyl acetate, toluene, respectively. The dispersion performance test results in Table-1.

TABLE-1
RESULTS OF THE MODIFIED MWNTS
MATERIALS DISPERSION PERFORMANCE

| Solvents | MWNTs | Carboxylic MWNTs | Esterified MWNTs |
|---|---|---|---|
| Deionized water | -- | +- | ++ |
| Chloroform | -- | ++ | ++ |
| Acetone | -- | +- | ++ |
| Ethyl acetate | -- | ++ | ++ |
| Toluene | -- | -- | -- |

Note: -- Almost can not be divided; +- well dispersed, but precipitation; ++ dispersed well in a week

The unmodified MWNTs cannot disperse in solvents (deionized water, chloroform, acetone, ethyl acetate and toluene) stability. Ultrasonic dispersion and then completed precipitation after standing a few minutes. The carboxyl modified MWNTs can be dispersed in water stability. There is only slightly precipitation after holding a week. Due to the introduction of organic functional groups, the modified esterification of MWNTs can disperse in different solvents stably and no significant precipitation after resting for a week. Besides, it can form a black stable suspension when dissolved in various solvents. It indicates that the dispersion capacity of modified esterification MWNTs materials is significantly improved in various solvents. But the modified MWNTs and unmodified MWNTs cannot disperse in toluene.

**Raman spectra of modified MWNTs materials:** In order to further characterize the modified success of esterification of MWNTs, the modified, unmodified, carboxylated and etherified MWNTs materials were conducted by Raman spectral characterization. Carbon atoms is in carbon nanotubes, adjacent to the other three carbon atom, mainly is in the $sp^2$ bonding, forming the six-angular mesh structure. The $sp^3$ bonded must exist in the reticular structure (Fig. 2).

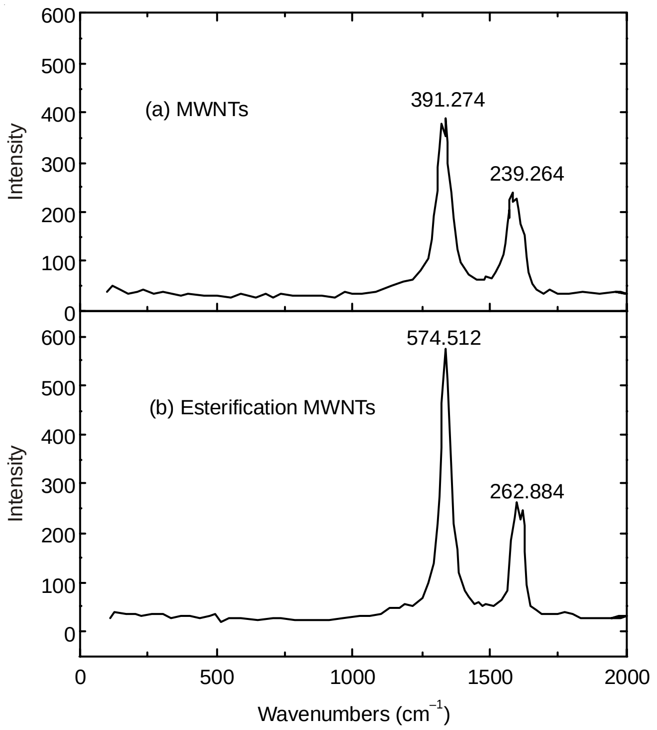

Fig. 2. Raman spectra of the modified MWNTs materials

The modified and unmodified MWNTs have two strong peaks. One is the G-band peak, appearing near 1580 cm$^{-1}$, which represents the $sp^2$ hybridized carbon. Another is D-band peak, appearing near 1330 cm$^{-1}$, representing the $sp^3$ hybridized carbon. The ratio of $sp^2$ hybridized carbon to $sp^3$ hybridized carbon, the peak intensity ratio of peak G-band and peak D-band, decreased from 0.612 to 0.458. This proves that the ester group has been successfully introduced to the MWNTs surface.

### Electrical production characteristics of microbial fuel cell before and after modification

**Start up time and repetitive firing properties of microbial fuel cell:** Start the peristaltic pump after inoculating the electrogenic bacteria into microbial fuel cell anode chamber. The microbial fuel cell runs with the three anode channel and the three cathode channel series. External resistor is 500 Ω. The output voltage changes with time as shown in Fig. 3.

From Fig. 3, after a period of stability, microbial fuel cell started to generate voltage. Different anode modified microbial fuel cell reached the maximum voltage platform at different times. The time, voltage output reached the first maximum in microbial fuel cell which MWNTs, graphite, esterification MWNTs as anode, are 127, 160 and 202 h. And the maximum voltage value reached, respectively 0.53308 V, 0.46209 V, 0.35787 V. microbial fuel cell ran for 3 cycles continuously, the duration is about 598 h. This figure also shows that the duration, the generated voltage in microbial fuel cell, is related to the amount of carbon source. Electrogenic bacteria can repeat electricity production.

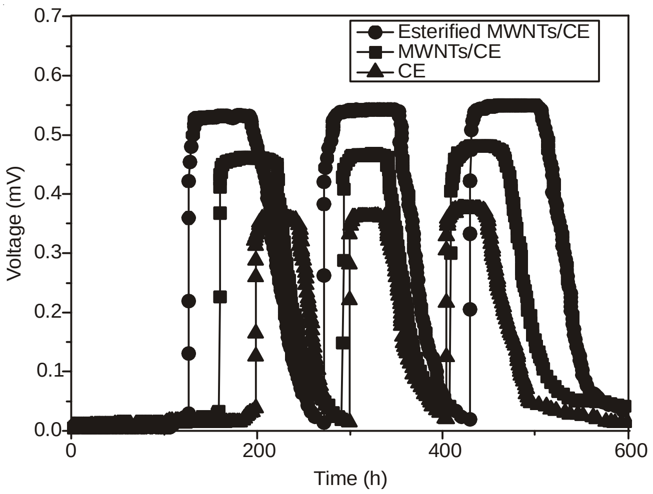

Fig. 3. Microbial fuel cells voltage curve of the modified MWNTs materials

**Performance test of the reactor in esterification MWNTs:** Start microbial fuel cell, after a period of time running and it reaches the stable, measuring the polarization curve and power density curve by steady discharge method in microbial fuel cell before and after modification (Fig. 4).

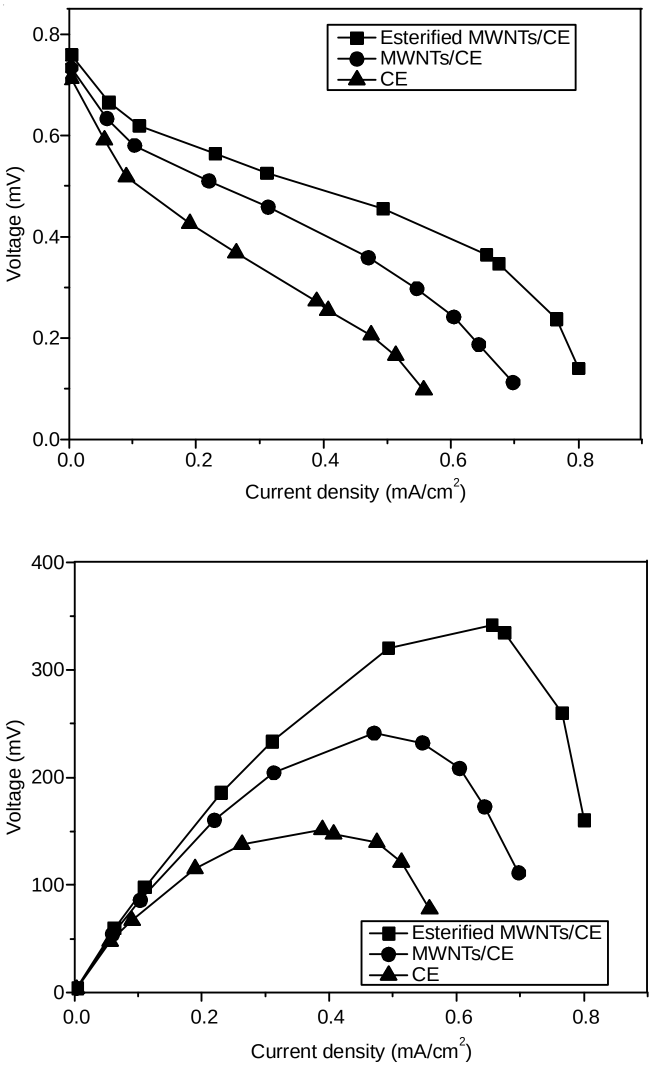

Fig. 4. Microbial fuel cells polarization curve and power density curve of the modified MWNTs materials

The polarization curve and power density curve can be obtained by changing the external resistance. In the same inoculation conditions and operating conditions, the maximum power density are 341.6, 241.1 and 151.4 mW/cm$^2$, when esterified MWNTs/CE, MWNTs/CE, CE as anode in microbial fuel cell. Compared with the ordinary graphite anode (CE) microbial fuel cell, the startup period is shorter, the power is increased 1.25 times when anode modified by esterification MWNTs in microbial fuel cell. The maximum power density increased by 59.2 %, when coarse graphite electrode as the anode in microbial fuel cell. The resistance of microbial fuel cell are 455, 568 and 819 Ω, when esterified MWNTs, MWNTs and graphite as the anode, respectively. The results show that, the resistance of microbial fuel cell can be reduced by modifying the MWNTs anode, accelerate the rate of electron transfer, thereby enhancing the electrical properties of microbial fuel cell.

**Comparing the biocompatibility of microbial fuel cells before and after modification:** As shown in Fig. 5, when esterification MWNTs is anode, the growth status of the microorganisms is good. Operating the reactor, running after a period of time, the microorganisms grow quickly, output current is also increasing rapidly. When the voltage of microbial fuel cell reached the maximum value, the growth of microorganisms can also achieve the best solution. Relative to unmodified MWNTs/CE anode and CE anode, the biocompatibility of esterification MWNTs/CE anode is the highest and the speed electrogenic bacteria attached on the anode surface is the fastest. The electron transfer rate is also highest. This shows that, in order to improve the power generation performance of microbial fuel cell, we can improve the hydrophilicity of the surface.

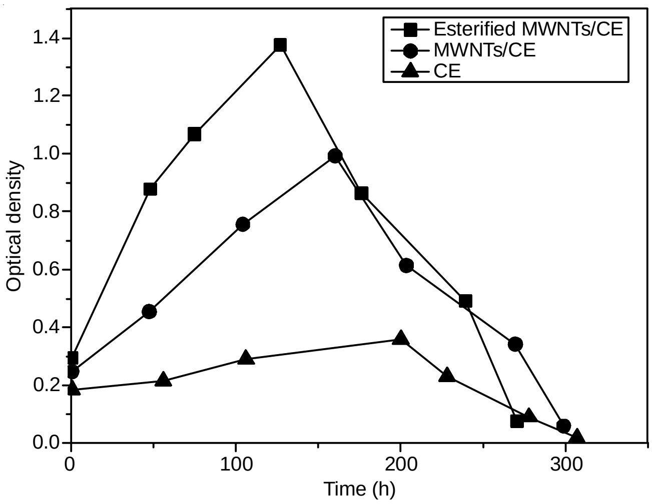

Fig. 5. Biocompatibility of microbial fuel cell anode materials

## Conclusion

The ester groups were successfully introduced onto the surface of MWNTs by both carboxylation and chlorination reaction. Esterification-modified materials were characterized by the dispersion test, infrared spectroscopy and Raman spectroscopy to confirm the covalent successful modification. When using esterified MWNTs, MWNTs and graphite as anode, the maximum power density in microbial fuel cell are 341.6, 241.1 and 151.4 mW/cm$^2$ and the resistance is 455, 568 and 819 Ω, respectively. Therefore, the esterification of MWNTs can reduce the resistance and the ohmic resistance of microbial fuel cell. The hydrophilicity of esterification MWNTs is better than MWNTs and the biocompatibility is also higher than MWNTs. The electrogenic bacteria can adhere and growth on the surface of esterification MWNTs anode. So the electricity generation performance of esterified MWNTs is better. Comparing to the esterification of MWNTs anode, the hydrophilic nature of MWNTs anode and graphite anode are not high. Because of the factors such as the specific surface area and conductivity of MWNTs are better than graphite anode, so the production performance of MWNTs is better than graphite.

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## REFERENCES

1. B.E. Logan, *Nat. Rev. Microbiol.*, **7**, 375 (2009).
2. H. Liu, R. Ramnarayanan and B.E. Logan, *Environ. Sci. Technol.*, **38**, 2281 (2004).
3. H. Liu, S. Cheng and B.E. Logan, *Environ. Sci. Technol.*, **39**, 5488 (2005).
4. B.E. Logan, B. Hamelers, R. Rozendal, U. Schröder, J. Keller, S. Freguia, P. Aelterman, W. Verstraete and K. Rabaey, *Environ. Sci. Technol.*, **40**, 5181 (2006).
5. M. Zhou, M. Chi, H. Wang and T. Jin, *Biochem. Eng. J.*, **60**, 151 (2012).
6. K. Rabaey, G. Lissens, S.D. Siciliano and W. Verstraete, *Biotechnol. Lett.*, **25**, 1531 (2003).
7. H. Liu, S. Cheng and B.E. Logan, *Environ. Sci. Technol.*, **39**, 658 (2005).
8. S.K. Chaudhuri and D.R. Lovley, *Nat. Biotechnol.*, **21**, 1229 (2003).
9. Z. He, N. Wagner, S.D. Minteer and L.T. Angenent, *Environ. Sci. Technol.*, **40**, 5212 (2006).
10. P. Aelterman, K. Rabaey, H.T. Pham, N. Boon and W. Verstraete, *Environ. Sci. Technol.*, **40**, 3388 (2006).
11. K. Rabaey, P. Clauwaert, P. Aelterman and W. Verstraete, *Environ. Sci. Technol.*, **39**, 8077 (2005).
12. Z. He, S.D. Minteer and L.T. Angenent, *Environ. Sci. Technol.*, **39**, 5262 (2005).
13. Q. Deng, X. Li, J. Zuo, A. Ling and B.E. Logan, *J. Power Sources*, **195**, 1130 (2010).
14. K.B. Gregory, D.R. Bond and D.R. Lovley, *Environ. Microbiol.*, **6**, 596 (2004).
15. M. Zhou, M. Chi, J. Luo, H. He and T. Jin, *J. Power Sources*, **196**, 4427 (2011).
16. F.A. Alatraktchi, Y. Zhang, J.S. Noori and I. Angelidaki, *Bioresour. Technol.*, **123**, 177 (2012).
17. J.J. Sun, H.-Z. Zhao, Q.-Z. Yang, J. Song and A. Xue, *Electrochim. Acta*, **55**, 3041 (2010).
18. G. Lepage, F.O. Albernaz, G. Perrier and G. Merlin, *Bioresour. Technol.*, **124**, 199 (2012).
19. Y. Qiao, C.M. Li, S.-J. Bao and Q.-L. Bao, *J. Power Sources*, **170**, 79 (2007).
20. P. Liang, H.Y. Wang, X. Xia, X. Huang, Y. Mo, X. Cao and M. Fan, *Biosens. Bioelectron.*, **26**, 3000 (2011).
21. T. Sharma, A. Mohanareddy, T. Chandra and S. Ramaprabhu, *Int. J. Hydrogen Energy*, **33**, 6749 (2008).