phys. stat. sol. (a) **205**, No. 6, 1484–1487 (2008) / **DOI** 10.1002/pssa.200778136

physica status solidi a
www.pss-a.com
applications and materials science

Biofilm growth from wastewater on MWNTs and carbon aerogels

Anca Dumitru[*,1], **Adina Morozan**[**,1], **M. Ghiurea**[2], **K. Scott**[3], **and S. Vulpe**[1]

[1] Polymer Science Group, University of Bucharest, P.O. Box MG-40, 077125, Bucharest-Magurele, Romania
[2] ICEHIM Bucharest, 202 Independence Street, District 6, P.O. Box 35-174, 060021 Bucharest, Romania
[3] School of Chemical Engineering and Advanced Materials, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU, UK

Received 30 September 2007, revised 9 April 2008, accepted 15 April 2008
Published online 3 June 2008

PACS 81.07.De, 82.20.Fk, 82.45.Cc, 82.45.Yz, 87.64.Ee

[*] Corresponding author: e-mail anka.dumitru@gmail.com, Phone: +40 21 457 49 49, Fax: +40 21 457 44 18
[**] e-mail adina@psg.unibuc.ro, Phone: +40 21 457 49 49, Fax: +40 21 457 44 18

The improving carbon nanostructure bioactivity related to a specific microorganism community is an important research goal of bionanotechnology. The functionalized CNTs are used to improve the bioaffinity of working electrode in microbial fuel cells and wastewater treatment. Beside CNTs, carbon aerogel is another carbon material with high surface area, high open porosity and controllable morpho-structural characteristics. In the present work we investigate the biofilm growth from wastewater on functionalized CNTs and carbon aerogels. The biofilm growth on CNTs and carbon aerogel was monitored by optical and scanning electron microscopy. The influence of the bacterial growth phase on carbon electrode was assessed by cyclic voltammetry measurements in wastewater and 0.5 M potassium phosphate buffer solution.

© 2008 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

1 Introduction Wastewater resulting from different industries is nowadays of great concern as it poses not only severe risks to human health, but also potentially unacceptable ecological risks to plants and animals. With the increasing demand for economic large-scale water treatment applications, the development of novel, low-cost and efficient treatment technologies is of great significance.

In recent years, microbial fuel cells (MFCs) have emerged as potential sustainable biotechnological solutions to future energy needs. MFCs convert microbial reducing power (generated by the metabolism of organic substrates) into electrical energy. It is also possible to produce electricity in a MFC from domestic wastewater as a fuel, due to environmental concerns and the need to reuse waste, while at the same time accomplishing biological wastewater treatment (removal of chemical oxygen demand, COD). The benefits of using MFCs as a practical method of wastewater treatment include: clean, safe, quiet performance, low emissions, high efficiency, and direct electricity recovery [1, 2]. The main challenge in constructing of MFC is first to identify materials and architectures that maximize power generation and Coulombic efficiency [3]. The efficient electron transfer between microorganism and anode seems to play a major role in the performance of MFCs, that can take place directly or indirectly with the use of soluble redox mediators. All MFCs researches invariably have to deal with the interface between microorganisms and electrodes.

The requirements of anode materials are: highly conductive, non-corrosive, high specific surface area and high porosity. One approach has been taken to increase performance of the MFCs anode by using different high specific surface area of carbon structures. A variety of anode materials have been used in MFCs, including plain graphite [4], carbon paper [5], carbon cloth, felt, or foam [6], reticulated vitreous carbon (RVC) [7], or graphite granules [8]. There appears to be general trend of increased power production with higher surface area materials used instead of standard materials. Using advanced materials has a good history in biofilm technology [9], a strategy especially valuable with biofilm systems, because biofilms can accumulate on the surface of the advanced material. Carbon nanotubes (CNTs) have been envisioned as promising materials for potential application in many fields because of their remarkable properties [10, 11]. Proteins and enzymes immobilized on carbon nanotubes (CNTs) have been used as biosensors and in methanol fuel cells. Given that CNTs are also suitable supports for cell growth, one could also

WILEY InterScience® DISCOVER SOMETHING GREAT

© 2008 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

use them as CNT-based electrodes in MFCs [12]. Beside CNTs, carbon aerogels (CA) are carbon materials with high surface area, high open porosity and controllable morpho-structural characteristics. The structure of CA with fully interconnected mesopores has attracted high interest, mainly in its application as catalyst support in fuel cell technology [13].

The papers aims to develop new bio-electrodes based on high surface area carbon materials for efficient electron-exchange with bacterial consortium from wastewater. In this purpose the study will be focused on monitoring biofilm growth on CNTs and CA and evaluation of bio-compatibility of carbon materials with bacterial consortium from wastewater, using a basic type of two-chamber MFC. One of the greatest challenges for using MFCs for wastewater treatment is increasing the specific surface area of the anode open structure to avoid biofouling. The biofilms' growth on carbon anodes was monitored by optical microscopy and scanning electron microscopy. The influence of the bacterial growth phase on carbon anode surface was assessed by cyclic voltammetry measurements in wastewater and 0.5 M potassium phosphate buffer solution.

2 Experimental The CNTs used in this work were purchased multi wall carbon nanotubes (MWNTs) obtained from Shenzhen Nanotech PortoCo., Ltd., China ($d < 10$ nm, length: 5–15 μm, P: ≥95%, specific surface area: 40–300 m^2/g). The desired CNTs properties are usually obtained after chemical modification [14, 15]. In order to improve the carbon nanostructure bioactivity, related to a specific microorganism community, the MWNTs were chemical activated by refluxing with HNO_3 (3M) (Merck reagent) for 24 h, followed by washing with deionised water until neutral pH and dried in air for 60 min at 80 °C. The modified CNTs have a much larger surface area than conventional electrodes and showed promising electrochemical activity toward several systems. Thus, the functionalized CNTs are used to improve the bioaffinity of working electrode in MFCs for wastewater treatment.

CA with of meso- and macroporosity were obtained by sol–gel syntehsis method presented elsewhere [16]. By polycondensation of resorcinol (R) and formaldehyde (F), wet resorcinol-formaldehyde (RF) gels were prepared. Using CO_2 supercritical drying, the RF gels were transformed into organic aerogels, then to CA by pyrolysis under nitrogen atmosphere. In this work we used mesoporous CA with high BET surface area of 860 m^2/g.

The biofilms growth on MWNTs and CA were studied using a basic type of two-chamber MFC separated by a polymeric proton exchange membrane. The anode chamber contains wastewater and it is tightly sealed to prevent oxygen diffusion into the chamber. The bacteria must be grown in an anaerobic environment in order to produce electricity. The cathode compartment was filled with 0.5 mM potassium phosphate buffer solution (pH = 7). The wastewater was replaced one time to allow a biofilm to form on the anode surface.

Optical and scanning electron microscopy was used to monitor the bacterial morphology and biofilms growth on the MWNTs and CA from wastewater. Biostar B4SPT-K71660 microscope was used for optical microscopy images. Images were taken digitally with Canon G6 Power Shoot camera connected to a PC. SEM investigations have been performed with a FEI-Quanta 400 scanning electron microscope.

The electrochemical measurements were carried out with VoltaLab 40 potentiostat (PGZ301, RADIOMETER) using a conventional three-electrode electrochemical cell at 25 °C. Pt mesh and CR5/Hg_2SO_4 – MMS were used as the counter and reference electrode, respectively. The working electrode was prepared by painting of 20% teflonised toray carbon paper surface with carbon ink. The carbon ink was prepared by sonicating the carbon material in distilled water for 60 min. After drying, a working electrode was obtained. Electrochemical measurement of biofilm growth onto MWNTs and CA were carried out in wastewater and 0.5 M potassium phosphate buffer solution.

3 Results and discussion In order to determine the presence of microbial activity in MFC reactor, a period of equilibration is performed to monitor the open circuit potential (Fig. 1).

It was observed that 2–3 days are needed to equilibrate the MFC system until the anodes were colonised by the bacterial population from the wastewater. In Fig. 2 is presented the biofilm growth on MWNTs investigated by optical microscopy. From Fig. 2 (b, d) can be observed that using MWNTs or CA substrate, under anaerobic conditions, the development of biofilm is not inhibited. The biofilms formed on the substrate are uniform and no significant differences are observed related to the nature of substrate, MWNTs or CA. To have a better view of the biofilm formation on MWNTs, in Figs. 3 and 4, are presented the results obtained from scanning electron microscopy, at different magnification.

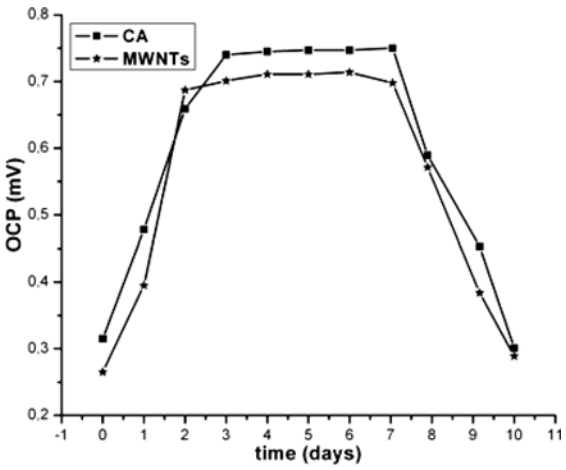

Figure 1 Equilibration period showing open circuit potential over time.

© 2008 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

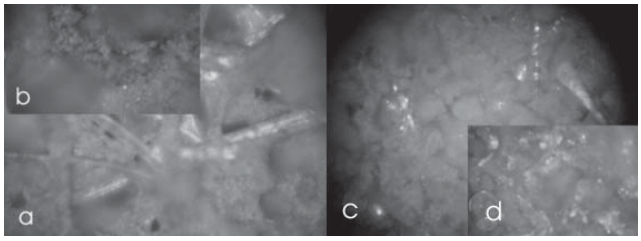

Figure 2 Optical microscopy images: a) MWNTs, b) biofilm growth on MWNTs, c) CA, d) biofilm growth on CA.

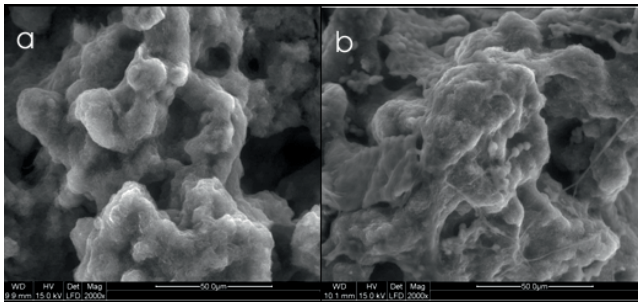

Figure 3 SEM images: a) MWNTs, b) biofilm growth on MWNTs.

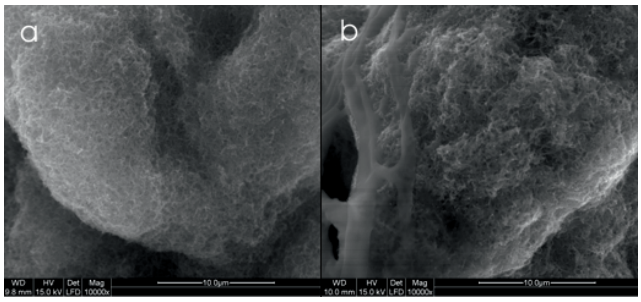

Figure 4 SEM images at higher magnification: a) MWNTs, b) biofilm growth on MWNTs.

The SEM images from Fig. 3 show the uniform biofilm growth on the substrate. In the case of the higher magnification of SEM images, Fig. 4, the biofilm is formed by an interconnected fibre like structure which covers the electrode surface.

In Figs. 5 and 6 are presented the SEM images, at different magnification, of biofilm growth on CA.

The images reveal that in the same experimental conditions, the nature of biofilm growth on CA is strongly different compared to that of biofilm growth on MWNTs. The biofilm growth on MWNTs (Fig. 3) has the appearance of uniform films which cover the substrate. In the case of CA, the biofilm is made of bacteria with different shapes. The predominant shape of bacteria has the appearance of tubules with ~10 μm in length and with a very good dispersion on the substrate.

The influence of the bacterial growth phase on the electrochemical measurements were achieved to obtain the cyclic voltammograms of the bacterial consortium growth on carbon electrode in wastewater (Fig. 7) and 0.5 M

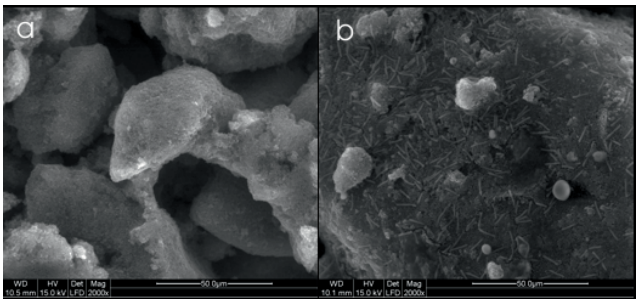

Figure 5 SEM images: a) CA, b) biofilm growth on CA.

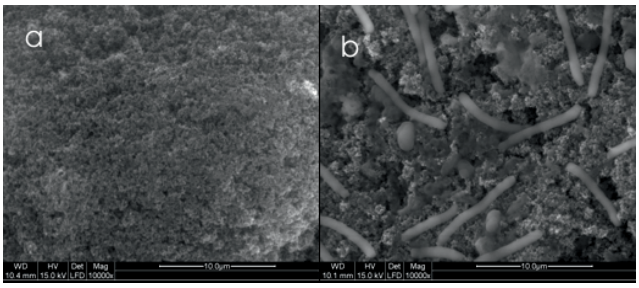

Figure 6 SEM images at higher magnification: a) CA, b) biofilm growth on CA.

potassium phosphate buffer solution (Figs. 8, 9) with 20 mV/S scanning rate. Cyclic voltammetry was generally performed by starting from −0.7 V and going up to 0.7 V and back versus MMS. If components were oxidized or reduced during this potential sweep of the culture, current peaks are revealed on the voltammogram shape. Every component that could be reversibly oxidized or reduced had a peak on both the upper and lower curves. In the both cases, the results obtained in wastewater (Fig. 7) at time zero show featureless voltammograms and no Faradic peaks can be observed between −0.7 V to +0.7 V (vs. MMS).

About 50 cycles in the same conditions, the voltammograms are keeping the same shape with a little current

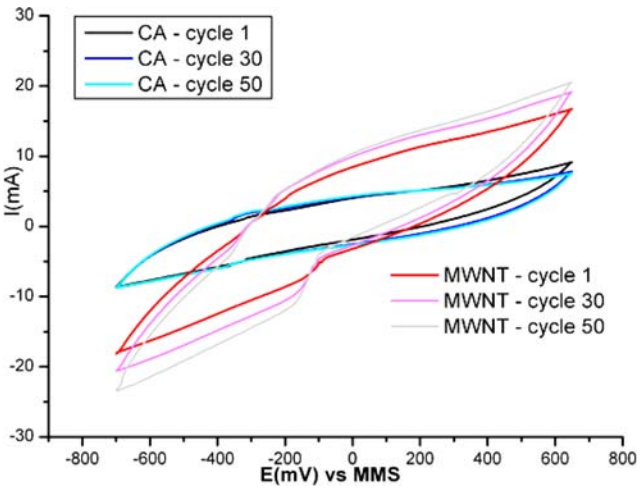

Figure 7 (online colour at: www.pss-a.com) Cyclic voltammetry of biofilm growth on CA and MWNTs after multiple cycling in wastewater.

© 2008 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

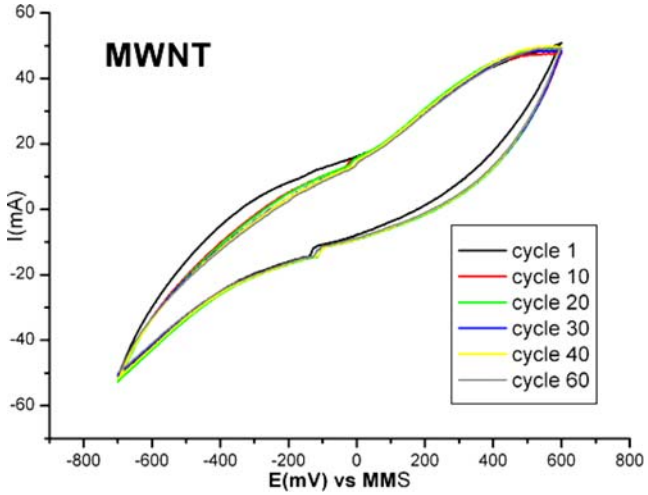

Figure 8 (online colour at: www.pss-a.com) Cyclic voltammetry of biofilm growth on MWNTs after multiple cycling in buffer solution.

increase for MWNTs. Similar results are shown in potassium phosphate buffer solution for biofilm growth on the MWNTs (Fig. 8). In the case of CA, an oxidation peak is observed around 0.3 V (Fig. 9) and no reduction peaks were found in reverse scans. The general voltammogram increased with time – number of cycling, which proves a good biocompatibility between biofilm and CA electrode.

4 Conclusion The monitoring of microbial activity of CNTs- and CA-based bioelectrode by open circuit potential show that 2–3 days are needed to equilibrate the MFCs system until the anodes were colonised by the bacterial population from wastewater, with a stabilization time about 4–5 days. The OCPs of the CNTs- and CA-based bioelectrode are different from each other, indicating that different electron transfer reactions occur at each of these anode types. The optical microscopy and SEM results indicated that both materials used as MFC anode favor the growth of the biofilm from wastewater. Bacteria growing on the MWNTs substrate appeared as uniform film. In contrast, the biofilm that grew on the CA was highly heterogeneous and many bacteria grew as tubules. Optical and SEM images show that the CA- and MWNTs-based electrodes are a real alternative for biofilm growth from wastewater and MFCs activity. The electrochemical measurements in wastewater or buffer solution show a featureless voltammograms without oxidation or reduction peaks. The exception is the result obtained for CA in buffer solution, where one oxidation peak appears around 0.3 V and increases in the intensity with number of cycling. About 50 cycles in the same conditions, the developed CNTs- and CA-based bioelectrodes showed very stable cyclic voltammetric behavior in buffer solution. These results suggest that the electrode surface area and morphologies play important roles in the electron transport properties of the interface between electrode and biofilm, thus having a significant impact on the power generation of MFCs.

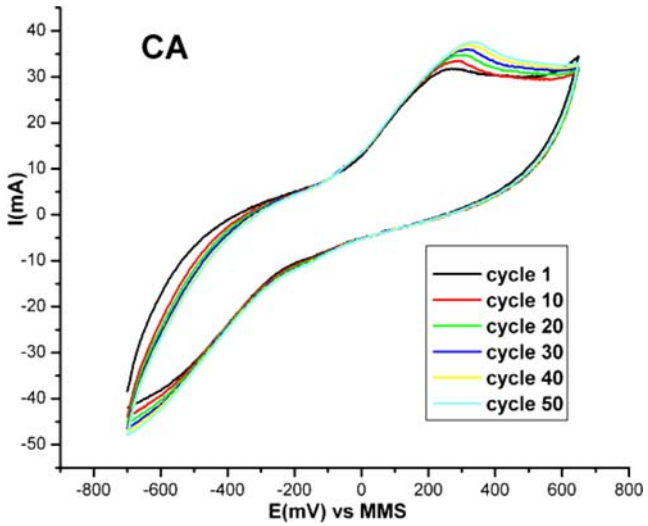

Figure 9 (online colour at: www.pss-a.com) Cyclic voltammetry of biofilm growth on CA after multiple cycling in buffer solution.

Acknowledgements This work he was supported by BMFC-MTKD-CT-2004-517215 contract and CNCSIS At 144/2007 grant.

References

[1] H. Liu, R. Ramnarayanan, and B. E. Logan, Environ. Sci. Technol. **38**, 2281 (2004).
[2] B. Min and B. E. Logan, Environ. Sci. Technol. **38**, 5809 (2004).
[3] B. E. Logan, Microbial Fuel Cell (John Wiley and Sons, New York, 2007).
[4] K. Rabaey, G. Lissens, S. D. Siciliano, and W. Verstraete, Biotechnol. Lett. **25**, 1531 (2003).
[5] H. Liu, S. Cheng, and B. E. Logan, Environ. Sci. Technol. **39**, 658 (2005).
[6] S. K. Chaudhuri and D. R. Lovley, Nature Biotechnol. **21**, 1229 (2003).
[7] K. Rabaey, P. Clauwaert, P. Aelterman, and W. Verstraete, Environ. Sci. Technol. **39**, 8077 (2005).
[8] Z. He, N. Wagner, S. D. Minteer, and L. T. Angenent, Environ. Sci. Technol. **40**, 5212 (2006).
[9] B. E. Rittmann and P. L. McCarty, Environmental Biotechnology: Principles and Applications (McGraw-Hill Book Co., New York, 2001).
[10] W. Huang, S. Taylor, K. Fu, Y. Lin, D. Zhang, T. W. Hanks, et al., Nano. Lett. **2**(4), 311 (2002).
[11] I. Heller, J. Kong, H. A. Heering, K. A. Williams, S. G. Lemay, and C. Dekker, Nano Lett. **5**(1), 137 (2005).
[12] M. C. Gutierrez, Z. Y. Garcia-Carvajal, M. J. Hortigüela, L. Yuste, F. Rojo, M. L. Ferrer, and F. del Monte, J. Mater. Chem. **17**, 2992 (2007).
[13] D. Hongda, L. Baohua, K. Feiyu, F. Ruowen, and Z. Yuqun, Carbon **45**(2), 429 (2007).
[14] S. S. Wong, E. Joselevich, C. M. Lieber, et al., Nature **394**, 52 (1998).
[15] J. Liu, A. G. Rinzler, H. Dai, et al., Science **280**, 1253 (1998).
[16] L. C. Cotet, A. Roig, I. C. Popescu, V. Cosoveanu, E. Molinis, and V. Danciu, Rev. Roum. Chem. **52**(11), 1077 (2007).

© 2008 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim