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Promising Graphene Modified Electrode Using Layer-by-Layer Method for Microbial Biofilm Connection

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Abstract: In order to enhance the power output of microbial fuel cell as well as its stability, the development of a new type of anode is essential. The purpose of this work is to modify a stainless steel foam, using the layer-by-layer self-assembly technique, with rGO (reduced graphene oxide) and PEI (polyethyleneimine). The efficiency of this kind of modification has been investigated to determine the supply of graphene in term of electricity generation and stability. Under an applied voltage, which is used to form an electroactive biofilm, the modified stainless steel foam (SSF/(PEI/rGO)5) exhibited a current 50 times higher than the blank anode. The roughness of the SSF/(PEI/rGO)5 observed by SEM (scanning electron microscopy) is more favorable to attach more bacteria on it. Also, graphene improved the stability of the electrode as no response where observed for the blank anode after 18 days meanwhile the SSF/(PEI/rGO)5 was still running after 54 days.

Key words: Microbial fuel cell, layer-by-layer, graphene, stainless steel.

1. Introduction

MFCs (microbial fuel cells) are bio-electrochemical devices in which microorganisms convert organic substances into electricity in a single step in an anaerobic environment [1]. MFCs have been considered, due to the usage of greener and renewable fuel as well as substrates, as a superior sustainable energy system over the conventional fuel cell [2-4]. A potential biotechnological application of this process is the harnessing of energy resources, for example from wastewaters, whilst degrading wastes [1, 5, 6] and also as biosensors [7-9].

It has been demonstrated for a few years that micro-organisms contained in different natural environments such as marine sediments [10, 11], waste water [12-14] and soils [15] can form electroactive biofilms on graphite anodes, oxidize the dissolved organic matter contained in the environment and use the electrode as the final electron acceptor. MFCs present promising advantages with respect to standard abiotic fuel cells [16]. They offer the possibility of harvesting electricity from organic waste and renewable biomass, as the catalyzing bacteria can adapt to different organic matters contained in a large variety of "dirty" environments such as wastewaters or sediments.

The fabrication of the materials for the anode plays a profound role in influencing the power generation by determining the actual accessible area for bacteria to anchor and affect the interfacial electron transfer resistance. Therefore, a high-performance anode material is most essential to improve the power outputs of MFCs.

Traditionally, carbon materials such as carbon cloth, carbon paper, graphite granules and graphite felt tend to be suitable as anodes in MFCs due to their chemical stability, high conductivity and high specific surface area. However, the pores within such materials can be clogged by the entering bacteria, resulting in cell death and significant reduction of the electrochemical reaction surface [17], thus much decreased electrocatalytic activity for the electrode microbial reactions can be subsequently observed. CNTs (carbon nanotubes) have also been explored to

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improve the power output of MFCs [18]. However, CNTs have a cellular toxicity that could lead to proliferation inhibition and cell death [19, 20].

Recently, graphene has been considered as the intriguing material, attracting strong scientific and technological interest with great application potentials in various fields, such as lithium ion batteries [21], solar cells [22] and electrochemical super-capacitors [23], for its unique nanostructure and extraordinary properties (high surface area [24], excellent conductivity [25], outstanding mechanical strength [26] and extraordinary electrocatalytic activities, etc.). Graphene can be synthesized by the chemical oxidation-reduction treatment of graphite [25, 27, 28], during which toxic metal catalysts are not used, which is a desired property for application in the MFCs.

In a previous study Zhang et al. [29] demonstrated the increase of performance of microbial fuel cell by using modified graphene electrode. In their work, *E. coli* has been used as catalytic microbial and connected to the anodic electrode by adding an electrochemical mediator: HNQ (hydroxy-1,4-naphtoquinone). The increase of power generation is attributed to the high surface area which is allowed to obtain a larger number of bacteria attached to the electrode and therefore a thicker biofilm coated the electrode.

In this study, the effect of another kind of graphene deposition (using polyelectrolyte with layer by layer method) at the anode has been evaluated in term of direct electrical connection of bacteria with the electrode. The influence of graphene on the electron transfer between bacteria and electrode has been investigated by performance of microbial fuel cell measurement.

2. Experimental Sections

2.1 Reduced Graphene Oxide Fabrication and Characterization

GO (graphene oxide) was synthesized from graphite by a modified Hummers method suspended in MilliQ water [30, 31]. Hydrazine and ammonia solution (purchased from Fluka) were used to chemically reduce GO [32]. rGO (reduced graphene oxide) obtained had a concentration of 0.25 mg/mL. 1 mg/mL solution of PEI (polyethyleneimine) (Aldrich) was prepared in an aqueous solution of 0.5 M NaCl (Fluka).

rGO was characterized by XPS (X-ray photoelectron spectroscopy) before and after hydrazine reduction to quantify the ration of carbon to oxygen containing groups in the final dispersion. As shown in Fig. 1, a significant decrease in C-O content (286 eV) relative to the C=C/C-C peak (284 eV) can be observed after chemical reduction of the GO dispersion [33, 34]. The remaining oxygen containing groups observed at 288 eV corresponds to the C=O groups which are responsible for keeping the rGO dispersion electrostatically stable [32]. The electrical resistivity of the material is directly attributed to the number of sp^2 bonded carbon site and so to the oxygen defects removed.

2.2 Electrode Modification

SSF (stainless steel foam) was modified with rGO using a layer-by-layer method (Fig. 2a) [35]. In order to adsorb the rGO, negatively charged, onto the surface of the electrode, it is important to form a positive layer on its surface. Indeed, the surface charge of stainless steel tends to be negative. The first layer was made by adsorption of PEI (positively charged polymer) onto the surface of the electrode by soaking it in a solution of PEI during 15 min. The electrode was then washed with distilled water and dried with azote. After that, the electrode was dipped into the solution of rGO during 15 min too, cleaned with distilled water and dried with azote. This process was repeated 5 times in the aim of making 5 bilayers of (PEI/rGO). This electrode is thus named SSF/(PEI/rGO)5.

The SEM (scanning electron microscopy) used is a S4800 FEG-HR from Hitachi (Montpellier University,

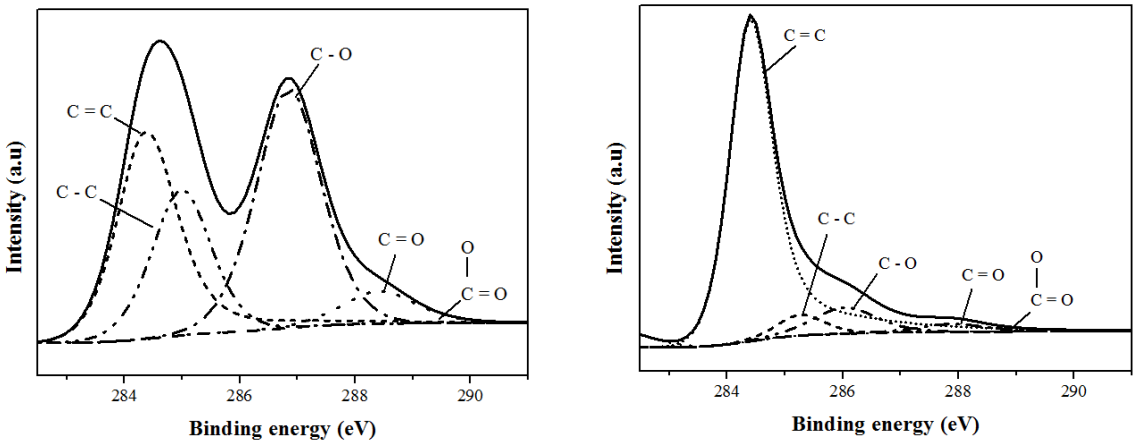

Fig. 1 XPS data for GO dispersions before (a) and after (b) hydrazine reduction.

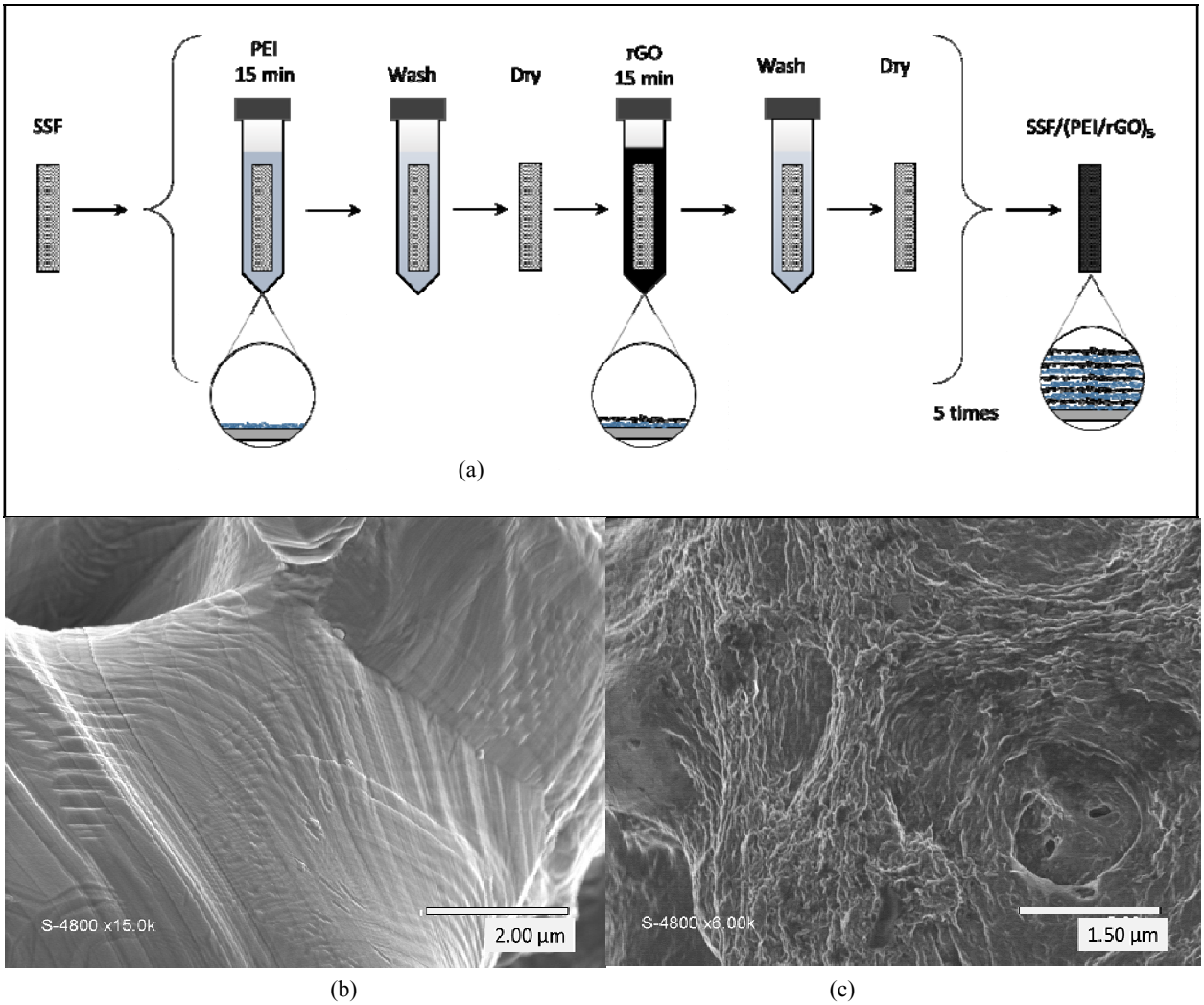

Fig. 2 (a) scheme of layer-by-layer electrostatic self-assembly of PEI and rGO on the SSF electrode: assembling positively charged PEI onto negatively charged SSF; assembling negatively charged rGO; repeating the two steps to control the desired number of layers (5 times); (b) SEM images of SSF; (c) SEM images of SSF/(PEI/rGO)5.

France). Before analysis, all samples were washed with ultra-pure water and attached to steel discs with double side-scotch carbon tape. Then, samples were coated with a thin layer of Pt/Pd (4 nm) using an evaporating technique. SEM images (Figs. 2b and 2c) show the electrode surface before and after Lyaer-by-Layer modification with rGO. It can be observed the roughness is higher in presence of rGO opposite to electrode without rGO which is smooth. This modification may provide a better adhesion of the biofilm onto the electrode.

2.3 Electroactive Biofilm Growths

Compost garden leachate was made with 1 L of commercial garden compost (Botanic France) and 1 L of 60 mM potassium chloride (Fluka). The mixture was allowed to maturate for 48 h and was filtered. A solution of potassium acetate (20 mM, Fluka) was used as fuel for the electrode. The growth of an electroactive biofilm was followed by chronoamperometry with a potentiostat (SP 50 Biologic France). In a three electrodes system, the working electrode (SSF/(PEI/rGO)5 or SSF) was immersed in a solution of leachate and sodium acetate (1:1). The potential applied to the counter electrode (graphite) was −0.246 V vs Ag/AgCl [36] and was applied for a period of 11 days. An Ag/AgCl electrode was used as reference electrode.

3. Results and Discussion

3.1 Polarization of the Electrode

Working electrodes were polarized in order to obtain an electroactive biofilm on the surface of the electrode. Polarization was first recorded in a solution without any micro-organisms (60 mM KCl and 20 mM potassium acetate) for the SSF/(PEI/rGO)5 electrode.

The polarization of the electrode does not influence the evolution of the current density. Indeed, no proper increase in current density has been observed without the presence of micro-organisms. Moreover, when an electrode is set in leachate compost garden and let it for few days (5 days) in the solution, a biofilm is formed. However, this biofilm is not electroactive as no current was observed when connected to a resistance.

Two electrochemical reactors were run in parallel with the same inoculum: first one was for the blank SSF electrode and the second one was for the modified SSF/(PEI/rGO)5 electrode. Reactors were initially fed with 20 mM acetate and working electrodes were polarized at −0.246 V vs Ag/AgCl [36]. The evolution of current density is shown in Fig. 3.

The current density showed similar evolution for the both electrodes. In both cases, current density increase with the biofilm growth onto the surface of the electrodes which is then followed by an important decrease of the current density due to a lake in acetate. When depletion was observed, acetate (20 mM) was added to the batch to provide fuel to the system which resulted by an increase of current density. However, it can be observed that the growth of the biofilm is much faster in the case of the modified SSF/(PEI/rGO)5 electrode. The first peak observed on the curve of the SSF/(PEI/rGO)5 corresponds to the instant growth of the biofilm which was not observed with the SSF electrode. Indeed, time was needed for the growth of the biofilm on the blank electrode. rGO seems to accelerate the process of biofilm formation on the surface of the electrode. Also, The current obtained with the SSF/(PEI/rGO)5 electrode is much higher than the unmodified electrode. The highest current obtained with rGO was about 2.8 A/m² (day 1.5), which is 50 times higher than without any rGO modification.

3.2 Debit of the Working Electrode

In the aim of knowing the stability and the power output of the formed electrode, the debit on a resistance of 1 kΩ was investigated over a period of 3 months. The working electrode with the biofilm on its surface was connected to the counter electrode in the same reactor were occurred the growth of the biofilm. Evolution of the current density in function of time is reported in Fig. 4.

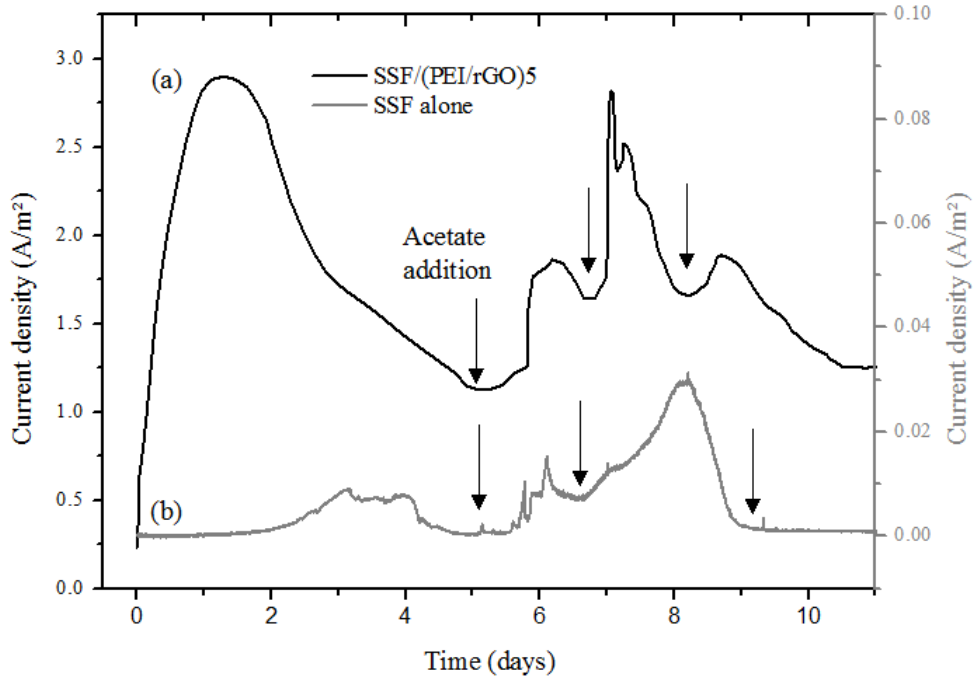

Fig. 3 Polarization under a potential of −0.246 V vs Ag/AgCl of the SSF/(PEI/rGO)5 (a) and SSF alone (b) for the growth of an electroactive biofilm. For each current's depletion, acetate was added to provide fuel to the electrode (indicated by the red arrows).

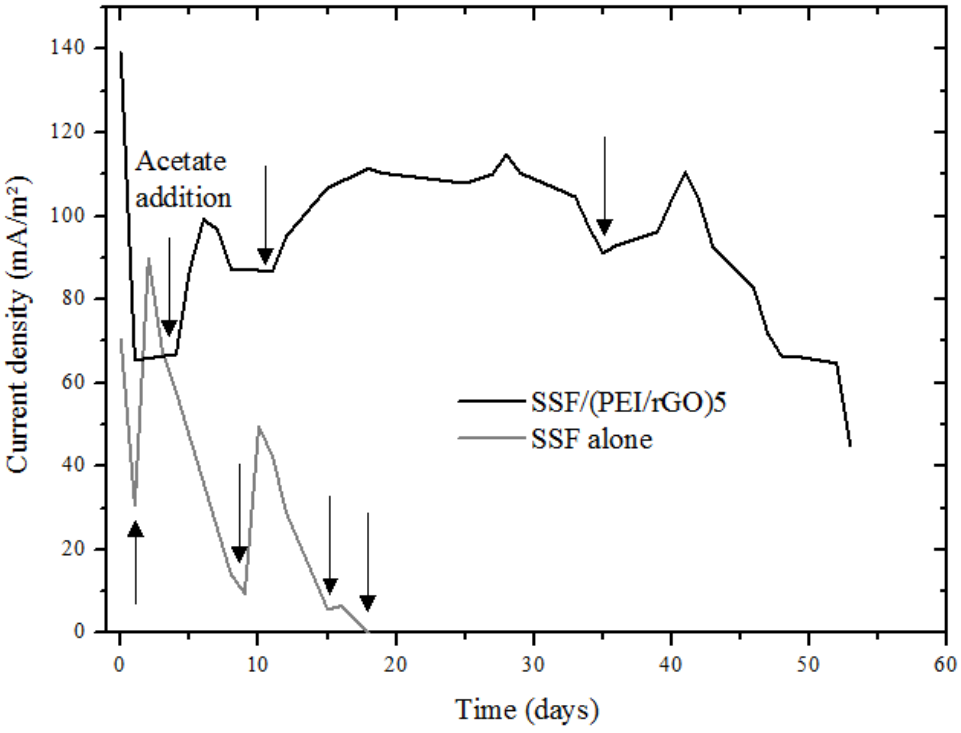

Fig. 4 Debit of the electrode with a resistance of 1 kΩ between the working electrode (either SSF/(PEI/rGO)5 or SSF). The addition of acetate is reported through the red arrows.

It appears that after 18 days, the SSF electrode was enabled to produce any current contrary to the SSF/(PEI/rGO)5 which showed a current of 110 mA/m². This could mean that the biofilm, adhered to a modified surface of rGO, presents a better connection to the electrode than with only stainless steel. At a time of 30 days, the power output obtained was 8.19 mW/m² for the SSF/(PEI/rGO)5 electrode whereas no power output was observed for the SSF alone.

3.3 Biofilm's Characterization

Blank SSF and SSF/(PEI/rGO)5 electrodes were both characterized by cyclic voltammetry in a solution of acetate (20 mM) and KCl (60 mM) before and after the growth of the biofilm. As shown in Fig. 5, voltammograms of the SSF/(PEI/rGO)5 electrode revealed a larger current responses compared to the SSF blank electrode in the potential scan range of −1.0 V to +0.6 V. The increase in the electron transfer efficiency can be attributed to the synergic properties of rGO. In addition, the same behavior is observed with the biofilm onto the surface of electrodes. A current increase is observed for both electrodes when the biofilm is formed although the current is higher for the modified electrode. The oxidation potential is shifted to the right (higher potential). Furthermore, SEM images (Fig. 7) have been realized on the electrodes at the end of the experiments in presence of biofilm. Sample (cut from the anode) was immersed in 4% glutaraldehyde (Sigma) solution for 4 h. It was then rinsed with de-ionized water for 3 times, followed by dehydration with increasing concentration of ethanol (20%, 40%, 60%, 80% and 98%) for 10 min each times and further rinsed in isoamyl acetate twice (10 min each time). Sample was then dried at CO_2-critical point for 3 h [37]. In both cases (Fig. 6a and Fig. 6c), the biofilm is fully covering electrodes and is also presented inside the porosity of the material. No difference between the blank and the modified electrode can be observed with the biofilm.

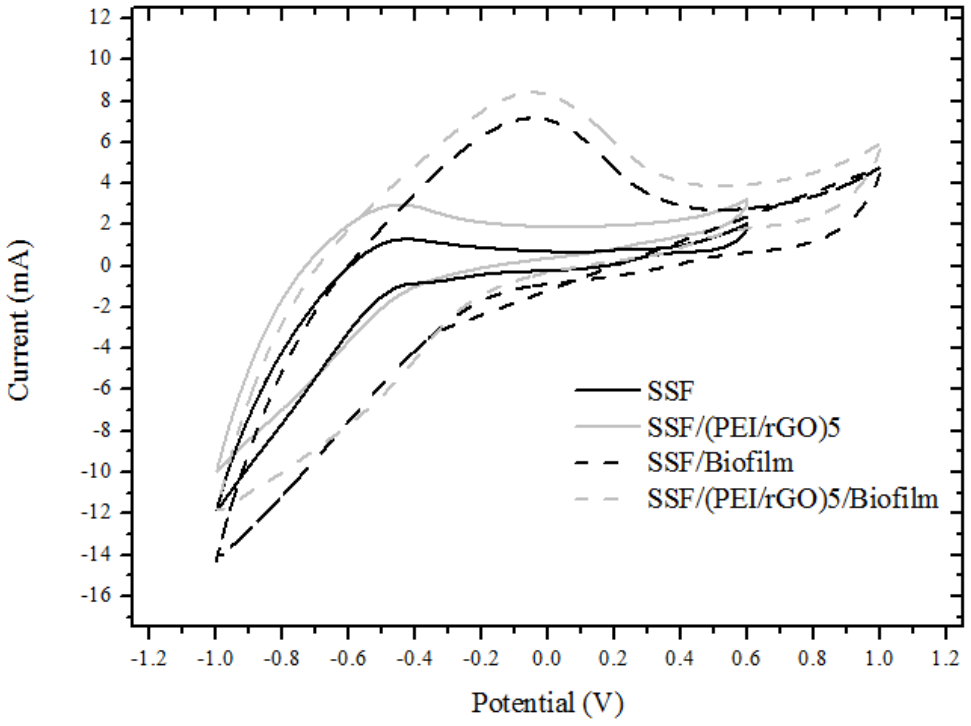

Fig. 5 Cyclic voltammetry of both electrodes (blank and modified) without and with biofilm realized in a solution of acetate (20 mM) and KCl (60 mM) at 50 mV/s vs Ag/AgCl.

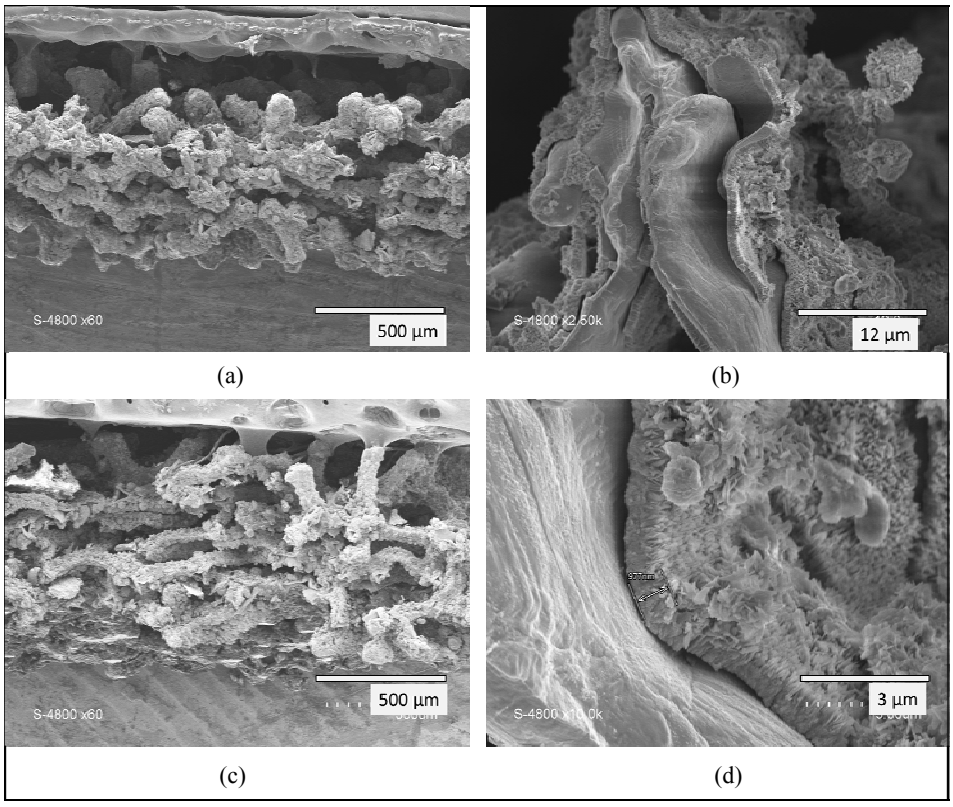

Fig. 6 SEM images of (a&b) SSF/biofilm and (c&d) SSF/(PEI/rGO)5/biofilm.

Further images have been taken (Figs. 6b and 6d) to see if the rGO modification can have an impact on the thickness of the biofilm. However, for both electrodes, the thickness of the biofilm is in the same range of order (985 nm for the SSF electrode an 977 nm for the SSF((PEI/rGO)5 electrode). This means that rGO does not have any influence on the growth of the biofilm in term of thickness and general appearance.

4. Conclusions

This study proposed a facile approach to modify the anode of MFCs with rGO which enhance the power output and the stability of the anode. The rGO presents a large specific surface area which is beneficial to the attachment of bacteria onto the electrode as well as for the electron transfer which is larger. Also, the current density and the power increase. rGO allows the biofilm to grow faster and can reduce the time of latent time. The study might present a great interest to the construction of new electrodes by an easy method with a small amount of graphene.

Acknowledgments

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