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Electrochemical micro-structuring of graphite felt electrodes for accelerated formation of electroactive biofilms on microbial anodes

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ABSTRACT

Electroactive biofilms were formed on graphite felt anodes under constant potential polarisation using compost leachate as the inoculum and dairy waste as the substrate. A new surface treatment is presented that consists of creating micro-cavities on the electrode surface by strong anodisation. This surface treatment, combined with pre-adsorption of the dairy waste, proved very efficient in accelerating the formation of electroactive biofilms and increasing the current values up to 1600 mA/m^2 for dairy waste oxidation.

1. Introduction

Microbial fuel cells (MFC) are a promising way of producing current directly from the chemical energy contained in organic matter [1]. The currents provided by microbial anodes remain low and increasing them is an essential challenge, especially when raw complex fuels are used. Food industry effluents are promising fuels for MFC because their organic matter can easily be oxidised by micro-organisms [2]. Dairy compounds [3] and dairy wastewaters [4,5] have already been tested in MFC. Our previous work has shown that electrochemically active biofilms formed from compost leachate are more efficient for dairy waste oxidation than biofilms formed only from the endogenous microflora of the dairy waste [6]. The system "compost leachate as inoculum/dairy waste as fuel" was consequently used in the present study.

The interaction between biofilm and electrode surface is crucial for the performance of microbial anodes. The importance of the carbon-type material has recently been evidenced [7]. Pre-treatment of the electrode surface is a complementary way to enhance anode performance. Treatment of graphite anodes by ammonia, oxidation in sulphuric acid or activation with HNO_3 has been reported [8,9]. Modification with carbon nanotubes has recently been shown to significantly increase current densities [10–12]. The choice of the anode potential is important to enrich electroactive consortia [13] or to select for electroactive strains [14]. It has been stated that the electrochemical pre-oxidation of graphite affects the biofilm micro-

bial composition [15] and a recent study has shown that surface pre-oxidation favours electron transfer due to the generation of carboxyl-containing functional groups [16].

The present work describes the design of a microbial anode suitable for dairy waste oxidation, which is based on the electrochemical pre-treatment of a graphite felt. For the first time, it is shown that electrochemical pre-treatment can enhance the performance of a microbial anode by micro-structuring the electrode surface.

2. Materials and methods

Electrochemical cells of 50 or 150 mL were equipped with a three-electrode setup monitored by a multichannel potentiostat (Bio-Logic Science Instruments). Working electrodes were made of graphite felt (RVG, Carbone Loraine, France) of 2 cm × 2 cm or 2 cm × 5 cm projected area and electrically connected with a titanium wire. Platinum mesh was used as the counter-electrode and a saturated calomel electrode (SCE, potential = 0.242 V vs. SHE) as the reference. Biofilms were grown under constant polarisation at +0.1 V/SCE at room temperature for the preliminary experiments and at 50 °C for all others.

The electrochemical pre-treatment called "low anodisation" consisted of 5 successive cycles. In each cycle the potential of the working electrode was maintained at +1.5 V/SCE for 2 min and then cyclically scanned from −1.0 V to +1.0 V at 30 mV/s. "Extended anodisation" was composed of 24 similar cycles, and in each cycle the constant potential step was 1 h long. Both pre-treatments were performed in 50 mM phosphate buffer pH 6.7. When indicated, the graphite felt electrode was pre-treated by adsorption of dairy waste as described previously [6].

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Leachate from garden compost was produced by adding a 10 mM NaCl solution to 1 L of garden compost to give a final volume of 3 L. This mixture was stirred for 24 h in Erlenmeyer flasks and then filtered through felt cloth.

Dairy waste was collected from a dairy industry and stored in bottles of 1.5 L at 3 °C for a short period or was frozen for long-term storage. Substrate dilutions of 20-, 100- and 200-fold were made with 50 mM phosphate buffer solution pH 6.7 containing 10 mM NaCl (conductivity 5.2 mS/cm).

Total chemical oxygen demand (COD) was determined using the standard method 8000 for 0–1500 mg L^{-1} COD (HACH France S.A.S.). Scanning electron microscopy (SEM) micrographs were taken with a Leo 435VP microscope (Germany). Samples were fixed with 4% glutaraldehyde solution, post-fixed with 2% OsO$_4$ solution, gradually dehydrated with acetone 50%, 70%, 100% solutions, and finally coated with gold for SEM imaging.

3. Results and discussion

3.1. Preliminary determination of standard experimental conditions (room temperature)

Graphite felt electrodes immersed in 150 mL compost leachate were polarised at +0.1 V/SCE. The initial current was always less than 17 mA/m^2 and fell to near zero after 2 days (Fig. 1A). The compost leachate was replaced by solutions of dairy wastes diluted 20-, 100- and 200-fold, corresponding to COD of 6700, 1360 and 730 mg/mL respectively. Low COD of dairy wastes (dilution 200) induced fast current increase and the highest current density, up to 93 mA/m^2, while high COD amounts (dilution 20) stressed the biofilms, which did not provide more than 21 mA/m^2 (Fig. 1A). Experiments were repeated, but dairy waste was added directly into the compost leachate at day 2 instead of emptying and refilling the reactors as
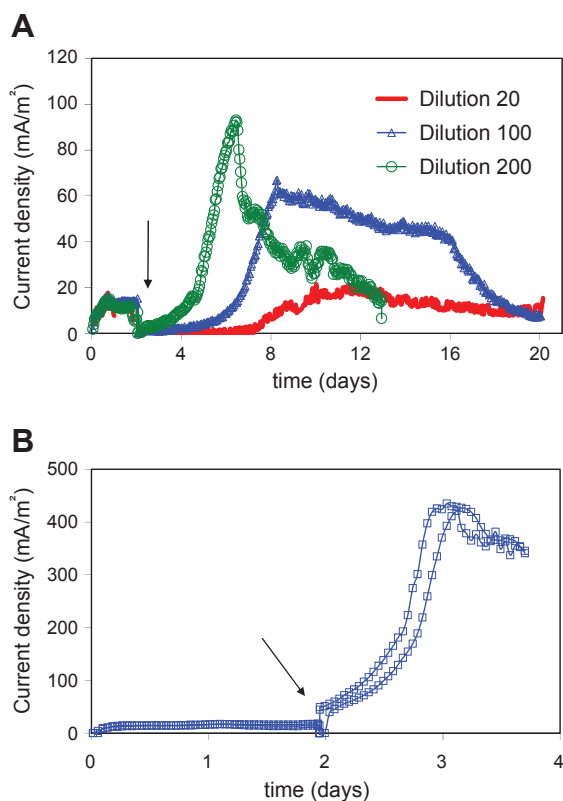
previously. Current densities reached 450 mA/m^2 after only 1 day for a final dilution ratio of 500 (Fig. 1B). The complete change of solution made in the first attempts imposed a drastic stress on the biofilms that was detrimental to the anode performance. It is also possible that a synergetic effect occurred between the microbial population coming from compost and electroactive microorganisms contained in dairy products [17,18]. The latter procedure was consequently chosen for the continuation of the experiments.

3.2. Anode pre-treatments by anodisation and dairy waste adsorption (50 °C)

Graphite felt anodes were pre-treated by adsorption of dairy waste before being used to form a biofilm. The biofilm growth phase in compost leachate gave a continuous increase of the current up to values around 500 to 600 mA/m^2 after 6 days. The current was due, in this case, to the consumption of the organic matter that had been pre-adsorbed on the electrode surface. On day 6, dairy waste was added at final dilution ratio of 500. Only a slight current increase was observed. The same behaviour was observed in four separate experiments (data not shown).

Graphite felt anodes were pre-treated by "low" or "extended" anodisation in phosphate buffer solution. The electrodes were first characterised by chronoamperometry at +0.1 V/SCE in the phosphate buffer solution. The current with the low-anodised electrodes was almost zero. The extended-anodised electrodes generated current density near 20 mA/m^2 in the first 5 h, which then dropped slowly to zero over 15 h. This evolution indicated the oxidation of surface compounds that were formed on the electrode surface by the pre-treatment. Cyclic voltammetry curves recorded at 1 mV/s (Fig. 2) with low-anodised electrodes were identical to the control curves obtained with non-treated felt graphite. The peak reduction at −0.4 V/SCE, which vanished after 1 h of nitrogen bubbling, was due to the reduction of dissolved oxygen. Cyclic voltammetry of extended-anodised electrodes showed up more drastic modifications, with high capacitive currents and the appearance of a new redox couple. Scanning electron microscopy showed that the extended-anodisation created numerous clearly observable micro-cavities on the surface of the fibres that made up the electrodes (Fig. 3A), while low-anodisation did not affect this aspect (Fig. 3B). Most of these cavities were less than 1 μm in diameter.

Anodes treated by both anodisation procedures were used to form biofilms with compost leachate. Four experiments were run simultaneously in separate reactors with the same inoculum, the same dairy waste load, and with low-anodised or extended-anodised electrodes (Fig. 4A). The low-anodised electrodes ensured fast biofilm growth (maximum current obtained in less than 1 day) but the addition of dairy waste did not increase the current, which never rose beyond 600 mA/m^2. The extended-anodised electrodes gave remarkably fast biofilm growth and a current density of 1100 mA/m^2 was reached in

Fig. 1. Chronoamperometry at +0.1 V/SCE with compost leachate as inoculum and dairy waste as fuel. Arrow indicates A) replacement of the inoculum medium by dairy waste, and B) dairy waste addition into the inoculum medium.
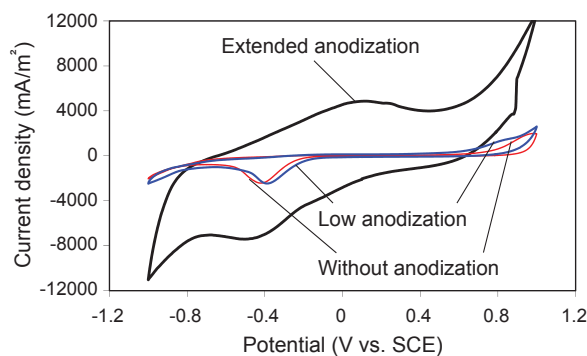

Fig. 2. Cyclic voltammetry (1 mV/s) with graphite felt electrodes after low- or extended-anodisation in phosphate buffer.

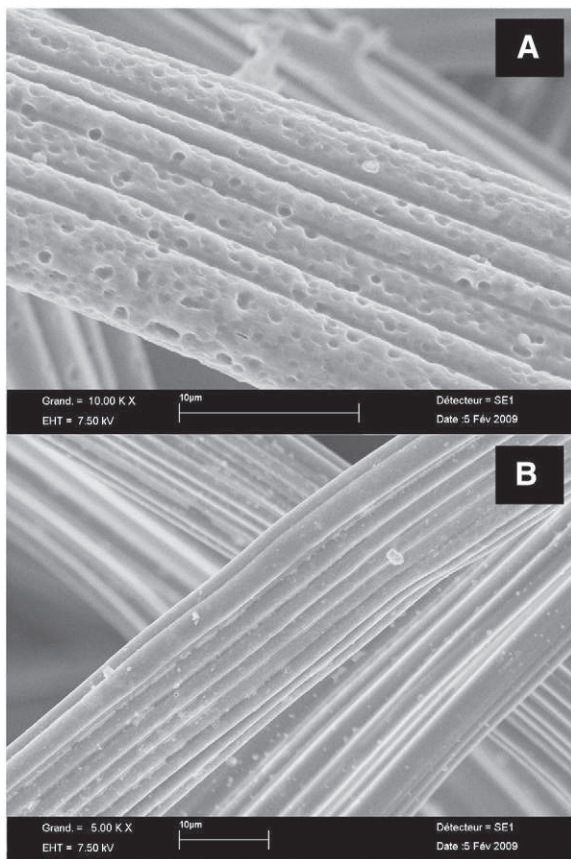**Fig. 3.** SEM images of graphite felt fibres after anodisation. A) Extended anodisation, and B) low anodisation.

Fig. 4. Chronoamperometry at $+0.1$ V/SCE of graphite felt in compost leachate. A) Low- and extended-anodised, and B) extended-anodised electrodes with adsorbed dairy waste. Arrows indicate dairy waste addition; two similar experiments were performed simultaneously for each case.

1 day. Addition of dairy waste at the peak of current did not have any noteworthy effect, the current fell to 50% of the peak value and then increased again to reach about 1150 mA/m² in 3.5 days. The micro-cavities created by extended-anodisation certainly favoured the anchorage of the microbial cells, resulting in fast biofilm settlement. Nevertheless, these biofilms then needed time to adapt to the oxidation of dairy waste.

Finally, the anodes were implemented in similar experiments after two successive pre-treatments: first extended-anodisation and then adsorption of dairy waste (Fig. 4B). The biofilm formation phase was similar to the previous one without pre-adsorption, but with a lower peak current (480 mA/m²). Addition of dairy waste on day 2 at a final dilution ratio of 500 caused an immediate increase in current up to values of 1600 mA/m². The current fell after 2 days and a new addition of dairy waste at day 5 led to a similar current increase.

The phase of biofilm formation gave average "current density/time" slopes around 60 mA/m² per day with pre-adsorbed raw electrodes, 750 mA/m² per day with low-anodised electrodes, 1200 mA/m² per day with extended-anodised electrodes (Fig. 4A) and 400 mA/m² per day with extended-anodised and pre-adsorbed electrodes (Fig. 4B). The preliminary adsorption of dairy waste tended to decrease the currents recorded during biofilm formation, as already observed in raw graphite electrodes [6], but pre-adsorption combined with extended anodisation resulted in biofilms that were more efficient for dairy waste oxidation. Certainly a synergetic effect occurred between extended anodisation and pre-adsorption of dairy waste.

It is has been widely demonstrated that anodisation of carbon or graphite electrodes leads to the incorporation of atomic oxygen, forming functional groups like phenol, carbonyl, carboxyl and quinone on the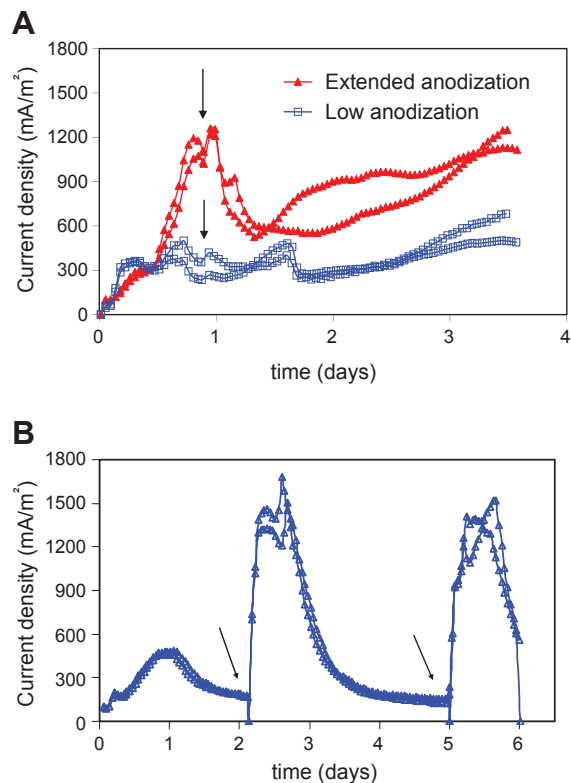 surface [19–22] that favour electron transfer with microbial cells [16]. Here, the low- and extended-anodisation procedures differed only by the number and duration of the electrolysis steps. Both types of surfaces should consequently have had quite close chemical compositions, the difference in which cannot explain such different voltammetry characteristics (Fig. 2). In contrast, the creation of micro-structure by the extended anodisation can explain the high capacitive behaviour, due to a higher ionic density of the interface and, by correlation, higher electron density in the material. Moreover, the pre-adsorption of dairy waste after anodisation certainly annihilated the effect of the surface composition. The remarkable current increase obtained by extended anodisation and further pre-adsorption must consequently be attributed to the creation of the micro-sized cavities rather than the formation of specific surface groups. The micro-cavities also explain the efficiency of biofilm formation because of easier anchorage of the cells on the surface.

4. Conclusions

Strong anodisation of a graphite electrode coupled with pre-adsorption of dairy waste resulted in an increase of the current densities by a factor of around 3 (reaching 1600 A/m²) with respect to pre-adsorbed but not-anodised electrodes. It is well known that the oxidation of graphite electrodes generates surface oxygenated functional groups that favour the performance of electroactive biofilms but this was the first time to our knowledge that electrochemical micro-modification of the structure was shown to favour the performance of microbial anodes. Studies now need to go ahead with characterising this new pre-treatment with pure microbial cultures fed with acetate.

Acknowledgements

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