Acetate to enhance electrochemical activity of biofilms from garden compost

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Abstract

Dimensionally Stable Anodes embedded in garden compost and maintained under constant polarization at 0.50 V/SCE for several days progressively became covered by a microbial biofilm that gave them the capability to oxidize the organic matter contained in the compost. The effect of acetate supply on the electrochemical activity of biofilms was investigated either by adding acetate after biofilm formation or mixing it into the compost initially. Addition of acetate allowed the current density values to increase up to 545 mA/m^2. Six individually monitored electrodes set up in the same reactor showed very good reproducibility, indicating that discrepancies observed between the different experiments were mainly due the different batches of compost. A numerical treatment of the epifluorescent microscopy pictures allowed the biofilm coverage ratios to be assessed. Comparing the variations of current density during chronoamperometry with the biofilm surface coverage ratios and with the current obtained by cyclic voltammetry led us to propose a mechanism based on two different steps that corresponded to different time-scales: slow acetate oxidation through the cell metabolism and fast electron transfer between the cell and the electrode surface.

Keywords: Electrochemically active biofilm; Compost; Acetate; Biofilm coverage; Cyclic voltammetry

1. Introduction

Electrochemically active biofilms that develop on the anodes of microbial fuel cells (MFCs) have recently revealed their crucial role in the efficiency of these systems [1,2]. They have been shown to be able to oxidize various substrates under anaerobic conditions, directly transferring electrons to the anode. Acetate is commonly used as a nutrient for anaerobic bacteria [3–7] and, in particular, to grow anaerobic active consortia in MFCs [8–13]. It has been shown to be a more efficient electron donor than butyrate, glucose, starch or dextran, since it supplies the largest power and current densities in the MFCs [8,9]. Acetate also leads to high coulombic efficiency, *i.e.* high conversion of acetate to electrons supplied to the external system, ranging from 65 to 92% [8,12]. In most cases the acetate is completely oxidized to CO_2, yielding 8 electrons to the anode [5–8,10]:

$$CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + 9H^+ + 8e^- \quad (1)$$

Acetate has been reported to have an impact on the composition of the microbial community attached to anodes of MFCs [14]. Feeding an MFC with acetate has been found to enrich the biofilm formed on the anode surface in electrochemically active bacteria, which come from the activated sludge used as the inoculum [10].

Our previous studies have shown that compost is a suitable environment for providing a source of electrochemically active biofilms able to oxidize organic compounds contained in the compost and transfer electrons to a Dimensionally Stable Anode (DSA) electrode [15,16]. DSA electrodes were chosen because of the high catalytic efficiency, mechanical properties and long-term stability they show in many oxidation processes on the industrial scale, and the promising opportunity they would thus offer to scale up very fast to large scale MFC pilots [17]. In a previous study, DSA electrodes were directly embedded in compost and polarized for some days at potential values in the range of 0.10–0.70 V/SCE. The current increase observed after a few days corresponded to the development of an electrochemically active biofilm on the electrode surfaces, which gave them the ability to oxidize the organic matter contained in compost. At 0.50 V/SCE, current densities varied within a

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range of 19–385 mA/m^2 depending on the compost batches and operating conditions [16].

In this paper, acetate was supplied to compost in order to increase the efficiency of the electrochemically active biofilm developed on DSA electrodes polarized at 0.50 V/SCE. It was used as an electron donor additional to the organic matter already present in the compost in order to encourage the biofilm growth and to define the most favourable conditions of biofilm formation for subsequent isolation of bacteria. In parallel with chronoamperometry, cyclic voltammetry was performed at different steps of the biofilm development with the objective of better understanding the mechanism of electron transfer and to look for a correlation between current density and the biofilm coverage ratio.

2. Experimental

2.1. Compost and chemicals

Garden compost (Eco-Terre) was purchased from a garden center in a 40 L bag and stored in the laboratory at ambient temperature. It was mixed with a 10 mM NaCl solution in the proportion of 2 vol. of compost to 3 vol. of NaCl solution. Sodium acetate solution was either mixed with the NaCl solution at a concentration of 10 mM or added to compost during the experiment. For control experiments, the compost was sterilized either by 20-min autoclaving at 121 °C or by γ-ray irradiation (Cobalt 60 irradiator, 40 kGy for 12 h, "CIGAL" cell, CEA Cadarache, France). NaCl and acetate solutions were also sterilized by 20-min autoclaving.

2.2. Electrochemical instrumentation and set-up

Experiments were performed under potentiostatic control (chronoamperometry) using a three-electrode set-up consisting of a Dimensionally Stable Anode as the working electrode, a saturated calomel reference electrode (SCE) and a graphite rod as the counter electrode. The experiments were conducted at 0.50 V/SCE with a multipotentiostat (VMP2 Bio-Logic SA, Software EC-Lab v.8.3, Bio-Logic SA). When mentioned, an N'Stat was used to allow individual polarization of several working electrodes with respect to the same reference and counter electrodes. All experiments were carried out at room temperature.

2.3. Working electrode materials and preparation

The Dimensionally Stable Anode electrodes (2.5 mm × 10 mm, 0.5 mm thickness) were made of titanium coated with iridium and tantalum oxides. The working surface area was either 20 cm^2 when the electrode was set vertically in the compost and connected with a flat alligator clip, or 25 cm^2 when the electrode was completely embedded horizontally in the compost and connected with a titanium rod. Before each experiment, the DSA electrodes were cleaned by 5-h galvanostatic electrolysis at 20 mA/cm^2 in a 0.1 M sulphuric acid solution. When indicated, the biofilm was removed from the electrode surface with a paper wetted with ethanol; the electrode was then rinsed for 10 min with distilled water.

2.4. Microscopy and image analysis

The biofilms were stained with acridine orange (0.03%, A6014, Sigma) for 10 min. The samples were left to dry in ambient air and observed using a 50× lens on a Carl Zeiss Axiotech 100 microscope equipped for epifluorescence with an HBO 50/ac mercury light source and the Zeiss 09 filter (excitor AP 450-490, reflector FT 510, barrier filter LP 520). Images were acquired with a monochrome digital camera (Evolution VF) and processed with the Image-Pro Plus v.5 software. Images were analysed by grey scale interpretation in order to calculate the proportion of the electrode surface covered by the biofilm (Fig. 1A). The grey intensity threshold between the areas covered by the biofilm and the non-covered areas was set manually. Grey levels greater than the threshold value were considered as biofilm-covered areas (white in Fig. 1B), while grey levels lower than the threshold were considered as clean areas (black in Fig. 1B). The threshold value was set by trial and error until the visual aspect of the black/white image obtained appeared consistent with the initial picture. With the biofilm considered here, this procedure limited deviation to less than 3.5% among all the operators. At least 5 different fields were processed per electrode to obtain an average value of the coverage ratio.

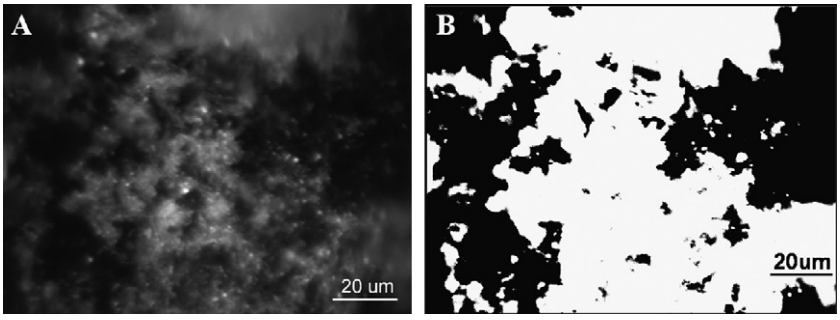

Fig. 1. (A) Epifluorescence microscopy image of biofilm developed on the surface of a DSA electrode embedded in compost for 5 days and polarized at 0.50 V/SCE. The light-grey area corresponds to the biofilm and the dark-coloured area to the non-colonized area. (B) Image after processing. The colonized areas have been overlaid with white and the non-colonized areas with black.

3. Results and discussion

3.1. Supply of acetate to a mature electrochemically active biofilm

A 20 cm^2 Dimensionally Stable Anode electrode was embedded in compost mixed with 10 mM NaCl and polarized at 0.50 V/SCE. The current density increased from the 5th day and reached a maximum of 62 mA/m^2 after 10 days (Fig. 2). This behaviour was similar to the current increase already reported to result from the formation of an electrochemically active biofilm on the electrode surface [15,16]. On the 11th day, adding 10 mM sterile solution of sodium acetate (arrows on Fig. 2) caused the current to increase to 260 mA/m^2 in less than 2 days. Then the current stabilized in the range of 250–260 mA/m^2 over 6 days. The same procedure repeated with compost that had previously been sterilized by autoclaving (Fig. 2) revealed no current increase after 7 days and adding 10 mM sterile acetate on the 7th day did not cause any variation in the low current recorded, showing that the electrochemically active biofilm was made up of the indigenous flora contained in the compost.

Five electrodes were electrically connected together, embedded in the same reactor and polarized at 0.50 V/SCE for 17 days. Two successive additions of 10 mM sodium acetate solution at days 3 and 14 made the current rise from 5 to 279 mA/m^2 (first addition) and from 300 to 619 mA/m^2 (second addition) (Fig. 3). Therefore, acetate was an effective substrate for the bacteria that constituted the biofilm formed from compost. These results were in accordance with what has been observed with electrochemically active biofilms formed from pure cultures of *Geobacter sulfurreducens* [5] and *Desulfuromonas acetoxidans* [4], where the current was enhanced by addition of acetate to the medium.

Among the five DSA electrodes embedded in compost, three electrodes were successively removed from the reactor after 3, 5 and 12 days to measure the biofilm coverage ratio (Fig. 4). Representative pictures obtained by epifluorescence microscopy after staining with acridine orange are reported in Fig. 4A–C. After 3 days' polarization, the biofilm was composed of a few colonies and scattered bacteria adhering to the electrode surface (Fig. 4A). Then the biofilm extended over the whole surface area (Fig. 4B) and finally became thicker and more dense at day

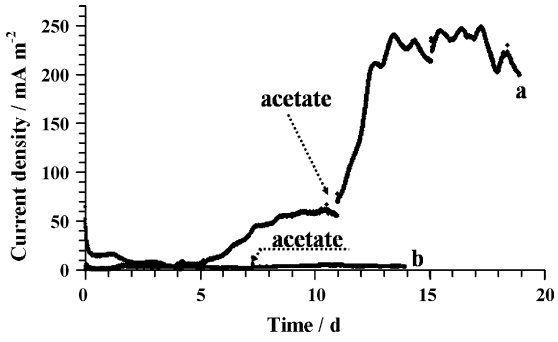

Fig. 2. Chronoamperometry at 0.50 V/SCE performed with DSA electrode embedded in compost (curve a) and in sterilized compost (curve b). Arrows indicate addition of 10 mM of sterile acetate to the compost.

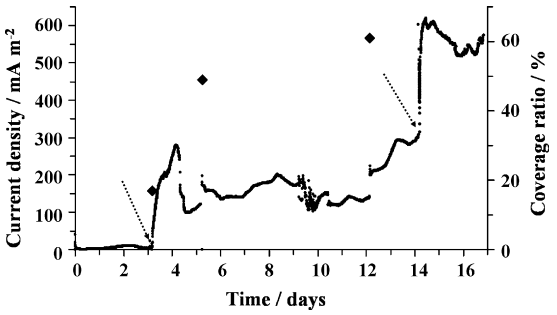

Fig. 3. Chronoamperometry at 0.50 V/SCE performed with five DSA electrodes embedded in compost (vertical axis on the left) and surface coverage ratio of the biofilm (represented by the points, vertical axis on the right). Arrows indicate addition of 10 mM acetate to the compost. The current density was always calculated with respect to the surface area embedded in compost (*i.e.* 100, 80, 60 and 40 cm^2 successively, after successive removal of electrodes).

12 (Fig. 4C). Because of the development of an extracellular polymeric substance that formed the biofilm matrix, the pictures were more difficult to focus from A to C. High concentrations of bacteria embedded in the matrix appeared as bright areas in these images, but parasite light was emitted from the bacteria embedded in planes which were different from the focal plane. The coverage ratio of the biofilm was defined as the percentage of the electrode surface area coated in biofilm with respect to the total geometric surface area embedded in compost (20 cm^2). It was assessed for each electrode by defining a threshold in the grey levels of the pictures that could be considered as the frontier between the clean areas and the coated areas (see Section 2). This procedure was applied to five different plots to get an average coverage ratio for each electrode. The mean value of the coverage ratios with the corresponding standard deviation at days 3, 5 and 12 are reported in Table 1, together with the current density values recorded just before extracting the electrode from the reactor. Current density resulting from chronoamperometry was calculated with respect to the total electrode surface area present in the compost (*i.e.* 100 cm^2 between days 0 and 3, 80 cm^2 between days 3 and 5, 60 cm^2 between days 5 and 12, 40 cm^2 between days 12 and 17).

The fifth electrode was used to achieve cyclic voltammetry (CV) at 10 mV/s at days 0, 3, 5, 12, 14 and at the end of experiment, after the biofilm had been removed from the electrode surface (Fig. 5). Once the electrode had been cleaned, it was put back at exactly the same place in the compost reactor to run CV. Cyclic voltammograms showed a low ionic resistance, certainly due to the compost medium, and significant currents characteristic of the charge and discharge of the oxide species that composed the DSA electrodes [17]. CV revealed an increase in anodic currents with a maximum at day 12 (Fig. 5A). The current intensities recorded on the CV at 0.90 V/SCE after subtracting the base current recorded on the initial CV are reported in Fig. 5B, showing that the difference increased from 0 to 2300 mA/m^2. The highest anodic current, recorded at day 12, corresponded to the well-formed biofilm (Fig. 4C). In addition, the cyclic voltammogram obtained with the cleaned electrode showed the same behaviour as the initial electrode before biofilm formation (Fig. 5A). As the cleaned electrode was put back at exactly the same place in the medium, this behaviour confirmed

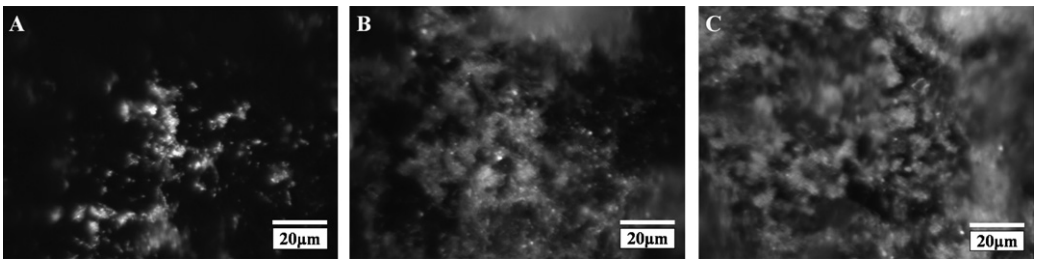

Fig. 4. Representative epifluorescence microscopy images of biofilms developed on a DSA electrode surface polarized at 0.50 V/SCE and embedded in compost for 3 (A), 5 (B) and 12 (C) days.

Table 1
Biofilm coverage ratio, current density and current density calculated with respect to the surface area covered by biofilm for chronoamperometry represented in Fig. 3

Days	Biofilm coverage ratio (%)	Current density during chronoamperometry (mA/m^2)	Current density calculated with respect to the surface area covered by the biofilm (mA/m^2)	Current density at 0.90 V/SCE in cyclic voltammetry (mA/m^2)
3	(17 ± 2)%	4	24	677
5	(49 ± 13)%	121	247	1225
12	(61 ± 8)%	142	233	2300
14	/	301	/	1472

The current density values recorded at 0.90 V/SCE in CV were extracted from Fig. 5B.

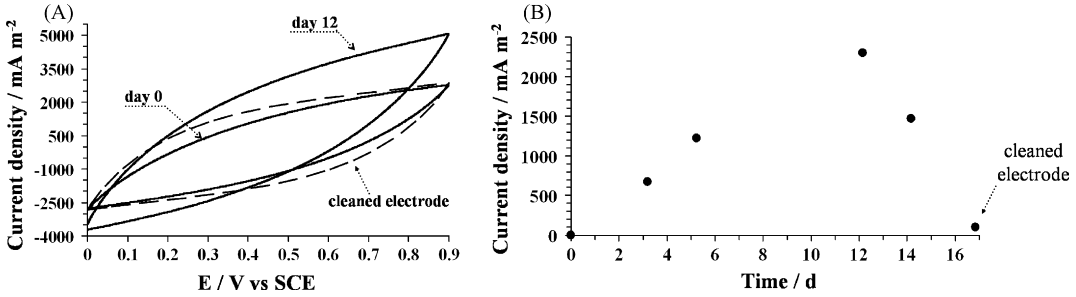

Fig. 5. (A) Cyclic voltammograms at 10 mV/s performed with the fifth electrode of the chronoamperometry represented in Fig. 3 (DSA polarized at 0.50 V/SCE). (B) Current intensities taken from the CV at 0.90 V/SCE, after subtraction of the current recorded in CV at day 0, for days 0, 3, 5, 12, 14 and with the cleaned electrode at the end of the chronoamperometry.

that the current was not due to metabolites produced by bacteria and that the presence of the biofilm was necessary for the oxidation reaction occur. No anodic peak was detected in the cyclic voltammograms, suggesting that mediators were probably not involved in the electron transfer. The transfer would thus require direct contact between the bacterial cells and the electrode and may be due to compounds bound to the cell membrane. As far as we know, only one previous study has reported cyclic voltammetry performed with an anode colonized by an electrochemically active biofilm. This took place in an MFC inoculated with domestic effluent [9]. In this case, the comparison of cyclic voltammograms obtained with the colonized electrode and a new electrode without biofilm also suggested that the electron transfer between bacterial cells and the electrode was direct and occurred through enzymes attached to the cell membrane.

3.2. Addition of acetate from the beginning of chronoamperometry

Six DSA electrodes were set up horizontally, completely embedded in the compost mixed with acetate 10 mM (geometric surface area 25 cm^2). Three electrodes were immersed at 15 cm depth and three others at 2 cm depth, for a total height of compost of 17 cm. Each electrode was individually polarized at 0.50 V/SCE. Current density increased from day 1.5 (Fig. 6) while, in comparison, current density in compost without an initial addition of acetate increased from the 5th day (Fig. 2). In

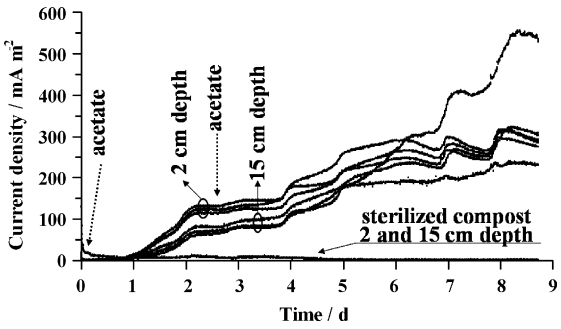

Fig. 6. Chronoamperometry performed with DSA electrodes individually polarized at 0.50 V/SCE and embedded in the same reactor containing either compost or sterilized compost. In each case DSA electrodes were immerged at 2 or 15 cm depth. Arrows indicate addition of 10 mM sterile acetate to the compost.

this case, adding acetate from the beginning of the experiment allowed the lag period before current increase to be shortened. Nevertheless, it should be noted that such short lag periods have already been observed in rare cases even in compost without acetate [16]. The current density increase showed a significant reproducibility for the 6 electrodes embedded in the same compost batch, indicating that the differences observed in chronoamperometry performed with different batches of compost were certainly due to the microbial origin of the phenomenon. However, between days 1 and 4, current densities of the electrodes embedded at 15 and 2 cm from the upper surface were clearly distinct. At day 4, electrodes closer to the surface showed current densities between 108 and 129 mA/m^2, whereas the deepest electrodes gave higher current densities, in the range of 153–182 mA/m^2. This difference was assumed to be due to the presence of oxygen in the first centimetres of the compost bulk, which disturbed the metabolism of electrochemically active bacteria forming the biofilm on the electrode surface. This is consistent with the electrochemical investigations performed with bacteria isolated from electrochemically active biofilm, which showed that the catalysis of acetate oxidation was certainly achieved by strictly anaerobic bacteria [18]. After day 4, when the aerobic bacteria contained in the compost had consumed the oxygen, the current densities of all electrodes evolved to similar values. Final current densities revealed more diversity for the 15-cm-embedded electrodes with 235, 290 and 545 mA/m^2 than for the 2-cm-embedded electrodes, which gave a final current density around 290 mA/m^2 (Fig. 6). These differences may be attributed to the unequal acetate diffusion between electrodes close to the surface and the more deeply embedded electrode when acetate was added above the surface of the compost. In contrast to the experiments of Figs. 2 and 3, adding acetate to compost already amended with acetate did not cause a rapid rise of the current density, suggesting that, above a certain threshold value of acetate concentration, acetate no longer had an effect on current increase (Fig. 6).

A control experiment was carried out with the same procedure, *i.e.* a 2-cm-deep electrode and a 15-cm-deep electrode in compost sterilized by γ-ray irradiation. Sterilized acetate was also added at days 0 and 2.5. In this case no current increase was observed for 10 days, confirming that the electroactive biofilm was formed with the indigenous flora of the compost.

3.3. Controlling steps of electron transfer

Theoretical conclusions should be drawn with care, as the experimental results proved to be strongly dependent on the batch of compost. Moreover, the experiment described in Figs. 3 and 5 was performed with 5 different electrodes, which were extracted from the compost successively. Measuring the surface coverage ratio, which required staining and operating in air, inevitably destroyed the biofilm electroactivity. Consequently, it was not possible to follow the biofilm coverage and its electrochemical behaviour strictly on the same electrode, but care was taken during the experiment to put the four electrodes in exactly the same configuration with respect to the reference and auxiliary electrodes. Bearing these warnings in mind, a few general comments can be made.

The current recorded under chronoamperometry was clearly limited by the amount of nutrient contained in the compost, as revealed by the immediate current increase caused by acetate addition (Figs. 2 and 3). Similarly, in Fig. 3, the current density decrease that was observed approximately 1 day after each acetate addition (day 4 and days 14–15) can logically be attributed to acetate depletion in the vicinity of the electrode surface. Moreover, each extraction of an electrode (days 5 and 12 in Fig. 3), which induced mixing of the compost, resulted in an immediate current increase. Acetate was certainly depleted in the vicinity of the electrode surface and moving the compost around it enhanced mass transfer from the bulk. Column 4 in Table 1 gives the current density values calculated if the electrode surface was fully covered by the biofilm (100% coverage ratio). The current density increased from 24 mA/m^2 just before acetate addition (day 3) to 10 times that value after acetate addition. Acetate addition consequently had a direct effect on the electron transfer rate. From day 5 to day 12, the current density evaluated for 100% biofilm coverage decreased slightly. Assuming that 8 electrons were released per mole of acetate (Eq. (1)), the current integration up to day 12 showed that only 1 mmol, *i.e.* 10% of the initial amount of acetate, was consumed during this period. This low consumption was consistent with the small decrease in the current calculated for 100% biofilm coverage. In consequence, the current increase observed between days 5 and 12 was not linked to an increase in the electron transfer rate, but was controlled by the increase in biofilm coverage. All these observations led to the first conclusion that the current obtained in chronoamperometry was controlled by the amount of substrate and its mass transfer rate to electrode surface.

In contrast, addition of acetate at day 2.5 had no effect on the 6 electrodes embedded in compost when acetate was present in the compost from the beginning of the chronoamperometry (Fig. 6). In this case, each biofilm supplied around 65–130 mA/m^2 at day 2.5, while biofilms of the same age supplied less than 5 mA/m^2 when the compost was not initially amended with acetate. It may be thought that the initial thorough mixing of acetate in compost ensured a more homogeneous distribution of acetate than pouring the acetate solution on the upper surface of the compost, as was done when acetate was added during the chronoamperometry.

As a second main conclusion, it can be stated that cyclic voltammetry and chronoamperometry addressed definitively different phenomena. The acetate addition at day 3 did not lead to any visible step in the continuous smooth increase of the CV current (Fig. 5B), as was the case for the chronoamperometry current (Fig. 3). Cyclic voltammetry was consequently not affected by the acetate mass transfer limitation. Moreover, between days 12 and 14, the CV current decreased, while the chronoamperometry current was multiplied by a factor of almost 2. Finally, the CV current (after subtraction of the current due to the charge/discharge of the surface oxides of the DSA) reached very high values, up to 2300 mA/m^2. CVs were recorded during the chronoamperometry experiment, just after

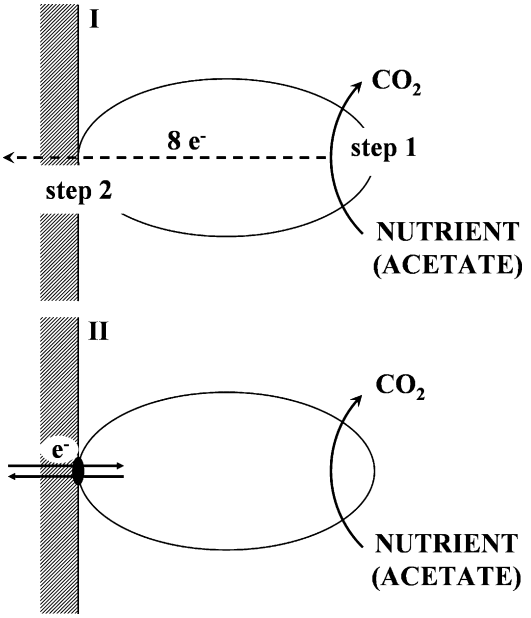

Fig. 7. Scheme of the two controlling steps identified with chronoamperometry and cyclic voltammetry. Pathway I, observed through chronoamperometry, is controlled by the microbial metabolism and affected by acetate mass transfer to the electrochemically active bacteria. Pathway II, observed through cyclic voltammetry, is controlled by fast electron transfer kinetics.

relaxation of the constant polarization, *i.e.* while the diffusion layer was fully established. In consequence, high current density values cannot be explained by transient currents due to the establishment of the diffusion layer, as would be the case for traditional CV recorded in a quiescent liquid with an initial uniform concentration of reactant. CVs more probably indicated here that electron transfer occurred with a species adsorbed on the electrode surface. A clear reduction phenomenon was observed at potential values less than 0.35 V/SCE, indicating that this electron transfer was reversible.

All these comments are summed up in the scheme presented in Fig. 7. The biofilm-driven catalysis of the oxidation of acetate appeared to be controlled by two different steps that corresponded to two different time-scales: the oxidation of acetate through the microbial metabolism (step 1) and the electron transfer between microbial cells and the electrode surface (step 2). Step 1, which corresponds to microbial kinetics, controlled the long-term chronoamperometry, and was obviously affected by acetate limitation and biofilm coverage (pathway I in Fig. 7). Step 2, which corresponds to electrochemical kinetics, controlled the fast transient behaviour that was addressed by CV (pathway II in Fig. 7). In this case, electron transfer depended solely on the transient capacity of bacteria to exchange the electrons stored in their internal electron transfer system with the electrode, a mechanism that was not affected by acetate diffusion. Indeed, scanning the potential range from 0.20 to 0.90 V/SCE at a rate of 10 mV/s lasted 70 s. During this short time, step 1, controlled by microbial metabolism kinetics, could not have an influence on electron transfer. CVs were consequently not sensitive to acetate limitation but only to the redox state of the cells.

4. Conclusions

Adding acetate to garden compost proved to be a promising way of encouraging the development of efficient electrochemically active biofilms, as has been previously observed with other sources of microorganisms. Coupling chronoamperometry, measurement of biofilm coverage ratios, and CV led us to propose a new mechanism that should now be more thoroughly assessed. It may also be supposed that acetate caused enrichment of the biofilm in electrochemically active microorganisms, similarly to what has been demonstrated previously with biofilms formed on the anode of a microbial fuel cell. When fed with a complex fuel, biofilm showed a more diverse bacterial distribution than when fed with acetate only [10]. Growing electroactive biofilms with acetate-amended compost will now be used to identify and to isolate the bacteria responsible for electron transfer with a view to reproducing the microbial electrocatalysis in well controlled conditions with pure microbial strains.

Acknowledgements

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