Forming electrochemically active biofilms from garden compost under chronoamperometry

Sandrine Parot, Marie-Line Délia, Alain Bergel *

Laboratoire de Génie Chimique-CNRS-INPT, 5 Rue Paulin Talabot, 31106 Toulouse, France

Abstract

Dimensionally stable anodes (DSA) were polarized at different constant potential values for several days in garden compost. After an initial lag period ranging from 1 to 10.5 days, the current increased fast and then stabilized for days. Current densities higher than 100 mA m^{-2} and up to 385 mA m^{-2} were obtained with the sole organic matter contained in compost as substrate. Control experiments performed with sterilized compost, oscillations of the current with the temperature, kinetics of the exponential phase of current increase and observations of the surface of electrodes by epifluorescence microscopy showed that the current was controlled by the colonization of the electrode surface by a biofilm which originated the indigenous flora of compost. Three individually addressed electrodes polarized at different potentials in the same reactor led to identical current evolutions on each electrode, which underlined the key role of the microbial flora of the compost in the discrepancy observed in the other experiments. Chronoamperometry revealed a promising technique to check natural environments for new electrochemically active microbial species.

Keywords: Electrochemically active biofilm; Biofilm electrochemistry; Compost; Dimensionally stable anode; Chronoamperometry

1. Introduction

It has been recently demonstrated that several microorganisms have the ability to form efficient electro-catalytic biofilms on electrode surfaces. In several cases the electrocatalytic properties of biofilms have been clearly related to the presence of some strains that are able to exchange electrons with the electrode either directly (*Geobacter sulfurreducens*, *Rhodoferax ferrireducens*, etc.) or through natural electron mediators (*Shewanella* spp., *Geothrix fermentans*, etc.) (Lovley, 2006). Nevertheless, most studies deal with complex microbial consortia, which are implemented in microbial fuel cells (MFCs). MFCs have been set-up in natural sites such as marine or freshwater sediments (Holmes et al., 2004; Tender et al., 2002) or in laboratories with inoculums coming from a wide range of different microbially-rich environments: anaerobic sewage sludge (He et al., 2005; Jong et al., 2006), activated sludge (Kim et al., 2004; Rabaey et al., 2004), industrial and domestic effluents (Heilmann and Logan, 2006; Logan, 2005), and animal waste (Min et al., 2005). At the moment natural soils have not been studied as a potential source of electrochemically active microorganisms yet, but it has been reported that heat treated soils could be used successfully as a source of spore forming bacteria that produce hydrogen (Niessen et al., 2006; Rosenbaum et al., 2006).

The purpose of this work was to propose a chronoamperometry procedure suited for identifying electrochemically active biofilms in natural environments. Until now chronoamperometry has been implemented only very rarely to investigate natural environments, although it should avoid any disturbance and artefact due to the second electrode or the internal resistance of the set-up, as may be the case with microbial fuel cells. As microbial biofilms are involved in many biogeochemical cycling of metals (Fe, Mn, and trace metals) it was expected that compost would constitute a

* Corresponding author. Tel.: +33 (0) 5 34 61 52 48; fax: +33 (0) 5 34 61 52 53.
E-mail address: Alain.Bergel@ensiacet.fr (A. Bergel).

promising environment for investigation. Moreover a preliminary study by our group has suggested that garden compost may be a pertinent environment in which to find electrochemically active biofilms (Dulon et al., 2007). Experiments were run under well-controlled electrochemical conditions, by imposing a constant anodic potential through a traditional three electrodes system. Most previous studies have been carried out with graphite and carbon anodes, with differing morphologies (cloths, foams, etc.) and sometimes modified with catalytic compounds. Here, dimensionally stable anode (DSA) electrodes were used. This kind of electrode made of titanium covered by iridium and tantalum oxides has been designed to ensure high electro-catalytic activity (Trasatti, 2000), and they have proved very efficient in many industrial oxidation processes (Comninellis and Nerini, 1995; de Andrade et al., 1998; Menini et al., 2005). Because of their rough surface and their optimal electro-catalytic properties it was expected that DSA would be an efficient tool to detect new electrochemically active biofilms.

2. Methods

2.1. Chemicals and materials

The compost for biological cultivation (Eco-Terre) was purchased from a garden centre. 2 L of a sodium chloride NaCl 10 mM (S-9625, Sigma) solution were mixed with compost to obtain a 3 L final working volume. The temperature in the reactor was measured with a PT100 temperature sensor (Sofraico), the oxygen concentration with a Mettler-Toledo Ingold InPro 6800 O_2 sensor (detection limit 6 ppb) and pH with a Radiometer Analytical PHM210 standard pH Meter. All measurements were carried out at room temperature. Control experiments were performed in compost sterilized by 20-min autoclaving at 121 °C.

2.2. Electrochemical measurements

A conventional three-electrode system was implemented with a multi-potentiostat (VMP2 Bio-Logic SA) interfaced to a computer (software EC-Lab v.8.3, Bio-Logic SA). The working electrode was a $10 \times 2.5 \times 0.1$ cm dimensionally stable anode electrode (DSA®, Electro Chemical Service) with a 20 cm^2 working surface area. 8 cm of the DSA were embedded vertically in the compost and 2 cm were above the level of the compost and connected with a flat alligator clip. Before each experiment, the electrode was cleaned by 5-h galvanostatic electrolysis at 20 mA cm^{-2} in a 0.1 M sulphuric acid solution. The counter electrode was a 35 cm^2 surface area graphite rod (Alfa Aesar, stock#10134). All potentials were monitored against a saturated calomel standard reference electrode (Radiometer Analytical, TR100) protected with a second porous frit. For experiments carried out with three DSA electrodes in the same compost reactor, a system was used which made it possible to individually address three working electrodes with respect to one reference electrode and one counter electrode (Bio-Logic SA).

For control experiments, electrodes were cleaned with ethanol and rinsed with distilled water.

3. Results

3.1. Current generation in compost

The general procedure used here for the formation and detection of electrochemically active biofilms consisted in chronoamperometry at constant potential. A constant potential value was imposed for days to DSA electrodes (surface area 20 cm^2) dipped in compost mixed with a 10 mM NaCl solution and the current evolution was monitored as a function of time. Twelve experiments were spread over a period of one year with different batches of the same brand of compost (Table 1). Chronoamperometries were performed either with only one working electrode in 3 L reactors (experiments I–IV and VII–XII in Table 1), or with working electrodes set-up in 10 L reactors and electrically individually addressed (experiments V–VII in Table 1). Fig. 1 shows four chronoamperometries characterizing the current density increase (experiments I–IV in Table 1). The typical pattern of the current evolution remained unchanged whatever the potential value, with 3 successive steps: a lag period for around 5 days, a rapid increase and a stabilization phase, which could go on for 10 days. Five parameters were listed to describe the current density evolution (Table 1). The current density was considered to start to increase when it rose to more than 1 mA m^{-2} in 1 day (D_{start} in column 3). Column 4 gives the maximal current density reached and the corresponding date. The average value of current density at final plateau and the total duration of the stabilization period are reported in column 5. The parameter D_{start} varied within a range of 1–10.5 days, but all experiments led to significant current increase. Maximal current density was on average around 105 mA m^{-2} for the 10 electrodes polarized at 0.50 V/SCE, and it went up to 385 mA m^{-2} (experiment VIII). The stabilization phase presented quite different features. In some cases current remained close to the maximal value for days, while it sometimes decreased continuously from the maximal value (experiment VI). The variation in the compost batches and in operating conditions certainly explains these discrepancies. It is important to note that 3 the electrodes dipped in a single reactor and independently polarized at 0.50 V/SCE (experiment V) showed exactly the same evolution and gave equal values of current and D_{start}, showing that the environmental conditions (composition of the compost, organic matter available, indigenous flora, temperature and hygrometry during the experiments, etc.) controlled the current increase to a large extent.

The pH of the environment was initially 7.7 and dropped to 7.4 after the current increase. After 1 day, the value of the concentration of oxygen in the reactor was

Table 1
Current density characteristics determined from 12 chronoamperometries achieved with DSA electrodes embedded in compost (NaCl 10 mM added) at 0.10, 0.30, 0.40, 0.50 and 0.70 V/SCE

Experiments (roman numerals) and electrodes		Polarization (V/SCE)	Start of current increase D_{start} (day)	Maximum current density (mA m^{-2}); date of maximal current (day)	Average value at stable state (mA m^{-2}); duration of stabilization (days)
I		0.10	5	51; 17	49; 10
II		0.40	6	24; 12.5	21; 7
III		0.50	4	62; 19	52; 6.5
IV		0.70	5	70; 17	66; 4
V	Electrode 1	0.50	3	105; 9	67; 4.5
	Electrode 2	0.50	3	106; 9	68; 4.5
	Electrode 3	0.50	3	106; 9	68; 4.5
VI	Electrode 1	0.10	4	17; 13	No stabilization
	Electrode 2	0.50	4	19; 13	No stabilization
	Electrode 3	0.50	4	19; 13	No stabilization
VII	Electrode 1	0.30	10	145; 13.5	129; 2
	Electrode 2	0.50	10	128; 13.5	114; 2
	Electrode 3	0.70	10	92; 15.5	88; 2
VIII		0.50	4	385; 9	229; 9
IX		0.50	1	53; 3	36; 2.5
X		0.70	No significant increase		
XI		0.50	5	62; 10	59; 2
XII		0.70	8	76; 18	Electrode removed before stabilization

Experiments V–VII were performed with 3 electrodes in the same reactor.

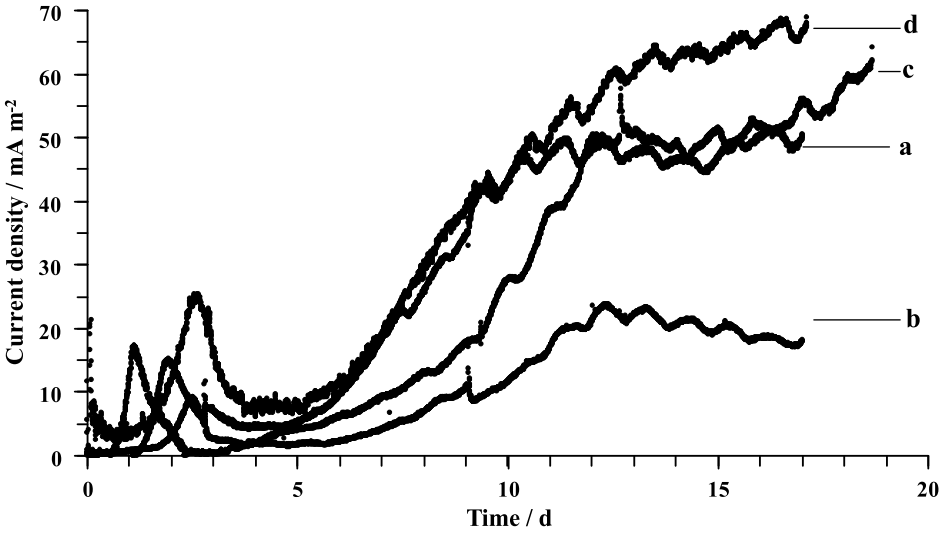

Fig. 1. Current density plotted against time for 4 DSA electrodes polarized at 0.10; 0.40; 0.50 and 0.70 V/SCE (curves a–d, respectively) in 4 different reactors containing compost and NaCl 10 mM (experiments I–IV).

inferior to the detection threshold of the sensor (6 ppb). The compost was hence considered as an anaerobic environment. At the end of current increase, epifluorescent microscopy images showed that the electrode surface was fully covered by a biofilm composed by bacteria which formed colonies spread all over the electrode (data not shown). A temperature sensor put in the compost in experiment VI revealed a complete correspondence between the temperature oscillations and those of the current density (Fig. 2). The oscillations that were often observed during the current density increase (Fig. 1 for instance) were therefore due to the circadian temperature variations.

Four independent control experiments were performed at 0.10, 0.40, 0.50, 0.70 V/SCE (Fig. 3) with compost which was preliminary sterilized by autoclaving. A low reduction current was observed at 0.10 V/SCE while oxidation occurred at more positive potential values (inset in Fig. 3), but the current density dropped close to zero in two days. No current was then obtained even after 10 days whatever the polarization. The presence of the indigenous flora was consequently required in order that the electrochemical oxidation occurred.

3.2. Influence of polarization

In order to avoid the discrepancies due to the different compost batches, the influence of polarization on the current increase was studied with three working electrodes

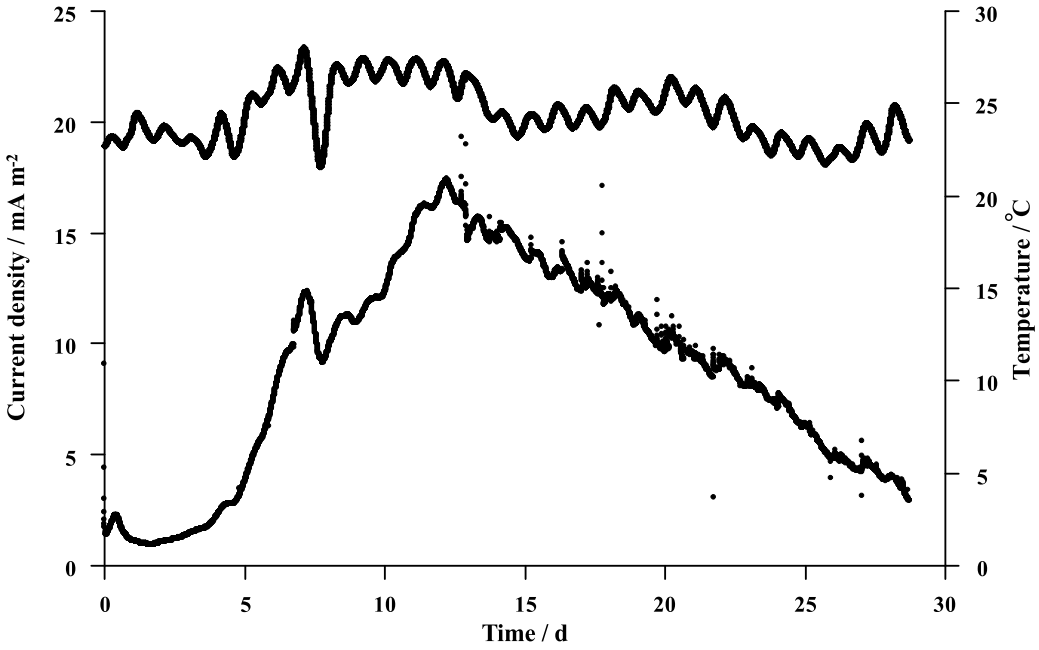

Fig. 2. Temperature and current density evolution plotted against time in a reactor containing compost and a DSA electrode polarized at 0.50 V/SCE (experiment VI). The *Y*-axis on the left represents the current density in mA m^{-2} (curve down) and the *Y*-axis on the right represents the temperature in Celsius degrees (curve up).

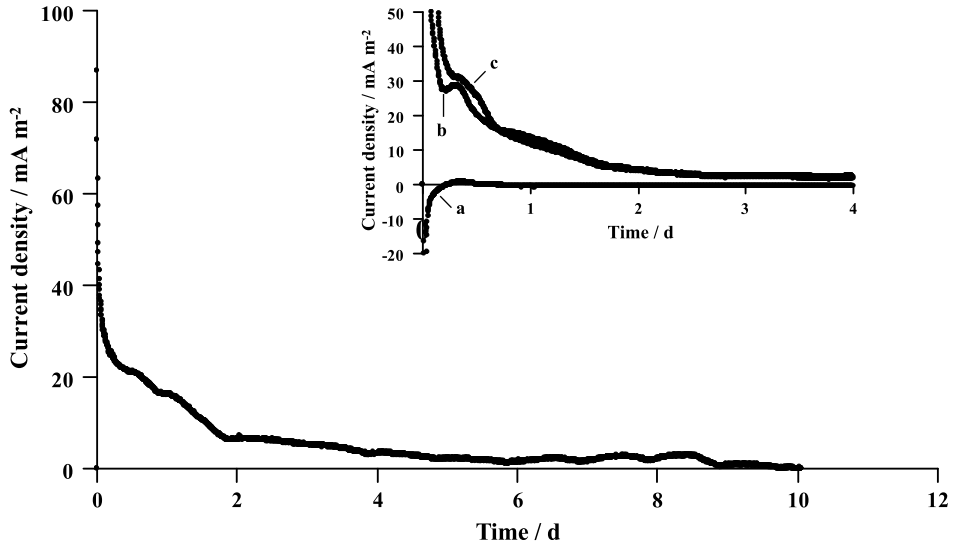

Fig. 3. Current density evolution plotted against time with a DSA electrode polarized at 0.50 V/SCE embedded in sterilized compost. Inset gives the beginning of the control experiments, before current density reached 0 mA m^{-2}, for polarization values of 0.10 V/SCE (curve a), 0.40 V/SCE (curve b), and 0.70 V/SCE (curve c).

individually addressed in a single 10 L reactor (experiment VII) and polarized at 0.70, 0.50 and 0.30 V/SCE. For the three electrodes there was an identical initial lag phase of 10 days and then the current increased from the 10th to the 13th day. Stabilization occurred during the last two days (Fig. 4). The general shape of the curves was similar for the three electrodes. The potential value actually had only a significant effect on the final current density values: on average 129, 114 and 88 mA m^{-2} at the stable plateau for 0.30, 0.50 and 0.70 V/SCE respectively (these average values are different from the values given in Table 1 that reports the maximum values).

As seen in Table 1, the polarization performed at 0.50 V/SCE provided the most reproducible values of current density, whereas polarizations at 0.70 V/SCE did not supply higher currents or were sometimes found to produce lower or slower current increase. Experiments IX and X were run in parallel at the same time with the same batch of compost, but in two independent reactors. In experiment X the DSA polarized first at 0.70 V/SCE during 8 days

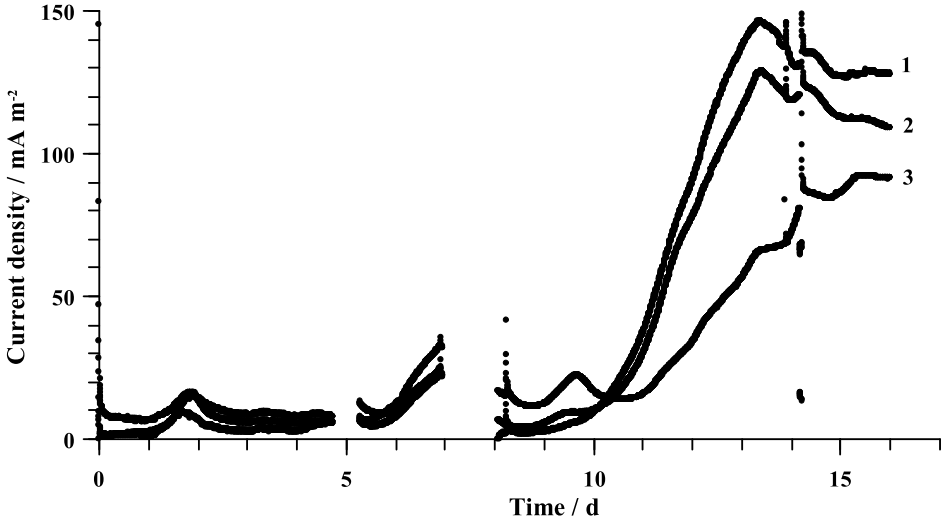

Fig. 4. Current density plotted against time from current recorded with 3 individually addressed DSA electrodes dipped in the same compost reactor. Curves 1–3: imposed potential values were 0.30; 0.50 and 0.70 V/SCE, respectively (experiment VII).

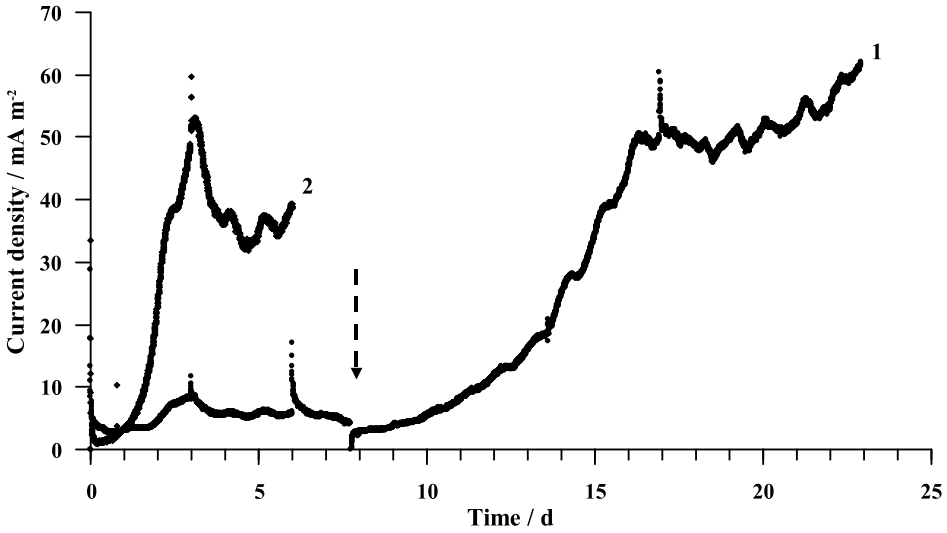

Fig. 5. Effect of polarization on current density evolution. Curve 1 (experiment X) the imposed potential initially set to 0.70 V/SCE was switched to 0.50 V/SCE at day 7 (arrow). Curve 2 (experiment IX) the imposed potential was 0.50 V/SCE from the beginning. Both experiments were performed concurrently in two different reactors.

gave a weak current density (Fig. 5). Then, when the potential value was switched to 0.50 V/SCE (arrow in Fig. 5), the current density increased for more than 8 days and reached 60 mA m^{-2} only 15 days after the polarization change. On the contrary, in experiment IX the electrode initially polarized at 0.50 V/SCE showed a fast current increase from the first day to around 50 mA m^{-2} on day 3. This confirmed that the potential value of 0.50 V/SCE favoured the formation of electrochemically active biofilm more consistently than higher values of potential.

4. Discussion

The general trend of current evolution recorded with DSA electrodes polarized in compost was coherent with the four-phase pattern growth that is characteristic of the formation of a bacterial biofilm (Costerton et al., 1999; Watnick and Kolter, 2000). The initial lag phase of 1–10.5 days (Table 1) was consistent with the first phase of bacteria approach and attachment to the electrode surface. During this period, the bacteria were adapting to the sessile conditions at the surface and to the local physicochemical conditions induced by polarization. The compactness of the medium and certainly local heterogeneity in composition and bacterial contents may explain the difference in length of this step. In microbial fuel cells, biofilm formation has often been reported to be longer, between 6 and 15 days (Chaudhuri and Lovley, 2003; Jang et al., 2004; Liu et al., 2005), therefore the compost may be the source of microorganisms that are able to adapt pretty fast to the anode conditions. A transient peak often appeared during the initial lag phase (easily visible in Fig. 1 between

days 1 and 4, or in Fig. 4 before day 2, for instance) when the compost turned anaerobic. This peak might be therefore due to the oxidation of some compounds produced by the anaerobic bacterial metabolism, in the same manner that sulphide compounds resulting from the sulphate reducing bacteria metabolism are oxidized in sediments.

The current increase that followed the lag period corresponded to the active growth phase of the biofilm and a stationary phase occurred when the surface coverage was maximal or the nutrient supply became limiting. The experiments did not last long enough to enter the fourth phase of biofilm decline, but the final slow current decrease was more likely due to substrate depletion. For instance, the current decrease of a few tenths of milliamps that was generally observed during one day just after reaching the maximal value may express the slow depletion of substrate in the vicinity of the electrode surface (Figs. 4 and 5). It is consistent with what was previously observed when the addition of acetate (10 mM) to compost as a supplementary nutrient caused a rapid rise of the current density (Dulon et al., 2007).

The microbial origin of the catalysis was confirmed by the control experiments performed after compost autoclaving, which did not result in current increase whatever the polarization value. The weak current observed at the beginning of the polarization, which decreased quickly (Fig. 3), may be due to the formation by autoclaving of a compound that directly reduced on the DSA at 0.10 V/SCE (negative current, see curve a in inset of Fig. 3) and oxidized at higher potential values (positive current, see curves b and c in inset of Fig. 3). This was similar to what has previously been observed in autoclaved aquatic sediments with graphite anodes where current has been measured during the first hours of polarization (Holmes et al., 2004).

The small circadian oscillations of the current (Fig. 2) have also been observed with electrochemically active biofilms formed from drinking water on platinum micro-electrodes (Dulon et al., 2007). It perfectly correlated with the high influence of the temperature on microbial metabolisms. All these observations confirmed that the electrochemical process was controlled by the microbial activity of the biofilms formed from the indigenous flora of compost. The biofilm revealed itself able to oxidize organic compounds present in compost and to transfer the electrons to the DSA electrode.

Considering all experiments, it appeared that polarization at 0.70 V/SCE gave smaller currents (experiment VII) or longer initial lag phase (experiment X). It may be speculative to compare other experiments that were not achieved simultaneously and with exactly the same compost batch, as the dispersion due to environmental conditions does not allow to drawn firm conclusions on the effect of potential. Nevertheless, the results obtained at 0.70 V/SCE were never significantly better than at 0.50 V/SCE and appeared less reproducible. Optimal conditions to be sure to form an electrochemically active biofilm were hence to impose a potential around 0.50 V/SCE. Similarly, it has already been demonstrated that the polarization of gold electrodes influenced the morphology and the biofilm structure of *Pseudomonas fluorescens* which developed on their surface (Busalmen and de Sánchez, 2005). In this case there was no electron transfer between electrode and bacteria, but it was observed that the biofilm developed at 0.50 V vs. Ag/AgCl following the same phases as observed here in compost: a lag phase during 30–40 min followed by exponential growth. On the contrary, polarization at 0.80 V vs. Ag/AgCl stopped the biofilm development.

For further considerations on polarization influence, it was supposed that the current I is proportional to the biofilm growth. In these conditions the traditional microbial growth equation:

$$dX/dt = \mu X$$

where X represents the biomass concentration and μ the specific growth rate, can be expressed through the current I:

$$dI/dt = \mu I$$

The specific growth rate μ (d^{-1}) was calculated from the current obtained with the 3 electrodes polarized at 0.30, 0.50 and 0.70 V/SCE in the same reactor in order to avoid the discrepancies due to the different compost batches (experiment VII). Plotting the logarithm of current density against time for the increasing phase (starting around day 10 for 0.30 and 0.50 V/SCE and day 11 for 0.70 V/SCE) gave $\mu = 1.2\ d^{-1}$ (straight line a in Fig. 6) for the potential values 0.30 and 0.50 V/SCE, while polarization at 0.70 V/SCE led to $\mu = 0.8\ d^{-1}$. These values are fully coherent with traditional bacterial growth rates (Bailey and Ollis, 1986). Electrochemically active biofilms formed on graphite anodes polarized at 0.20 V vs. Ag/AgCl from pure culture of *Geobacter sulfurreducens* have also shown specific growth rate around $0.96\ d^{-1}$ during their exponential increasing phase (Bond and Lovley, 2003). As observed in traditional microbial cultures, the curve derived from the linear regression due to nutrient limitation. A common value $\mu = 0.4\ d^{-1}$ fitted this nutrient-deprived phase, whatever the potential value imposed to the electrode (straight line b in Fig. 6). It may be concluded that the growth phase of the electrochemically active biofilms revealed to be fully controlled by biochemical parameters and to be independent of the potential as long as too high value (0.70 V/SCE) did not interfere in its formation.

The current densities often higher than 100 mA m^{-2} and up to 385 mA m^{-2} obtained without any addition of substrate in the compost were among the highest values reported previously for compact media. Current density of 34 mA m^{-2} (Holmes et al., 2004) have been obtained with three different sediment types in laboratory MFC, and values around 40 mA m^{-2} (Ryckelynck et al., 2005) and 100 mA m^{-2} (Tender et al., 2002) have been reached with graphite electrodes embedded *in situ* in marine sediments. Obviously, higher current densities have been obtained in liquid media. Chronoamperometry performed

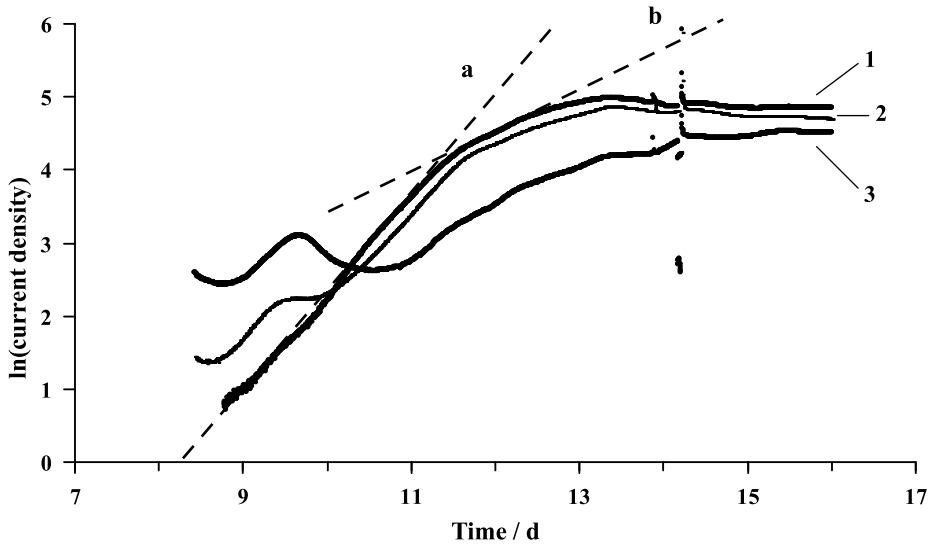

Fig. 6. Calculation of the specific growth rate coefficient μ for 3 DSA electrodes in the same reactor (experiment VII) and polarized at curve 1: 0.30 V/SCE; curve 2: 0.50 V/SCE; curve 3: 0.70 V/SCE. Dotted straight lines indicate the linear regression curves.

with graphite electrodes in pure cultures of microorganisms isolated from sediments have given a large range of results such as 165 mA m^{-2} with *Desulfuromonas acetoxidans*, 268 mA m^{-2} with *Geobacter metallireducens* (Bond et al., 2002) and even 1143 mA m^{-2} with *Geobacter sulfurreducens* (Bond and Lovley, 2003). Actually, it seems that some of the highest current densities, higher than 16 A m^{-2} (Niessen et al., 2006) or 30 A m^{-2} (Rosenbaum et al., 2006), have been achieved with spore forming bacteria coming from heat treated compost. Heat treatment was a way to select hydrogen producing microorganisms that were then used in solution. The hydrogen produced in solution was oxidized abiotically on platinum or tungsten carbide anodes. The principle of the fuel cell was therefore basically different from work presented here, as it did not implement a direct biofilm-driven catalysis.

5. Conclusion

This study confirms that soils are an interesting and easy-to-handle source of electrochemically active microorganisms which is "*available everywhere, free of charge*" (Niessen et al., 2006). Work is now in progress to identify and isolate the predominant bacterial species that form the most efficient biofilms. It should be noted that none of the 18 chronoamperometry experiments failed in forming electrochemically active biofilms. Chronoamperometry can be considered as a promising tool to drawn new electrochemically active biofilms from all kind of natural and industrial environments that has not been checked yet.

Acknowledgements

The authors are most grateful for technical contributions from Luc Etcheverry, engineer CNRS, Laboratoire de Génie Chimique (Toulouse, France), and for the kind advice from Pr. André Savall and Dr. Karine Groenen-Serrano from Laboratoire de Génie Chimique, Université Paul Sabatier (Toulouse, France). They deeply thank Dr. Alfonso Mollica from CNR-ISMAR (Genoa, Italy) for the design of the electrochemical set-up and for his efficient advice. This work was part of the EA-Biofilms project supported by European Community (FP6-NEST508866).

References

Bailey, J.E., Ollis, D.F., 1986. Biochemical Engineering Fundamentals, second ed. McGraw-Hill, New York.

Bond, D.R., Holmes, D.E., Tender, L.M., Lovley, D.R., 2002. Electrode-reducing microorganisms that harvest energy from marine sediments. Science 295, 483–485.

Bond, D.R., Lovley, D.R., 2003. Electricity production by *Geobacter sulfurreducens* attached to electrodes. Appl. Environ. Microbiol. 69, 1548–1555.

Busalmen, J.P., de Sánchez, S.R., 2005. Electrochemical polarisation-induced changes in the growth of individual cells and biofilms of *Pseudomonas fluorescens* (ATCC 17552). Appl. Environ. Microbiol. 71, 6235–6240.

Chaudhuri, S.K., Lovley, D.R., 2003. Electricity generation by direct oxidation of glucose in mediatorless microbial fuel cells. Nat. Biotechnol. 21, 1229–1232.

Comninellis, Ch., Nerini, A., 1995. Anodic oxidation of phenol in the presence of NaCl for wastewater treatment. J. Appl. Electrochem. 25, 23–28.

Costerton, J.W., Stewart, P.S., Greenberg, E.P., 1999. Bacterial biofilms: a common cause of persistent infections. Science 284, 1318–1322.

de Andrade, A.R., Donate, P.M., Alves, P.P.D., Fidellis, C.H.V., Boodts, J.F.C., 1998. Ethanol electro-oxidation in ruthenium-oxide-coated titanium electrodes. J. Electrochem. Soc. 145, 3839–3843.

Dulon, S., Parot, S., Délia, M-L., Bergel, A., 2007. Electroactive biofilms: new means for electrochemistry. J. Appl. Electrochem. 37, 173–179.

He, Z., Minteer, S.D., Angenent, L.T., 2005. Electricity generation from artificial wastewater using an upflow microbial fuel cell. Environ. Sci. Technol. 39, 5262–5267.

Heilmann, J., Logan, B.E., 2006. Production of electricity from proteins using a microbial fuel cell. Water Environ. Res. 78, 531–537.

Holmes, D.E., Bond, D.R., O'Neil, R.A., Reimers, C.E., Tender, L.R., Lovley, D.R., 2004. Microbial communities associated with electrodes

harvesting electricity from a variety of aquatic sediments. Microb. Ecol. 48, 178–190.

Jang, J.K., Pham, T.H., Chang, I.S., Kang, K.H., Moon, H., Cho, K.S., Kim, B.H., 2004. Construction and operation of a novel mediator- and membrane-less microbial fuel cell. Process Biochem. 39, 1007–1012.

Jong, B.C., Kim, B.H., Chang, I.S., Liew, P.W.Y., Choo, Y.F., Kang, G.S., 2006. Enrichment, performance, and microbial diversity of a thermophilic mediatorless microbial fuel cell. Environ. Sci. Technol. 40, 6449–6454.

Kim, B.H., Park, H.S., Kim, H.J., Kim, G.T., Chang, I.S., Lee, J., Phung, N.T., 2004. Enrichment of microbial community generating electricity using a fuel-cell-type electrochemical cell. Appl. Microbiol. Biotechnol. 63, 672–681.

Liu, H., Cheng, S., Logan, B.E., 2005. Production of electricity from acetate or butyrate using a single-chamber microbial fuel cell. Environ. Sci. Technol. 39, 658–662.

Logan, B.E., 2005. Simultaneous wastewater treatment and biological electricity generation. Water Sci. Technol. 52, 31–37.

Lovley, D.R., 2006. Bug juice: harvesting electricity with microorganisms. Nat. Rev. Microbiol. 4, 497–508.

Menini, R., Henuset, Y.M., Fournier, J., 2005. Electrochemical oxidation of phenolic compounds using a flow-through electrolyser with porous solid electrodes. J. Appl. Electrochem. 35, 625–631.

Min, B., Kim, J.R., Oh, S.E., Regan, J.M., Logan, B.E., 2005. Electricity generation from swine wastewater using microbial fuel cells. Water Res. 39, 4961–4968.

Niessen, J., Harnisch, F., Rosenbaum, M., Schröder, U., Scholz, F., 2006. Heat treated soil as convenient and versatile source of bacterial communities for microbial electricity generation. Electrochem. Commun. 8, 869–873.

Rabaey, K., Boon, N., Siciliano, S.D., Verhaege, M., Verstraete, W., 2004. Biofuel cells select for microbial consortia that self-mediate electron transfer. Appl. Environ. Microbiol. 70, 5373–5382.

Rosenbaum, M., Zhao, F., Schröder, U., Scholz, F., 2006. Interfacing electrocatalysis and biocatalysis using tungsten carbide: a high performance noble-metal-free microbial fuel cell. Angew. Chem. 118, 1–4.

Ryckelynck, N., Stecher III, H.A., Reimers, C.E., 2005. Understanding the anodic mechanism of a seafloor fuel cell: interactions between geochemistry and microbial activity. Biogeochemistry 76, 113–139.

Tender, L.M., Reimers, C.E., Stecher III, H.A., Holmes, D.E., Bond, D.R., Lowy, D.A., Pinobello, K., Fertig, S.J., Lovley, D.R., 2002. Harnessing microbially generated power on the seafloor. Nat. Biotechnol. 20, 821–825.

Trasatti, S., 2000. Electrocatalysis: understanding the success of DSA®. Electrochim. Acta 45, 2377–2385.

Watnick, P., Kolter, R., 2000. Biofilm, city of microbes. J. Bacteriol. 182, 2675–2679.