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Specific and efficient electrochemical selection of *Geoalkalibacter subterraneus* and *Desulfuromonas acetoxidans* in high current-producing biofilms

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Abstract

Two different saline sediments were used to inoculate potentiostatically controlled reactors (a type of microbial bioelectrochemical system, BES) operated in saline conditions (35 g_{NaCl} l^{-1}). Reactors were fed with acetate or a mixture of acetate and butyrate at two pH values: 7.0 or 5.5. Electroactive biofilm formation lag-phase, maximum current density production and coulombic efficiency were used to evaluate the overall performance of reactors. High current densities up to 8.5 A m^{-2} were obtained using well-defined planar graphite electrodes. Additionally, biofilm microbial communities were characterized by CE-SSCP and 454 pyrosequencing. As a result of this procedure, two anode-respiring bacteria (ARB) always dominated the anodic biofilms: *Geoalkalibacter subterraneus* and/ or *Desulfuromonas acetoxidans*. This suggests that a strong electrochemically driven selection process imposed by the applied potential occurs in the BES system. Moreover, the emergence of *Glk. subterraneus* in anodic biofilms significantly contributes to broaden the spectrum of high current producing microorganisms electrochemically isolated from environmental samples.

Key words: Microbial bioelectrochemical systems, 454 pyrosequencing, *Geoalkalibacter subterraneus, Desulfuromonas acetoxidans*, saline wastewater

1 Introduction

Microorganisms embedded in electroactive biofilms convert organic matter into electrical current through electron transfer mechanisms occurring in microbial bioelectrochemical systems (BES). Such microorganisms are also commonly referred to as anode respiring bacteria (ARB) due to their ability to transfer electrons to an electrode material via direct or indirect electron transfer. ARB includes representatives of α, β, γ, δ-*Proteobacteria*, *Firmicutes*, *Acidobacteria* and *Actinobacteria* which are able to transfer electrons to electrode materials [1]. While urban/domestic wastewater (WW) is a common source of microorganisms for the development of electroactive biofilms in BESs, its application is consequently limited to the treatment of domestic WW [2]. Hence, the selection of halophilic ARB is an essential prerequisite for the treatment of saline WWs which represent more than 5% of the WWs generated worldwide and considered a high risk effluent for soils, surfaces and groundwater salinization [3]. Although it is well known that saline conditions increase the conductivity of electrolyte solutions and thus facilitate the proton transport with the consequent increase of the overall BES performance [3], this has been rarely tested. Liu *et al.* observed that an increase from 1.7 to 6.8 g_{NaCl} l^{-1} induced an 85% increase in power densities in domestic wastewater-based BESs [4]. More recently, Lefebvre *et al.* reported an increase of power densities by progressively using higher concentrations of NaCl from 0.0 up to 20.0 g_{NaCl} l^{-1} [3]. However, higher salinity concentrations proved to be detrimental to the overall performance of BESs in those studies. Nevertheless, such cited studies were conducted with domestic WW as inoculum and the ARB enriched were highly impacted at relatively low salinities. Accordingly, saline sediments could be a better source of suitable halophilic ARB [5-7], an issue scarcely investigated. Erable *et al.* obtained a current production of 2.5 A m^{-2} using a natural marine biofilm on a graphite electrode polarized at -0.1 V vs. SCE and fed with 10mM acetate under saline conditions (20 g_{NaCl} L^{-1}) [8]. Recently, Rousseau *et al.* observed a sharp increase in the current density up to 85 A m^{-2} in a potentiostatically-controlled system (a type BES where the electrodes' potential is controlled by a potentiostat). Their BES was operated under saline conditions and fed with 40 mM acetate at 45 g_{NaCl} l^{-1} by developing an electroactive biofilm on a porous carbon felt anode [9]. A decrease of the current production was observed at 60 g_{NaCl} l^{-1} despite a significant performance (30 A m^{-2}). Unfortunately, little was known about the microbial community involved. The present work aims at characterizing the microbial community structure in high current-producing biofilms formed from saline sediments. We investigated the strength of the selection pressure occurring in BESs under different experimental conditions such as pH and electron donor.

2 Materials and methods

2.1 General experimental procedure and description of Phase 1 and 2

The present work is part of a project aiming at feeding BESs with dead-end metabolites resulting from the production of hydrogen by dark fermentation effluents. Consequently, the tested pH values were acidic or neutral to compare conditions of dark fermentation and BESs, respectively. In order to study the uptake of metabolites from a dark fermentation effluent, the main organic acids were used here. To get close to characterisation of dark fermentation effluent composition in saline conditions [14], acetate alone or a mixture of acetate and butyrate were used as electron donors. In the case of reactors fed with a mixture of acetate and butyrate, a complete conversion of butyrate into acetate was firstly observed. Then, acetate consumption started. For all experimental units, biofilm sampling took place at the maximum current density. Therefore, no complete substrate removal was observed (data not shown). Experiments were divided in two phases. Phase 1 focused on the effects of pH and substrates on biofilm formation

and performance. Thus, a set of experiments was carried out using one saline sediment as inoculum (Inoculum 1) in potentiostatically-controlled reactors (hereafter, reactors) operated under different experimental conditions, *i.e.*: pH 7.0 or 5.5 and acetate only or acetate/butyrate as carbon sources (please see Table 1). Once an experimental protocol was established in the laboratory, a second set of experiments was conducted. This series, called Phase 2, was focused on the effect of inoculum on biofilm formation and performance. Therefore, two different saline sediments (Inoculum 1 and 2) were tested under the best conditions to produce current identified in Phase 1, *i.e.*: pH 7.0 and acetate only as carbon source. Finally, biofilms were analyzed at the maximal current density production to describe the microbial selection within the anodic biofilm.

2.2 Inocula source

The two microbial inocula used here were sampled from two different locations: Inoculum 1 corresponded to sediments collected at the salt lake of Gruissan (France) and Inoculum 2 corresponded to sediments from a lagoon collecting the wastewaters of a salt factory (Salins de Saint Martin, France). Both inocula presented similar physicochemical properties, *i.e.*, pH of 7.8±0.2; 36.1±3.5 gram of volatile solids per gram of sediments and a conductivity of 93.6±12.1 mS cm^{-1}. Those physicochemical similarities and operating conditions might explain why regardless of the precedence of the inocula, similar ARB were highly enriched within the anodic biofilms (Table 1).

2.3 Bioelectrochemical set-up and operating conditions

Reactor design and materials were exactly the same as those described by Carmona-Martínez *et al.* [10]. In brief, experiments were carried out using electrochemical reactors under potentiostatic control (VSP Bio-Logic SA) monitored with a computer (EC Laboratory v.10.1 software, Bio Logic SA). The set-up consisted of a working electrode (graphite plate), a SCE reference electrode (KCl 3.0 M, +240 mV vs. SHE, Materials Mates, La Guilletière 38700 Sarcenas, France) and a counter electrode (platinum grid). The working electrode, *i.e.* the anode, was a graphite planar electrode with the following dimensions: 2.5x2.5x0.2 cm (C000440/15, Goodfellow SARL). To ensure an electrical connection, a 2 mm diameter and 12 cm long titanium rod (TI007910/13, Goodfellow SARL) was used. The counter electrode, *i.e.* the cathode, was a Platinum Iridium grid (90%/10%) (Heraeus) cleaned by heating in a blue flame as previously reported [11]. The anode potential was fixed at +200 mV vs. SCE during all chronoamperometric (CA) growth. CA maximum current densities (j_{max} in A m^{-2}) of mature microbial biofilms were calculated considering the total immersed electrode surface area and regardless of the orientation of the working electrode towards the counter electrode (15 cm^{-2}). Coulombic efficiencies (CE) were calculated for each experiment according to Call *et al.* [12]. The inoculum was added into the culture media (10% v/v for a final working volume of 400mL) containing 50mM of MES buffer and mineral solution [13]. Reactors were fed with acetate (10 mM) or a mixture of acetate (5 mM) and butyrate (5 mM) as described in Table 1. Concentrations in butyrate and acetate were determined according to Pierra *et al.* who reported the fermentative production of acetate and butyrate in a saline environment [14]. All media compositions were supplemented with 35 g_{NaCl} L^{-1} to simulate the conductivity and salinity conditions commonly observed in sea water. The initial pH was adjusted to 5.5 or 7.0 using NaOH (1 M). To ensure anaerobic conditions, reactors containing growth medium and sediments were flushed with high purity N_2 (≥99.9999%) for at least 30 min. The final composition of the gas phase was typically a mixture of N_2:98.56 ± 1.18% and O_2:1.41 ± 1.16%. Such traces of oxygen might have caused the lag phase times observed in Fig. 1. Reactors were incubated at 37°C since mesophilic conditions usually provide a favorable environment for fast

biofilm formation and optimum current density performance [15]. Acetic (C2) and butyric (C4) acids were determined with a gas chromatograph (GC-3900 Varian) equipped with a flame ionization detector, as previously described elsewhere [16].

2.4 Microbial community analysis of electrochemically derived anodic biofilms

Genomic DNA was extracted and purified using a previously described protocol [17]. Total extracted DNA was purified using a QiAmp DNA microkit (Qiagen, Hilden, Germany). Then, the region V3 of 16S rRNA genes were amplified using universal primers for bacteria (W49 and W104) according to Wéry *et al.* [18]. Each PCR mixture (50µL) contained 5µL of 10x Pfu Turbo DNA buffer, 200 nMf of dNTP, 500 nMf of each primer, 2.5 U μl^{-1} of Pfu Turbo DNA polymerase (Stratagene) and 10 ng of genomic DNA. Reactions were performed in a Mastercycler thermal cycler (Eppendorf). The 16S rRNA genes were amplified as follows: initial denaturing step at 94°C for 2 min, followed by 25 cycles performed at 94°C for 30 s, 61°C for 30 s and 72°C for 30 s, with a final elongation at 72°C for 10 min. Reactions were stopped by cooling the mixture to 4°C. A capillary electrophoresis single-strand conformation polymorphism (CE-SSCP) method was used for PCR products diversity characterization (see Fig. S1). Samples were heat-denatured at 95°C for 5 min and re-cooled directly in ice for 5 min. CE-SSCP electrophoresis was performed in an ABI Prism 3130 genetic analyzer (Applied Biosystems) in 50 cm capillary tubes filled with 10% glycerol, conformation analysis polymer and corresponding buffer (Applied Biosystems). Samples were eluted at 12kV and 32°C for 30 min, as described elsewhere [18]. CE-SSCP profiles were aligned with an internal standard (ROX) to consider the inter-sample electrophoretic variability. CE-SSCP profiles were normalized using the StatFingerprints library [19] in R software version 2.9.2 (R. Development Core Team 2010). Simpson diversity index was calculated from SSCP profiles to estimate the complexity of the community [20-22]. DNA samples were sequenced on a 454 GS-FLX Titanium sequencer (Roche Life Sciences, USA) by the Research and Testing Laboratory (Lubbock, USA). The sequences of the most abundant bacteria found in each biofilm were deposited in the NCBI Genbank database under the following accession numbers: KF573509 to KF573519 for B1 to B9 biofilm respectively.

3 Results and discussion

3.1 Chronoamperometric performance in terms of biofilm growth lag-phase, maximum current density and coulombic efficiency

3.1.1 Phase 1, effect of pH and substrate on biofilm formation and performance

In Phase 1, reactors were inoculated with saline lake sediments (Inoculum 1). The representative chronoamperometric (CA) curves represented in Fig. 1 illustrate how lag phase and maximum current density were calculated (see also Fig. S2). The Lag-phase for every biofilm reported in Table 1 was determined taking into account the beginning of the exponential increase in current density production. As depicted in Fig. 1, the lag-phase for each anode was not identical. Notwithstanding, this difference might have been caused by the distinct experimental parameters used here such as pH/substrate (phase 1) and inocula (phase 2). Furthermore, each electroactive biofilm depicted a slightly different exponential growth. Typically, shorter lag-phases led to shorter exponential growth phases (see Fig. S3).

-Please insert Figure 1 here-

By using the same inoculum source (Inoculum 1), two pH values and two different electron donors (Table 1), the maximum current density (j_{max}) ranged from 1.9 to 4.2 A m^{-2}, for reactors

B1 to B4, respectively. The Coulombic Efficiency (CE) varied between 11 % to 70 % either with a mixture of acetate and butyrate or acetate as sole carbon source, at pH 7.0 or 5.5. Interestingly, when reactors were fed with a mixture of acetate and butyrate, a complete conversion of butyrate into acetate was first observed before acetate consumption started (data not shown). The low CE achieved is an indication of either other reactions than direct conversion into current or the presence of internal electron loops [23]. Among those 4 reactors (B1-4), the highest j_{max} (4.2 A m^{-2}), the highest CE (70 %) and the shortest lag phase (1.5 days) were obtained at neutral pH and with acetate as the sole electron donor, as commonly reported in the literature (see Fig. S4) [15, 24]. Hence, these conditions (pH 7.0 and acetate as carbon source) were selected to perform further experiments in Phase 2.

-Please insert Table 1 here-

3.1.2 Phase 2, effect of inoculum on biofilm formation and performance

In Phase 2, five additional reactors were inoculated with sediments from a salt lake or from an industrial salt-producing platform at pH 7.0 with acetate as carbon source (see Table 1). On average, j_{max} values were 7.6±0.9 A m^{-2} and 5.0±1.1 A m^{-2} and CEs were 63±12 % and 76±5 % with reactors inoculated with sediments from site 2 and site 1, respectively. Those results show that experiments carried out in phase 2 outperformed those in phase 1 in terms of j_{max} and CE, except for the case of B4 (pH 7.0, In 1, fed with acetate). This observation confirms that pH 7.0 and acetate as an electron donor are the most appropriate conditions tested in phase 1 for the enrichment of anode-respiring bacteria (ARB) from saline sediments.

When added alone, acetate proved to be an effective electron donor for bacteria that compose the electroactive biofilm enriched from sediments in terms of lag-phase, j_{max} and CE (see Table 1). This observation is consistent with previous studies in which environmental samples, regardless of the origin of the inoculum, have been tested under well potentiostatically controlled conditions as a source to develop high current-producing biofilms [5, 6, 25]. Freguia *et al.* fed BESs with a mixture of more than 7 different volatile fatty acids (VFAs). They tested the harvest of electrons by an electroactive biofilm from different substrates. They found out that acetic and propionic acids were the preferential electron donors for ARB enriched in those microbial biofilms [26]. Consistently, an enriched electroactive biofilm in a BES fed with acetate generated 66% more power density than a BES fed with butyrate [4].

In the present work, the overall performance was higher at pH 7.0 than at pH 5.5 in terms of CE (see Table 1). For reactors fed with acetate, CEs were 70% and 12% at pH 7.0 and 5.5, respectively. This is consistent with previous results in which electroactive biofilms showed a better performance in terms of CE at neutral pH [24].

As shown in this study, the pH is an important parameter for microbial electron transfer. When substrate oxidation occurs at the anodic microbial biofilm, protons are produced and released to the media. From there, they migrate to the cathode where they react with the electrons from the external driven flow to finally produce hydrogen. A pH imbalance in both anode and cathode environments could cause irreversible anodic biofilm degradation and thus severely affect the reduction reaction [24].

3.2 Microbial community analysis of anodic biofilms

3.2.1 Electrochemically driven selection of anode-respiring bacteria

The microbial community of electroactive biofilms resulted in simple communities structures with an average Simpson index of 0.86±0.06. In comparison, the inoculum was more diverse with a Simpson diversity index of 0.98±0.00. This confirms the high selection of ARB which occurred within the anodic biofilms in the present study. Such strong and specific selection of

ARB species from environmental samples has been extensively and almost exclusively reported in previous studies using WW as a source of inoculum [5, 26, 27]. In the present study, the analysis of 16S ribosomal DNA genes by 454 pyrosequencing showed a pronounced dominance of bacteria from the δ subgroup of *Proteobacteria* with 85% to 97% of the 16S rDNA sequences from the anodic biofilms belonging to this group. Interestingly, the most abundant ARB species found in all electroactive biofilms were closely related and were up to 97% DNA similar to either *Desulfuromonas acetoxidans* or *Geoalkalibacter subterraneus* in all reactors (see Table 1).

Of special interest is the presence of *Glk. subterraneus*, an ARB that has only been reported in a recent study which showed that *Glk. subterraneus* dominated electroactive biofilms derived from environmental anaerobic samples [6]. Therefore, the present results are consistent with previous studies showing a high ARB selection and a decrease of diversity [6]. On the other side, multiple studies have reported that under such well-potentiostatically controlled conditions, a high microbial enrichment is very likely [27-29]. This is conferred by the ability of several *Deltaproteobacteria* to transfer electrons to an electrode material [30].

More precisely, such high microbial selection in the anodic biofilms resulted from several specific and constant experimental conditions, *e.g.*: (i) the fixed anodic applied potential (+200 mV vs. SCE), (ii) the constant temperature (37°C), (iii) the neutral pH conditions (7.0), (iv) the homogeneous mass transfer in the bulk medium due to continuous stirring, (iv) the use of a synthetic medium containing a non fermentable substrate such as acetate or butyrate, (v) the anaerobic conditions and more importantly (vi) the moderately halophilic conditions (35 g/L NaCl).

In the literature, several examples can be found where such a high microbial selection has occurred. For instance, in a recent work conducted under very similar experimental conditions, Miceli *et al.* used diverse environmental samples to enrich (~90%) several putative anode-respiring bacteria (including *Geobacter* spp. and *Geoalkalibacter* spp., among others) that converted acetate to current densities higher than 1 A/m^2 [6]. Consistently with the work of Miceli *et al.*, Yates *et al*. (2012) obtained highly enriched electroactive biofilms from different municipal wastewaters [28]. Biofilms were mainly composed of *Geobacter* spp. (≥80%), a well-known ARB capable of producing high current densities and strongly attach to electrodes via the formation of ≥50μm thick biofilms [31]. Similarly, Harnisch *et al.* demonstrated with flow-cytometry that besides the high microbial diversity of wastewater as inoculum, *Geobacter sulfurreducens* can be systematically enriched as an ARB by employing well potentiostatically controlled conditions similar to those used in the present work [27].

Although a high selection of only a few ARB occurred in the present study, this might not always be desirable, especially when the system is intended to treat real effluents like saline WWs. If such WWs are the target of a treatment process by an ARB biofilm based technology, then a more diverse microbial community would probably show higher adaptation and resistance to the usual changing operating conditions observed in real WW treatment.

Among the δ-*Proteobacteria*, many species, including *Desulfuromonas* spp. and *Geoalkalibacter* spp., are able to oxidize organic compounds and reduce insoluble Fe(III) oxides at the same time [5, 32]. The present study shows that the enriched ARB effectively used the electrode material as an electron acceptor, as they do in a natural environment when using other insoluble final electron acceptors such as iron or manganese oxides [33]. Consequently, it can be proposed that for the ARB found here, especially for *Geoalkalibacter* spp., the capacity to reduce insoluble electron acceptors (such as iron oxides) does confer the ability to transfer electrons to electrode materials.

3.2.2 *D. acetoxidans* selection in butyrate-acetate fed reactors and its implications for the overall performance and the anodic microbial composition of BESs

The simultaneous use of two substrates to feed a BES might have important implications in terms of the overall performance of the system and the microbial composition of the anodic community. It was previously shown that microbial composition and performance of electroactive biofilms are highly dependent on the nature of the substrate. According to Chae *et al.*, a higher CE was found when the anodic biofilm was enriched from an anaerobic digester sludge fed with acetate (72%) than with butyrate (43%) at pH 7.0 [26]. Those results are consistent with the results presented here at neutral pH fed with acetate and with an acetate and butyrate mix (Table 1). Moreover, in Freguia *et al*., the microbial composition of the anodic biofilm was also more diverse. In butyrate-fed reactors, there was a predominance of α, β and δ–*Proteobacteria* and *Firmicutes*, with a majority of β-*Proteobacteria*. In acetate-fed reactors, there was a predominance of α, β and δ *Proteobacteria* with a majority of β and δ-*Proteobacteria* [26]. Therefore, in the study by Freguia *et al*. [26], a lack of *Geobacteraceae* in the butyrate fed-system suggests that this group of microorganisms is not able to harvest electrons from butyrate oxidation.

Interestingly, *Glk. subterraneus* is able to oxidize butyrate whereas *D. acetoxidans* is not [32, 33]. The emergence of *D. acetoxidans* in acetate-butyrate fed reactors (B1 and B2 in Table 1) was likely favoured by the conversion of butyrate into acetate that occurred before current production took place (data not shown). In addition, *D. acetoxidans* is an ARB that was firstly isolated from marine sediments and is known to grow anaerobically by oxidizing acetate with the reduction of elemental sulphur or Fe(III) [32]. Additionally, an organism close to *D. acetoxidans* was repeatedly identified in enriched anodes by Bond *et al.* [5]. In their work, a pure culture of *D. acetoxidans* provided a current density of 0.157 A m^{-2} on a graphite electrode poised at +200 mV vs. AgAgCl.

3.2.3 Selection of *Glk. subterraneus* as an anode-respiring bacterium capable of producing high current densities

Whereas *D. acetoxidans* has been well described in the literature as an ARB [6, 23], members of the *Geoalkalibacter* genus have only recently been found in electroactive biofilms. Only one study so far reported *Glk. subterraneus* as a dominant ARB in the population of electroactive microbial biofilms enriched from shoreline and mangrove sediments [6]. Biofilms highly enriched in *Geoalkalibacter* sp. reported by Miceli *et al*. reached a current density ranging from 3.87 to 8.73 A m^{-2} after a first CA growth cycle on a graphite electrode polarized at -0.3 V vs. Ag/AgCl [6]. Our results are analogous even if applied potentials differ. Such similarities in terms of j_{max} are likely due to the saline conditions used in both cases (from 20 to 35 g_{NaCl} L^{-1}) in which the inocula grew in both studies. Besides the high anodic microbial selection of *Glk. subterraneus* that occurred in anodic biofilms under saline conditions, Miceli *et al*.'s work and the present work have shown that it is indeed possible to obtain high conversion of organic matter into current even under such saline conditions, usually considered to inhibit bacterial metabolism (j_{max} >4 A m^{-2}).

Additionally, the recent electrochemical and microscopic characterization of pure cultures of *Glk. subterraneus* provides further information to explain the appearance of such ARB in the anodic biofilms of the present study [10, 34]. Moreover, *Glk. subterraneus* is considered as either alkalitolerant or alkaliphilic (here the pH of the inocula was 7.8±0.2) and is able to use a wide panel of substrates as electron donors such as acetate and ethanol [33]. Furthermore, *Glk. subterraneus* belongs to the *Geobacteraceae* family (90% of similarity). This similarity between this ARB and the well-known ARB *Glk. subterraneus* in terms of current density production, electron transfer mechanism and biofilm formation might explain the significant performance of *Glk. subterraneus* [10].

4 Conclusions

In summary, among all conditions tested, *D. acetoxidans* and *Glk. subterraneus* were the main dominant anode-respiring bacteria in all anodic electroactive biofilms, alone or together. This reveals a high selectivity of the method used, very likely due to the saline conditions, neutral pH, readily degradable substrate and applied potential, among others. Moreover, efficient electroactive biofilms were obtained in terms of significantly high current densities that reached up to 8.5 A m^{-2}. Hence, a high substrate conversion with coulombic efficiency up to 83% was obtained. The later clearly indicates that most of the substrate was effectively converted into current.

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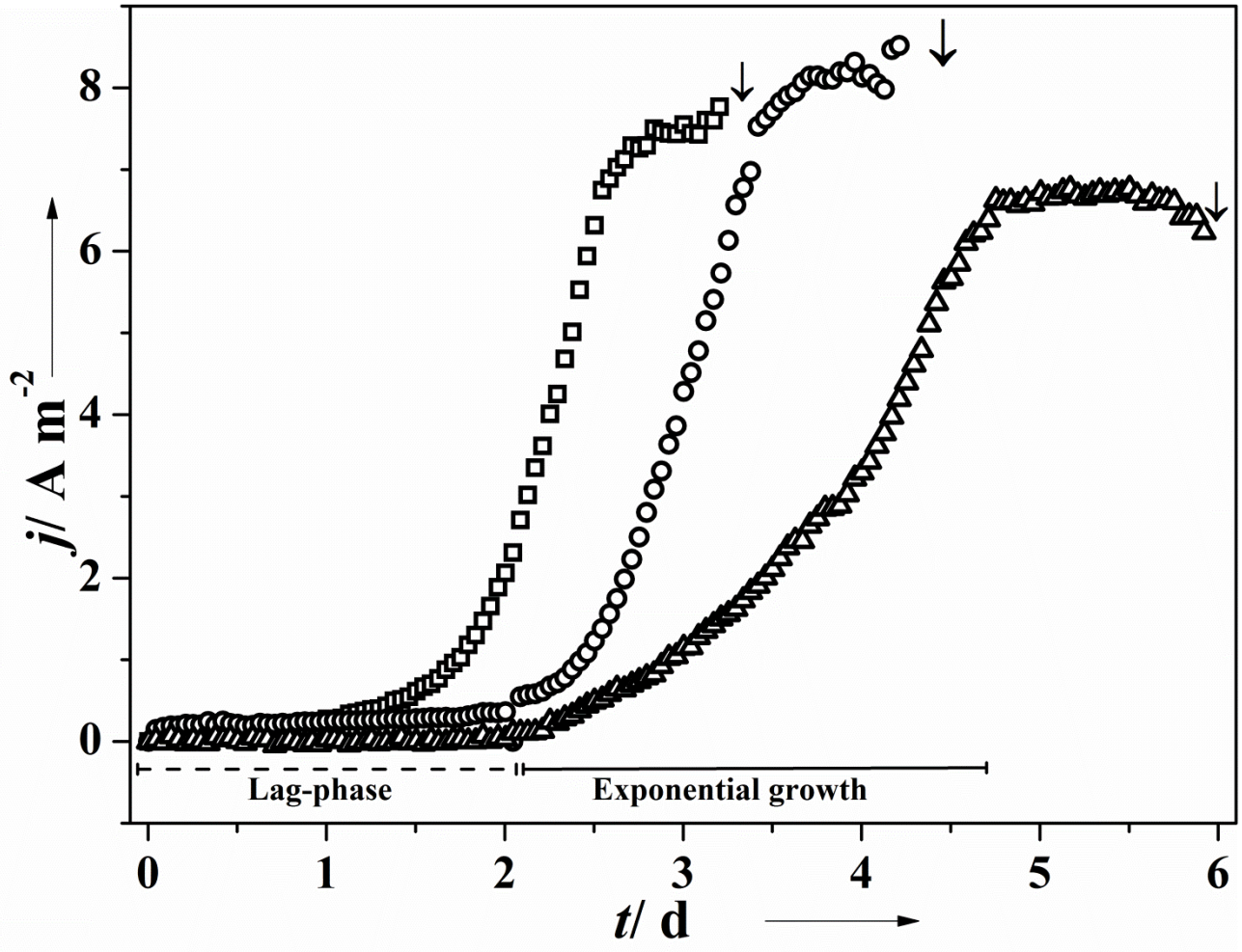
Table 1 Performance of electrochemically derived biofilms grown under saline conditions

Experimental conditions*					Simpson diversity index	Lag phase/ days	j_{max}/ A m^{-2}	CE/ %	% Main dominant species in the biofilm (closest phylogenetical known sequences identified)†
B.	pH	In.	Ac.	Bu.					
Phase 1, effect of pH and substrate on biofilm formation and performance									
B1	7.0	1	Yes	Yes	0.75	2.9	1.9	11	*D. acetoxidans* (86%)‡
B2	5.5	1	Yes	Yes	0.90	9.4	2.2	21	*D. acetoxidans* (98%)
B3	5.5	1	Yes	No	0.84	8.7	2.7	12	*D. acetoxidans* (99%)
B4	7.0	1	Yes	No	0.90	1.5	4.2	70	*Glk. subterraneus* (39%) *D. acetoxidans* (47%)
Phase 2, effect of inoculum on biofilm formation and performance									
B5	7.0	1	Yes	No	0.87	2.2	4.5	80	*Glk. subterraneus* (95%)
B6	7.0	1	Yes	No	0.81	1.8	6.3	78	*Glk. subterraneus* (99%)
B7	7.0	2	Yes	No	0.93	1.9	6.7	60	*Glk. subterraneus* (73%)
B8	7.0	2	Yes	No	0.91	0.3	7.7	53	*Glk. subterraneus* (20%) *Desulfuromonas* spp. (58%)
B9	7.0	2	Yes	No	0.76	1.9	8.5	77	*Glk. subterraneus* (91%)

Notes: *B.: Biofilm, In.: inoculum type, Ac: acetate and Bu.: butyrate. †Names in *italics* correspond to the closest phylogenetical known sequence based on the percentage of identity of dominant species (>20% of total sequences). ‡Numbers in parentheses represent the relative abundance obtained from 454 pyrosequencing analysis.

Fig. 1: Representative chronoamperometric batch cycles of electrochemically derived biofilms B7 (Δ), B8 (□) and B9 (○). Arrows indicate anodic biofilm collected for microbial analysis.



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