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Marine aerobic biofilm as biocathode catalyst

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A B S T R A C T

Stainless steel electrodes were immersed in open seawater and polarized for some days at -200 mV vs. Ag/AgCl. The current increase indicated the formation of biofilms that catalysed the electrochemical reduction of oxygen. These wild, electrochemically active (EA) biofilms were scraped, resuspended in seawater and used as the inoculum in closed 0.5 L electrochemical reactors. This procedure allowed marine biofilms that are able to catalyse oxygen reduction to be formed in small, closed small vessels for the first time. Potential polarisation during biofilm formation was required to obtain EA biofilms and the roughness of the surface favoured high current values. The low availability of nutrients was shown to be a main limitation. Using an open reactor continuously fed with filtered seawater multiplied the current density by a factor of around 20, up to $60 \mu\text{A}/\text{cm}^2$, which was higher than the current density provided in open seawater by the initial wild biofilm. These high values were attributed to continuous feeding with the nutrients contained in seawater and to suppression of the indigenous microbial species that compete with EA strains in natural open environments. Pure isolates were extracted from the wild biofilms and checked for EA properties. Of more than thirty different species tested, only *Winogradskyella poriferorum* and *Acinetobacter johnsonii* gave current densities of respectively 7% and 3% of the current obtained with the wild biofilm used as inoculum. Current densities obtained with pure cultures were lower than those obtained with wild biofilms. It is suspected that synergetic effects occur in whole biofilms or/and that wild strains may be more efficient than the cultured isolates.

Keywords:

Seawater
Stainless steel
Oxygen reduction
Biocathode
Microbial fuel cell

1. Introduction

According to a considerable number of authors, the low efficiency of the cathode process is a major limitation in microbial fuel cells (MFCs) [1]. Oxygen is the most usual final electron acceptor for the MFC cathode reaction because of its high redox potential ($+0.82$ V vs. SHE) and its low cost thanks to its abundance in air. Platinum is the most efficient catalyst for oxygen reduction in MFCs [2–5] but Pt application remains limited because of its excessive cost and its possible poisoning by components of the substrate or by-products from the anodic compartment. In addition, the pH value, which is commonly in the range of 6.0 to 8.0 in MFCs, reduces the catalytic activity of Pt [6,7]. Other metals deposited on a variety of carbon supports and chemically modified electrodes including pyrolysed iron (II) phthalocyanine (FePc) and Cobalt tetramethyl phenylporphyrin (CoTMPP) have been studied to replace Pt [6,8,9]. Nevertheless, Pt is unfortunately the only catalyst proven to date to withstand thousands of hours of operation.

Microbial cathodes have been attracting increasing attention as an inexpensive and sustainable alternative to abiotic cathodes. Biocathodes can be classified as aerobic or anaerobic, depending on the terminal electron acceptors. Using nitrate as electron acceptor, maximum power densities of around $10 \text{ W}/\text{m}^3$ have been obtained [10], while open air biocathodes have provided powers up to 83 and $65 \text{ W}/\text{m}^3$ for batch fed and continuous systems respectively. Microbial graphite felt cathodes catalysing the oxygen reduction and inoculated with sludge and river sediments have reached $2.2 \text{ A}/\text{m}^2$ [11] in a continuously recirculated MFC system.

The catalysis of oxygen reduction by microbial biofilms deposited on metallic materials has been widely studied in the domain of aerobic corrosion for a long time. In this framework, it has been stated that biofilms formed in natural seawater efficiently catalyse oxygen reduction on stainless steels [12]. From fundamental studies carried out in the field of corrosion, it has been reported that forming seawater biofilm on stainless steel may be a promising track for designing new low-cost microbial cathodes for fuel cells. This approach was initiated in 2005 with a biofilm-covered cathode that supported current densities of up to $1.89 \text{ A}/\text{m}^2$ [13]. Since this date, several attempts have been made to implement marine microbial cathodes in MFCs in a sea environment [14,15]. Different technical problems have been identified

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but an extremely important limitation is the requirement for large volumes of fresh seawater. Actually, developing efficient seawater biofilms requires stainless steels to be exposed under constant polarisation for several days in large volumes of seawater that is continuously renewed [16]. Experiments performed in closed vessels containing only a few litres of fresh seawater did not give electrochemically effective biofilms.

This study aimed first to reconstruct electrochemically active (EA) biofilms in easy-to-handle laboratory conditions, i.e. in closed electrochemical vessels. The second objective was to progress in understanding the mechanisms of microbial catalysis by identifying and isolating microbial strains that made up the EA seawater biofilms and to check the electrochemical efficiency of each isolate in pure cultures.

2. Materials and methods (or Experimental section)

2.1. Biofilm growth on site

Wild EA biofilms were formed in natural seawater at the ISMAR marine station, located in the port of Genoa, Italy. Stainless steel electrodes were immersed in a tank containing 100 L seawater, which was continuously renewed at a rate of about $1.5\text{--}2\text{ L min}^{-1}$ with seawater directly pumped from the sea. Tests were performed during January 2006, a period when seawater had the following main characteristics: $11\text{ }^\circ\text{C}$ < temperature < $13\text{ }^\circ\text{C}$; 6 mg/L < dissolved oxygen < 8 mg/L ; $8 < \text{pH} < 8.2$; 33.5% < salinity < 36.5% . An Ag/AgCl electrode filled with KCl 3 M (INGOLD 103633040) was used as the reference electrode. Stainless steel electrodes ($95\text{ mm} \times 25\text{ mm} \times 1\text{ mm}$) were cut from a plate of super-austenitic alloy 254 SMO (UNSS 31254: Cr = 19.5–20.5%, Ni = 17.5–18.5%, Mo = 6–6.5%, N = 0.18–0.22%, Cu = 0.5–1%, S < 0.01%, Si < 0.8%, P < 0.03%, Mn < 1%, C < 0.02%, Fe = balance) and treated with emery papers of up to P1200. Electrical connections between electrode samples in the solution and wires outside were made with titanium rods ($\varnothing = 2\text{ mm}$) screwed on to the electrodes. The area of titanium rod exposed to seawater was a negligible fraction of the connected stainless steel electrode surface area ($S_{Ti}/S_{SS} < 5\%$). Electrodes covered with EA biofilms were sent from Genoa and stored at $4\text{ }^\circ\text{C}$ in seawater for several days before being used to prepare the bacterial suspension or isolate the strains.

2.2. Preparation of bacterial suspension from wild biofilms

Stainless steel electrodes covered with marine aerobic biofilm and seawater used for the transport were placed in a 50 mL glass cell. The system was exposed to ultrasounds in an ultrasonic bath (Transsonic 950/H, Prolabo, frequency 35 kHz) for 3 periods of 5 min separated by 5-minute periods of cooling in order to disrupt the biofilm structure. The bacterial suspension obtained was centrifuged for 10 min at 10,000g (Sigma 3k20, Bioblock Scientific) and the pellet was resuspended in 10 mL fresh seawater.

2.3. Electrochemical setups

Experiments were carried out in reactors containing 500 mL seawater, continuously stirred (magnetic barrel 150 rpm) and/or bubbled with air. The reactors were inoculated with a 10 mL suspension of wild biofilm (details in Section 2.2). The top of the reactor comprised six sampling ports. One to four working electrodes of 254 SMO stainless steel having a projected surface area of 25 cm^2 were placed in the same reactor and connected to the same counter and reference electrodes through a multi-potentiostat (VMP2, software EC-Lab v.8.3, Bio-Logic SA). Each working electrode was monitored independently by means of an N-STAT device (Bio-Logic SA). The auxiliary electrode was a platinum grid of large surface area and an Ag–AgCl electrode was used as the reference electrode. The stainless steel was cleaned before each experiment by immersion in

2% HF 0.5 M HNO_3 solution for 20 min and rinsed for 1 h with distilled water. The potential of the working electrodes was fixed at -200 mV/Ag-AgCl during chronoamperometry. In continuous experiments, the continuous seawater flow was installed on the bottom of the reactor and the outlet was an overflow system. The seawater flow was 1.5 L/day and seawater was filtered with a $0.2\text{ }\mu\text{m}$ filter before entering the system.

2.4. Fluorescent microscopy and 3D imaging

Microbial colonisation and 3D biofilm structure were investigated on the electrode surface by epifluorescence microscopy. Electrodes were extracted from the reactors and washed carefully with seawater to remove all materials except the attached biofilms. The biofilm was stained with 0.03% orange acridine (A6014, Sigma) for 10 min. The samples were then left to dry in ambient air and analysed with a Carl Zeiss Axiotech 100 microscope equipped for epifluorescence with an HBO 50/ac mercury light source and the Zeiss 09 filter (excitor HP450–490, reflector FT 10, barrier filter LP520). Images were acquired with a monochrome digital camera (Evolution VF) and processed with the Image-Pro Plus 5.0 software.

The average surface roughness (Ra) of the cleaned electrodes was measured using a Zygo New View 100 OMP-0348K white light interferometer.

2.5. Population analysis of wild biofilms

The biofilm was removed from the stainless steel cathode by sonication (Branson™ 3200) and cell suspensions were used for cultivation experiments. Marine Agar and Marine Broth media from Difco were used to isolate heterotrophic marine bacteria. These media contain minerals that nearly duplicate the major mineral composition of seawater, with the addition of peptones and yeast extract that provide a good source of nutrients. The inoculated culture media were incubated aerobically at $20\text{ }^\circ\text{C}$ for several days. Pure cultures were obtained from all growth media and isolates were stored at $-80\text{ }^\circ\text{C}$ in MicroBank™ vials. Population analysis of wild biofilm has been described in detail by Vandecandelaere et al. (this issue). Shortly, all isolates were grouped and tentatively identified (MIS, Newark, USA) using whole cell fatty acid methyl ester analysis (FAME) as described by Mergaert et al. [17]. The FAME profiles obtained were grouped using the BioNumerics 4.61 (Applied Maths, Belgium) software. Subsequently, 16S rRNA gene sequence analysis was performed on selected representatives of each of the FAME clusters to elucidate their exact taxonomic position as described previously [17].

3. Results and discussion

3.1. Formation of wild biofilms on site

Type 254 SMO stainless steel (SS) was used for this study because of its excellent corrosion resistance in seawater due to the synergistic action of the alloying elements [18]. Six 254 SMO SS electrodes of 25 cm^2 projected surface area each were immersed under constant polarisation at -200 mV/Ag-AgCl for 15 days in an open seawater area of a marine station located at the Genoa harbour. The current density recorded during polarisation is reported in Fig. 1a. No current was detected during the first two days because oxygen reduction is very slow on a clean SS electrode without any catalyst. Starting from the third day, the current increased exponentially until day 10. This phenomenon has been correlated to the development of a microbial biofilm on the SS surface able to catalyse oxygen reduction [13,19]. From day 10 to day 15, a constant current density around $2.2\text{ }\mu\text{A/cm}^2$ was recorded. Comparing these data to analogous experiments that have been performed in recent years (Fig. 1b), the maximal value of current density obtained here was significantly lower; usual current

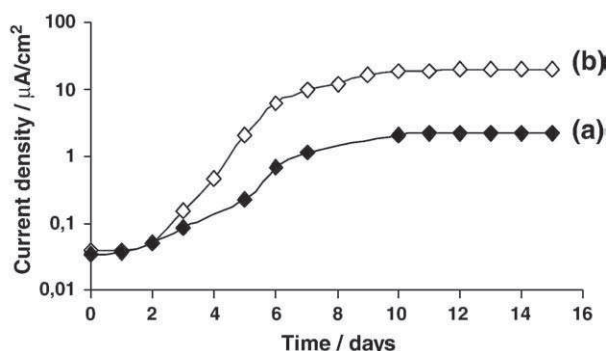


Fig. 1. Cathodic current (absolute value, and logarithmic scale) evolution of SS electrode polarised at -200 mV vs. Ag/AgCl in open natural seawater ($T=11-12$ °C) at the CNR-ISMAR marine station located in the port of Genoa, Italy. (a) This study, and (b) usual analogous experiment [16].

densities were about one order of magnitude higher ($10-20$ $\mu\text{A}/\text{cm}^2$) [13,16]. Pollution of SS surfaces by other non-specific organisms such non-EA microorganisms, algae, zooplankton... may be suspected. The low temperature of the sea in winter may also be a possible cause of the lower current density obtained here. Nevertheless, the biofilms obtained here were scraped from the electrode surfaces and resuspended in fresh seawater to be used as the inoculum for the laboratory experiments.

3.2. Biofilm reconstruction in closed laboratory reactors

Two SS electrodes were placed in the bioreactor containing 0.5 L seawater. One of them was continuously polarised at -200 mV/Ag–AgCl as in the experiments performed in open seawater, the other was left at open circuit potential. No current was detected on the polarised electrode for 1 day before the bacteria inoculation. Adding 10 mL of a wild biofilm suspension led to an almost instantaneous production of a small weak current that increased exponentially during the second day (Fig. 2), following the kinetics of microbial EA biofilm growth [20]. At the same time, no current was detected when the electrode kept at open circuit was polarised for a few minutes at -200 mV/Ag–AgCl, indicating that constant polarisation at -200 mV/Ag–AgCl was required for EA biofilm formation on the electrode surface.

Several control experiments were performed to confirm the real implication of the aerobic biofilm on oxygen reduction. First, a clean electrode was introduced in the bioreactor when maximal cathodic current was detected on the working SS electrode to verify the role of planktonic bacteria on oxygen reduction catalysis. This control did not show any current at all. A second control experiment was performed with a SS electrode under constant polarisation in 0.5 L fresh natural

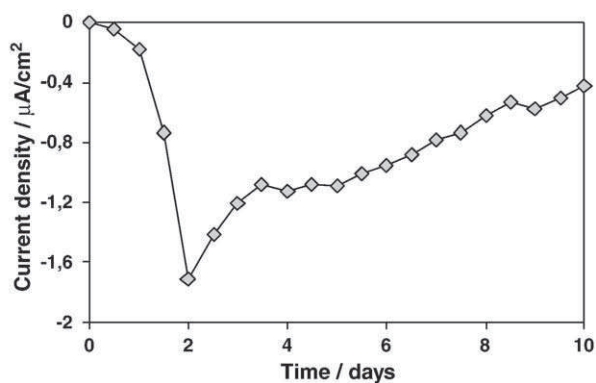


Fig. 2. Current evolution obtained in closed reactors containing 0.5 L fresh seawater with SS electrodes polarised at -200 mV vs. Ag/AgCl with or without (control) inoculation with resuspended wild biofilm.

seawater without addition of the wild biofilm suspension. No current was obtained during 8 days of polarisation (Fig. 2), confirming that EA biofilms could not be formed in only natural seawater. Consequently, the current obtained was due to the wild biofilm suspension added into the reactor and not to indigenous microorganisms naturally present in the seawater used as the medium.

The exponential increase in the current obtained on the polarized electrode stopped at the end of the second day and the current then gradually decreased over 6 days at a rate averaging 0.2 $\mu\text{A}/\text{cm}^2$ per day. It may be suspected that the population of electroactive bacteria attached on the surface was substantially eliminated by the intervention of Protozoa, such as *amoeba*, known to be fierce predators of bacteria [21]. The current drop may also have been due to a limitation of the bacterial growth by the depletion of essential nutrients such as carbon, nitrogen or phosphorous sources in the closed reactor.

To discern the reasons for this current fall, several electrochemical reactors were run in parallel with SS electrodes polarised at -200 mV/Ag–AgCl using the following media:

- synthetic sterile seawater (composition g/L: NaCl 24.53, MgCl_2 5.20, Na_2SO_4 4.09, CaCl_2 1.16, KCl 0.695, NaHCO_3 0.021, salinity 35 g/L, pH 8.0) containing no trace of organic nutrients or living microorganisms.
- natural seawater without any special treatment,
- sterile seawater that was 0.2 μm filtered to remove microorganisms naturally present in water, such as sea heterotrophic bacteria, cyanobacteria, algae or zooplankton [22].

The maximum current density obtained using natural seawater was of the same order as that observed in the previous experiment (Fig. 3b). In filtered seawater (Fig. 3c), the current increased faster than in fresh seawater. This can be explained by less intense microbial competition to colonize the SS surface because the seawater was previously cleared of its microbial population. The maximal current density was much higher, since it reached 2.0 $\mu\text{A}/\text{cm}^2$, but the phenomenon of current collapse was still observed.

The charges Q calculated by integrating the current over time (Coulomb) that were consumed for 8 days were relatively close (around 5800 mC) when natural or filtered seawater was used. This tends to prove that the system was regulated by the availability of nutrients present in the media. The experiment performed with synthetic seawater confirmed this explanation since it contained no nutrients, and no cathodic current was detected (Fig. 3a). It was confirmed here that the availability of a sufficient quantity of growing nutrients that allow bacteria to multiply was a key factor in the formation of efficient EA marine biofilms.

Similar experiments were reproduced in natural seawater with two different surface finishes of the SS electrodes: (a) finish as delivered with an average surface roughness $R_a=0.3$ μm , (b) wet

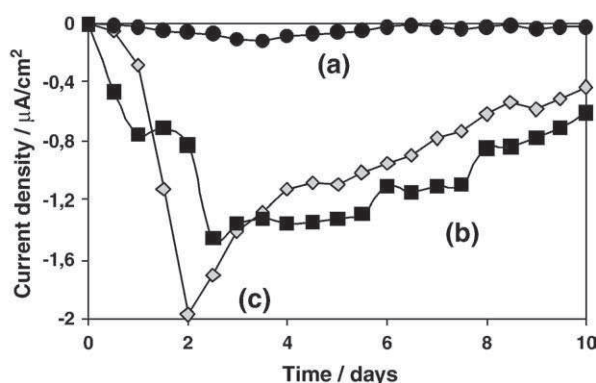


Fig. 3. Current evolution obtained in closed reactors inoculated with 10 mL resuspended wild biofilm with SS electrodes polarised at -200 mV vs. Ag/AgCl; effect of medium: synthetic seawater (a), natural seawater (b) and filtered natural seawater (c).

polished leaving a mirror-like surface with $Ra < 0.05 \mu\text{m}$. A maximal current density of $1.7 \mu\text{A}/\text{cm}^2$ was obtained with the non-polished SS electrode after 2 days, while less than $0.5 \mu\text{A}/\text{cm}^2$ was recorded on the polished surface (Fig. 4). Epifluorescent pictures showed that the non-polished electrode had a greater bacterial colonisation, even if the difference in colonisation did not seem sufficient to explain a tripling of the current. The influence of surface roughness on the adhesion of microbes is still under debate. It seems that the dominant trend is to assume that roughness values of the order of the size of bacterial cells favour bacterial settlement [23–25]. Nevertheless, Hilbert et al. [26] have demonstrated that the adherence of *Pseudomonas* sp., *Listeria monocytogenes* and *Candida lipolytica* to stainless steel is not affected by surface roughness ranging from polished stainless steel ($Ra < 0.01$) to ground stainless steel ($Ra 0.9$). Here, polishing the surface slightly decreased the bacterial settlement but had a more marked effect on the current density. The literature on the influence of stainless steel roughness on corrosion resistance is unanimous [26]. Similarly, polishing the electrodes affected bacterial colonisation here but it also decreased the electrochemical properties of the material, certainly by decreasing the current exchanges.

Finally, a similar experiment was performed in continuous mode with an open 0.5 L bioelectrochemical reactor equipped with four individually addressed working electrodes. The reactor was first put in a closed loop for 48 h to allow the pioneer bacteria of the wild biofilm suspension to adhere to the electrode surface, then seawater was renewed continuously with an 8-hour residence time. An increasing cathodic current was detected after a few hours of polarisation and it began to fall after 24 h. Feeding the reactor continuously with fresh seawater made the current increase again over more than 35 days. The current density increased overall with fluctuations due to changes in temperature or accidental flow fall. Current densities of the order of $45 \mu\text{A}/\text{cm}^2$ were reached in these conditions, with a maximum peak value of $60 \mu\text{A}/\text{cm}^2$. The accidental flow falls that provoked current decrease proved that continuous feeding with nutrients was a requirement to sustain high current densities (Fig. 5).

In parallel, the biofilm development on the surface of the stainless steel electrode was followed by epifluorescence microscopy by extracting one of the working electrodes at days 7, 19 and 36. After 7 days' polarisation, the biofilm coverage rate was 8%. It was 26% after 19 days and reached 63% at the end of the experiment (day 36). Several images of the same plot were taken at different focal planes z in the direction perpendicular to the electrode surface (thickness) in order to rebuild the 3-dimensional structure of the biofilm using an image processing software.

Fig. 6a shows a z -profile of the 3D-reconstruction, indicating an average of $20 \mu\text{m}$ for the biofilm thickness. The voids and the cavities in the biofilm are visualised in Fig. 6b corresponding to the projected volume of the biofilm. The biofilm structure consisted of microbial cell clusters covering 60–65% of the surface. The clusters were loaf-

shaped, with a basal diameter of $10\text{--}20 \mu\text{m}$. In a study on microbial corrosion, Mattila et al. [27] used confocal microscopy analysis to describe the development of a marine biofilm on stainless steel coupons left at open circuit in natural seawater. The development of aerobic marine biofilms is known to induce an increase (ennoblement in terms of corrosion) of the free potential at open circuit, which may shift the material to potential values where the passive layer can be disrupted, leading to local corrosion. The authors linked the start of potential ennoblement to the formation of mushroom-like microbial structures $50 \mu\text{m}$ in diameter and $10 \mu\text{m}$ in height, covering 1–5% of the steel surface. Biofilm mushrooms grew in height to reach $100 \mu\text{m}$ after 21 days while the coverage of the steel surface approached 20%. There is a similarity between the biofilm formed on a non-polarised steel surface and the biofilm obtained here under polarisation, but the EA biofilms formed here were thinner and covered a higher proportion of the electrode surface area. The higher availability of electrons that the metal can provide due to polarisation may explain the higher biofilm coverage. On the other hand, in quiescent conditions, the lack of nutrients may incline the biofilm to increase its surface growth towards the bulk to favour nutrient transfer from the bulk, which can explain the formation of high mushrooms. Differences in electron and nutrient availability were seen to drastically affect the structure of the biofilm observed here, which was composed of many compact, thin clusters that covered a large part of the electrode surface.

3.3. Isolates from wild biofilms

Analysis of the microbial population of the wild biofilms is described in detail by Vandecandelaere et al. (this issue). The goal was not to characterize all the strains from the biofilm but only to extract the easily-cultivable bacteria. Then, heterotrophic marine bacteria were isolated on a specific solid medium for cultivable marine bacteria. Serial growths of bacterial strains were limited to a minimum number of steps in order to not lose completely the electro-activity of the isolated strains. In general, the majority of the isolates were identified as Gram-negative bacteria. The isolates proved to belong mainly to five different phylogenetic groups: Alphaproteobacteria, Gammaproteobacteria, Firmicutes, Actinobacteria and Flavobacteriaceae.

Bacterial communities were investigated during biofilm formation in coastal seawater by Lee et al. [28]. Identification of major populations by 16S rRNA gene sequences indicated that gamma-Proteobacteria (*Pseudomonas*, *Acinetobacter*, *Alteromonas*, and uncultured gamma-Proteobacteria) were predominant in the community for approximately 9 h, while the ratio of alpha-Proteobacteria (*Loktanella*, *Methylobacterium*, *Pelagibacter*, and uncultured alpha-Proteobacteria) increased approximately 4.8 fold during approximately 36 h of the biofilm formation, emerging as the predominant group. Results of this study

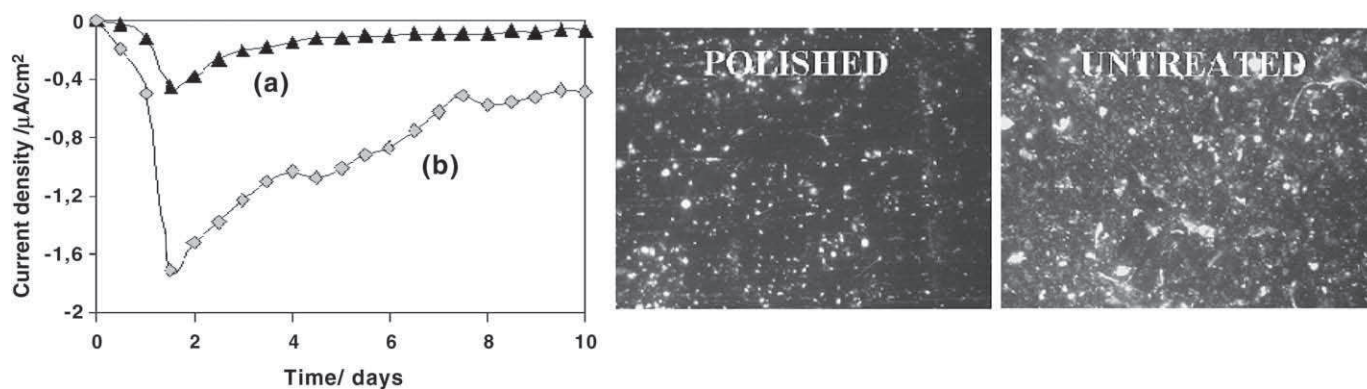


Fig. 4. Current evolution obtained in 0.5 L natural seawater inoculated with 10 mL resuspended wild biofilm with SS electrodes polarised at -200 mV vs. Ag/AgCl ; effect of the surface roughness polished (a) or not (b) on both current and colonisation.

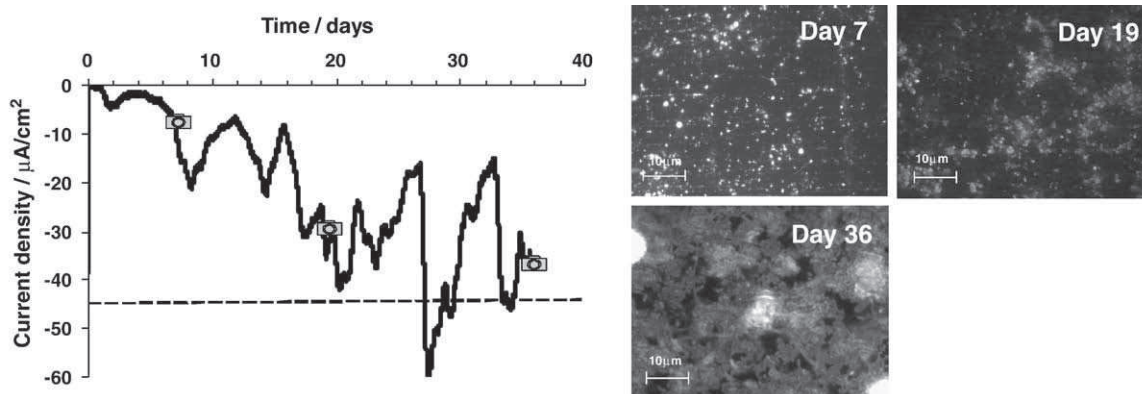


Fig. 5. Current evolution obtained in continuous reactor inoculated with 10 mL resuspended wild biofilm and fed with natural seawater since day 2; SS electrodes polarised at -200 mV vs. Ag/AgCl. Epifluorescent microscopy pictures of stainless steel surface at different stages of biofilm formation.

indicated that some species of gamma-Proteobacteria were more important as the pioneering population.

A large number of aerobic bacterial strains were isolated from the wild EA biofilms. Pure cultures of all the predominant isolates that could be suspected of being implicated in the generation of current were tested in closed electrochemical reactors. The list of bacteria tested here is presented in Table 1.

After 24 h of incubation at 28 °C, the fresh bacteria were centrifuged and recuperated in filtered natural seawater. The bacterial suspensions obtained were then used to inoculate the electrochemical bioreactors containing 500 mL filtered seawater and equipped with SS electrodes polarised at -200 mV/Ag–AgCl. Seven different *Roseobacter* species of Alphaproteobacteria had been tested in a previous work and did not give any current (paper in preparation). Among the 24 isolates tested here (Table 1), only two strains generated cathodic current (Fig. 7): *Winogradskyella poriferorum* and *Acinetobacter johsonii*. They gave maximal cathodic current densities of 0.1 $\mu\text{A}/\text{cm}^2$ and 0.04 $\mu\text{A}/\text{cm}^2$ corresponding respectively to only 7% and 3% of the current obtained in similar batch conditions with the whole wild biofilm inoculum. To the best of our knowledge, for now only two isolates have been reported to possess the capability to

catalyse the electrochemical reduction of oxygen in pure cultures: *Sphingobacterium* sp. and *Acinetobacter* sp. [11]. As observed here, the current densities that were obtained with pure cultures of these two species were lower than the current provided by the whole wild biofilm (14 and 31% respectively).

There were two main differences between wild biofilm inoculum and bacterial isolates: i) wild biofilms were composed of complex mixed populations, ii) wild biofilms were scraped and resuspended without any intermediate planktonic culture. In consequence, two different or complementary assumptions can explain that only 2 strains exhibited EA properties, and that these two strains exhibited lower efficiency than whole biofilms. On the one hand, it may be thought that the high efficiency of natural mixed biofilms is due to synergetic interactions between different strains. Conversely, or in complement, it may be suspected that most wild strains lose their EA properties during the intermediate planktonic cultures.

4. Conclusions

This work has presented the first case of reconstruction, in a small closed volume, of a marine biofilm that was able to efficiently catalyse the reduction of oxygen. Up to now, it had not been possible to form EA biofilms directly on polarised electrodes in small volumes of seawater. It has been shown here that inoculating the reactor with a wild EA biofilm allows marine biofilms to be formed that keep their activity for oxygen reduction. Using a continuously fed reactor

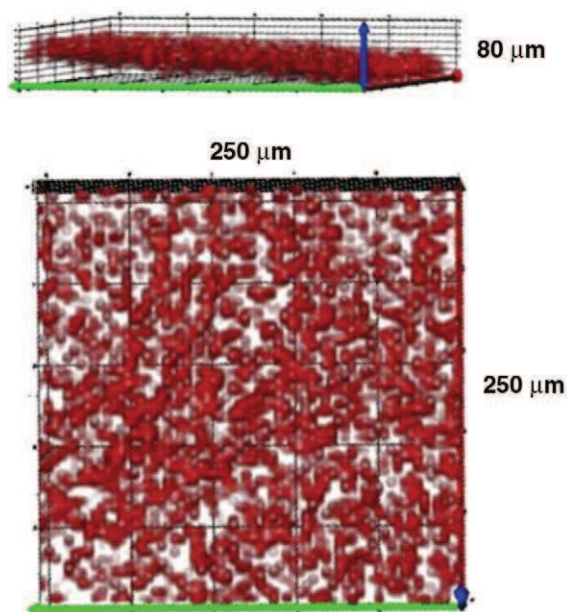


Fig. 6. 3D structure of the aerobic biofilm formed on stainless steel after 36 days of constant polarisation at -200 mV vs. Ag/AgCl; a) z-profiles, and b) top view of the rebuilt structure.

Table 1
List of isolates tested in electrochemical reactors.

Bacterial class	Bacterial isolates
Actinobacteria	<i>Actinobacterium</i> sp.
	<i>Arthrobacter agilis</i>
	<i>Arthrobacter oxydans</i>
	<i>Frigoribacterium</i> sp.
Gammaproteobacteria	<i>Aeromonas ichtiosmia</i>
	<i>Acinetobacter johsonii</i>
	<i>Acinetobacter calcoaceticus</i>
	<i>Idiomarina loihiensis</i>
	<i>Marinobacter hydrocarbonoclasticus</i>
	<i>Pseudoalteromonas tetradonis</i>
Flavobacteriaceae	<i>Maribacter goseongensis</i>
	<i>Winogradskyella poriferorum</i>
Firmicutes	<i>Bacillus cohnii</i>
	<i>Bacillus firmus</i>
	<i>Bacillus pumilus</i>
	<i>Exiguobacterium lactigenes</i>
	<i>Paenibacillus</i> sp.
	<i>Staphylococcus cohnii</i>
	<i>Staphylococcus saprophyticus</i>

sp.: species.

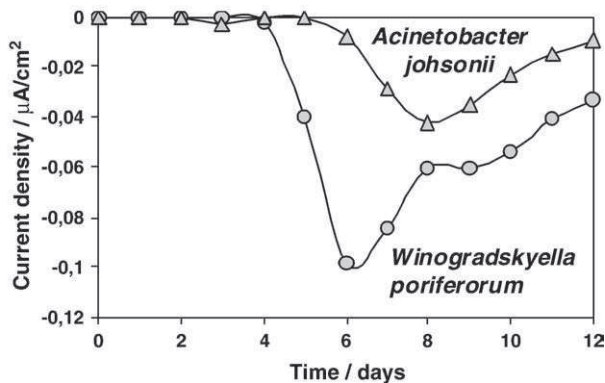


Fig. 7. Current evolution obtained in closed reactors with SS electrodes polarised at -200 mV vs. Ag/AgCl; reactors were inoculated with *Winogradskyella poriferorum* and *Acinetobacter johsonii*.

demonstrated that the availability of nutrients was the key factor that limited the development of EA biofilms in closed reactors. The reactor continuously fed with fresh seawater provided current densities in laboratory conditions higher than the values obtained initially with the wild biofilm in natural open environments. The continuous provision of the nutrients contained in natural seawater to the system was one of the reasons for the high current density reached. On the other hand, filtering the seawater avoided competition from indigenous common strains with the EA strains coming from the inoculum. These results represent a major step in improving the efficiency of marine EA biofilms for oxygen reduction. Checking the isolates for EA properties confirmed that *Acinetobacter* spp. are promising candidates. The current densities obtained with pure cultures were still lower than those obtained with wild whole biofilms. Synergetic effects can be suspected in whole biofilms and wild strains may be more efficient than the cultured isolates.

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References

- [1] H. Rismani-Yazdi, S.M. Carver, A.D. Christy, O.H. Tuovinen, Cathodic limitations in microbial fuel cells: an overview, *J. Power Sources* 180 (2008) 683–694.
- [2] H. Liu, B.E. Logan, Electricity generation using an air-cathode single chamber microbial fuel cell in the presence and absence of a proton exchange membrane, *Environ. Sci. Technol.* 38 (2004) 4040–4046.
- [3] S.E. Oh, B. Min, B.E. Logan, Cathode performance as a factor in electricity generation in microbial fuel cells, *Environ. Sci. Technol.* 38 (2004) 4900–4904.

- [4] T.H. Pham, J.K. Jang, I.S. Chang, B.H. Kim, Improvement of the cathode reaction of a mediator-less microbial fuel cell, *J. Microbiol. Biotechnol.* 14 (2004) 324–329.
- [5] E.H. Yu, S. Cheng, K. Scott, B.E. Logan, Microbial fuel cell performance with non-Pt cathode catalysts, *J. Power Sources* 171 (2007) 275–281.
- [6] F. Zhao, F. Harnish, U. Schröder, F. Scholz, P. Bogdanoff, I. Hermann, Challenges and constraints of using oxygen cathodes in microbial fuel cells, *Environ. Sci. Technol.* 40 (2006) 5193–5199.
- [7] B. Erable, L. Etcheverry, A. Bergel, Increased power from a two-chamber microbial fuel cell with a low-pH air-cathode compartment, *Electrochem. Commun.* 11 (2009) 619–622.
- [8] B. Slijkic, C.E. Banks, R.G. Compton, Oxygen reduction catalysis at anthraquinone centres molecularly wired via carbon nanotubes, *J. Iran. Chem. Soc.* 2 (2005) 60–64.
- [9] S. Cheng, H. Liu, B.E. Logan, Power densities using different cathode catalysts (Pt and CoTMP) and polymer binders (Nafion and PTFE) in single chamber microbial fuel cells, *Environ. Sci. Technol.* 40 (2006) 364–369.
- [10] P. Clauwaert, D. Van Der Ha, N. Boon, K. Verbeken, M. Verhaege, K. Rabaey, W. Verstraete, Open air biocathode enables effective electricity generation with microbial fuel cells, *Environ. Sci. Technol.* 41 (2007) 7564–7569.
- [11] K. Rabaey, S.T. Read, P. Clauwaert, S. Freguia, P.L. Bond, L.L. Blackall, J. Keller, Cathodic oxygen reduction catalyzed by bacteria in microbial fuel cell, *ISME J.* 2 (2008) 519–527.
- [12] V. Scotto, R.D. Cintio, G. Marcerano, The influence of marine aerobic microbial film on stainless steel corrosion behaviour, *Corros. Sci.* 25 (1985) 185–194.
- [13] A. Bergel, D. Féron, A. Mollica, Catalysis of oxygen reduction in PEM fuel cell by seawater biofilm, *Electrochem. Commun.* 7 (2005) 900–904.
- [14] C. Dumas, A. Mollica, D. Féron, R. Basseguy, A. Bergel, Marine microbial fuel cell: uses of stainless steel electrodes as anode and cathode materials, *Electrochim. Acta* 53 (2007) 468–473.
- [15] C. Dumas, A. Mollica, D. Féron, R. Basseguy, A. Bergel, Checking graphite and stainless anodes with an experimental model of marine microbial fuel cell, *Bioresour. Technol.* 99 (2008) 8887–8894.
- [16] M. Faimali, F. Chelossi, F. Garaventa, C. Corrà, G. Greco, A. Mollica, Evolution of oxygen reduction current and biofilm on stainless steels cathodically polarised in natural aerated seawater, *Electrochim. Acta* 54 (2008) 148–153.
- [17] J. Mergaert, A. Verhelst, M.C. Cnockaert, T.L. Tan, J. Swings, Characterization of facultative oligotrophic bacteria from polar seas by analysis of their fatty acids and 16S rDNA sequences, *Syst. Appl. Microbiol.* 24 (2001) 98–107.
- [18] E.A. Abd El Meguid, A.A. Abd El Latif, Electrochemical and SEM study on type 254 SMO stainless steel in chloride solutions, *Corros. Sci.* 46 (2004) 2431–2444.
- [19] A. Mollica, E. Traverso, D. Thierry, European Federation of Corrosion Publication 22, The Institute of Materials, 1997, p. 51.
- [20] A.K. Marcus, C.I. Torres, B.E. Rittman, Conduction-based modeling of the biofilm anode of a microbial fuel cell, *Biotechnol. Bioeng.* 98 (2007) 1117–1182.
- [21] K. Pedersen, Factors regulating microbial biofilm development in a system with slowly flowing seawater, *Appl. Environ. Microbiol.* 44 (1982) 1196–1204.
- [22] S.J. Giovannoni, U. Stingl, Review of molecular diversity and ecology of microbial plankton, *Nature* 437 (2005) 343–348.
- [23] S.H. Flint, P.J. Bremer, J.D. Brooks, Biofilms in dairy manufacturing plant – description, current concerns and methods of control, *Biofouling* 11 (1997) 81–97.
- [24] C. Dumas, R. Basseguy, A. Bergel, DSA to grow electrochemically active biofilms of *Geobacter sulfurreducens*, *Electrochim. Acta* 53 (2008) 3200–3209.
- [25] C. Dumas, R. Basseguy, A. Bergel, Electrochemical activity of *Geobacter sulfurreducens* biofilms on stainless steel anodes, *Electrochim. Acta* 53 (2008) 5235–5241.
- [26] L.R. Hilbert, D. Bagge-Ravn, J. Kold, L. Gram, Influence of surface roughness of stainless steel on microbial adhesion and corrosion resistance, *Int. Biodeterior. Biodegrad.* 52 (2003) 175–185.
- [27] M. Mattila, L. Carpen, T. Hakkarainen, M.S. Salkinoya-Salonen, Biofilm development during ennoblement of stainless steel in Baltic Sea water: a microscopic study, *Int. Biodeterior. Biodegradation* 40 (1997) 1–10.
- [28] J.W. Lee, J.H. Nam, Y.H. Kim, K.H. Lee, D.H. Lee, Bacterial communities in the initial stage of marine biofilm formation on artificial surfaces, *J. Microbiol.* 46 (2008) 174–182.