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Oxygen-reducing bidirectional microbial electrodes: A mini-review

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ABSTRACT

Aerobic bidirectional microbial electrodes (ABME) oxidize matter in anodic conditions (substrate supply, no oxygen) and reduce oxygen in cathodic conditions (oxygen supply, no substrate). The different experimental protocols used to design them, their electrochemical performance and the microbial populations involved are reviewed here. In spite of a limited number of studies, ABMEs have already shown promising capabilities to solve the issues of oxygen crossover and pH control in microbial fuel cells. They may also broaden the field of efficient electroactive bacteria to phyla other than *Proteobacteria*.

1. Introduction

At the beginning of the microbial fuel cell (MFC) story, things seemed clear: the microbial anode had to be anaerobic. The truth of this statement has become less obvious with the demonstration that some microbial anodes can tolerate oxic environments [1] and can even benefit from micro-aeration [2,3]. The initial rule has also been challenged by a recent study reporting an MFC that produces higher power density ($6.4 \text{ W}\cdot\text{m}^{-2}$) than ever reported before, with an air-cathode only a few millimetres from the microbial anode and without any separator between them [4].

Finally, microbial anodes have been fully reconciled with oxygen by the advent of aerobic bidirectional microbial electrodes (ABME). ABMEs are able to oxidize organic matter when implemented in anodic conditions (supply of electron donor(s), no oxygen) and to reduce oxygen in cathodic conditions (oxygen supply, no electron donor). Actually, the principle of bidirectional microbial electrodes has been known for a long time but it has generally been developed in fully anaerobic conditions, i. e. by using electron acceptors other than oxygen, such as nitrate, sulfate, fumarate or carbon dioxide [5–7].

Aerobic bidirectional microbial electrodes may offer elegant alternatives to improve MFC performance. They should also be at the core of microbial electrochemical snorkels, which are to be implemented in the fuzzy oxic/anoxic zone of wastewater treatment tanks [8]. Here, the state of the art of ABMEs is reviewed in order to reveal their specific advantages and to point out the directions in which there is an urgent need for research development.

2. Materials and methods

2.1. Material, medium, inoculum and reactors

All the ABME reported so far have been designed on carbon-based electrode materials (Table 1). Most studies have used synthetic media [9–12], only two have used natural media: compost leachate [13] or wastewater [14]. All the media, synthetic or natural, were supplemented with acetate (in the range of 1 to 20 mM) [11–15] or glucose (around 0.5 g/L) [9,10,16] as the carbon source.

Two types of inoculum were used: raw ones – compost [13] or wastewater [14] – and mature ones, such as electroactive biofilms coming from a previous MFC [9,11,12], or a pre-acclimated algal-bacterial biofilm with *Chlorella vulgaris* mixed with sludge [10]. Only one study implemented a pure culture (*Comamonas testosteroni*) [15].

Most studies used double-chamber MFCs [9,10,12,15,16]. One study also used a single-chamber MFC, for comparison [15]. A few studies implemented 3-electrode set-ups to control the potential applied to the electrode [11,13,14]. The values of the applied potential are grouped in a narrow range: -0.2 V/SCE [13], $-0.3 \text{ V vs Ag/AgCl}$ [11], $-0.2 \text{ V vs Ag/AgCl}$ [14]. An attempt to use $+0.2 \text{ V vs Ag/AgCl}$ [11] did not give any encouraging result. A specific system has recently been designed and consists of a circular rotating electrode with one half immersed in the solution and the other half exposed to air [14].

2.2. Biofilm growth protocols and polarity reversal

The design of ABMEs is generally articulated in two phases. The first

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Table 1 Characteristics and electrochemical performance of ABMEs reported in the literature; EAB: electroactive biofilm from previous MFC; SM: synthetic medium; WW: wastewater.

Purpose	Reactor	Electrode	Medium & inoculum	Polarity reversal	J_{anode} ($\text{mA}\cdot\text{m}^{-2}$)	J_{cathode} ($\text{mA}\cdot\text{m}^{-2}$)	E_{anode} (V /ref)	E_{cathode} (V /ref)	Ref
Design O_2 -reducing biocathode	3-electrode	Carbon cloth 8 cm^2	Compost leachate, acetate	Alternating supply of acetate and oxygen	16 000	-400	-0.2 V/ECS (imposed)	-0.2 V/ECS (imposed)	[13]
Design O_2 -reducing biocathode & pH control		Granular graphite 156 cm^3	SM, acetate EAB		1 231 No significant current	-513	-0.3 V vs Ag/AgCl (imposed) +0.2 V vs Ag/AgCl (imposed)	-0.3 V vs Ag/AgCl (imposed) +0.2 V vs Ag/AgCl (imposed)	[11]
Azo dye treatment & pH control	Reversible MFC	Carbon felt 30 cm^2	SM, glucose EAB, sludge	Anode chamber becomes cathode one and vice versa	150	-150	-0.35 V/ SCE	-0.15 V/ECS	[9]
Design O_2 -reducing biocathode			SM, Congo red, glucose Pre-acclimated algal-bacterial biofilm with <i>Chlorella vulgaris</i> and sludge	Dark anode chamber becomes cathode under illumination and vice versa	200	-200	-0.45 V/ SCE	-0.2 V/ECS	[10]
Design O_2 -reducing biocathode			SM, glucose and graphene oxide	Anode becomes the cathode and vice versa	230 vs 200 (w/o graphene)	-230 vs -200 (w/o graphene)	-0.4 V/SCE	+0.1 V/ECS	[16]
pH control	MFC with one abiotic chamber	Graphite felt 290 cm^2	Activated sludge SM, acetate Effluent from previous MFC (anode) and WW sludge (cathode)	Aerobic biocathode becomes the ABME with an abiotic anode	10	-3.3	-0.45 V vs Ag/AgCl	+0.12 V vs Ag/AgCl	[12]
Mechanisms of <i>C. testosteroni</i>	MFC & 3-electrode	Carbon cloth 12 cm^2	SM, acetate <i>Comamonas testosteroni</i>	Anaerobic conditions, 10 min aeration at the end only	-	-41.6	-	-0.7 V/SCE (voltammetry)	[15]
Aerobic rotating electrode for WW treatment	Single rotating electrode & 3-electrode	Graphite fibre brushes 10.9 cm^2	Real WW, acetate	Anode and cathode reactions on the same rotating electrode	1600 vs 400 (w/o modification) 6 500	-2000 vs -500 (w/o modification)	-0.2 V vs Ag/AgCl (imposed)	-0.2 V vs Ag/AgCl (imposed)	[14]
							-0.55 V vs Ag/AgCl (OCP)		

phase is most often anodic with the objective of favouring the development of a robust electroactive biofilm. When the substrate (electron donor) is fully depleted, the bioanode is reversed to the cathodic condition by supplying oxygen. Anodic and cathodic phases can then be alternated: oxygen supply is stopped and substrate is added to launch the anode phase, while oxygen is supplied again to drive the cathode phase when the substrate is depleted.

Many variations of this core protocol are possible depending on the systems. For instance, when a potential is applied, depletion of the substrate can be easily detected by the current dropping to zero [11,13]. The duration of the alternate anode–cathode periods can be a few hours to several days. In the study that implemented *C. testosteroni* as a pure culture, aeration was only performed for 10 min at the end of the experiment [15]. In solar powered MFCs, which implement algae, the cathode phase is driven by illumination of the algae, which produce oxygen.

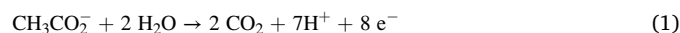
In one case, the ABME was designed by starting in cathodic conditions. [12]. The ABME was formed as the biocathode of an MFC that contained algae, firstly with oxygen supply and then under periodic illumination.

The rotating electrode bioreactor is a recent, original set-up that runs simultaneously in both anodic and cathodic conditions (Fig. 1). There is no polarity reversal and the ABME is continuously polarized at -0.2 V vs Ag/AgCl [14]. The biofilm is organized in two layers, catalysing the anodic and cathodic reactions simultaneously: the internal exoelectrogen layer, in contact with the electrode surface, and the external aerobic layer.

3. Applications

3.1. pH management in MFC

In an MFC, protons are produced at the anode by oxidation of the organic matter (e.g. acetate):



and hydroxide anions are produced at the cathode by the reduction of oxygen:



These reactions result in anode acidification and cathode alkalization, which are both strongly detrimental to MFC performance [17].

Prior to the development of real bidirectional electrodes, in 2008, the team of S. Freguia proposed a precursor system, where the anolyte outlet was used to feed the cathode compartment. The protons produced at the anode were thus removed from the anode compartment and used to mitigate cathode alkalization [18]. After this seminal study, other teams tackled the pH control issue in microbial electrochemical systems by designing ABME.

The first MFC using ABME in sequential anode–cathode configuration, reported in 2010, succeeded in stabilizing the pH at 7 without addition of buffer, by using aeration periods of 30 min every 2.5 h [11]. Other studies clearly confirmed the beneficial action against pH drift. In a double-chamber MFC, the pH of the anode compartment decreased from 7 to 5 and was corrected back to 7.0–7.5 by 10 days of cathodic phase. Conversely, the pH of the cathode, which rose to 7.6–7.7, was decreased to 5.2–5.4 by polarity reversal [9]. Similar success was confirmed later [10]. This pH self-neutralization promoted long-term stability. Up to 125 days of operation was ensured with a polarity reversal every 10 days [9,10]. In the same way, an MFC using algae to produce oxygen showed a pH varying from 6.2 at the end of the anode phase to 7.2 at the end of cathode phase, which lasted 14 h per day. The pH was kept between these two values for 141 days [12].

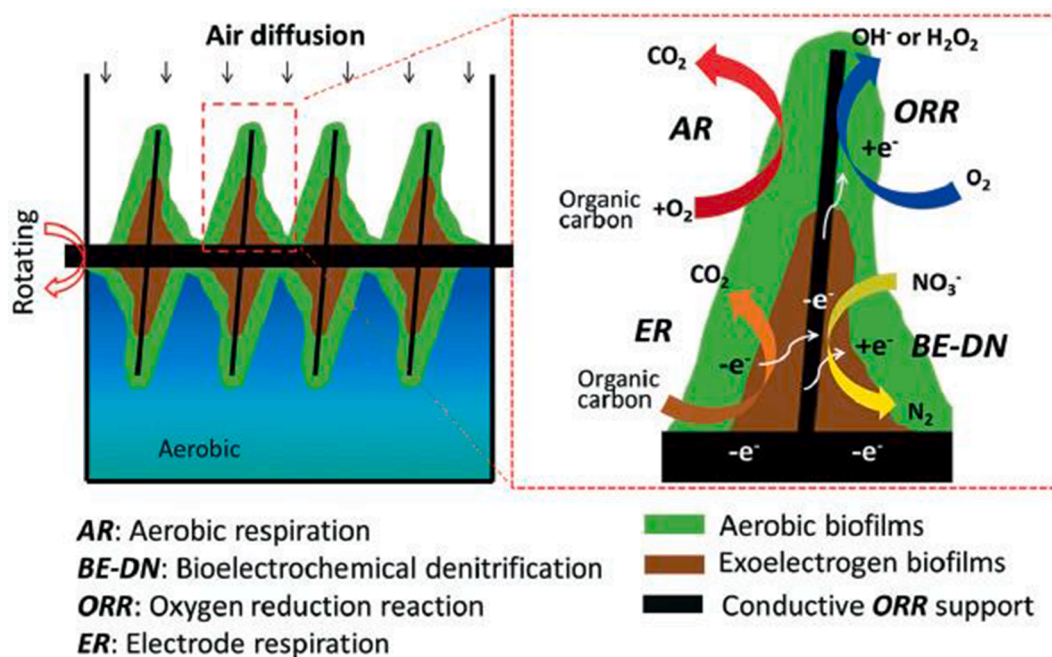


Fig. 1. Schemes of the aerobic rotating electrode and the possible biological and bioelectrochemical biofilm-catalysed reactions; from [14].

3.2. Effluent treatment

The use of ABMEs has been contemplated for anaerobic–aerobic azo dye treatment, based on dark/light reactions alternating in a solar MFC equipped with an algal-bacterial biofilm. In the dark, anodic phases, respiration of the algae maintained the anaerobiosis and improved the treatment of Congo Red. Conversely, during the cathode phases under illumination, the oxygen produced by the algae was used by the bacteria to aerobically degrade the by-products resulting from the Congo Red degradation. This sequential decolourization process with the alga *Chlorella vulgaris* resulted in a 93% increase in decolourization rate and an 8% increase in mineralization [10], compared to the same system without algae.

The recent concept of an aerobic rotating electrode (Fig. 1) has shown promising capability for the treatment of domestic wastewater [14]. It ensured a COD removal rate of $103.6 \pm 11.9 \text{ g O}_2 \cdot \text{L}^{-1} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$, in contrast with the $11\text{--}47 \text{ g O}_2 \cdot \text{L}^{-1} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ obtained with traditional non-conductive rotating contactor. This comparison shows how the electrical conductivity of the support is essential to enhance the performance of the treatment. When the potential of -0.2 V vs Ag/AgCl was applied to the rotating electrode, removal of $1400 \pm 124 \text{ mg O}_2 \cdot \text{L}^{-1} \text{ COD}$ took less than 0.7 days (i.e. removal rate was around $1300 \text{ g O}_2 \cdot \text{L}^{-1} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$).

3.3. Fundamental studies

Several studies devoted to ABME have had the specific objective of designing oxygen-reducing biocathodes and increasing the current they produce [11,13]. In this context, one original approach consisted of using the bidirectional capacity to integrate graphene into the biofilm during its formation [16]. Basic investigation of the bidirectional electron transfer mechanisms has also been carried out with a pure culture of *Comamonas testosteroni* [15]. Actually, in this case the aerobic conditions were approached only in the last part of the study, by 10 min of aeration.

4. Electrochemical performance

The values of the maximum current density reached by ABMEs and the related potentials, in anode and cathode conditions, are reported in Table 1. These values were extracted from studies performed in 3-

electrode cells or in MFCs. The 3-electrode cells allow the anode and cathode performance to be compared at the same value of applied potential. In contrast, in an MFC, the current is the same for the anode and the cathode but the potential of the anode is lower than that of the cathode. The performance of the anode is thus reported at a lower potential than that of the cathode. This situation is detrimental to both electrodes in comparison to characterization performed at the same potential by using 3-electrode cells. Consequently, the values extracted from MFC studies underestimate the capabilities of ABMEs, as can be observed in Table 1.

Logically, the highest values of current density were obtained in 3-cell set-ups for both anode and cathode conditions. The highest current density reported in anode operation was $16 \pm 1.7 \text{ A/m}^2$ [13]. Table 1 shows that, in MFCs, the current densities recorded for the bioanodes were clearly limited by the low current provided by the biocathodes. As discussed elsewhere [19], MFCs are not appropriate systems to characterize the intrinsic kinetics of an electrode.

In cathode operation, the highest current densities, of 2 A/m^2 (Table 1), have been obtained by applying the potential of -0.2 V vs. Ag/AgCl at the rotating electrode [14]. This system had the great advantage of ensuring continuous aeration of the electrode by rotating it with one half exposed to air (Fig. 1). Actually, one major issue of oxygen reduction is the low solubility of oxygen in aqueous solution (0.28 mM at $20 \text{ }^\circ\text{C}$), which severely limits its mass transport to the biocathode. If the (modest) cost of the energy required to rotate the system is considered acceptable, this issue can be alleviated by implementing a rotating electrode. It should be noted that the N, P co-doped coating of the graphite brush also had a great effect as, without this surface modification, the current was only 0.5 A/m^2 .

When no potential was applied to the rotating electrode, and after adding 10 mM acetate, the potential dropped from 0 to -0.55 V vs Ag/AgCl while the current increased in the anodic direction from 0 to $6.5 \text{ A} \cdot \text{m}^{-2}$. The ABME left at open circuit thus showed a globally anodic behaviour while the electrode was continuously exposed to air by the rotation. This means that this system was able to develop efficient exoelectrogen capabilities although continuously exposed to aerobic conditions.

With stationary electrodes, the highest current densities obtained for oxygen reduction were 0.4 [13] and 0.513 A/m^2 [11]. Both studies explain that these high values were due to the accumulation of protons

during the anode phase (Eq. 1), which promoted oxygen reduction when the electrode was turned to the cathode phase (Eq. 2).

Integrating graphene into the biofilm led to a small increase of the current density, from 200 mA/m² without graphene to 230 mA/m² with graphene, at potentials of +0.1 V/ECS for the cathode and - 0.4 V/ECS for the anode [16]. Li et al., comparing a reversible MFC with a conventional one, noted a 36% increase of the power density due to the reversible operation [9]. The maximum power density was 38 W/m² (current density of 0.15 A/m²) compared to 28 W/m² for the conventional MFC.

The two solar powered ABMEs gave disparate results. In one case, current density remained very low. During the illumination phase the cathode produced - 3.3 mA/m² at the potential of +0.12 V vs Ag/AgCl. During the dark phase, the current was 10 mA/m² with a potential of -0.45 V vs Ag/AgCl [12]. This was the only case where the ABME was initially formed in cathodic conditions. It could be feared that forming an ABME from a biocathode may not be a great solution. Nevertheless, other studies will be necessary before drawing firm conclusions. The second study dealing with a solar powered ABME implemented a reversible MFC and commonly obtained values of current density up to 200 mA/m², for potential values of -0.45 and -0.2 V/SCE for the anode and the cathode, respectively [10].

5. Microbial population and mechanisms

5.1. Microbial populations

In the case of solar powered MFCs, different groups of microorganisms, such as unicellular green algae, cyanobacteria or protozoa, such as *Trinema enchelys*, have been identified, without it being possible to determine which contributed to the anodic or cathodic reactions [12].

Two ABMEs, which produced among the highest values of current intensity for oxygen reduction, showed the selection of a given dominant genus or phylum. The genus *Ignavibacterium* was dominant in the photo-ABME designed with *C. vulgaris* algae. This genus, from the *Chlorobi* phylum, is known to include electroactive bacteria. Similarly, the ABMEs formed from compost leachate were strongly dominated by the *Chloroflexi* phylum (49 ± 1%), frequently found in bioelectrochemical systems. The species from this phylum can use a large number of electron acceptors and are aero-facultative bacteria.

The graphene-modified ABME showed a large abundance of *Proteobacteria*, the most usual phylum in electroactive biofilms [16]. The abundance of *Proteobacteria* was 24% before the polarity reversal and 63% after. In contrast, the abundance of *Firmicutes*, another phylum often enriched in electroactive biofilms [25], decreased from 49% to 15% after the polarity reversal. The presence of graphene in the ABME increased the abundance of anaerobic bacteria such as *Geobacter* (2% with graphene vs 0.04% without). It was speculated that some bacteria may have been coated by graphene, which protected them from oxygen.

A model of growth was proposed by Chen et al. to explain the self-organization of the ABME on the aerobic rotating electrode [14]. At the beginning, an aerobe biofilm, dominated by *Neomegalonema* (40% ± 5%) grew on the fibres of the carbon brush alongside a few exoelectrogens dominated by *Arcobacter* (15% ± 4%). These two genera belong to the *Proteobacteria* phylum. When the aerobe biofilm was thick enough to block oxygen diffusion, the exoelectrogen biofilm could develop, leading to a two-layer biofilm, with an internal anaerobic layer and an aerobic surface layer (Fig. 1).

5.2. Mechanisms

The molecular mechanisms of bidirectional extracellular electron transfers have been intensively investigated and have been reviewed in several recent articles [5–7]. It would be useless to plagiarize these extensive review works here. Nevertheless, almost all studies deal with fully anaerobic bidirectional electron transfer, i.e. using electron

acceptors other than oxygen, for example nitrate, sulfate, fumarate, or carbon dioxide [6,7]. It is not yet known whether or not electron transfer follows the same molecular pathways as those already described in anaerobic conditions when oxygen is the electron acceptor.

So far, very few studies have tried to unravel the molecular pathway of ABME. *Shewanella oneidensis* [26] and *Comamonas testoteroni* [15] have been used as model microorganisms in this objective. In the first case, the cathodic electron flow is speculated to result from reversal of multiple extracellular electron transport routes driven by proton gradients. In the second case, in contrast, two redox species have been identified. One, bound to the outer membrane, is involved in anodic electron transfer, while the other is soluble and catalyses oxygen reduction. The two redox species are speculated to have totally different behaviours for “inward” and “outward” electron transfer.

6. Concluding remarks and perspectives

Despite the limited number of studies, some clear conclusions can be extracted. First of all, ABMEs have definitely demonstrated that some efficient microbial anodes can accept regular expositions to air. The anodic current densities reported to date are smaller than the highest values reached with conventional microbial anodes, but they probably suffer from being evaluated in MFCs, i.e. under limitation by the cathode kinetics. Further studies in 3-electrode set-ups are advisable to characterize the kinetics of ABME over large ranges of potential.

Several studies have confirmed very interesting capacity for pH control in MFCs, resulting in long-term stability for up to several months. ABME may thus be the absolute weapon to solve the problems of oxygen crossover and pH control in MFCs. Nevertheless, these weapons must be handled with care. In particular, the two phases, of substrate addition on the one hand and aeration on the other, must be well separated in time, otherwise heterotrophic aerobic bacteria could develop and compete for oxygen reduction with the electrotroph bacteria on the cathode [11]. The balance between anodic and cathodic reactions must also be regulated accurately. It has already been observed that the anode acidification can be higher than the cathode alkalization, sometimes leading to acidification of the cathode [9].

In a general framework, beyond the scope of ABMEs, the most efficient biocathodes for oxygen reduction have reached current densities above 2 A/m². For example, current densities up to 2120 ± 64 mA/m² have been reached with a carbon felt electrode polarized at -0.1 V/ECS [20], 2200 mA/m² at + 0.2 V/SCE in hypersaline media [21] and up to 3000 mA/m² at + 0.4 V vs Ag/AgCl with forced aeration at 1.2 L/min air flow rate [22]. In the scope of ABMEs, the rotating electrode produced cathodic currents at the same level, thanks to the improved mass transport. The other ABMEs were slightly behind but many ensured current densities of several hundreds of mA/m² [9–11,13], similarly to many conventional O₂-reducing biocathodes [23,24].

Analyses of the microbial populations involved in ABMEs are still rare but they have already identified various dominant genera, with suspected electroactivity. Some of them belong to phyla different from the *Proteobacteria*, which are a ubiquitous source of anaerobic electroactive species. ABMEs should consequently broaden the field of microorganisms of high interest for microbial electrochemical technologies.

Most studies have been conducted in synthetic media and always with the addition of acetate or glucose. Further efforts must now be made to use real wastewater if the objective is an application for wastewater treatment. Obviously, the question about a single reversible or multiple extracellular electron pathway(s) remains a key basic issue to be dealt with.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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