

Minireview

Outlook for benefits of sediment microbial fuel cells with two bio-electrodes

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Summary

The benefits of sediment microbial fuel cells (SMFCs) go beyond energy generation for low-power applications. Aside from producing electrical energy, SMFCs can enhance the oxidation of reduced compounds at the anode, thus bringing about the removal of excessive or unwanted reducing equivalents from submerged soils. Moreover, an SMFC could be applied to control redox-dependent processes in sediment layers. Several cathodic reactions that may drive these sediment oxidation reactions are examined. Special attention is given to two biologically mediated cathodic reactions, respectively employing an oxygen reduction and a manganese cycle. Both reactions imply a low cost and a high electrode potential and are of interest for reactor-type MFCs as well as for SMFCs.

Introduction

Sediment microbial fuel cells (SMFCs) are a relatively new technology which involves multidisciplinary developments. The application of SMFCs is conventionally regarded in relation to sustainable energy generation. In this outlook, we broaden the application field to encompass the enhanced oxidation of organic compounds in sediments and a means to control redox-dependent processes, by relying on the anode potential.

In order to serve the aforementioned applications (energy generation, enhanced oxidation and redox

control), i.e. to achieve rapid and intense oxidation reactions at the anode, and in order to attain high anodic electrode potentials, a very reactive and preferably low-cost cathode is needed. The lack thereof has caused cathode catalysis to be one of the limiting factors for MFC operation at the moment. Nevertheless, cathodes for SMFCs are largely unexplored up till now. The second part of this outlook therefore overviews cathodic processes applicable for SMFCs, with a focus on the more recent biologically mediated cathode reactions. In this context, an oxygen reduction reaction and a manganese cycle will be addressed. As these forms of microbially influenced electrochemistry on cathodes basically originate from the phenomenon of steel biocorrosion, reference will be made to the latter concept.

MFC principle

Fuel cells convert the energy contained in either organic or inorganic electron donors into electrical energy using chemical catalysts (Friauf, 1961). Biological fuel cells (biofuel cells) use biocatalysts to achieve either the anodic, the cathodic or both reactions. These catalysts can be either enzymes or whole cells, in which case the fuel cells are respectively called enzymatic fuel cells and MFCs (Katz *et al.*, 2003). The MFCs require microorganisms to switch from their natural electron acceptors, such as oxygen and nitrate, to an insoluble electron acceptor, the anode (Fig. 1) (Rabaey and Verstraete, 2005). Transfer of electrons to an anode can proceed through several mechanisms, which can be categorized as direct or mediated (Rabaey *et al.*, 2007). Direct transfer implies direct contact between membrane-bound electro-active compounds, such as cytochromes, and the anode. It was recently suggested that conductive pili or pilus-like structures, also referred to as nanowires, might establish a longer distance link with the electrode (Reguera *et al.*, 2005; Gorby *et al.*, 2006). This phenomenon is however not yet unequivocally demonstrated due to the lack of longitudinal conductivity data for the pili. Mediated electron transfer implies active shuttling of soluble redox mediators between the microorganism and the anode. These mediators can either be externally added, such as neutral red, or microbially produced, such as phenazines (Park and Zeikus, 2000; Rabaey *et al.*, 2004; 2005a). As

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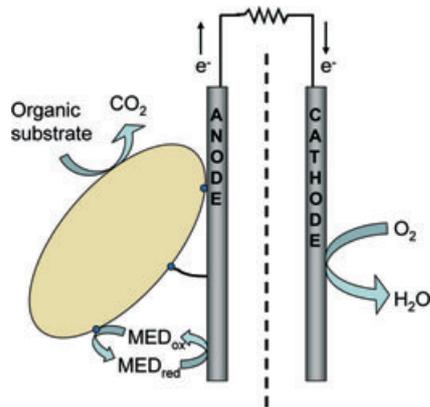


Fig. 1. Scheme of an MFC in which an organic substrate is oxidized at an anode. Electrons are transferred from the microorganisms to the anode through three possible mechanisms: direct contact, nanowires (a still controversial concept) and redox mediators. They then pass an electrical circuit and end up at the cathode, where they reduce the final electron acceptor, in a chemically or microbially mediated reaction. The dashed line represents a membrane in case of reactor-type MFCs and the sediment–water interface in case of sediment MFCs. MED, a soluble redox mediator shuttle.

soon as the electrons are delivered to the anode, they are conveyed through an electrical circuit containing a current user towards the cathode, where they reduce a terminal electron acceptor. Because of the voltage that is gener-

ated between the anodic oxidation and the catalysed cathodic reduction, electrical energy can be produced. The organic substrate (electron donor) utilized at the anode varies from simple carbohydrates, such as glucose (Rabaey *et al.*, 2003; Cheng *et al.*, 2006a) and acetate (Bond and Lovley, 2003; Liu *et al.*, 2005), to more complex compounds, such as starch (Niessen *et al.*, 2004), wastewater (Min and Logan, 2004; Rabaey *et al.*, 2005b) and sediment organic and inorganic constituents (Reimers *et al.*, 2001). The oxidation of sulfide at MFC anodes has been described as well, but the involvement of microorganisms is as yet unclear (Rabaey *et al.*, 2006; Reimers *et al.*, 2006).

The SMFCs

Principles of SMFCs

Energy can be harvested from naturally occurring electropotential differences (Whitfield, 1972; Reimers *et al.*, 2001), through the burial of an anode in a sediment and the immersion of a cathode in the overlying body of water (Fig. 2A). Such a set-up is called an SMFC or benthic MFC (Reimers *et al.*, 2006). An open circuit voltage (the maximum obtainable voltage when no current flows through the SMFC) of about 750 mV is typically

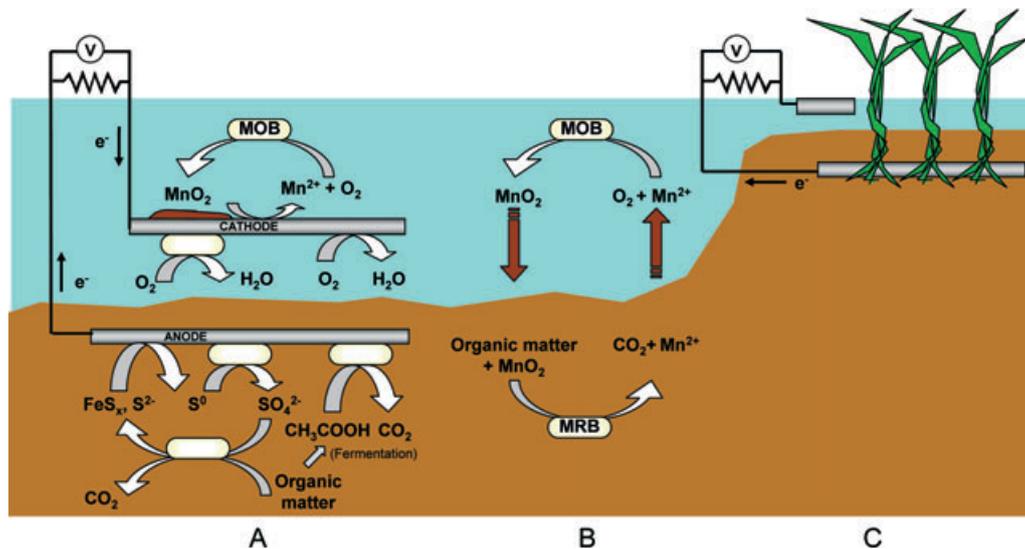


Fig. 2. Overview of discussed oxidation strategies in sediments.

A. An SMFC with an anode buried in sediment and a cathode floating on or in the overlying waterlayer. The anode oxidizes reduced compounds such as sulfides and fermentation products, while the cathode reduces oxygen, the final electron acceptor. In practice, both electrodes are positioned a few decimetres apart from each other. A direct cathodic reduction of oxygen (either chemically or microbially mediated) is depicted at the bottom of the cathode, while a cathodic cycle involving manganese is depicted on top of the cathode. In the latter, manganese oxides are reduced by electrons delivered from the cathode and are re-oxidized and deposited by manganese-oxidizing bacteria (MOB). These reactions presumably take place in a biofilm on the surface of the cathode. Microorganisms are represented by ovals.

B. Manganese cycle occurring throughout the sediment–water system. The oxidation of manganese by MOB is performed in an oxic zone, while the manganese reduction – with concomitant oxidation of organic matter – is performed by manganese-reducing bacteria (MRB) in an anoxic zone (De Schamphelaire *et al.*, 2007).

C. Application of the SMFC system in a (constructed) wetland. The anode matrix is positioned in the root zone, the cathode in the overlying waterlayer.

obtained (Lowy *et al.*, 2006). Microorganisms create reducing power in the sediment directly through the oxidation of organic matter or through redox reactions involving inorganic reduced compounds, such as sulfur species. The cathodic reaction of the SMFC involves the reduction of high redox electron acceptors, such as oxygen dissolved in the water body.

Anodic process of SMFCs

As pointed out above, research on SMFCs has indicated that several reactions contribute to the generation of electrical current: (i) the chemical oxidation of microbially produced reductants, such as humic acids, Fe^{2+} , but especially sulfur compounds at the anode, (ii) the microbial oxidation of organics such as acetate and (iii) the microbial oxidation of S^0 to SO_4^{2-} (Reimers *et al.*, 2001; Bond *et al.*, 2002; Tender *et al.*, 2002; Holmes *et al.*, 2004a; Ryckelynck *et al.*, 2005; Lowy *et al.*, 2006). Through the release of electrons during these processes, captured by the anode, a current is generated in an electrical circuit. The responsible anodic microbial communities have been investigated by several authors and in a number of different aquatic sediments – marine, salt-marsh and freshwater. A clear enrichment of δ -*Proteobacteria*, more specifically of the family *Geobacteraceae*, was most often observed on anodes. The most closely related organism for anodes residing from marine sediments was *Desulfuromonas acetoxidans*. For freshwater sediments, the most related species were *Geobacter* species, of which *Geobacter sulfurreducens* is the most studied organism (Bond *et al.*, 2002; Tender *et al.*, 2002; Holmes *et al.*, 2004b; Lovley, 2006a). *Desulfuromonas acetoxidans* and *G. sulfurreducens* were found to be able to oxidize acetate with an anode as sole electron acceptor (Bond *et al.*, 2002). In the study of Holmes and colleagues (2004b), *Desulfobulbus/Desulfocapsa* species appeared to be especially enriched on anodes from estuarine sediments, while the Fe(III)-reducing *Geothrix* species were only found to be enriched on freshwater anodes. Moreover, Reimers and colleagues (2006) discovered that the anodic community diversity changed with anode depth: the top section of a rod shaped anode, positioned vertically in the sediment, was less diverse than the bottom section. Recently, marine sediment was used to enrich a thermophilic and anodophilic consortium, capable to produce 10-fold more current at 60°C than at 22°C. This consortium mainly consisted of the Gram-positive *Thermincola carboxydophila* (Mathis *et al.*, 2008).

Energy generation with SMFCs

The production of energy in the form of readily usable electrical power is the most direct application of SMFCs.

The energy can be generated for a long period in remote areas and from a sustainable source. They allow an *in situ* electricity generation for small electrical apparatuses. As such, SMFCs could be used to power sensors and data transmitters, for example, in the middle of the ocean. To overcome low currents and voltages, capacitors and converters can be added (Shantaram *et al.*, 2005). This principle was recently demonstrated with an SMFC with a bio-anode which could power a meteorological buoy and data transmitter with an average power consumption of 18 mW, by applying a power conditioner and a capacitor (Tender *et al.*, 2008).

The average sustainable power which has been obtained from SMFCs is in the order of 10 to 20 mW m⁻² of anodic electrode footprint area (EFA) (Reimers *et al.*, 2001; Tender *et al.*, 2002), with a sustainable maximum of 28 mW m⁻² EFA or 104 mA m⁻² EFA (Tender *et al.*, 2002; Ryckelynck *et al.*, 2005). The SMFCs described in literature thus far were mostly positioned in marine waters (Reimers *et al.*, 2001; 2006; Bond *et al.*, 2002; Tender *et al.*, 2002; Holmes *et al.*, 2004b; Ryckelynck *et al.*, 2005; Lowy *et al.*, 2006; Rezaei *et al.*, 2007). They have the advantage of high salt concentrations in the electrolyte and thus a high ion conductivity and low cathode passivation. Nonetheless, the output of SFMCs in general is restricted because of a number of limitations:

- (i) Kinetics at the anode. Lowy and colleagues (2006) could temporarily increase the current densities at the anode by increasing the kinetics of microbial reduction of the anode. This was done by applying microbial oxidants onto or into anode graphite disks as charge transfer mediators. An anode with adsorbed anthraquinone-1,6-disulfonic acid delivered a maximum, non-sustainable power density of 98 mW m⁻² EFA, while a maximum, non-sustainable power density of 105 mW m⁻² EFA was achieved using Mn^{2+} and Ni^{2+} incorporated in the anode. Current densities decreased with the square root of time, resulting from mass transfer limitations (Lowy *et al.*, 2006). In the context of kinetics, temperature should be mentioned as a significant factor, as Reimers and colleagues (2001) noticed, an abrupt drop in power resulting from a temperature decrease of 4.5°C.
- (ii) Mass transfer limitations for electron donors to reach the anode form a major limitation for power production from SMFCs. Lowy and colleagues (2006) stated that a sustained power generation of 100 mW m⁻² EFA should be possible in environments with high concentrations of anode reactants. Such a test was performed in a cold ocean sulfide seep (Reimers *et al.*, 2006), where the advective flow of sulfide- and

methane-rich fluids could indeed – initially – support a higher power production. A maximum power output, sustainable during 24 h, of 1100 mW m⁻² EFA was obtained. The anode used in this study was a long graphite rod, having a high ratio of electrode surface over EFA. A deposition of elemental sulfur on the anode, resulting in a passivation and hence deactivation of the anode surface, became apparent as an additional limitation of the SMFC system (Reimers *et al.*, 2006). Nielsen and colleagues (2007) installed benthic chambers, housing the anode, on top of the sediment. In order to increase the power production, advective flows of reductant-rich porewater were led through an anode with high surface area. These flows were either caused by natural processes or created through active pumping. Continuous power densities of 233 mW m⁻² EFA were reached.

- (iii) The organic matter content of sediments as such is a limiting factor as well. Rezaei and colleagues (2007) tried to increase the power generation through the addition of particulate organic matter (chitin and cellulose) in the anodic matrix, an approach which is also not yet sustainable.
- (iv) Cathode catalysis. Oxygen availability, as a limiting factor for cathode performance of SMFCs, was handled in a lab-scale SMFC by using a rotating cathode, increasing the power production with a factor 1.7 as compared with a non-rotating cathode. Sustainable outputs of 25 mW m⁻² EFA were reached with a rotating cathode and with sucrose fed at the anode (He *et al.*, 2007).

Although research on sediment fuel cells has focussed on marine environments, freshwater environments can also sustain electrical current production. Lower output values have been reported, likely due to the decreased conductivity and the fact that salt water – as opposed to freshwater – enhances ‘virtual’ corrosion at the cathode, which is beneficial for the cathode performance (Bergel *et al.*, 2005). Holmes and colleagues (2004b) obtained a sustainable current production of 20 mA m⁻² total anode surface for marine SMFCs versus 9 mA m⁻² total anode surface for freshwater environments. In laboratory freshwater SMFCs, the mass transfer limitation was attenuated by growing rice plants in the sediment (De Schampelaire *et al.*, 2008), which could continuously deliver fresh substrates at the anode, under the form of rhizodeposits, hence increasing the sustainable power production with a factor 7. A maximum sustainable output of 33 mW m⁻² EFA could be attributed to an oxidation of rhizodeposits. Kaku and colleagues (2008) also reported the application of an SMFC in a freshwater rice paddy field. However, a low sustainable output (3 mW m⁻² EFA) was reached with their system.

In order to allow a better comparison of the results discussed above, an overview of power and electrical currents obtained with SMFCs is presented in Table 1.

SMFCs for the oxidation of organic compounds in sediments

Organic overloading of submerged soils through anthropogenic and natural processes may result in negative consequences for the aquatic environment as well as the area immediately surrounding it. Such accumulation may affect the aquatic community, for example by leading to oxygen deficiency, and cause an increase in greenhouse gas (GHG) emissions. Methane emissions have medians between 0.3 and 300 mg CH₄ m⁻² day⁻¹, depending on the type of underwater soil and the possible cultivation type. A maximum of 2.9 g CH₄ m⁻² day⁻¹ was noted for rice paddies (Le Mer and Roger, 2001). Organic matter accumulation may also impede navigation in waterways. Apart from the general accumulation of sediment organic matter, sediments may also be contaminated with specific organic compounds, such as hydrocarbons, of which the removal is desired.

Human intervention, for example by active aeration, may enhance the oxidative breakdown of these various types of organics. However, the applicable techniques for such an intervention have several limitations. Recently, an alternative technique for sediment oxidation was proposed (De Schampelaire *et al.*, 2007), encompassing a microbial manganese cycle in the water body (Fig. 2B). Oxidized Mn can act as electron acceptor at the sediment, while the reduced Mn diffuses upwards and is re-oxidized in the oxidized parts of the water body. Manganese hence represents an electron shuttle between the sediment organic matter and the final electron acceptor oxygen, thereby increasing the oxidation rate in the sediment. Here, we bring forth an SMFC as alternative, but related technique.

By constructing an MFC in the sediment, the anoxic oxidation of sediment organic matter can be stimulated and possibly accelerated. The basis hereof lies within the spatial separation of the oxidative, electron-generating half-reaction at the anode and the electron-consuming half-reaction at the cathode. As a result, the oxidation of the reduced compounds within the sediment is no longer limited by the availability of electron acceptors within the sediment, but rather relies on the vicinity of an anode.

Before quantitatively discussing the attainable anodic sediment oxidation rate, we will go deeper into the processes involved. During biodegradation in sediments, the complex assemblage of organic matter in sediments is hydrolysed to long-chain fatty acids, aromatic compounds and fermentables. The latter, such as sugars and amino acids, are subsequently fermented to acetate and minor

Table 1. Overview of current and power results^a obtained with SMFCs.

Operating characteristics of the SMFC ^b	Power density (mW m ⁻² EFA)	Power density (mW m ⁻² ES)	Current density (mA m ⁻² EFA)	Current density (mA m ⁻² ES)	Reference
• Marine sediment in laboratory					Reimers <i>et al.</i> (2001)
• Platinum mesh or carbon fibres	10				
• Marine environments					Tender <i>et al.</i> (2002); Ryckelynck <i>et al.</i> (2005)
Estuarine (constant voltage)	28	9	104	35	
Salt marsh (constant current)	26	9	100	34	
• Graphite disk with holes					Bond <i>et al.</i> (2002)
• Marine sediment in laboratory					
• Graphite disk		16			
• Laboratory incubations					Holmes <i>et al.</i> (2004b)
Marine				20	
Salt marsh				7	
Freshwater				9	
• Graphite disk					
• Coastal site					Lowy <i>et al.</i> (2006)
• Graphite disk with AQDS	98	47	560	266	
with Mn ²⁺ and Ni ²⁺	105	47	350	158	
(maximum non-sustainable results ^a)					
• Ocean cold seep					Reimers <i>et al.</i> (2006)
• Vertical graphite rod	1100	34	2647	82	
• Estuarine environment					Nielsen <i>et al.</i> (2007)
Forced advection	233	0.18	466	0.35	
Natural advection	140	0.71	350	1.77	
• Carbon brush					
• Laboratory seawater incubation					Rezaei <i>et al.</i> (2007)
• Pillow-shaped carbon cloth filled with Chitin 80	51	8	184	31	
• Laboratory incubation of river sediment amended with sucrose solution and a rotating cathode	25	12.5	5	2.5	He <i>et al.</i> (2007)
• Carbon cloth					
• Freshwater matrix planted with rice plants					De Schampelaire <i>et al.</i> (2008)
• Graphite felt	33	16	55	26	Kaku <i>et al.</i> (2008)
• Freshwater rice paddy field					
• Graphite felt	3	1.5	15	7	
• Field deployment in salt marsh, powering a meteorological buoy	387	16	1105	46	Tender <i>et al.</i> (2008)
• Array of vertical graphite plates					

a. All results represent average or sustainable values, except for the Lowy and colleagues (2006) reference, in which maximum non-sustainable values are given.

b. The operating characteristics of the SMFC respectively mention: type of sediment or set-up and the type of anode electrode. ES, (anodic) electrode surface; AQDS, anthraquinone-1,6-disulfonic acid.

fermentation products, such as ethanol and H₂. Finally, these fermentation products, as well as the long-chain fatty acids and aromatic compounds, can be further microbially oxidized. Microorganisms such as *Geobacteraceae* can be responsible for this oxidation, using Fe³⁺, Mn⁴⁺ or humic acids as electron acceptor in natural conditions. If an MFC is present, *Geobacteraceae* are able to use the solid anode as an alternative electron acceptor, hence generating an electrical current. The fact that *Geobacteraceae* are 10-fold more enriched on anodes generating current than on control anodes suggests that direct oxidation of organic matter is an important current generating process in SMFCs. The process can be mediated through a direct contact of the microorganisms with the electrode, involving outer-membrane c-type cytochromes (Lovley, 2006a,b). Other microorganisms enriched on sedimentary anodes, such as the freshwater bacterium

Geothrix fermentans, use electron shuttles to oxidize organic compounds at the anode (Bond and Lovley, 2005).

In sulfide-rich sediments, sulfur compounds can be a major electron donor for the anode, although these processes are related to organic matter oxidation as well. The sulfide present in these sediments results from the microbial sulfate reduction coupled to organic matter oxidation. Part of the sulfide formed reacts with Fe²⁺ to form reduced sulfur minerals, FeS and FeS₂. Dissolved sulfide, as well as sulfides trapped in iron minerals, can abiotically be oxidized to S⁰ at an anode (Ryckelynck *et al.*, 2005). The sulphate-reducing *Desulfocapsa* and *Desulfobulbus* genera were found to be enriched at an active anode in sulfide-rich sediments (Holmes *et al.*, 2004b), while it was demonstrated that *Desulfobulbus propionicus* can oxidize S⁰ to sulfate (SO₄²⁻) with an electrode as sole electron

acceptor (Holmes *et al.*, 2004a). Hence, the further oxidation of S^0 to SO_4^{2-} (microbially mediated or abiotic) is most likely one of the current generating processes in this type of sediments. Alternatively, *Desulfobulbus/Desulfocapsa* genera can disproportionate S^0 to SO_4^{2-} and S^{2-} , while the latter can be re-oxidized (Ryckelynck *et al.*, 2005). Overall, these processes result in the regeneration of sulfate as an electron acceptor for the oxidation of organic matter. Moreover, elemental sulfur can also act as an electron acceptor for the microbial degradation of organic matter (Ho *et al.*, 2004). Sulfur compounds can thus serve as electron shuttles for the oxidation of organic matter in the anodic half-cell and the amplification of a sulfur cycle at the anode can increase the oxidation rate of sediment organic matter (see also Fig. 2A).

Further evidence for the stimulating effect of an active anode can be found in the comparison of chemical profiles around active and control anodes in sediments. Tender and colleagues (2002) described a linear sulfide gradient above and below the active anode in a salt marsh in Tuckerton, New Jersey, with a sulfide depletion at the anode surface. An enrichment of dissolved iron and a modest enrichment of sulfate could also be observed near the active anode. These results indicate the regeneration of sulfur compounds acting as electron acceptors. In Yaquina Bay estuary, Ryckelynck and colleagues (2005) found a sulfate gradient near the active anode – sulfate concentrations decreased when approaching the active anode and increased again in a 3-cm-thick zone around this anode – but not near the control anode. Moreover, smaller increases in ammonia and phosphate were observed near the control anode. This implies that higher dissimilatory sulfate reduction rates occurred near the active anode. Second, although the organic carbon content was similar immediately above the active and control anodes, the organic matter content was higher near the sediment–water interface in case of the active anode. The steeper organic matter gradient around the active anode also suggests a higher oxidation rate of organic matter in the presence of a current generating anode. Reimers and colleagues (2007) applied marine plankton, representing the biofuel for SMFCs, in two-chambered reactor-type MFCs. For about 2 months, they operated six cells in closed circuit and one in open circuit as control. Although there was no statistical difference in the final level of total organic carbon between the active and control cells, the active cells did demonstrate significantly lower dissolved organic carbon levels. Recently, experiments were performed with model SMFCs, where a mixture of sand, starch and inoculum was applied around the anode (L. De Schampelaire and W. Verstraete, unpubl. results). Six cells were actively operated as SMFCs by closing the electrical circuit, while six other cells were held in open circuit and acted as control cells.

After approximately 1 month, the cells were dismantled. The actively operated SMFCs demonstrated levels of soluble chemical oxygen demand (COD) and volatile fatty acids which were significantly ($P < 0.01$) lower than in the control cells (respectively 20% and 50% lower).

The elements discussed above all argue for an increased rate of organic matter oxidation, stimulated by an active anode. The effect of the anode can mainly be noticed on dissolved organic matter oxidation, for which the availability of oxygen or sulfate is the limiting factor. The degradation of complex organic matter also involves hydrolysis and fermentation steps, which can be rate-limiting, and are, as far as known, not directly mediated by an active anode. The anodic process hence actually stimulates the final steps in the breakdown of complex organic matter, with acetate presumably as the most important electron donor because of its pivotal role in the degradation of organic matter by anaerobic microbial consortia (Lovley, 2006a).

At this moment, the process can be observed from a more quantitative point of view. Based on the values represented in Table 1, one can envisage an SMFC able to generate a sustainable electrical current of 100 mA m^{-2} of EFA. The current density is highly dependent on the anode design, as shown in Table 1. In the research of Tender and colleagues (2002), 40% of SMFC electron transfer in a salt marsh resulted from dissolved sulfide oxidation to elemental sulfur S^0 . As only modest oxidation of this S^0 occurred, 60% of the electron transfer was attributed to other processes, such as acetate oxidation or more generally, to carbon oxidation. In the work of Ryckelynck and colleagues (2005), 90% of the total electron transfer was due to a regeneration of SO_4^{2-} , which was originally consumed/reduced during the oxidation of organic matter. The regeneration of sulfate creates a new electron acceptor for organic matter oxidation. The observations from these studies indicate that at least 60–90% of SMFC electron transfer can be attributed to a direct or indirect oxidation of organic carbon. If the number of 100 mA m^{-2} EFA is related to sediment oxidation rates, one obtains a charge production of 8640 Coulomb per day, which equals a transfer of 89.5 mmol electrons per day. This correlates with a direct or indirect sediment oxidation rate of $13.4 \text{ mmol C (60%)}$ or $20.1 \text{ mmol C (90%) m}^{-2} \text{ sediment surface day}^{-1}$.

The values obtained can be compared with carbon accumulation rates: an organic carbon accumulation of $0.01\text{--}9.1 \text{ mmol C m}^{-2} \text{ day}^{-1}$ in highly productive marine areas was reported in Ryckelynck and colleagues (2005), while an excess oxidation demand for carbon oxidation of $0.21\text{--}2.4 \text{ mmol C m}^{-2} \text{ day}^{-1}$ at continental margin sediments was reported by Hartnett and Devol, (2003). A carbon burial rate of $1.8 \text{ mmol C m}^{-2} \text{ day}^{-1}$ in lake sediment was mentioned by Thomsen and colleagues (2004).

These numbers show that the present performance of SMFCs should suffice to oxidize all deposited organic carbon per m², assuming that the organics or their fermentation intermediates can be oxidized by anodophiles.

Besides the oxidation of organic matter, naturally residing in sediment layers, the anode reaction can also be of interest for the oxidation of organic contaminants in sediment layers, such as petroleum compounds. Adding oxygen to the subsurface to stimulate the aerobic microbial community is a typical strategy for accelerating contaminant degradation, but adding anaerobic electron acceptors can be a good strategy as well (Finneran and Lovley, 2001). The insertion of an anode as electron acceptor might have the same effect. Indeed, *Geobacter metallireducens* was shown to be capable to oxidize the aromatic contaminant benzoate at an electrode (Bond *et al.*, 2002).

Organic contaminants are a concern both in freshwater and marine systems. As for the oxidation of excess organic matter, this is often more crucial in freshwater than in marine environments. Due to the lower water depths in fresh waters, oxygen deficiency sooner affects the aquatic community and impediment of the waterway can sooner set in. Because of the proximity to rural areas, odour is also a more important issue. As more studies are performed on the better-performing marine SMFCs than on freshwater SMFCs, an expansion of the research focus is needed: the SMFCs should be engineered to perform as well in freshwater – with low salt concentrations – and with little or no water movement, which implies that an effective cathodic reaction should be ensured as well.

SMFCs for the oxidation of organic loading in constructed wetlands – an application

The aforementioned concept could be applied to enhance organic matter oxidation in the bottom layer of constructed wetlands. The latter, which are often reed beds, are used as a means of purifying pre-settled effluent from rural housing and agricultural waste streams before discharge into watercourses (McGechan *et al.*, 2005). Microorganisms living in the rhizosphere of the reed plants perform both anaerobic and aerobic degradation processes. Aerobic conditions are created as oxygen enters the rhizosphere through diffusion, convection and to a major extent through the aerenchyma of the stems and roots of the reed plants. The total oxygen transfer capacity lies in the range of 160–1400 mmol O₂ m⁻² day⁻¹ (McGechan *et al.*, 2005). In temperate climates, the reed plants are dormant during winter, which diminishes the oxygen supply through the aerenchyma to the root zone (Ouellet-Plamondon *et al.*, 2006). A general issue with wetlands in temperate and tropical regions is the fact that they

produce an effluent of limited quality due to background levels of organic matter, solids and nutrients generated within the wetland (Greenway and Woolley, 1999). To aid a wetland in meeting good effluent standards, an SMFC could be installed in the root zone of the constructed wetland (Fig. 2C). The electrode matrix of the SMFC anode, which will act as an additional electron acceptor, might thus compensate for the insufficient aeration of the root zone by the aerenchyma, dormant periods in plant growth, lower oxygen fluxes during the night (Armstrong and Armstrong, 1990) or lower oxygen dissolution at high temperatures. The resulting enhanced oxidation in the wetland might furthermore be accompanied by a reduced emission of methane from the wetland, as will be explained in the next section. A prerequisite for the installation of an independent SMFC system is the possibility for a sustainable operation of the cathode. Operation during winter might hence be hindered as low temperatures with possible ice formation can severely hamper or even harm the cathodic reaction. Water movement, through an aeration of the cathode, might be applied to prevent freezing of the cathode. An alternative option – instead of the SMFC – to overcome the lower performance of the wetlands is the artificial aeration of the entire root zone, which requires a substantial energy input and additional costs (Ouellet-Plamondon *et al.*, 2006).

Kern and Idler (1999) and Puigagut and colleagues (2007) reviewed the performances of several constructed wetlands throughout literature and obtained carbon removal rates of 137–1726 mmol C m⁻² day⁻¹. If artificial aeration would be required to sustain for example a carbon removal of 500 mmol C m⁻² day⁻¹, an aeration energy requirement of 16 Wh m⁻² day⁻¹ would be needed, assuming an oxygen transfer capacity of 2 kg O₂ kWh⁻¹ and an oxygenation capacity per load of 2. For an average household of four persons and 3 m² of reed bed per person, a daily energy requirement of 0.19 kWh would be needed. On the other hand, if an SMFC could be installed inside the wetland, it would require no net energy input but rather produce energy. Alternatively, a reactor-type MFC could be positioned at the outlet of the constructed wetland, in order to treat the wetland effluent before discharge in a watercourse.

Based on the values discussed in the last section, an SMFC could be envisaged which can oxidize approximately 17 mmol C m⁻² day⁻¹ in a sustainable way using a plain graphite disk as anode, during the production of 100 mA of current m⁻² EFA. This current density can be increased with at least a factor 25 (Table 1) through the combination of an increased ratio of electrode surface versus EFA and a high mass transfer. An affordable, granular – with high specific surface and thus high electrode surface over EFA ratio – and conductive material could be used to replace part of the support layer for

Table 2. Overview of redox-dependent processes which could be prevented by increasing the redox potential in sediments.

Process	Unwanted metabolite	Minimal inhibiting redox potential (V versus SHE)	Reference
Methanogenesis	CH ₄	CO ₂ /CH ₄ couple: -0.244 Inhibiting potential: -0.150	Stumm and Morgan (1996); Singh (2001)
Sulfate reduction	H ₂ S	SO ₄ ²⁻ /H ₂ S couple: -0.214 Inhibiting potential: c. -0.100	Devai and DeLaune (1995a); Stumm and Morgan (1996)
Reductive methylation	CH ₃ Hg	Hg ²⁺ /CH ₃ Hg : +0.15 (decrease from -0.22 to +0.15)	Compeau and Bartha (1984); DeLaune <i>et al.</i> (2004)
Phosphate reduction	PH ₃	P couples: -0.48 to -1.22 (does not refer to inhibiting potential)	Roels and Verstraete (2001)
Reductive dehalogenation	/	R-Cl/R-H couple: +0.25 to +0.6 Enzymatic: -0.36	Holliger <i>et al.</i> (1998)

SHE, standard hydrogen electrode.

growth of the reed plants, as this will allow sufficient COD oxidation rates. Alternatively, several parallel layers of electrode material with high specific surface area (e.g. carbon mats) could be installed in the root zone. Additionally, the flux of wastewater through the anode matrix could be increased, for example by installing a recirculation system, in order to increase the mass transfer. With electrical current densities a factor 25 higher, i.e. of the order of 2.5 A m⁻², 425 mmol C m⁻² day⁻¹ could be oxidized, which is fair within the range of COD removal rates in constructed wetlands. The total power which could be obtained from the system (with for example 0.5 V per SMFC electrode pair) is 1.25 W m⁻² constructed wetland. This means that this SMFC could daily produce 0.36 kWh instead of the 0.19 kWh energy requirement for artificial aeration.

SMFCs to control redox-dependent processes in sediments

The anode of an SMFC, buried in the sediment layer, is characterized by a specific potential. The latter is influenced by a number of factors (Rabaey and Verstraete, 2005a; Logan *et al.*, 2006; Rabaey *et al.*, 2007), such as: (i) the activation overpotential: this is a current-dependent potential drop between the electrode surface and the surrounding environment. The extent of catalysis of the anode, its surface area and its geometry are determining factors for this potential drop; (ii) the supply of electron donor, the efflux of breakdown products and both their respective redox potentials. This is mainly a diffusional problem, also influenced by the charge of the molecules; (iii) the internal resistance of the SMFC, which causes a potential loss between the anode and the cathode due to diffusional limitations for the cations; and (iv) the strength of the cathode. A well-catalysed cathode enables to increase the anode potential in conjunction with an adequate external resistance. Consequently, an anodic redox potential of a relatively high value can be obtained, which potentially turns this anode into an attractive elec-

tron acceptor in the sediment. This offers a way to manipulate redox-dependent metabolic processes in the sediment. An overview of such redox-dependent processes is given in Table 2. Notably the formation of nuisance compounds could hence be alleviated.

Methanogenesis and sulfate reduction, resulting in the formation of respectively the GHG CH₄ and the toxic and odorous H₂S, are commonly known anaerobic processes. They can be diminished at higher redox potentials (Devai and DeLaune, 1995a; Singh, 2001), such as by the presence of an anode with a redox potential sufficiently higher than the potential of the corresponding redox couples. The standard redox potential of the CO₂/CH₄ couple is -0.244 V versus SHE (Stumm and Morgan, 1996). The corresponding methanogenesis process is generally found not to occur at soil redox potentials above -0.150 V versus SHE (Singh, 2001). Some studies however demonstrate that a positive soil redox potential cannot inhibit the methanogenesis process as long as H₂ is sufficiently available (Conrad, 2002). As H₂ can however also be oxidized at an anode, with the use of a proper (bio)catalyst (Rosenbaum *et al.*, 2005), methanogenesis can even be decreased by an SMFC in this case. Ishii and colleagues (2008) successfully demonstrated that an electron-capturing anode can suppress methanogenesis. They showed this in reactor-type MFCs fed with cellulose and inoculated with rice paddy field soil. A comparison of electron fluxes to CH₄ and current generation suggested that the suppression of methanogenesis was not merely ascribable to simple competitive inhibition.

In case of sulfate reduction, the standard redox potential for the SO₄²⁻/H₂S couple is -0.214 V versus SHE (Stumm and Morgan, 1996). Devai and DeLaune, (1995b) found that in a range of relative redox potentials varying from -0.240 V to +0.220 V versus SHE, representing the anaerobic range of redox potentials in flooded soils, H₂S formation was significantly decreased from -0.100 V versus SHE and higher.

Mercury is a metal pollutant which can be transformed into methyl mercury in reducing conditions. The latter

is highly neurotoxic and is prone to biomagnification (Compeau and Bartha, 1984). The majority of CH_3Hg results from biomethylation of mercury (Compeau and Bartha, 1984), performed by sulfate reducers and as recently discovered, also by iron-reducing bacteria (Fleming *et al.*, 2006). Several researchers have reported a decrease in methylation (and an increase in demethylation), following an increase in redox potential from -0.22 V to $+0.15\text{ V}$ versus SHE (Compeau and Bartha, 1984; Matilainen *et al.*, 1991; DeLaune *et al.*, 2004). An oxidized sediment layer could serve as a barrier for the efflux of CH_3Hg from the sediment (Gagnon *et al.*, 1996; Mason *et al.*, 2006).

The anode of an SMFC can further be applied to influence redox reactions involved with other metals, in order to decrease the toxicity of sediments. The solubility and mobilization of arsenic are for instance decreased under conditions with high redox potential (Signes-Pastor *et al.*, 2007).

Another anaerobic process leading to unwanted emissions is the production of phosphine. The emission of this highly toxic gas has been detected from sediments and landfills among others. The redox couples involved in the reduction of phosphate to phosphine (PH_3) are extremely low (between -1.22 V and -0.48 V versus SHE at pH 7), which makes it highly unlikely that a dissimilatory energy metabolism is involved (Devai and DeLaune, 1995b; Roels and Verstraete, 2001). The presence of an anode could however still decrease the likelihood of anaerobic phosphine formation due to an increased redox potential in the anode environment.

Contrarily, reductive processes can lead to desired bioconversions such as reductive dehalogenation. For example, the R-Cl/R-H couple has a high redox potential in general: between $+0.25\text{ V}$ and $+0.6\text{ V}$ versus SHE (Holliger *et al.*, 1998), which turns halogenated compounds into interesting electron acceptors from a thermodynamic point of view. However, kinetics forces the processes to occur at lower redox potentials. For example, electron donors with a redox potential lower than -0.36 V versus SHE are required in the catalytic cycle of the *Desulfitobacterium* dehalogenase (Holliger *et al.*, 1998). In a reversed SMFC, the buried electrode could function as cathode, delivering electrons in order to stimulate the reductive dehalogenation and hence promote the breakdown of contaminants. This has been demonstrated in a reactor-type MFC, although in the presence of redox mediators (Aulenta *et al.*, 2007).

To achieve a reversed SMFC system, the redox potential of the cathode would have to be decreased by supplying electrons to the sediment through an external power supply. Gregory and Lovley (2005) demonstrated the bioremediation of uranium with such a system, by reducing the soluble and thus mobile U(VI) to the rela-

tively insoluble U(IV). This reduction could be accomplished by delivering electrons to microorganisms through a cathode buried in a soil layer and poised at -0.3 V versus SHE. The biologically reduced uranium was relatively stable and only re-oxidized and dissolved in the presence of oxygen. Therefore, this process could allow for example removal of uranium from groundwater.

Generally, it can be stated that the electrodes of an SMFC can be applied to influence redox-dependent processes, in order to decrease the adverse and/or harmful characteristics of sediments. Ryckelynck and colleagues (2005) demonstrated that the chemical profile of sediments was shifted in a zone of 3 cm around an active anode. The influence of the anode is thus limited in space, but is substantial, certainly in sediments with a forced water flow. An oxidized electrode grid could for instance be placed in the upper sediment layer, in order to form a protective barrier for nuisance or hazardous compounds. A well-catalysed and effective cathodic reaction would be required to pull the electrons through the electrical circuit and generate the required oxidizing force at the anode. In case the severity of a spill or the nature of the toxicant would require this, small amounts of energy could be applied to the system in order to poise the anode potential or to reverse the polarity of the system.

Chemically catalysed cathodes

Oxygen is in many cases the preferred terminal electron acceptor, due to its high potential and availability. However, reduction reactions of dissolved oxygen have slow kinetics and are restricted due to the limited solubility and diffusability of oxygen. To improve the kinetics of the oxygen reduction reaction, an obvious possibility is to apply a catalyst at the cathode, for instance by using a cathode from plain Pt (Reimers *et al.*, 2001) or by building a cathode from Pt coated carbon (Rezaei *et al.*, 2007). In the latter case, a binder, such as nafion or polytetrafluoroethylene, is required for the adhesion of the Pt catalyst (Cheng *et al.*, 2006b). So far, these prototypes of cathodes are expensive or difficult to make. Assuming an average catalyst loading of 0.5 mg Pt cm^{-2} , the cost of a platinum/carbon catalyst for an electrode surface of 1 m^2 amounts up to €700 (10 wt% Pt/C, E-TEK). Furthermore, as platinum is susceptible to poisoning through reaction with among others sulfide and organics, catalyst protection would be required as well. This could involve the application of an alloy of platinum and for instance ruthenium instead of pure platinum (Urgeghe, 2006).

Conventional fuel cells often use platinum in the construction of the cathode, but the limited lifetime, the high price and the overall environmental cost of platinum push research towards alternative catalysts, even for conventional fuel cells (Wang, 2005). The fact that an MFC

operates at ambient temperature and neutral pH results in higher activation energy. However, for MFCs, which demonstrate lower current and power levels, alternative electrode materials/assemblages with a lower performance suffice.

Recent attempts to replace the Pt at the cathode involved among others a coating of carbon cloth with iron(II)phthalocyanine or cobalt tetramethoxyphenylporphyrin (CoTMPP) as catalyst suspended in an aerated buffer solution (Zhao *et al.*, 2005). Park and Zeikus, (2003) developed a cathode from a mixture of graphite powder, ferric sulfate, kaolin and nickel chloride, baked as a tile and with Fe^{3+} as bound electron mediator. For an alternative, such as CoTMPP, used with the same loading as for Pt, the price comes to $\text{€}90 \text{ m}^{-2}$ (94%, Acros). With an equal loading (2 mg cm^{-2}), the performance of both catalysts is similar at the current densities observed in reactor-type MFCs (1.5 mA cm^{-2}) (Zhao *et al.*, 2005). Other substances are necessary to fix the catalysts, which will raise the price with a factor two. However, even with the application of an expensive catalyst, the rates of oxygen reduction remain limiting. Ambient temperature, low ionic strength and a mostly neutral pH pose thermodynamic and kinetic constraints on the performance of the cathode catalyst (Zhao *et al.*, 2006). An alternative strategy to diminish the overpotential towards oxygen reduction is the use of high specific surface areas to increase the reaction interface instead of applying a catalyst (Freguia *et al.*, 2007).

In case of SMFCs, the cathodes applied so far mainly consisted of extruded graphite disks, platinum mesh, carbon/graphite fibres or mats and a combination of carbon and titanium or platinum materials (Reimers *et al.*, 2001; 2006; Bond *et al.*, 2002; Tender *et al.*, 2002; Holmes *et al.*, 2004b; Ryckelynck *et al.*, 2005; Lowy *et al.*, 2006; He *et al.*, 2007; Nielsen *et al.*, 2007; Rezaei *et al.*, 2007; De Schamphelaire *et al.*, 2008). These cathodes require the reduction of dissolved oxygen in the overlying water bodies. This is analogous to seawater batteries that apply an oxygen reduction in the seawater. The movement of the water body is responsible for the delivery of fresh oxygen-rich seawater to the cathode and for the removal of alkaline reaction products (Hasvold *et al.*, 1997).

Pure graphite cathodes operate at higher open circuit potential than copper or stainless steel (Wilcock and Kauffman, 1997). As such, it would be the most interesting material to be used for the cathode of an (S)MFC. Wilcock and Kauffman (1997) stressed that a cathode of a marine fuel cell needs to have a very open structure to maximize the advection of oxygen towards the cathode and to stimulate the removal of reaction products. Graphite has a very brittle nature, which makes it less suited for the construction of a relatively tough cathode with open struc-

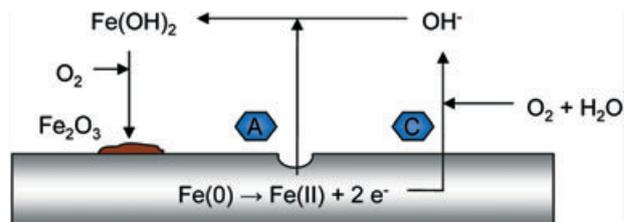


Fig. 3. Principle of steel corrosion with pH above 7 and in the presence of oxygen. Fe(0) is oxidized at an anodic site (A), resulting in dissolution of iron as Fe(II) in the overlying water layer. At the cathodic site (C), the released electrons are transferred to O_2 , resulting in formation of OH^- . The dissolved Fe(II) produced by the corrosion of the steel is transformed to Fe(III) oxide or rust by autoxidation in the presence of OH^- .

ture. More readily available and easily manipulated materials, such as stainless steel wools, may hence be more useful in this context.

Microbially catalysed cathodes – biocathodes

The use of microbially mediated cathodic reactions perhaps offers most perspectives for sustainable and sustained cathode operation. The processes can be natural, affordable, sustainable and show acceptable kinetics. Several research groups studied for example the possibility of a cathode-driven microbial denitrification (Gregory *et al.*, 2004; Clauwaert *et al.*, 2007a). A process of cathodic iron reduction could be made more sustainable through a continuous biological ferrous iron oxidation (Ter Heijne *et al.*, 2007). The latter two processes are mainly suited for reactor-type MFCs. Microbially catalysed cathode processes involving oxygen reduction or a manganese cycle are suited for both sediment-type and reactor-type MFCs and will be discussed in the following sections.

Underlying corrosion principle

The principle of corrosion (Bockris and Drazic, 1972; Pletcher and Walsh, 1984; Brett and Brett, 1993; Borenstein, 1994) is represented in Fig 3. A spatial variability in redox potential develops on the surface of steel because of factors such as impurities or microbial growth on the steel surface. Contact with a layer of a conductive solution, for example a rain drop, will allow electron flow through the steel from a place with a low potential (anodic site) to a place with a high potential (cathodic site), while (negative) ions will be conducted through the overlying conducting solution in the opposite direction. At the anodic site, the energetically favourable oxidation of Fe(0) to Fe(II) occurs, during which Fe(II) dissolves. Simultaneously, a reduction reaction will occur at the cathodic site, which will through the production of OH^- eventually

lead to the formation of Fe_2O_3 or rust. Iron molecules therefore dissolve at one place and will eventually precipitate as a porous iron oxide elsewhere.

Corrosion of steel is facilitated when differences in potential between different surface sites arise. Microorganisms can enhance the corrosion process in several ways (Borenstein, 1994). This is referred to as microbiologically induced corrosion (MIC). The underlying principles for MIC encompass a shielding from oxygen, excretion of acidic compounds (Borenstein, 1994), reduction of the protective iron oxide layer (Lovley, 1991) and under anaerobic conditions, the removal of the protective passivation layer of hydrogen by sulfate reduction (Borenstein, 1994) or nitrate reduction (De Windt *et al.*, 2003).

As discussed further, corrosion is often related to spontaneous natural processes involving Mn oxides and/or oxygen-reducing biofilms. These processes render stainless steels into well-performing cathodic electrodes. In case they are used in an SMFC, they will be connected to anodes installed into the sediment. This means that the electrons that are delivered to the cathodic site on the stainless steel could come from anodic reactions in the sediment instead of from an anodic oxidation of iron(0) in the steel itself. This could prevent the steel from degrading, and could be seen as a form of cathodic protection. The latter principle has been successfully applied by Orfei and colleagues (2006). Therefore, both graphite and stainless steel covered with manganese oxides sustained by a microbial community or an oxygen-reducing biofilm could be successfully used as a cathode in (sediment) MFCs, as demonstrated in the following sections.

Biocathodes for oxygen reduction

In the area of marine corrosion, the catalysis of oxygen reduction has been studied for many years. When stainless steel is immersed in aerobic seawater, a biofilm develops on the metal surface, increasing the rate of oxygen reduction. The required electrons originate from the metal, causing an increase of the free corrosion potential. This can, through a disruption of the protective oxide layer, finally lead to an increased corrosion probability and propagation (Bergel *et al.*, 2005).

The corrosion-related observations led Bergel and co-workers to the development of a low-cost stainless steel cathode for a fuel cell. A two-compartment fuel cell was developed with a platinum anode for the oxidation of hydrogen gas, and a stainless steel cathode for the reduction of oxygen (Bergel *et al.*, 2005). A biofilm was grown on the cathode through the immersion of stainless steel coupons in a tank with continuously renewed seawater and a polarization at constant potential value. The electrical current obtained started to increase exponentially in

the first week, which was assumed to be due to the biofilm growth. Power outputs could be compared between a cathode with biofilm and the same cathode with the biofilm removed. In the presence of the biofilm, power densities were up to a factor 100 higher. The open circuit potentials of the cathodes were also substantially higher in the presence of a biofilm (+0.44 V versus SHE as opposed to +0.01 V versus SHE). The highest power and current densities were respectively 325 mW m^{-2} and 1.89 A m^{-2} . It should be noted that these were obtained with a H_2 -saturated NaOH solution at pH 12.5 at the anode and with a cathode of 1.8 cm^2 .

The concept was further tested in a marine sediment fuel cell and a laboratory-type fuel cell (Dumas *et al.*, 2007). For the first set-up, stainless steel anodes were inserted in marine sediments and stainless steel cathodes in aerated seawater of 13°C . An increase in the current density (from 5 to 25 mA m^{-2}) and cathode potential could again be noted after several days. The system was capable to deliver 4 mW m^{-2} but suffered from technical problems such as grazing by fish. In the laboratory set-up, a stainless steel anode was placed in a closed tank with marine sediments and the stainless steel cathode in a container with continuously renewed seawater heated to 25°C . In this set-up, a higher performance could be obtained (up to 140 mA m^{-2} and 23 mW m^{-2}), but the anode was identified to be a limiting factor.

Dulon and colleagues (2007) performed microsensor experiments in aerated drinking water with small stainless steel wires as cathodes and a platinum wire as counter electrode. Under constant polarization at -0.06 V versus SHE, they were able to demonstrate the catalysis of oxygen reduction at the stainless steel wires. A current increase was observed with time and was attributed to the formation of a biofilm. Their findings suggest that a biofilm could form on stainless steel and catalyse the cathodic reduction of oxygen in water with low salt concentrations.

Experiments performed by our group (Clauwaert *et al.*, 2007b; Rabaey *et al.*, 2008; De Schampelaire *et al.*, 2008) demonstrate that graphite as cathode material can also support the growth of a biofilm which catalyses the reduction of oxygen. Clauwaert and colleagues (2007b) tested such a biocathode in a set-up with an acetate-fed tubular reactor-type of MFC surrounded with a cathodic graphite felt and obtained maximum power densities of 1.2 W m^{-2} and cathodic electrode potentials higher than $+0.4 \text{ V}$ versus SHE. Bacteria were shown to directly change the catalysis of the cathode, leading to strongly decreased potential losses. Members of the genera *Pseudomonas* and *Novosphigobium* were more enriched at the air-oriented side of the cathodic felt. In the work of Rabaey and colleagues (2008), using two-compartment reactor-type MFCs and carbon fibre and graphite-felt

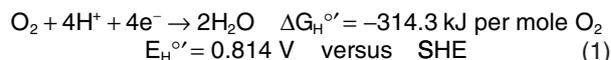
cathodes, microbially mediated cathodic current densities of 2.2 A m^{-2} were reached. The cathodic community was dominated by *Sphingobacterium*, *Acinetobacter* and *Acidovorax* sp.

A similar type of biocathode was used in laboratory set-ups with freshwater SMFCs (De Schamphelaire *et al.*, 2008). Graphite anodes (with a total geometric surface of 216 cm^2) were placed in an open container filled with potting soil, which was immersed in a basin filled with tap water and added nutrients. A graphite-felt cathode with a geometric surface of 62.5 cm^2 , on which a biofilm had developed, was positioned in the tap water, which was aerated in the surroundings of the cathodic graphite felt. Cathodic current densities of 570 mA m^{-2} could be obtained, as well as power densities of 160 mW m^{-2} . The cathodic potential had a value of $+0.49 \text{ V}$ versus SHE. These results demonstrate that a biofilm on graphite can successfully catalyse the reduction of oxygen in a freshwater SMFC. High anodic electrode potentials (up to $+0.3 \text{ V}$ versus SHE) are possible when applying such a cathode.

It appears likely that a similar type of biologically mediated cathodic reaction has played an important role in the performance of SMFCs described in literature to date. Observations about the cathode are however only occasionally mentioned. Holmes and colleagues (2004b) found that the cathodes of current-generating SMFCs were enriched in members of the γ -*Proteobacteria* (mainly of the *Cycloclasticus*/Type I Methanotroph cluster) in case of marine SMFCs and α -*Proteobacteria* (*Rhodobacter* family) in case of salt-marsh SMFCs. A cycling nitrogenous compound at the cathode or a physicochemically altered environment were preliminarily suggested to explain these enrichments. Reimers and colleagues (2006) found a cathodic community dominated by *Pseudomonas fluorescens* (and to a lesser extent by *Janthinobacterium lividum* and *Aeromonas encheleia*) at a sulfide seep. They repeatedly observed that cathode potentials of new carbon fibre or solid graphite electrodes rose sigmoidally over 6–25 days when inserted in raw but non-sterile seawater systems, and this with or without electrical current flowing. A maximum cathode potential of 0.580 V versus SHE was observed in the absence of current flowing. The cathodic potential and dominance of *P. fluorescens* suggested that manganese oxides and oxyhydroxides and/or phenazines played a major role in maintaining the activity of the cathode in the low-oxygen bottom waters (Reimers *et al.*, 2006). As mentioned before, cathodes of SMFCs are generally considered to react with oxygen dissolved in the seawater. From the past paragraph, it seems likely that the naturally developing biofilms on these cathodes will further mediate the cathodic process.

Finally, in discussing oxygen as cathodic reactant, some theoretical considerations concerning the electrode

potentials should be made. A system which reduces oxygen, for instance at a platinum electrode, will be governed by the following redox reaction (1):



The standard potential ($E_{\text{H}^+}^{\circ}$) for this reaction is 1.228 V versus SHE according to Stumm and Morgan (1996). With a pH of 7 and a maximum partial pressure of 0.2 atm – because of the composition of air – a maximum theoretical redox potential E_{H} of 0.804 V versus SHE can be reached. For all the bio-catalysed oxygen-reducing cathodes described earlier in this section, cathodic potentials higher than 0.4 V versus SHE were obtained.

Biocathodes with a manganese cycle to drive SMFCs

It was found that sites on the surface of (stainless) steel where corrosion takes place often are covered with precipitates that contain manganese oxides (Dickinson *et al.*, 1996; Olesen *et al.*, 2000; Geiser *et al.*, 2002; Kielemoes *et al.*, 2002; Shi *et al.*, 2002; 2003). These oxides, which are deposited by manganese-oxidizing bacteria, are in direct electrical contact with the metal and therefore raise the open circuit potential of the steel to higher values, according to the corresponding redox reactions of the manganese oxides. This will eventually undermine the integrity of the material (Borenstein, 1994; Heitz *et al.*, 1996). The high potentials caused by the manganese oxides will draw the electrons faster through the steel on the one hand, while the reduction of manganese oxides to divalent manganese is a spontaneous reaction on the other hand (as Mn^{2+} is more stable than Mn_xO_y). Manganese reduction therefore forms an excellent cathodic reaction. Experimental observation of stainless steels in water show that biofouling with manganese-oxidizing bacteria increases the cathodic current with 2–3 log units (Olesen *et al.*, 2000).

Bearing in mind the manganese-related corrosion observations and the difficulties in chemically reducing dissolved oxygen, Lewandowski and co-workers used biodeposited manganese oxides as an electron acceptor at the cathode of an MFC (Rhoads *et al.*, 2005; Shantaram *et al.*, 2005). The working principle for the cathodic reaction involves a manganese cycle between the cathode on the one hand and oxygen on the other hand. Manganese-oxidizing bacteria form a biofilm on top of the cathode and precipitate manganese oxides on the surface of the electrode. Electrons from the anode reduce the manganese oxides to Mn^{2+} which dissolves in the overlying aqueous phase. The manganese-oxidizing bacteria, residing on the cathodic surface, re-oxidize the Mn^{2+} with the formation of fresh manganese oxides (Fig. 2A). This involves a two-step process in which MnOOH and MnO_2

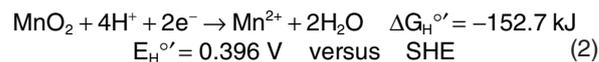
are formed (Shi *et al.*, 2002). In the end, the manganese is recycled and acts as a redox mediator.

Rhoads and colleagues (2005) tested some prototypes of MFCs holding a cathode involving a manganese cycle. They started by applying the principle in a two-compartment reactor-type MFC in which the anode gathered electrons coming from the oxidation of glucose. In the cathodic compartment, manganese oxides – deposited by *Leptothrix discophora* SP-6 – were electrochemically reduced at a porous graphite cathode. The open circuit of this system (without external resistance) reached 809 ± 19 mV, while in closed circuit (with external resistance, allowing current generation), the power density reached an average value of 126 ± 31 mW m⁻². This value was obtained after the addition of a redox mediator, 2-hydroxy-1,4-naphthoquinone, to the anodic compartment. It should be taken into account that the electrical circuit of the fuel cell was only closed at separate points in time. As the authors pointed out, this only gives peak values for power and current due to a discharge of capacitance and reduced/oxidized compounds built up at the electrodes. In addition to this, long-term sustainability was not achieved. It needs to be determined whether the manganese-oxidizing bacteria are able to maintain the manganese cycle for the cathodic reaction, and what the loss of Mn²⁺ was over time. The set-up of Rhoads and co-workers illustrates an advantage of deposited manganese over dissolved oxygen: the cathode potential had a value which was approximately 300 mV higher in case of manganese and the (peak) power density in case of oxygen only reached 3.9 ± 0.7 mW m⁻², which is 1/30 of the value for manganese mentioned earlier.

The manganese system has also been used in a set-up using sediments (Shantaram *et al.*, 2005). A sacrificial anode was used, in the form of a corroding (i.e. undergoing oxidation) magnesium plate on the bottom of a freshwater creek. Stainless steel electrodes at the surface of the water were covered by a deposit of naturally formed MnO₂ during a period of several weeks, during which the cathodic open circuit potential increased from +190 mV versus SHE to +640 mV versus SHE. The fuel cell as such was again only tested for short connection times, during which a pseudo-steady state of only 10 min was reached. The electrode pair was used to charge a capacitor, which for its part could power electrochemical sensors and a small telemetry system to transmit acquired data. The experiment illustrates how a (S)MFC can be used to deliver the required energy to keep electrical equipment running. Unfortunately, insufficient data were provided to substantiate the sustainability of the manganese cycle as a cathodic reactant. Further research must be performed to document the overall feasibility of this approach. This includes the continuous operation of an MFC with a manganese-catalysed cathode in closed electrical circuit,

so that the cathodic MnO₂ is continuously reduced. A microbial community should then be enriched, capable of re-oxidizing the reduced manganese at least at the same rate as the cathodic reduction process, leading to an overall steadiness of the cathodic potential.

If the process is sustainable, manganese would be a well-suited cathodic reactant. To corroborate this concept, theoretical redox potential values can be considered. When using cyclic Mn redox reactions at the cathode, a key reaction is:



$E_{\text{H}^{\circ}}$ for this reaction amounts to 1.223 V versus SHE (Stumm and Morgan, 1996). With a pH of 7 and a culture medium for manganese-oxidizing bacteria in which the concentration of Mn²⁺ can be 0.3 mmol at the highest, an $E_{\text{H}^{\circ}}$ of 0.5 V versus SHE is obtained. Through the action of the manganese-oxidizing bacteria, the concentration of Mn²⁺ is lowered and the open circuit redox potential therefore is expected to be higher. In nature, aqueous manganese concentrations in the range of 0.010–500 μM Mn²⁺ can occur. At a concentration of 1 μM Mn²⁺, the corresponding redox potential would be 0.573 V versus SHE. The final redox potential will furthermore be influenced by the intermediate reduction reaction of MnO₂ to MnOOH. Shantaram and colleagues (2005) obtained open circuit cathode potentials of 0.64 V versus SHE in a freshwater creek, while Rhoads and colleagues (2005) obtained 0.62 V versus SHE in a reactor-type MFC.

Also from an economic point of view, manganese forms an interesting catalyst. Due to the low cost, a cathode could be provided with high quantities of catalyst. For example, at a catalyst loading of 5 mg Mn cm⁻² electrode surface (as opposed to 0.5 mg Pt cm⁻²), the price of the required Mn(II)SO₄ would only be about €4 (purum p.a. ≥ 98% (T), Fluka) for a 1 m² cathode. The need of a binder is omitted, as the Mn-oxidizing microorganisms accomplish the attachment of the catalyst to the electrode. Even with the recent findings that Pt loadings of 0.1 mg cm⁻² perform almost as well as 0.5 mg cm⁻² (Cheng *et al.*, 2006a), prices for chemical cathode catalysts are a factor 20 to 30 higher than those for a cathode with a microbially mediated manganese cycle.

Finally, when discussing manganese as a cathodic reactant, it should be mentioned that MnO₂ is widely used as a cathodic reactant both in aqueous and non-aqueous chemical batteries (Cao *et al.*, 2003). Especially in alkaline metal-air batteries, MnO₂ is a commonly used catalyst. Cathodes with impregnated MnO₂ catalyse the reduction of oxygen in an alkaline solution (Calegari *et al.*, 2006). Cao and colleagues (2003) concluded that during the process, MnO₂ would be reduced to MnOOH

and re-oxidized. Manganese can thus function as a cathodic catalyst, both in a pure chemical way and in a microbially mediated way, with an effective operation at circumneutral pH.

Outlook

The high potentials attainable during moderate current generation through oxygen reduction reactions or manganese cycles could be well-suited to drive an SMFC. They allow to pull the electrons from the reduced substances in the sediment to a well-functioning cathode. This simultaneously creates the opportunity for energy harvesting and the oxidation of sediment organics. Both bio-catalysed cathodic processes have (preliminarily) been shown and have furthermore been demonstrated to develop spontaneously in SMFC systems. Progress in terms of manganese microbiology and cathodic biofilms might be a valuable tool for the development of efficient and low-cost cathodic processes, which might both contribute to the design of SMFCs as well as reactor-type MFCs. Additional research is however required to assure a sustained performance of the manganese cathode.

In considering an SMFC, we can state that several benefits are inherently connected to the system. Energy can be harvested, allowing *in situ* powering of small-scale electrical equipment. Research on SMFCs demonstrated that significant sediment oxidation rates can be attained by the application of an anode in the sediment. These electron fluxes allow to enhance the biological oxidation of reduced substances in sediments and wetlands. Among other gains, the latter may decrease the accumulation of sludge and increase the removal of organic contaminants. The electrical circuitry of the SMFC furthermore offers the opportunity to influence redox-dependent processes. The oxidation process could be controlled, for example by applying different values of external resistance or by steering the anodic potential, in order to decrease methanogenesis, avoid the formation of unwanted chemicals (H₂S, CH₃Hg etc) and decrease toxicity.

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