Possibilities for extremophilic microorganisms in microbial electrochemical systems

Mark Dopson¹,*, Gaofeng Ni¹ and Tom HJA Sleutels²

¹Centre for Ecology and Evolution in Microbial Model Systems (EEMiS), Linnaeus University, SE-391 82 Kalmar, Sweden and ²Wetsus, European Centre of Excellence for Sustainable Water Technology, 8911 MA Leeuwarden, The Netherlands

Corresponding author: Centre for Ecology and Evolution in Microbial Model Systems (EEMiS), Linnaeus University, SE-391 82 Kalmar, Sweden. Tel: +46 (0)480-447334; Fax: +46 (0)480-447305; E-mail: mark.dopson@lnu.se

One sentence summary: This review highlights the use of microbial electrochemical systems to catalyze environmental processes coupled to the production of energy or valuable resources and how utilizing extremophilic microorganisms opens up new possibilities such as bioremediation of environmentally hazardous wastes.

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ABSTRACT

Microbial electrochemical systems exploit the metabolism of microorganisms to generate electrical energy or a useful product. In the past couple of decades, the application of microbial electrochemical systems has increased from the use of wastewaters to produce electricity to a versatile technology that can use numerous sources for the extraction of electrons on the one hand, while on the other hand these electrons can be used to serve an ever increasing number of functions. Extremophilic microorganisms grow in environments that are hostile to most forms of life and their utilization in microbial electrochemical systems has opened new possibilities to oxidize substrates in the anode and produce novel products in the cathode. For example, extremophiles can be used to oxidize sulfur compounds in acidic pH to remediate wastewaters, generate electrical energy from marine sediment microbial fuel cells at low temperatures, desalinate wastewaters and act as biosensors of low amounts of organic carbon. In this review, we will discuss the recent advances that have been made in using microbial catalysts under extreme conditions and show possible new routes that extremophilic microorganisms open for microbial electrochemical systems.

Keywords: anode-respiring bacteria; bioanode; bioelectrochemical systems; electricity generation; extremophiles; microbial electrolysis cells; microbial fuel cells; MEC; MFC

INTRODUCTION

Microbial electrochemical systems (MESs) describe all electrochemical devices that make use of microbial catalysts to drive or accelerate electrochemical reactions at the anode, the cathode or both electrodes (Rabaey and Rozendal 2010; Logan and Rabaey 2012). An MES in which energy is recovered is a microbial fuel cell (MFC), while a system which requires the input of electrical energy to drive a reduction reaction is a microbial electrolysis cell (MEC) (Logan et al. 2006, 2008).

The discovery that an electrical current can be derived directly from organic electron donors (e.g. acetate) led to the testing of a nearly endless number of substrates for microbial-assisted production of electrons (Pant et al. 2010). At first, this production was mainly used to (i) extract the energy available from wastewaters (Logan et al. 2006), (ii) remove recalcitrant organic and inorganic compounds (Kim et al. 2008; Catal, Bermek and Liu 2009; Mu et al. 2009; Ter Heijne et al. 2010; Virdis et al. 2010), (iii) extract energy from plant rhizodeposits...
GENERAL PRINCIPLES OF MESs

Oxidation of a substrate can be spontaneous as well as chemically or microbiobally catalyzed reaction to form one or multiple products, and in an electrochemical reaction, the electrons are released (Fig. 1a). The derived electrons are transferred to the solid anode either directly or indirectly via mediators wherefrom they flow through an external electrical circuit to the cathode. At the cathode, the electrons are combined with an electron acceptor to form a product. Again, similar to the anodic reaction, this can be catalyzed by microbes or metals (Fig. 1b).

Reactions at the anode

Microorganisms that predominantly act as catalysts in MESs and pass electrons to the anode are termed ‘electricigens’ (the term used in this review), ‘electrogens’, ‘anodophiles’ or ‘anode respiring bacteria’. Electricigens have been identified from a range of phyla but those commonly identified in high abundance are from the Proteobacteria and Firmicutes. The two most studied species are Geobacter sulfurreducens (Caccavo et al. 1994) and Shewanella oneidensis (Venkateswaran et al. 1999). However, many other species have been identified depending on the conditions in the anodic chamber. In this review, we will discuss alternative electricigens that have been reported to live under extreme conditions.

In ideal conditions, the anode compartment lacks any alternative external electron acceptors such that the cells pass their electrons to the anode via either the ‘direct’ or ‘indirect’ mechanisms (Fig. 1a). The direct mechanism involves anaerobic electron transport down the electron transport chain before transfer to the insoluble electron acceptor (the anode). This transfer is mediated by c-type cytochromes and iron-sulfur proteins localized to the cell surface (Liu et al. 2014). An additional proposed method of direct electron transfer (at a distance) is via nanowires (Reguera et al. 2005) that are produced by many electricigens. The mechanism by which electricigens transfer electrons to the anode via cytochromes and nanowires is under intense scrutiny (Lovley and Malvankar 2015; Malvankar et al. 2015). Indirect transfer of electrons to the anode involves soluble redox shuttles, termed ‘mediators’. Mediators can be either organic (e.g. humic acids) or inorganic (e.g. the S\(^2\)/H\(_2\)S shuttle) and in a mixed microbial culture, electron transfer to the anode by mediators is not necessarily limited to the species that produces the shuttle. Microbial populations within the anode chamber can exist as planktonic cells that utilize mediators, as a biofilm formed on the surface of the anode that facilitates direct electron transfer, or a combination thereof. These populations can be single species or mixed populations that can act in a synergistic manner to break down complex organic compounds to simple compounds that can be utilized by the electricigens. However, competing microbial metabolisms that divert electrons from being transferred to the anode, such as methanogenesis, can also occur.

Reactions at the cathode

Electrons from the anode flow to the cathode where the reduction reaction takes place (Fig. 1b). The final electron acceptor varies depending on the type of system and its application. In an MFC for electricity production, oxygen is commonly used as the electron acceptor as it is abundant and sustainable due to its reduction product being water (Logan et al. 2006; Clauwaert et al. 2007; Virdis et al. 2008). On the other hand, MECs rely on the input of additional external energy supplied by a power supply and can be used for production of valuable resources, such as hydrogen at the cathode (Logan et al. 2008). Major advantages of hydrogen-producing MECs is that the energy consumption is substantially less than that required for conventional electrolysis of water (Liu, Grot and Logan 2005; Rozendal et al. 2006) and a much higher yield of hydrogen can be reached (Logan et al. 2008).

Figure 1. Schematic of a bioelectrochemical system. The system typically consists of anode (where substrate is oxidized (a)) and cathode (where the product is formed (b)) compartments separated by a membrane. Energy is applied by means of a power supply in MECs (c) or harvested from the system in MFCs (d). The anode and cathode chambers can be separated by various types of membranes (e–g) or be a single chamber without an ion exchange membrane (h).
Separators

In many cases the electrodes are separated by an ion exchange membrane that allows the anolyte and catholyte to be of different compositions. The ion exchange membrane also prevents product and substrate crossover, makes production of a pure product in both anode and cathode possible, and enables transport of specific ionic charge and the rejection of the opposite ionic charge. An anion exchange membrane allows negatively charged ions to be transported and positive ions are rejected (Fig. 1e), while a cation exchange membrane transports positive ions and rejects anions (Fig. 1f). With the expanding field of MES applications, more severe conditions are being employed in operating these cells. For example, a bipolar membrane can be used to maintain a large pH gradient between the anode and cathode (Fig. 1g) (Ter Heijne et al. 2006; Harnisch, Schröder and Scholz 2008; Harnisch and Schröder 2009). In some cases, this charge separation is not required and the ion exchange membrane can be left out or a non-selective barrier is used (Fig. 1h).

Thermodynamics

Depending on the value of the Gibbs free energy change (\(\Delta G_r\)) of both the oxidation reaction at the anode and the reduction reaction at the cathode, energy needs to be applied by means of a power supply (\(\Delta G_r > 0\); Fig. 1c) or energy can be harvested from the system (\(\Delta G_r < 0\); Fig. 1d). For the reaction \(v_A A + v_B B \rightarrow v_C C + v_D D\), the Gibbs free energy change can be calculated using equation 1:

\[
\Delta G_r = \Delta G_r^0 + RT \ln\left(\frac{[C]^{v_C} [D]^{v_D}}{[A]^{v_A} [B]^{v_B}}\right). \tag{1}
\]

where \(\Delta G_r^0\) is the Gibbs free energy change under standard conditions (298 K, 1 bar and 1 M concentration for all species), \(R\) is the universal gas constant (8.31 J mol\(^{-1}\) K\(^{-1}\)), \(T\) is the temperature in degrees Kelvin, \([\cdot]\) is the concentration of a specific reactant (mol L\(^{-1}\)) and \(v_i\) is the reaction coefficient. In MESs, it is more convenient to express the Gibbs free energy change (kJ mol\(^{-1}\)) of the overall reaction as the potential difference between the oxidation (anode) and reduction (cathode) reaction (\(\Delta E; V\)) which is related to the Gibbs free energy of the overall reaction according to equation 2:

\[
E_r = -\frac{\Delta G_r}{nF}. \tag{2}
\]

where \(n\) is the amount of electrons involved in the reaction. These anode (\(E_{an}\)) and cathode (\(E_{cat}\)) potentials can be calculated in a similar way as the energy change of the total reaction in an MFC but then related to a reference potential. For these anode and cathode potentials, the cell voltage can be calculated using equation 3:

\[
E_{cell} = E_{cat} - E_{an} \tag{3}
\]

From a microbial perspective, it is interesting to see the amount of energy that is available to generate ATP. A schematic representation of the electron flow from microorganism to the electrode is given in Fig. 2. The microorganisms oxidize a substrate to a product from which electrons are released into the electron transfer chain. The energy level of these electrons is determined by the Gibbs free energy change of this oxidation reaction. Finally, the electrons are transferred to the electrode surface by a redox active enzyme or cytochrome. The energy at which the electrons are released is determined by the final step in the electron transfer chain and the enzyme that catalyzes this final step (\(E_{cat}\)). Figure adapted from Hamelers et al. (2011).

**Figure 2.** Schematic representation of the electron flow from microorganism to the electrode. The microorganisms oxidize a substrate (\(S\)) to a product (\(P\)) from which electrons are released into the electron transfer chain. The energy level of these electrons is determined by the Gibbs free energy change of this oxidation reaction (\(E_{ox}\)). Finally, the electrons are transferred to the electrode surface by a redox active enzyme. The energy at which the electrons are released is determined by the final step in the electron transfer chain and the enzyme that catalyzes this final step (\(E_{cat}\)). Figure adapted from Hamelers et al. (2011).

**EXTREME CONDITIONS AND EXTREMOPHILES**

Extreme conditions are those that are harmful for most life on earth and encompass both physical (e.g. high and low temperatures) and geochemical (e.g. high metal concentrations or under extreme nutritional limitations) conditions (Fig. 3). Extreme milieu are defined taxonomically (rather than anthropocentrically) as environments with low species diversity where whole taxonomic groups are missing (Bott and Brock 1969). These environments can be natural in origin such as Mono Lake, California, that is both alkaline and hypersaline ( Antony et al. 2013); polar sites that have low temperatures and in some cases are arid (Cowan et al. 2014); and hydrothermal vents that are high temperature (Dick et al. 2013). In addition, extreme environments
can be anthropogenic in origin such as metal laden, acidic mine waters (Dopson and Holmes 2014) or nuclear waste sites high in radioactivity (Morozkina et al. 2010). Extremophilic microorganisms that inhabit these environments have been identified from all three domains of life (Pikuta, Hoover and Tang 2007). These organisms are adapted to live and thrive in these challenging conditions and in most cases are incapable of growth under non-extreme conditions. One example is the extreme acidophile *Picrophilus oshimae* that has an optimum growth pH of 0.7 and the cells burst above pH 4 (van de Vossenberg et al. 1998). At least 15 classes of extremophiles have been defined such as cryptoendoliths that live in spaces between rocks and xerophiles that are able to survive in very dry conditions. The extremophile groups that have been utilized in MESs and are described in this review include acidophiles and alkaliphiles, psychrophiles and thermophiles, halophiles and oligotrophs.

Acidophiles are defined as having an optimum growth pH < 5 (extreme acidophiles have an optimum pH < 3) and include Bacteria, Archaea and Eukaryota. Species range from low temperature adapted acidophiles to thermoacidophiles. Various species can oxidize ferrous iron (Bonnefoy and Holmes 2012), sulfur and inorganic sulfur compounds (Dopson and Johnson 2012), and organic carbon for energy (Sato and Atomi 2011) and in some cases fix CO$_2$ for organic carbon (Dopson 2012). Acidophiles utilize several mechanisms to cope with low pH including membranes that are resistant to the influx of protons, an internal negative membrane potential that inhibits the influx of protons, as well as primary and secondary proton transporters (reviewed in Slonczevski et al. 2009). As a result of catalyzing metal sulfide dissolution, acidophiles are also often required to be tolerant to very high metal loads and the underlying mechanisms are a combination of both biotic and abiotic systems (reviewed in Dopson and Holmes 2014; Dopson et al. 2014).

Alkaliphiles grow in a pH range of 8.5–11 and also include examples from all three domains of life. They are found in environments that include soda lakes, alkaline soils and the Red Sea and are often also halophilic, termed ‘haloalkaliphiles’. Alkaliphiles maintain their internal pH ≥ 2 pH units below that of the external milieu in part by exploiting a sodium motive force (sodium cannot be replaced by potassium) to conserve energy...
EXTREMOPHILES IN MESs

Several different classes of extremophilic microorganisms have been utilized in MESs and this section (see categories below) will describe their use as well as comparing the different electrochemical efficiencies achieved.

The energy that is available for microbial activity is highly dependent on the electrode potential, the type of microorganism, the electron transfer chain it possesses and its ability to deal with environmental conditions. The Gibbs free energy change is dependent on temperature, pressure, concentrations/activity and pH of the electrolyte that is altered when calculating the actual Gibbs free energy under extreme conditions compared to normal conditions. In non-dilute systems, the activity of a substance has to be used instead of the concentration in dilute systems. The correct value for the pH and the pressure of a certain gas can be directly used in the equation. As an example, the Gibbs free energy change for the oxidation of acetate was calculated at different pH values, assuming that the microorganisms are able to convert acetate at this entire pH range. This Gibbs free energy change not only alters because of the different conditions caused by the pH but also because the reaction equation changes due to the presence of different species (equations 4–6):

\[
2\text{CO}_2 + 8\text{H}^+ + 8\text{e}^- \rightarrow \text{CH}_3\text{COOH} + 2\text{H}_2\text{O}; \text{pH} 2
\]

\[
1.68\text{HCO}_3^- + 0.32\text{CO}_2 + 8.68\text{H}^+ + 8\text{e}^- \rightarrow \text{CH}_3\text{COO}^- + 3.68\text{H}_2\text{O}; \text{pH} 7
\]

\[
\text{HCO}_3^- + \text{CO}_2^+ + 10\text{H}^+ + 8\text{e}^- \rightarrow \text{CH}_3\text{COO}^- + 4\text{H}_2\text{O}; \text{pH} 10
\]

At standard conditions the Gibbs free energy change for the oxidation of acetate is 0.187 V (vs NHE) (Logan et al. 2006), while at practical conditions (pH 7 and 0.005 M for reactive species) the value changes to −0.291 V (Fig. 4). At acidic conditions (pH 2), the anode potential is −0.022 V while the anode potential halophiles experience at pH 10 is −0.609 V (Fig. 4). When the anode reaction is coupled to a cathode reaction and the cell voltage is calculated (equation 3), an increase in cell voltage is seen at

\[
\text{e}^- + \text{H}^+ \rightarrow \frac{1}{2}\text{H}_2
\]

\[
0.187 \ 	ext{V} \ (\text{vs NHE})
\]

\[
-0.022 \ 	ext{V}
\]

\[
-0.291 \ 	ext{V}
\]

\[
-0.609 \ 	ext{V}
\]
increasing anolyte pH due to the additional pH gradient also representing a source of energy (chemical potential). The practical implication of an elevated cell voltage is that more energy can be gained from these systems at higher pH values. When more of this energy is available to the microorganisms, they can expend more maintenance energy to withstand the extreme conditions in which they live or produce more current.

To date, few studies have been performed that systematically compare the performance of MESs (maximum power densities, Coulombic efficiencies, anode overpotentials, current densities and cell voltages) at extreme conditions. In addition, the reactor cell architecture and choice of electrolyte have a significant impact on performance. Therefore, it is difficult to draw hard conclusions from the reported effects of different extremes on the system performance.

**Acidophiles**

In this review, MES studies that exploit moderate and extreme acidophiles in the anode (e.g. *Acidiphilium* spp.; Borole et al. 2008) as well as biocatalysts in the cathode (e.g. *Acidithiobacillus ferrooxidans*; Ter Heijne, Hamelers and Buisman 2007) are discussed (Table 1). An advantage of a low pH MES is that the protons will not cause diffusion limitations in the cathode compartment for reduction of oxygen and therefore, will not limit the current production (Erable, Etcheverry and Bergel 2009). However, a consequence of growth at low pH is that the cells have to maintain a near neutral cytoplasm (reviewed in Slonczewski et al. 2009). This consumes a portion of the energy derived from electron transport for other processes such as proton export, increasing the anode overpotential (reducing whole cell voltage outputs) and lowering power generation. Despite a higher chemical oxygen demand removal rate at pH 4, lower voltage outputs and power generation are observed for neutrophilic microbe catalyzed MFCs operated at pH 7. This low Coulombic efficiency is attributed to a poor quality biofilm on the anode (Zhang et al. 2011a). This indicates that current generation at these acidic conditions from acetate is possible but many electrons from the carbon source are directed to alternative electron acceptors than the electrode.

The first acidophile demonstrated to generate electricity was *Acidiphilium cryptum* that oxidizes glucose coupled to the reduction of ferric iron at pH ≤ 4 (Borole et al. 2008). However, accumulation of the electron acceptor ferric iron (potentially as Fe(OH)₃) restricted current output. In order to eliminate this effect, the alternative electron mediators nitroliotriacetic acid and phenosafranin were added to the system that resulted in an increased power output of 12.7 mW m⁻². Thus, the current generation in this study was based on electron transfer by the artificial mediators rather than direct electron transfer from the microorganisms to the electrodes (Borole et al. 2008). In contrast, *Acidiphilium* sp. strain 3.2 sup 5 isolated from Rio Tinto, Spain, mediates direct electron transfer from glucose metabolism to the anode at pH 2.5 at a rate of 3 A m⁻² even in the presence of air (Malki et al. 2008). The ability of *Acidiphilium* sp. strain 3.2 sup 5 to donate electrons directly to the anode in the presence of air facilitates the MES design as a membrane halting oxygen diffusion from the cathode to anode compartments is unnecessary. This strain grows on carbon cloth and graphite anode materials, produces extracellular polymeric substances, forms a biofilm between the carbon microfibers and in pores on the graphite rod surface (Tapia et al. 2009), and preferentially attaches to graphitic flakes as opposed to glass (Malki et al. 2013). *Acidiphilium* strain 3.2 sup 5 forms a closely packed multilayered biofilm that Table 1. Comparison of the performance of selected bioelectrochemical systems under acidic conditions.

| Condition | Inoculum | Electron donor | Voltage | Power density | Current density | CE | Reference |
|-----------|-----------|----------------|---------|---------------|----------------|----|-----------|
| MFC pH 2.0 | Acidophilus fermentans | FeSO₄ | 0.18 V | 18 mW m⁻² | 2.5 A m⁻² | 37% | Carbiajo et al. (2010) |
| MFC pH 2.5 | Acidophilus fermentans | Acetate | 0.248 V | 433 mW m⁻² | 18 A m⁻² | 20% | Ter Heijne, Hamelers and Buisman (2008) |
| MFC pH 3.0 | Acidophilus fermentans | Glucose | 0.30 V (OCV) | 425 mW m⁻² | 29 mA m⁻² | 30% | Li et al. (2013) |
| MFC pH 4.0 | Acidophilus fermentans | Organic carbon compounds | 0.28 V | 454 mW m⁻² | 22 A m⁻² | 27% | Ter Heijne, Hamelers and Buisman (2008) |
| MFC pH 4.5 | Acidophilus fermentans | Ethanol | 0.20 V | 45 mW m⁻² | 26 mA m⁻² | 20% | Ter Heijne, Hamelers and Buisman (2008) |
| MFC pH 5.0 | Acidophilus fermentans | Wastewater | 0.20 V | 43 mW m⁻² | 26 mA m⁻² | 20% | Ter Heijne, Hamelers and Buisman (2008) |

*Acidiphilium* fermentans was acclimated in the cathode for regeneration of Fe⁴⁺ to Fe⁴⁺. The anode contained a mixed culture.
contains iron potentially in c-type cytochromes that may mediate electron transport to the anode (Malki et al. 2013). Although Acidiphilium strain 3.2 sup 5 reduces soluble ferric iron in the presence of air, thick biofilms of acidophilic microorganisms become anaerobic (Baker-Austin et al. 2010). Therefore, electron transfer to the anode may occur in anaerobic conditions as oxygen cannot penetrate deeply into the biofilm. The electrically Acidiphilium strain PM genome has been sequenced but no genes are ascribed to mediate its ability to reduce ferric iron in this species (San Martin-Uriz et al. 2011) or other acidophiles (Osorio et al. 2013). A microcosm sediment–water interface MFC demonstrated electricity generation in a natural acidic system at pH 3 with Acidiphilium spp. predominantly colonizing the anode while A. ferrooxidans and Leptospirillum spp. colonize the cathode (Garcia-Munoz et al. 2011). Due to the available protons and high ionic strength of the medium, the current density and maximum power generation are between 2- and 20-fold higher than similar sediment–water interface MFCs operated at neutral pH, respectively (Garcia-Munoz et al. 2011).

Microorganisms from pH neutral environments have been inoculated into moderately acidic MESs (anode pH 4 to ∼5.5) to treat organic wastewaters such as from distilleries (Kim et al. 2014) and food waste (Li et al. 2013). Other MESs have been designed to remove sulfate in acidic wastewaters (pH 2.5–4.5) using organic carbon-fed neutrophilic microorganisms to produce sulfide that is chemically oxidized to elemental sulfur on the anode surface (Liang, Xiao and Zhao 2013; Zheng et al. 2014). However, many wastewaters also contain inorganic carbon and energy sources such as inorganic sulfur compounds in mining wastewaters. To be able to treat mining wastewaters through oxidation and electron release, it is necessary to develop MESs containing sulfur-reducing microorganisms in the anodic chamber able to oxidize inorganic sulfur compounds. An MFC utilizing tetrathionate as electron donor has a mixed culture of A. ferrooxidans and Ferroplasma spp. coupled to the reduction of ferric iron (Sunol et al. 2015). However, the low Coulombic efficiency (<5%) suggests that many electrons were not passed to the anode. Acidithiobacillus ferrooxidans has also been utilized to catalyze oxygen reduction at the cathode at pH 2 to overcome kinetic limitations associated with the consumption of four protons per oxygen molecule reduced (Carbajosa et al. 2010). Under practical conditions, the reduction of ferric iron on the cathode has a higher cathode potential than for the reduction of oxygen and the use of A. ferrooxidans to regenerate the ferric iron resulted in a 38% increase in power output compared to an MFC without the cathode biocatalyst (Ter Heijne, Hamelers and Buisman 2007). Of course, this increased power output cannot entirely be attributed to the presence of A. ferrooxidans as the elevated concentration of Fe^{3+} might help reduce the overpotential.

Looking at the general bioelectrochemical performance of systems employing acidophiles, it can be seen that the oxidation rate of substrate to produce current for these microorganisms is high and therefore, the removal rate is also high (e.g. 5 A m^{-2} for FeSO_{4} and 3 A m^{-2} for glucose). Unfortunately, overpotentials for the oxidation reaction are reported in very few studies. These overpotentials could give more insight into the energetic efficiency of the oxidation reaction and more solid conclusions about the process could be drawn.

**Alkaliphiles**

An overview of the studies reporting alkaliphiles in MESs is given in Table 2. In addition, alkaliphiles also capable of growth in high salt concentrations (making them haloalkaliphiles) that have

| Anode potential (vs SHE) | Reference |
|--------------------------|-----------|
| -                        | -         |

### Table 2. Comparison of the performance of selected bioelectrochemical systems under alkaline conditions.

| EES | Condition | Inoculum | Electron donor | Voltage | Power density | Current density | CE |
|-----|-----------|----------|----------------|---------|---------------|----------------|----|
| MFC | pH 9.0-11.0 | Acetate | - | 0.80 V | 0.80 W m^{-2} | 0.70 A m^{-2} | 90% |
| MFC | pH 9.0 | Synthetic waste | - | 0.60 V (OCV) | 1270 mW m^{-2} | 0.12 A m^{-2} | 63% |
| MFC | pH 9.0 | Compost | - | 0.60 V (OCV) | 559 mW m^{-2} | 0.15 A m^{-2} | 23% |
| MFC | pH 9.0 | Paper mill effluent | - | 0.60 V (OCV) | 6 W m^{-2} | 7.5 A m^{-2} | 33% |
been utilized for MESs are discussed in the multiple extremes section below. It is important to note that many MESs make use of alkaline medium as feed. However, since the anodic oxidation reaction is strongly acidifying, many of these systems actually operate under near pH neutral conditions. For example, urine is a well-known alkaline feed for MESs. While the urine itself has a pH of around 9, the effluent pH is around 7. Alkalization of the medium in this case is caused by hydroxyl transport from the cathode and decreased current production drastically (Kunke et al. 2014).

Operating the bioanode of MFCs at alkaline conditions has several advantages for electricity production. These include diminished competition for substrate by methanogenesis (Gutierrez et al. 2009) and at high pH, the lower anode potential results in an increased cell voltage (Erable, Etcheverry and Bergel 2009). During MFC operation, the anode becomes acidified while the cathode becomes more alkaline leading to a reduced cell voltage and power output. An acetate-fed, high current density bioanode inoculated with a garden compost inoculum also resulted in a pH increase to 9.0 that was attributed to the spontaneous evolution of compost leachate (Pocaznoi et al. 2012). To address this issue, an MFC with a pH 10 anode compartment and pH 2 cathode compartment was operated that had higher open circuit voltage, power density and the high anodic pH resulted in an increased efficient Coulombic efficiency than a MFC run at neutral pH (Zhuang et al. 2010). Of course it is not possible to sustain these pH levels in practice as addition of chemicals would be too costly and their addition is an unsustainable solution. Further studies showing enhanced MFC performance with alkaliphiles include an MFC treating urban wastewater that has improved power generation at pH 9.5 compared to neutral pH (Puig et al. 2010), a single-chamber air-cathode MFC operated at pH 9 that has 39% greater power density than that observed at pH 7 that is partly attributed to a reduction of charge-transfer and diffusion resistance when a high pH carbonate buffer is used compared to a lower pH phosphate buffer (Fan, Hu and Liu 2007) and a pH 9 MFC that has 29% and 89% higher power density compared to operation at pH 7 and 5, respectively (Yuan et al. 2011). Again, the increase in power can be attributed to the addition of chemicals, in this case buffer species that decreased the diffusion resistance for protons. As in the previous case, the addition of chemicals is costly and unsustainable. The molecular mechanism underlying increased electricity production at alkaline pH in S. oneidensis MR-1 has been investigated (Yong et al. 2013). Shewanella oneidensis MR-1 extracellular electron transfer to the anode is mediated by riboflavin and the amount synthesized increases between pH 6 and 9 and is directly proportional to electricity output (Yong et al. 2013). Reversible bioelectrodes that alternate between substrate addition and aeration have been designed to exploit proton accumulation to increase the efficiency of the bioanode. A reversible acetate and oxygen bioelectrode with a garden compost inoculum reached a maximum pH of 9.9 (the pH increase was attributed to compost leachate) and maintained its anodic efficiency despite the aeration periods (Blanchet et al. 2014). Analysis of the microbial population identified 49 ± 1% Chloroflexi, making this the first time this bacterial class has been identified as dominating bioanodes.

The first alkaliphile to be utilized in an MFC was a Bacillus sp. at pH 9.5–11 that requires the presence of a redox mediator (Akiba et al. 1987). Since then further alkaliphiles have been utilized in pure culture MFCs including the Gram-positive Corynebacterium strain MFC03 that degrades glucose and donates electrons to the anode in the absence of exogenous mediators (Liu et al. 2010). However, cyclic voltammetry suggested the involvement of exogenous mediators produced by the Corynebacterium sp. and the addition of anthroquinone-2,6-disulfonate increased the maximum power density (Liu et al. 2010). A second pure culture of an anode-respiring alkaliphile utilized in MFCs is Geoalkalibacter ferrhydriticus that generates a high current density by oxidation of acetate and yeast extract at pH 9.3 (Badalamenti, Krajmalnik-Brown and Torres 2013).

Further strains isolated as pure cultures from MFCs operating at high pH include Corynebacterium humireducens MFC-5 (Wu et al. 2011) and Bacillus pseudofirmus MC02 (Ma et al. 2012) both isolated from humic acid/humus-degrading MFCs. Biological humic acid reduction has environmental benefits and humic substances, once reduced, can shuttle electrons to insoluble electron acceptors. The Wu et al. (2011) study revealed the possibility of concurrent biological humic acid reduction and biochemical electricity generation within an MFC system oxidizing several organic carbon electron donors including lactate, ethanol and sucrose. Another species capable of electricity production at high pH, Pseudomonas alcaliphila, excreted phenazine-1-carboxylic acid that acts as an electron shuttle during oxidation of citrate (Zhang et al. 2011b). An MFC has also been developed to treat food wastes that comprise 30–55% (by weight) of all refuse in urban societies. The waste was first treated by anaerobic digestion (considered the most environmentally friendly treatment method) and the resulting food waste leachate was amended with 100 mM NaCl and electricity generated in a pH 9 MFC that had a maximum of 63% Coulombic efficiency (Li, Cheng and Wong 2013). Finally, MFCs have been used to treat alkaline paper mill effluents that reached an anode pH > 9 that was shown to contain Desulfurodomas acetexigens as the single dominant species in the tertiary microbial anodes (Ketep et al. 2013).

Psychrophiles

In this section, studies of both psychrophilic and psychrotolerant microorganisms are reported (Table 3). In general terms, temperature has a great impact on the performance of MESs. It impacts the functioning of microorganisms, the electrochemical reactions and the Gibbs free energy change of the reactions. The microbial enzyme machinery has an optimum temperature for performance while the electrochemical reaction rate increases at increasing temperature. Lowering the operating temperature has an adverse effect on MFC performance in terms of power and current (Larrosa-Guerrero et al. 2010), start-up time (Cheng, Xing and Logan 2011; Michie et al. 2011) and substrate oxidation rate (Larrosa-Guerrero et al. 2010; Michie et al. 2011). This negatively impacts MESs for processes such as wastewater treatment since these streams are generally at low temperature. However, an advantage of low temperature MFCs is that they typically produce higher Coulombic efficiencies (Jadhav and Changrekar 2009; Catal et al. 2011; Michie et al. 2011; Zhang et al. 2014).

The microbial community enriched from anaerobic sludge on the anode of an acetate-fed MFC operated at 15 °C has been analyzed. The selected strains have 16S rRNA gene sequences most similar to the psychrophiles Simplicispira psychrophila LMG 5408 and G. psychrophilus P35 (Liu et al. 2012). A second study of a 5°C–10°C MFC enriched for a community most similar to low-temperature microorganisms from the genera Arcobacter, Pseudomonas and Geobacter (Zhang et al. 2014).

One promising application of low-temperature MFCs, especially for low power consuming applications like sensors that are intended to last for a long period of time, is energy production from marine sediments that function by placing the anode in...
Table 3: Comparison of the performance of selected bioelectrochemical systems under low-temperature conditions.

| Anode potential (vs SHE) | Reference |
|-------------------------|-----------|
| +0.223 V                | Li et al. (2011) |
| +0.6 V                  | Lu et al. (2011) |
| +0.94 V                 | Lu et al. (2012) |
| +0.94 V                 | Motojima et al. (2014) |

The anaerobic sediment and the cathode in the aerobic seawater above (Bond et al. 2002). This technology represents an extremely large energy reserve, although long-term power generation can be limited by the flow of substrates to the anode (Reimers et al. 2006). Quantitative PCR of the 16S rRNA gene suggests marine, salt marsh and freshwater sediment energy-harvesting anode populations (incubated in the laboratory at 15 °C) are 53–70% enriched in δ-Proteobacteria (Holmes et al. 2004a). Of the identified δ-Proteobacteria, ~65 to 75% of the 16S rRNA gene sequences are further classified as aligning with the Geobacteraceae family (Holmes et al. 2004a). A psychrotolerant (grows between 4 °C and 30 °C) and optimum at 22 °C microbial isolate grown from a marine sediment anode represented a novel lineage in the Geobacteraceae and was named Geopsychrobacter electrodiphilus (Holmes et al. 2004b). Geopsychrobacter electrodiphilus grows utilizing the anode as sole electron acceptor and has a high Coulombic efficiency of approximately 90% while donating 90% of the available electrons from its substrate oxidation to the anode (Holmes et al. 2004b). An alternative electron donor for marine sediment MFCs is sulfide and methane-rich fluids from cold seeps (Reimers et al. 2006). The anode biofilm community in this environment altered according to the sediment depth that mimicked the geochemical environment. The shallow community (20–29 cm below the sediment surface) is dominated by strains similar to Desulfovibrioacetoxidans that changed with increasing depth (46–76 cm below the sediment) to comprise a more complex community of δ-Proteobacteria (including from the genus Desulfovibrio and Syntrophus) along with ε-Proteobacteria (Reimers et al. 2006). However, a drawback of this system is decreasing current over time that was partially attributed to sulfide oxidation products (i.e. non-conductive solid sulfur) passivating the anode surface.

Another example of low-temperature MESs is hydrogen production in an MEC with glucose (Lu et al. 2012), acetate (Lu et al. 2011), waste-activated sludge and acetate (Xu et al. 2014), or molasses wastewater (Wang et al. 2014) as the electron donors. The amount of hydrogen produced from glucose at 4 °C is similar to mesophilic MECs and has a microbial community carrying out negligible competing methanogenic and homoacetogenic reactions (Lu et al. 2012). This is because microorganisms mediating these pathways are generally inactive at low temperature. The dominating genus (37% of the total 16S rRNA gene reads) in the anode community at 4 °C is Dysgonomonas that ferments glucose to acid (but not hydrogen gas) that exists syntrophically with other community members including known electrogens (Lu et al. 2012). A second low-temperature study with acetate as the electron donor also has decreased methanogenesis as methanogenic archaea are suppressed at 4 °C and 9 °C while growth of G. psychrophilus was promoted (Lu et al. 2011). Trehalose is a compatible solute that, among other functions, protects microorganisms against low temperatures (Kawahara 2008). Addition of trehalose to a waste-activated sludge and acetate-fed hydrogen-producing MEC operating at 0 °C increases the energy recovery and Coulombic efficiencies (Xu et al. 2014). Finally, biodegradation of the antibiotic chloramphenicol in a biocathode MES has been demonstrated at 10 °C (Kong et al. 2014). The study shows antibiotic removal in an MES after a temperature switch from 25 °C to 10 °C, although the chloramphenicol reduction rate was decreased.

Thermophiles

An overview of MESs operated under thermophilic conditions can be found in Table 4. The benefit of operating biochemical systems at thermophilic conditions could include a higher
An acetate-fed MFC inoculated with a mixed culture from a methanogenic anaerobic digester (55 °C) was stable for 100 days with a Coulombic efficiency of 89% (Wrighton et al. 2008). 16S rRNA clone library characterization of the mixed community showed 80% of the population responsible for electricity production to be from the Firmicutes. Isolation of pure cultures from the MFC identified Thermincola strain JR, which is the first reported strain from this genus to directly transfer electrons to the anode (Wrighton et al. 2008). A pure culture of the ferric iron-reducing Gram-positive thermophile, Thermincola ferriacetica, is also able to donate electrons from acetate oxidation to the anode at 60 °C and maintain a stable current (400 mA m⁻²) for over 3 months with 97% Coulombic efficiency (Marshall and May 2009). Cyclic voltammetry suggests electron transport from acetate to the anode and cell-free medium scans did not have any significant peaks. This suggests that direct electron transfer occurs from the T. ferriacetica biofilm to the anode without soluble mediators. Thermincolaferriacetica biofilms grow to a thickness of ~38 μm and contain extracellular appendages similar to Geobacter sp., further supporting that electron transfer is via a solid conductive matrix (Parameswaran et al. 2013). A second species from the Thermincola genus, T. potens was isolated from the anode surface of a MFC at 60 °C (Wrighton et al. 2011). Characterization of the T. potens anode biofilm supports direct transfer of electrons from acetate to the anode mediated by several multiheme c-type cytochromes (Carlson et al. 2012), and a total of 32 multiheme cytochromes suggested to be located in the periplasm or on the cell surface are present on the T. potens genome (Byrne-Bailey et al. 2010). Further MFCs inoculated with pure cultures of thermophilic electricogens at 50 °C include B. thermoglucosidans and B. licheniformis fed with various carbon sources including glucose and lactose, respectively (Choi et al. 2004).

Several further studies utilize high-temperature anaerobic sludge as sources of thermophilic inocula in MFCs (Jong et al. 2006; Ha et al. 2012; Hussain et al. 2012; Fu et al. 2013a,b). The anode community of two separate studies of acetate-fed MFCs operated at 55 °C is dominated by strains related to the Gram-negative nitrate-reducing species Calditerrivibrio nitroreducens (Jong et al. 2006; Fu et al. 2013a). A pure culture of C. nitroreducens generated a maximum power density of 272 mW m⁻² in the absence of mediators (Fu et al. 2013a). The C. nitroreducens genome encodes 12 hypothetical proteins with c-type cytochrome domain(s) (Pitluck, Sikorski and Zeytun 2011). Of these, the gene ORF Caln1470 is predicted to encode a multiheme c-type cytochrome that is a candidate to mediate electron transfer to the anode as it is similar to the T. potens cytochrome C1150mediatingextracellularElectrontransfer. Anacetate-fedMFCoperatedat55°Cselectedforamixedpopulationofspecies from the Firmicutes, Synergistetes, Coprothermobacteria and Chloroflexi with the Firmicutes making up 87% of the clone library sequences (Fu et al. 2013b). The dominant-identified phylotype is similar to T. ferriacetica, while the second most abundant phylotype is similar to the genus Caloramator. A pure culture of

### Table 4. Comparison of the performance of selected bio-electrochemical systems under high-temperature conditions.

| BES | Condition | Inoculum | Electron donor | Voltage | Power density | Current density | CE | Reference |
|-----|-----------|----------|----------------|---------|---------------|----------------|----|-----------|
| MFC | 60 °C     | Marine marsh sediment | Acetate | 0.39 V (100 Ω) | 43 mW m⁻² | 254 mA m⁻² | 36% | Mathis et al. (2008) |
| MFC | 55 °C     | Thermophilic compost | Acetate | 0.64 V | 465 mW m⁻² | 100 mA m⁻² | 89% | Mathis et al. (2009) |
| MFC | 60 °C     | Thermophilic ferriacetica | Glucose | 0.39 V (100 Ω) | 146 mW m⁻² | 1030 mA m⁻² | 80% | Wrighton et al. (2006) |
| MFC | 55 °C     | Thermophilic ferriacetica | Water/acetate | 0.70 V (OCV) | 422 mW m⁻² | 2200 mA m⁻² | 89% | Wrighton et al. (2006) |
| MFC | 55 °C     | Anaerobic digester | Acetate | 0.68 V (OCV) | 1000 mW m⁻² | 2120 mA m⁻² | 89% | Wrighton et al. (2006) |
| MFC | 50 °C     | Waste water | Glucose/lactose | 0.68 V (OCV) | 34 mW L⁻¹ | 200 mA L⁻¹ | 41% | Mathis et al. (2008) |
| MFC | 50 °C     | Anaerobic digester | Acetate | 0.68 V (OCV) | 1000 mW m⁻² | 2120 mA m⁻² | 41% | Wrighton et al. (2006) |

- **Anode potential (vs. SHE)**: The difference in potential between the cathode and anode.
- **CE**: Coulombic efficiency, which indicates the percentage of electrons that are transferred.
- **Reference**: Source of the data or study cited.

**Microbial Activity and Electron Transfer:**

Microbial activity, better substrate solubility, high mass transfer rate and lowered risk of contamination. An example of an improved current generation at high temperature (60 °C) is a marine sediment MFC that generated 209–254 mA m⁻² compared to 10–22 mA m⁻² at 22 °C (Mathis et al. 2008). However, a drawback is higher rates of evaporation from MESs designed for applications at low or mesopholic temperatures. Two answers to this problem are possible, either to run the MES in continuous mode allowing replacement of the anolyte and catholyte or to utilize an MES that precludes evaporation, as designed by Carver, Vuoriranta and Tuovinen (2011).
Caloramator australicus reached a maximum current of 0.16 mA that is slightly lower than that attained with the mixed culture MFC (Fu et al. 2013b).

Potential applications of high-temperature MFCs include the treatment of alcohol distillery wastewater (Ha et al. 2012). Distillery wastewater usually contains high levels of organic carbon and sulfate, with temperatures typically ranging from 70°C to 80°C. In one study, the MFC system is able to achieve higher power output and Coulombic efficiency than previous wastewater MFCs at different temperatures while simultaneously reducing sulfate (Ha et al. 2012). 16S rRNA sequencing identified the anode mixed culture to be dominated by Bacteroidetes with a single operational taxonomical unit constituting almost 40% of the reads (Ha et al. 2012). Finally, an MFC for H2 production at 55°C had a rate of 377 ± 73 mmol day−1 m−2 and a cathodic recovery of approximately 70% (Fu et al. 2013c). 16S rRNA gene analysis of the biocathode population identified 21 phylogenotypes of which Firmicutes (77%) followed by Coprothermobacter (20%) dominate the community.

**Halophiles**

Reports of halophilic microorganisms in MESs are summarized in Table 5, although marine sediment MFCs have not been included in this section and those operating at low- and high temperatures are described in the psychrophile and thermophile sections, respectively. A high salt concentration generally has a positive effect on the performance of MESs. First of all high salt concentrations decrease the internal resistance of the system as the resistance of the electrolyte is the reciprocal of the electrolyte conductivity. Second, when salt is added in the form of buffering compounds, the speed at which for example protons are removed from the biofilm increases and thereby, the proton diffusion resistance decreases. However, addition of salt and/or buffering compounds is an unsustainable and costly method to increase the performance of MESs. In contrast, solutions with naturally occurring salt concentrations could be of great use for the practical application of these types of systems. When reporting on the performance of halophiles in these systems under high ionic strength, care should be taken that the performance increase is indeed due to the microbial activity and not due to a reduced internal resistance.

Raising the ionic strength (as well as decreasing the distance between the electrodes) in an acetate-fed, wastewater-inoculated single chamber MFC lowers the internal resistance and increases power generation and output (Liu et al. 2008). In a second study, electricity generation by S. marisflavi also increases due to lessened internal resistance in anolytes containing up to 1.5 M ionic strength (Huang, Sun and Zhang 2010), although the S. marisflavi type species is only a slight halophile (Yoon et al. 2004). Finally, adding 20 g L−1 (342 mM) NaCl increases the power production by 30% in an acetate-fed MFC inoculated with domestic wastewater (Lefebvre et al. 2012). These data demonstrate that halophiles are able to produce electrons, but the increase in performance is due to a reduction in internal resistance.

Other pure cultures of halophiles have been tested for electricity generation. These include testing the performance of halophilic Haloferax volcanii (anode ionic strength 2.68 M, pH 5.9) compared to the haloalkaliphilic Natralba magadii (anode ionic strength 3.63 M, pH 10; discussed in the multiple extremes section) and the neutrophilic Escherichia coli (ionic strength 0.09 M, pH 7) in dual chamber MFCs (Abrevaya et al. 2011). Of the three, H. volcanii has the highest maximum power and current
Table 6. Comparison of the performance of selected bioelectrochemical systems under low substrate conditions.

| BES          | Condition          | Inoculum              | Electron donor       | Voltage (mV) | Power density (W/m²) | Current density (A/m²) | CE (%) | Anode potential (vs SHE) | Reference               |
|--------------|--------------------|-----------------------|----------------------|--------------|----------------------|------------------------|--------|-------------------------|-------------------------|
| MFC          | –                  | River sediment        | Organics             | –            | –                    | –                      | –      | –                       | Moon et al. (2005)      |
| MFC          | –                  | River sediment        | Glucose/glutamate    | –            | –                    | –                      | 20     | –                       | Kang et al. (2003)      |
| MFC/BOD sensor | –                  | Starch wastewater     | Starch wastewater    | –            | –                    | –                      | –      | –                       | Kim et al. (2003)       |

(Abrevaya et al. 2011). A second pure culture halophile tested in MFCs is Ge. subterraneus (Badalamenti, Krajmalnik-Brown and Torres 2013; Carmona-Martinez et al. 2013). Geoalkalibacter subterraneus was selected from an MES inoculated with sediments from a salt plant and forms a 76 ± 7 µm thick biofilm on the anode where it is suggested to directly transfer electrons (Carmona-Martinez et al. 2013). This pure culture produced a high current density of 4.7 A m⁻² at a poised anode potential of +200 mV vs SCE. Finally, the arsenate-respiring halophiles B. seletitreduencrens and candidatus Halarasenatibacter silvermani strain SLAS-1 isolated from moderately hypersaline Mono Lake and extremely hypersaline (salt saturated) Searles Lake, respectively, generated similar power levels (Miller and Oremland 2008). The cultures oxidize lactate and donate electrons to the anode in the absence of added mediators (although it was not discerned if the microbes excreted endogenous mediators). The Coulombic efficiencies of the MFCs are 0.3% and 6.7%, respectively suggesting alternative electron acceptors are used or a large part of the substrate is consumed for other processes (Miller and Oremland 2008).

Mixed cultures of anode-respiring microorganisms have been selected in MFCs with inocula from various sources: a saline microbial mat from Cabo Rojo, PR, USA (Miceli et al. 2012); a Mediterranean Sea (Gruissan, France) salt marsh (Rousseau et al. 2013); and a salt lake or salt factory wastewater lagoon (Pierra et al. 2015). The anode community selected in the Cabo Rojo saline microbial mat enrichment contains a diverse community with approximately one third of each of Bacteroidia and Clostridia (Miceli et al. 2012); the microbial community from the salt marsh was dominated by Desulfuromonas and Marinobacter spp. (Rousseau et al. 2014); and the salt lake or salt factory wastewater population was dominated by one or both of Ge. subterraneus and D. acetoxidans (Pierra et al. 2015).

An application of MESs is for the desalination of saline waters that are produced by many industries (Lefebvre and Mitsui 2006) and these MESs can remove up to 95% of salt from sea water (e.g. Cao et al. 2009; Luo et al. 2012). A three-chamber microbial saline-wastewater electrolysis cell was designed that metabolized organic carbon in the anode compartment in the presence of 0.34 M NaCl (20 g L⁻¹) while simultaneously desalinating wastewaters (Kim and Logan 2013). The majority of the substrate was removed in the anode compartment with a Coulombic efficiency of approximately 100%.

Oligotrophs

Traditionally MFCs were looked at as wastewater treatment devices that have the ability to recover energy. Therefore, microorganisms are expected to have high metabolic rates to achieve high power and current. In contrast, MFCs have been utilized with oligotrophic organisms (Table 6) for low biochemical oxygen demand sensors that are more efficient, stable and accurate compared to conventional biochemical oxygen demand monitoring techniques (reviewed in Abrevaya et al. 2015). An MFC-based biosensor for wastewater BOD measurement was stable for over five years using an enrichment of microorganisms from a starch processing plant (Kim et al. 2003). Oligotrophic extremophiles have been enriched from surface waters containing 6 mg L⁻¹ BOD and artificial wastewater containing glucose and glutamate and used for an MFC-based sensor with good stability (Kang et al. 2003). Based upon 16S rRNA gene sequences, the microbial communities in the above biosensors contain distinct populations with 46% of the river water enriched sequences aligning with β-Proteobacteria while the artificial wastewater enriched for 64% α-Proteobacteria (Phung et al. 2004). Finally, MFC biosensors with oligotrophic microorganisms have been further developed for continuous online monitoring of BOD (Moon et al. 2005).

Multiple extremes

Microorganisms with multiple extremophilic characteristics utilized in MESs are haloalkaliphiles that grow in high salt and basic pH (Table 7). A pure culture of haloalkaliphilic N. magadii (ionic strength 3.63 M, pH 10) generated an electrical current in the absence of mediators (Abrevaya et al. 2011). A second study inoculated with a mixed culture from Soap Lake, Washington, enriched for a culture growing optimally at 7% salinity (1.2 M NaCl) and pH 11 that generated a current density of 12.5 mA m⁻² (Paul et al. 2014). 16S rRNA gene sequencing showed that the MFC was enriched with a single strain most similar (99%) to Halanaerobium hydrogeniformans. Finally, soil microorganisms from the high pH and saline Texaco Lake, Mexico, were enriched on a bioanode at pH 10.5–11 and 1.2 M NaCl (Sathish-Kumar et al. 2012, 2013). Low currents have been detected, and cyclic voltammetry suggests that electron transfer is mediated by alkaliphilic cytochromes.

FUTURE OPPORTUNITIES FOR EXTREMOPHILES IN MESs

The application of extremophiles in MESs has only just begun and oxidation of particular substrates in combination with specific inocula may reveal new applications for the recovery of energy or the remediation of polluted water streams. Bioremediation of wastewaters that represent an extreme environment offers a major opportunity for further application of these microorganisms in MESs. In this case, the energy costs of the remediation of these polluted streams might be compensated by the energy production of the MES. A key research need is the discovery of extremophile electricians to be able to treat the desired wastewaters and this will require sampling of diverse extreme environments likely to contain suitable microorganisms (e.g. those described in Fig. 3). However, further unanswered questions remain including which pollutants can be used as electrons donors are electron acceptors available to give sufficient energy gain when coupled to these donors to fulfill the energy need, and will both donor and acceptor be available at the same site.
Table 7. Comparison of the performance of selected bioelectrochemical systems under multiple extreme conditions.

| Condition          | Electron donor        | Voltage     | Power density | Current density | Anode potential (vs SHE) | Reference |
|--------------------|-----------------------|-------------|---------------|-----------------|--------------------------|-----------|
| MFC 10 g L⁻¹ NaCl/Pb | Yeast extract         | 0.58 V      | 54 mW m⁻²     | 138 mA m⁻²      | -0.091 V                 | Abrevaya et al. (2011) |
| MFC 10 g L⁻¹ NaCl/Pb | Yeast extract         | 0.8 V (OCV) | 1.66 mW m⁻²   | 12.5 mA m⁻²     | -                       | Paul et al. (2014)   |
| MFC 10 g L⁻¹ NaCl/Pb | Yeast extract         | 0.58 V      | 49 mW m⁻²     | 4.18 mA m⁻²     | -                       | Paul et al. (2013)   |
| MFC 10 g L⁻¹ NaCl/Pb | Yeast extract         | 0.58 V      | 49 mW m⁻²     | 4.18 mA m⁻²     | -                       | Satish-Kumar & et al. (2013) |
| MFC 10 g L⁻¹ NaCl/Pb | Yeast extract         | 0.58 V      | 49 mW m⁻²     | 4.18 mA m⁻²     | -                       | Satish-Kumar & et al. (2012) |

To date, MESs for sulfate removal from acidic wastewaters have utilized inocula from neutral pH environments (Li et al. 2013; Kim et al. 2014). These studies identified sulfate-reducing species typically growing at pH 7 and it would be interesting to test truly acidophilic sulfate reducers such as Desulfothrix acidiphilus (Jameson et al. 2010) in these systems. In addition, inocula from other anaerobic low pH environments could be tested in MESs to select for potential electricgens. For instance, acid mine drainage sludge possibly contains electricgens able to treat low pH, high metal concentration wastewaters. An example of this research is the European FP7 project ‘BioelectroMET’ where the oxidation of tetrahionate is coupled to the reduction of ferric iron (Sulonen et al. 2015). High pH wastewaters may also be treated with MESs that has advantages for electricity production (described above). However, there are only a few reports of the use of alkaliphiles in MESs and very few pure and mixed culture inocula have been tested. The possibilities for discovering new electricgens are good and their enrichment from high pH environments such as soda lake sediments and slag dumps may prove successful. Examples of wastewaters that could be treated with alkaliphilic electricgens are from the textile (Choe et al. 2005) and brewing industries (Rao et al. 2007).

Both psychrophiles and thermophiles can be applied to treat waste streams at different temperatures providing an economic advantage as the waters would not need to be heated or cooled, respectively. However, little is known about the actual performance of these systems in real-life conditions, and systematic studies are required to see how MESs perform over a longer period of time. The start-up temperature of low-temperature MESs employing psychrotolerant microbes has been suggested to be important (Cheng, Xing and Logan 2011), and further characterization of this parameter would also be warranted. Benthic low-temperature MESs have successfully been used to oxidize sulfide but the resultant deposition of sulfide on the anode surface causes deactivation (Reimers et al. 2006). Future research strategies to attempt to solve this issue include stripping the passivating layer on the anode surface and cycling periods of current harvesting and no harvesting (Reimers et al. 2006). For the thermophiles, one study has addressed the issue of evaporation (Carver, Vuoriranta and Tuovinen 2011) but further development will likely result in more efficient reactor designs.

Using halophiles for the desalination of water (e.g. Cao et al. 2009) has to date only been carried out in batch systems. Further development is needed as commercial use would require a continuous system. When it comes to the use of oligotrophs in biosensors, it is important to increase the signal-to-noise ratio, reproducibility, sensitivity, ability of the microbial consortia to degrade a wide range of substrates and to investigate the many novel species that were enriched (Abrevaya et al. 2015).

The use of MESs also offers possibilities to treat streams with different extreme conditions, as the anode and cathode are generally separated by an ion exchange membrane. This is best illustrated by the pH gradient that in most cases develops over the membrane and is considered as one of the major disadvantages of MESs (Rozendal et al. 2008a,b). The pH gradient develops since the oxidation (proton producing) and reduction (proton consuming) reactions are separated and do not take place in a single compartment (as in a bacterial cell). This charge difference is not compensated as the charge transport in the system is mainly by cations other than protons causing the pH to decrease in the anode and to increase in the cathode. The electrical potential loss associated with this pH gradient is 60 mV per pH unit and severely reduces the power output of MFCs or increases the required power input in MECs (Sleutels et al. 2012).
2013). When a pH 3 stream is fed to the anode, the chance of the charge transport through the membrane to be in the form of protons is 10 000 times higher than when a stream of pH 7 is used (Rozendal, Hamelers and Buisman 2006). Another potential advantage of extremophiles in MESs is to use a low pH catholyte in combination with an alkaline anolyte to create a negative pH gradient which may help the energetics of the system. Of course the pH gradient in these types of systems remains a serious topic of research. The use of a bipolar membrane, which in theory could sustain the pH in both compartments, has not shown its full potential due to the high-energy demand of water splitting (Ter Heijne et al. 2006; Harnisch and Schröder 2009). Further research on these types of membranes and especially how to make them more selective in the electrolyte conditions used in MESs is required to prove their purpose in MESs.

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