REVIEW ARTICLE

Possibilities for extremophilic microorganisms in microbial electrochemical systems

Mark Dopson[1,*], Gaofeng Ni[1] and Tom HJA Sleutels[2]

[1]Centre for Ecology and Evolution in Microbial Model Systems (EEMiS), Linnaeus University, SE-391 82 Kalmar, Sweden and [2]Wetsus, 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

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

(Strik *et al.* 2011) or (iv) power small-scale off-grid devices and sensors (Shantaram *et al.* 2005). These applications have evolved into chemical or microbial catalyzed generation of products with a higher added value from the produced electrons, such as hydrogen (Logan *et al.* 2008), methane (Cheng *et al.* 2009), hydrogen peroxide (Rozendal *et al.* 2009), and short- and medium-chain fatty acids (Steinbusch *et al.* 2011; van Eerten-Jansen *et al.* 2013). The more recent discovery that these electrons can be used to drive microbial metabolism opens up a whole new world of possible products of which we cannot yet see the full impact (Rabaey and Rozendal 2010; Nevin *et al.* 2011). Nowadays, even the ionic current generated with the production of the electrical current is used to drive separation processes such as the recovery of ammonia (Kuntke *et al.* 2012), the production of alkalinity (Sleutels, Hamelers and Buisman 2010; Modin *et al.* 2011) or to provide additional energy to drive microbial activity (Logan and Elimelech 2012).

GENERAL PRINCIPLES OF MESs

Oxidation of a substrate can be spontaneous as well as chemically or microbially 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
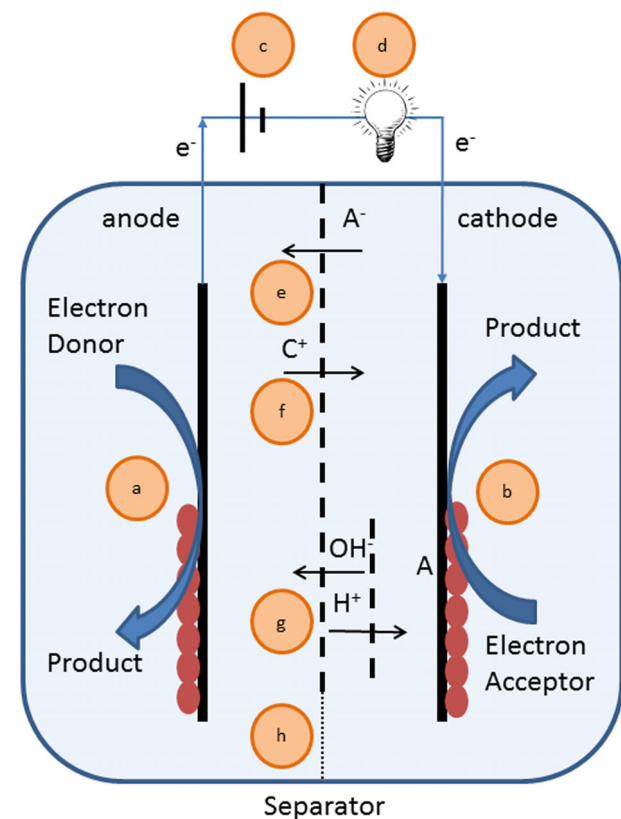

Figure 1. Schematic of a bioelectrochemical system. The system typically consists of anode (where substrate is oxidized (**a**)) and cathode (where the product is formed (**b**)) compartments separated by a membrane. Energy is applied by means of a power supply in MECs (**c**) or harvested from the system 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**).

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^0/H_2S 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).

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 (ΔG_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 ΔG_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, $[i]$ 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 (ΔE; 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. From 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
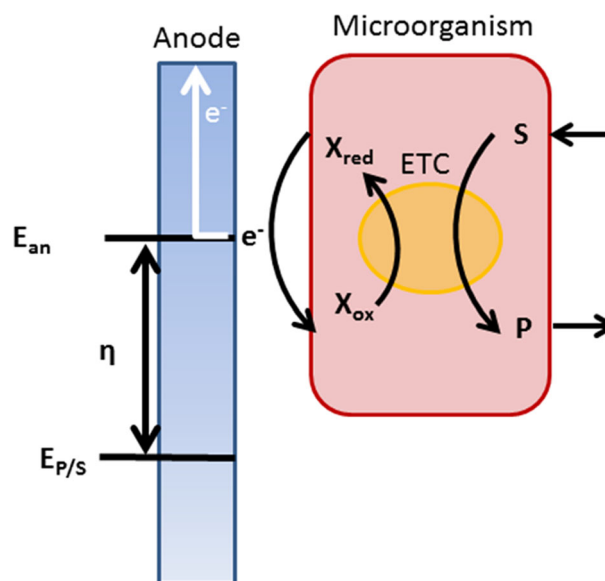

Figure 2. Schematic representation of the electron flow from microorganism to the electrode. The microorganisms oxidize a substrate (S) to a product (P) from which electrons are released into the electron transfer chain. The energy level of these electrons is determined by the Gibbs free energy change of this oxidation reaction ($E_{P/S}$). 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_{an}). Figure adapted from Hamelers *et al.* (2011).

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.

For the anode, the maximum amount of work that can be performed by the microorganisms is determined by the energy available from the substrate ($E_{p/s}$), the energy level at which these electrons are donated to electrode (E_{an}; anode potential) and the rate (I; current density) at which they are converted, which is in turn related to the anode potential. In more general terms, this potential difference between the thermodynamic potential and the actual potential is known as the electrode overpotential (η) and includes an activation and concentration term. The concentration term is determined by the concentration of the reacting species (both substrates and products) and the mass transfer in the system, which in most cases is limited by diffusion. The activation overpotential is mostly determined by the catalyst, in this case the microorganism, and the energy it requires to transfer the electrons.

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
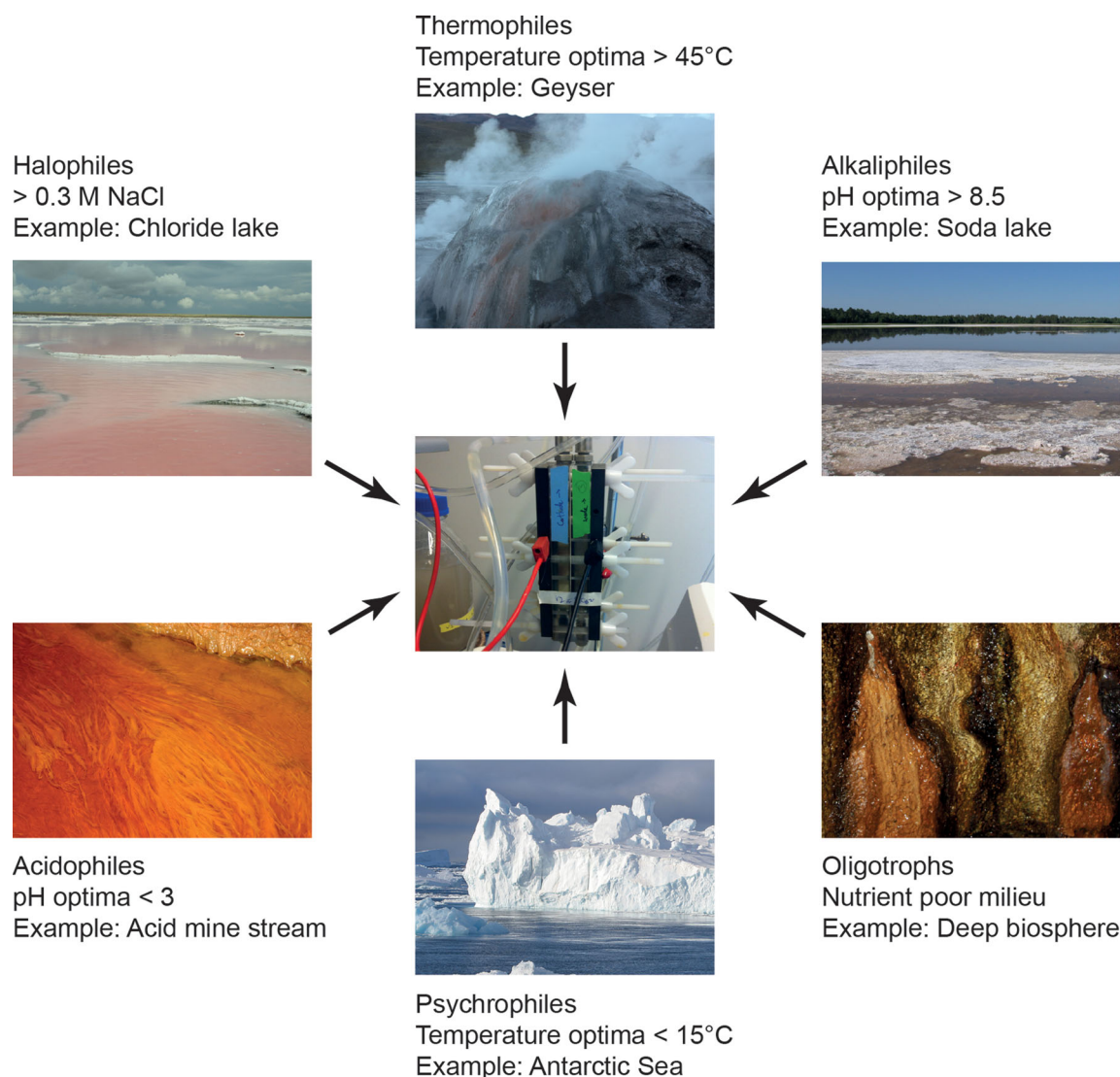
Figure 3. Classes of extremophilic microorganisms that have been utilized in MESs, their defining characteristic and an example of an environment where they grow. Photographs are by the authors except of the chloride and soda lakes (courtesy Dimitry Sorokin) and the Antarctic Sea (courtesy Daniel Bengtsson).

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 Slonczewski *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

(Slonczewski *et al.* 2009; Hicks *et al.* 2010). Other alkaliphile pH homeostatic mechanisms include the Mrp Na^+/H^+ antiporter, additional transport systems and a voltage-gated sodium channel, $Na_V BP$ (reviewed in Slonczewski *et al.* 2009). In contrast, some alkaliphiles (but not halophiles) grow best in the absence of sodium (Tiago *et al.* 2006). Alkaliphiles have been extensively used in other biotechnologies, primarily enzymes for detergents (Sarethy *et al.* 2011).

Psychrophiles (also known as cryophiles) are defined as having an optimum growth temperature <15°C and being able to replicate at <0°C while psychrotolerant species grow at low temperatures but their optimal temperature is >15°C. The absolute lowest temperature for life is unclear but cellular activity occurs as low as −20°C and cells can reproduce at −12°C (Rivkina *et al.* 2000). Natural low-temperature environments include the deep oceans, Antarctica and the Arctic, mountain areas and glacial ice, while man-made fridges and freezers are also permanently cold. Due to these diverse areas, low-temperature environments cover approximately 80% of the globe resulting in psychrophiles being the most common and diverse of the known extremophiles. Much research has been directed at their low-temperature adaptations that include areas of the genome with high GC; proteins with increased flexibility, decreased thermostability and higher activity; expression of genes associated with the cold shock response; and physiological adaptations in the membrane, cryoprotectants, anti-freeze proteins and production of extracellular polysaccharide (reviewed in De Maayer *et al.* 2014).

Thermophiles are predominantly archaea that grow at temperatures above 45°C and up to the present temperature maximum for growth, 122°C (Takai *et al.* 2008). They are often found in geologically active areas such as Yellowstone National Park from where the first thermophile was discovered (Brock 1967). Thermophilic adaptations to life at high temperature include increased salt bridges and a hydrophobic core to increase the heat stability of proteins, a higher percentage of membrane saturated fatty acids and adaptations in the genome such as high GC content and positive supercoiling of the DNA (reviewed in Lewin, Wentzel and Valla 2013).

Halophiles grow in high salt concentrations and are mostly found within the Archaeal domain. They are classified as slight (0.3–0.8 M sodium chloride), moderate (0.8–3.4 M NaCl) and extreme (>3.4 M NaCl) halophiles (Cavicchioli and Thomas 2000). These microorganisms are adapted to a high salt environment by increasing their cytoplasmic osmolarity, such as via importing potassium ions (Oren 2013). The average salinity of seawater is about 3.5% (wt/vol; 599 mM NaCl) resulting in marine microorganisms being classified as slight halophiles.

Oligotrophs grow in extremely nutrient poor environments (e.g. the deep biosphere and sandplains of southern Western Australia) and are typically characterized as having low growth rates. For instance, carbon turnover rates in the deep biosphere estimate generation times to be in the 100s to 1000s of years and these species are hypothesized to have low energy maintenance systems (Hoehler and Jorgensen 2013). Further adaptations to oligotrophy include small cell size and a streamlined genome (Luef *et al.* 2015).

EXTREMOPHILES IN MESs

Several different classes of extremophilic microorganisms have been utilized in MESs and this section (see categories below) will
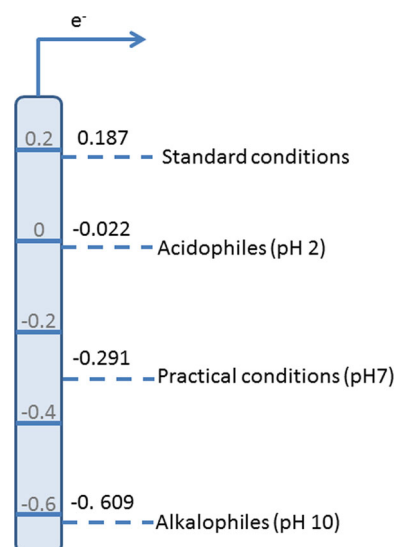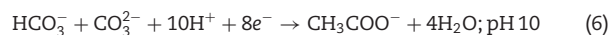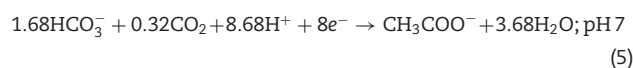

Figure 4. Example of the change in anode potential according to changes in the pH based on thermodynamics. Potentials at standard conditions are calculated with a concentration of 1 M, while the practical conditions are calculated with a concentration of 0.005 M. All potentials are reported vs NHE.

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 possess 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):

$$2CO_2 + 8H^+ + 8e^- \rightarrow CH_3COOH + 2H_2O; pH\,2 \tag{4}$$

$$1.68HCO_3^- + 0.32CO_2 + 8.68H^+ + 8e^- \rightarrow CH_3COO^- + 3.68H_2O; pH\,7 \tag{5}$$

$$HCO_3^- + CO_3^{2-} + 10H^+ + 8e^- \rightarrow CH_3COO^- + 4H_2O; pH\,10 \tag{6}$$

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 alkaliphiles 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

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 $\leq$ 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 nitrilotriacetic 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.

BES	Condition	Inoculum	Electron donor	Voltage	Power density	Current density	CE	Anode potential (vs SHE)	Reference
MFC	pH 2.0	*Acidithiobacillus ferrooxidans**	Electrode	–	–	5.0 A m^{-2}	–	–	Carbajosa et al. (2010)
MFC	pH < 2.5	*Acidithiobacillus ferrooxidans**	FeSO$_4$	–	1.2 W m^{-2}	4.4 A m^{-2}	–	–	Ter Heijne, Hamelers and Buisman(2007)
MFC	pH 2.5	*Acidiphilium* spp.	Glucose	–	–	3 A m^{-2}	–	+ 0.15 V (controlled)	Malki et al. (2008)
MFC	pH 2.5	Mining wastewater	Tetrathionate	0.18 V	18 mW m^{-2}	433 mA m^{-2}	<5%	0.248 V	Sulonen et al. (2015)
MFC	pH 3.0	Sediment	Glucose/glycerol	–	300 mW m^{-2}	3.5 A m^{-2}	–	–	Garcia-Munoz et al. (2011)
MFC	pH 4.0	Anaerobic sludge	Acetate	–	–	–	6.45%	–	Zhang et al. (2011a)
MFC	pH 4.0	*Acidiphilium cryptum*	Glucose	0.30 V (OCV)	12.7 mW m^{-2}	–	–	–	Borole et al. (2008)
MFC	pH 4.0	Anaerobic sludge	Synthetic food waste	0.14 V (OCV)	425 mW m^{-3}	–	18%	–	Li, Cheng and Wong (2013)
MFC	pH 4.4	Distillery wastewater	Distillery wastewater	0.88 V	12.9 W m^{-3}	29 mA m^{-2}	–	–	Kim et al. (2014)
MES	pH 4.5	Synthetic waste water	Organic carbon compounds	–	–	–	–	+0.003 V	Zheng et al. (2014)
MFC	pH 4.5	Anaerobic sludge	Ethanol	–	–	–	27%	–	Liang et al. (2013)
MFC	pH 4.7	Wastewater/sludge	Wastewater	–	454 mW m^{-3}	–	–	–	Li et al. (2013)

*$Acidithiobacillus ferrooxidans$ was inoculated in the cathode for regeneration of Fe^{2+} to Fe^{3+}. 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 that deeply into the biofilm. The electricigen *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 sulfide in acidic wastewaters (pH 2.5–4.5) using organic carbon-fed neutrophilic sulfate-reducing microbes 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 acidophilic electricigens 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 (Sulonen *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

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

BES	Condition	Inoculum	Electron donor	Voltage	Power density	Current density	CE	Anode potential (vs SHE)	Reference
MFC	pH 9.0–11.0	Bacillus spp.	Glucose	0.90 V	–	–	–	–	Akiba et al. (1987)
MFC	pH 10.0	Artificial wastewater	Organics	–	–	–	–	–	Wu et al. (2011)
MFC	pH 10.0	Bacillus pseudofirmus	Organics	–	–	–	–	–	Ma et al. (2012)
MFC	pH 10.0	Existing MFC	Brewery wastewater	1.04 V (OCV)	31 W m^{-3}	135 A m^{-3}	90%	–	Zhuang et al. (2010)
MFC	pH 9.5	Pseudomonas alcaliphila	Citrate	0.53 V (OCV)	–	70 mA m^{-2}	–	–0.094 V	Zhang et al. (2011b)
MFC	pH 9.5	Parental MFC	Urban sewage	0.65 V (OCV)	1.8 W m^{-3}	13.7 A m^{-3}	77%	–	Puig et al. (2010)
MFC	pH 9.5	Corynebacterium sp.	Glucose	0.45 V	7.3 mW m^{-2}	33 mA m^{-2}	5.9%	–	Liu et al. 2010
MEC	pH 9.3	Geoalkalibacter ferrihydriticus	Acetate	–	–	8.3 mA m^{-2}	84–95%	–	Badalamenti, Krajmalnik-Brown and Torres (2013)
MEC	pH 9.1	Operational MFC	Urine	–	–	23.1 A m^{-2}	97%	–	Kuntke et al. (2014)
MFC	pH 9.0	Waste water	Acetate	–	2770 mW m^{-2}	99 mA m^{-2}	–	–	Fan, Hu and Liu (2007)
MFC	pH 9.0	Activated sludge	Acetate	–	1170 mW m^{-2}	0.18 mA m^{-2}	–	–0.393 V (OCP)	Yuan et al. (2011)
MFC	pH 9.0	Anaerobic sludge	Synthetic waste	0.60 V (OCV)	5591 mW m^{-3}	–	63%	–	Li, Cheng and Wong (2013)
MFC	pH 9.0	Compost	Acetate	–	6 W m^{-3}	7 A m^{-2}	23%	–0,041 V	Pocaznoi et al. (2012)
MFC	pH 9.0	Compost	Acetate	–	–	16 A m^{-2}	33%	–0,041 V	Blanchet et al. (2014)
MFC	pH 9.0	Paper mill effluent	Acetate	–	–	7.5 A m^{-2}	–	–0.156	Ketep et al. (2013)

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. Alkalinization of the medium in this case is caused by hydroxyl transport from the cathode and decreased current production drastically (Kuntke *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 biocathode. 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 in-

volvement 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 ferrihydriticus* 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 *Desulfuromonas 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 Ghangrekar 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.

BES	Condition	Inoculum	Electron donor	Voltage	Power density	Current density	CE	Anode potential (vs SHE)	Reference
MEC	0°C	Activated sludge	Acetate	–	–	–	46%	–	Xu et al. (2014)
MFC	4°C	Marine sediment	Wastewater	–	98.1 mW m⁻²	–	25%	–	Larrosa-Guerrero et al. (2010)
MFC	4°C	Wastewater	Acetate	–	425 mW m⁻²	–	31%	–	Cheng, Xing and Logan (2011)
MFC	4°C	Seafloor sediment	Seafloor sediment	0.62 V	34 mW m⁻²	85 mA m⁻²	–	–0.223 V	Reimers et al. (2006)
MEC	4°C	Operational MFC	Glucose	–	–	–	82%	–	Lu et al. (2012)
MEC	4° & 9°C	Operational MFC/wastewater plant	Acetate	–	–	–	94%	–	Lu et al. (2011)
MFC	4–14°C	Domestic wastewater	Organics	0.44 V	602 mW m⁻²	41 mA m⁻²	38%	–	Catal et al. (2011)
MFC	5–10°C	Marine sediment	–	0.43 V (1000 Ω)	160 mW m⁻²	–	24%	–	Zhang et al. (2014)
MFC	8–22°C	Existing MFC	Synthetic wastewater	0.075	15 mW m⁻²	1.5 mA	5%	–	Jadhav and Ghangrekar (2009)
MFC	10°C	Anaerobic sludge	Wastewater	–	–	–	–	–	Michie et al. (2011)
MEC	9°C	Domestic wastewater	Molasses	0.60 V applied	–	77 A m⁻³	–	–	Wang et al. (2014)
MES	10°C	Activated sludge	Glucose	–	–	–	–	–	Kong et al. (2014)
MFC	15°C	Marine sediment	Organics	–	–	~30 mA m⁻²	–	–	Holmes et al. (2004a)
MFC	15°C	Salt marsh sediment	Organics	–	–	~29 mA m⁻²	–	–	Holmes et al. (2004a)
MFC	15°C	Marine sediment	Organics	–	–	~21 mA m⁻²	–	–	Holmes et al. (2004a)
MFC	15°C	Anaerobic sludge	Acetate	362 mV 791 mV (OCV)	591 mW m⁻²	2.8 A m⁻²	–	–	Liu et al. (2012)

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 *D. acetoxidans* that changed with increasing depth (46–76 cm below the sediment) to comprise a more complex community of δ-Proteobacteria (including from the genus *Desulfocapsa* 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 homoacetogic 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 electricigens (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

Table 4. Comparison of the performance of selected bioelectrochemical systems under high-temperature conditions.

BES	Condition	Inoculum	Electron donor	Voltage	Power density	Current density	CE	Anode potential (vs SHE)	Reference
MFC	60°C	Marine marsh sediment	Acetate	–	43 mW m^{-2}	254 mA m^{-2}	36%	–	Mathis et al. (2008)
MEC	60°C	Thermincola ferriacetica	Acetate	–	–	7.0–8.0 A m^{-2}	93%	–	Parameswaran et al. (2013)
MFC	60°C	Thermincola potens	Acetate	–	–	–	–	–	Wrighton et al. (2011)
MFC	60°C	Thermincola ferriacetica	Acetate	–	146 mW m^{-2}	400 mA m^{-2}	97%	–	Marshall and May (2009)
MFC	57°C	Thermophilic compost	Glucose	0.39 V (100 Ω)	375 mW m^{-2}	–	–	–	Carver, Vuoriranta and Tuovinen (2011)
MFC	55°C	Anaerobic digester	Acetate	0.64 V	436 mW m^{-2}	8.3 mA	–	–	Fu et al. (2013b)
MFC	55°C	Anaerobic digester	Acetate	–	823 mW m^{-2}	3.5 mA	–	–	Fu et al. (2013a)
MFC	55°C	Calditerrivibrio nitroreducens	Acetate	–	272 mW m^{-2}	2.5 mA	–	–	Fu et al. (2013a)
MFC	55°C	Anaerobic digester	Acetate	–	1030 mW m^{-2}	–	80%	–	Jong et al. (2006)
MFC	55°C	Methanogenic digester	Acetate	–	37 mW m^{-2}	–	89%	–	Wrighton et al. (2008)
MFC	55°C	Anaerobic digester	Waste water	–	1000 W m^{-2}	2300 mA m^{-2}	89%	–	Ha et al. (2012)
MEC	55°C	Thermophilic MFC	Acetate	–	–	1280 mA m^{-2}	–	–	Fu et al. (2013c)
MFC	50°C	Bacillus licheniformis/B. thermoglucosidansius	Glucose/lactose	0.70 V (OCV)	–	–	41%	–	Choi et al. (2004)
MFC	50°C	Enrichment culture	Synthetic syngas	0.68 V (OCV)	34 mW L^{-1}	200 mA L^{-1}	34%	–	Hussain et al. (2012)

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 mesophilic 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).

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 electricigens at 50°C include *B. thermoglucosidasius* 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 Calni_1470 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 that mediates extracellular electron transfer. An acetate-fed MFC operated at 55°C selected for a mixed population of species 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

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 MEC for H_2 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 phylotypes 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 elevated 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 *Natrialba 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 5. Comparison of the performance of selected bioelectrochemical systems under high salt conditions.

BES	Condition	Inoculum	Electron donor	Voltage	Power density	Current density	CE	Anode potential (vs SHE)	Reference
MEC	17 g L^{-1} NaCl	*Geoalkalibacter subterraneus*	Acetate/yeast extract	–	–	3.3 Am^{-2}	84–95%	–	Badalamenti, Krajmalnik-Brown and Torres (2013)
MFC	151 g L^{-1} NaCl	Saline sediments	Lactate/acetate/H$_2$	–	18 mW m^{-2}	–	–	–	Miller and Oremland (2008)
MFC	158 g L^{-1} NaCl	*Haloferax volcanii*	Yeast extract	0.57 V	510 mW m^{-2}	560 mA m^{-2}	–	–	Abrevaya *et al.* (2011)
MFC	91 g L^{-1} NaC	Costal sediment	Lactate	–	5.2 mW m^{-2}	–	–	–	Huang, Sun and Zhang (2010)
MES	45 g L^{-1} NaCl	Salt marsh	Acetate	–	–	85 A m^{-2}	24%	–	Rousseau *et al.* (2013)
MFC	18 g L^{-1} NaCl	Wastewater	Acetate	0.42 V (OCV)	22 W m^{-3}	52 mA m^{-2}	52%	–	Liu *et al.* (2008)
MFC	35 g L^{-1} NaCl	*Geoalkalibacter subterraneus*	Acetate	–	–	4.68 A m^{-2}	–	+0.441 V	Carmona-Martinez *et al.* (2013)
MFC	35 g L^{-1} NaCl	Salt lake/salt factory	Butyrate/acetate	–	–	8.5 A m^{-2}	70%	–0.047 V (controlled)	Pierra *et al.* (2015)
MFC	40 g L^{-1} NaCl	Domestic wastewater	Acetate	0.42 (OCV)	18 W m^{-3}	–	22%	–	Lefebvre *et al.* (2012)
MEC	20 g L^{-1} NaCl	Saline microbial mats	Acetate	–	–	4.45 A m^{-2}	–	–0.103 V	Miceli *et al.* (2012)
MEC	25 g L^{-1} NaCl	Existing MFC	Acetate	1.2 V (applied)	–	7.8 mA m^{-2}	100%	–0.197 V	Kim and Logan (2013)

Table 6. Comparison of the performance of selected bioelectrochemical systems under low substrate conditions.

BES	Condition	Inoculum	Electron donor	Voltage	Power density	Current density	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 $76 \pm 7 \mu$m 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. selenitireducens* and candidatus *Halarsenatibacter silvermanii* 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 march 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 Moletta 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 upon16S 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 electricigens 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.

BES	Condition	Inoculum	Electron donor	Voltage	Power density	Current density	CE	Anode potential (vs SHE)	Reference
MFC	210 g L^{-1} NaCl/pH 10	*Natrialba magadii*	Yeast extract	0.58 V	54 mW m^{-2}	560 mA m^{-2}	–	–	Abrevaya et al. (2011)
MFC	75 g L^{-1} NaCl/pH 11	Soap lake sediment	Yeast extract	0.8 V (OCV)	1.66 mW m^{-2}	12.5 mA m^{-2}	–	–	Paul et al. (2014)
MFC	60–80 g L^{-1} NaCl/pH 9	Texaco lake	Acetate	–	49 mW m^{-2}	–	–	0.094 V	Sathish-Kumar et al. (2013)
MFC	70 g L^{-1} NaCl/pH 11	Texaco lake	Acetate	0.294 V 0.6 V (OCV)	41 mW m^{-2}	138 mA m^{-2}	–	–0.091 V	Sathish-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 *Desulfosporosinus 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 electricigens. For instance, acid mine drainage sludge possibly contains electricigens able to treat low pH, high metal concentration wastewaters. An example of this research is the European FP7 project 'BioelectroMET' where the oxidation of tetrathionate 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 electricigens 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 electricigens 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.

FUNDING

REFERENCES

Abrevaya XC, Sacco N, Mauas PJD *et al.* Archaea-based microbial fuel cell operating at high ionic strength conditions. *Extremophiles* 2011;**15**:633–42.

Abrevaya XC, Sacco NJ, Bonetto MC *et al.* Analytical applications of microbial fuel cells. Part I: biochemical oxygen demand. *Biosens Bioelectron* 2015;**63**:580–90.

Akiba T, Bennetto HP, Stirling JL *et al.* Electricity production from alkalophilic organisms. *Biotechnol Lett* 1987;**9**:611–6.

Antony CP, Kumaresan D, Hunger S *et al.* Microbiology of Lonar Lake and other soda lakes. *ISME J* 2013;**7**:468–76.

Badalamenti JP, Krajmalnik-Brown R, Torres CI. Generation of high current densities by pure cultures of anode-respiring *Geoalkalibacter* spp. under alkaline and saline conditions in microbial electrochemical cells. *mBio* 2013;**4**:1–8.

Baker-Austin C, Potrykus J, Wexler M *et al.* Biofilm development in the extremely acidophilic archaeon 'Ferroplasma acidarmanus' Fer1. *Extremophiles* 2010;**14**:485–91.

Blanchet E, Pécastaings S, Erable B *et al.* Protons accumulation during anodic phase turned to advantage for oxygen reduction during cathodic phase in reversible bioelectrodes. *Bioresource Technol* 2014;**173**:224–30.

Bond DR, Holmes DE, Tender LM *et al.* Electrode-reducing microorganisms that harvest energy from marine sediments. *Science* 2002;**295**:483–5.

Bonnefoy V, Holmes DS. Genomic insights into microbial oxidation and iron homeostasis in extremely acidic environments. *Environ Microbiol* 2012;**14**:1597–611.

Borole AP, O'Neill H, Tsouris C *et al.* A microbial fuel cell operating at low pH using the acidophile *Acidiphilium cryptum*. *Biotechnol Lett* 2008;**20**:1367–72.

Bott TL, Brock TD. Bacterial growth rates above 90°C in Yellowstone hot springs. *Science* 1969;**164**:1411–2.

Brock TD. Life at high temperatures. *Science* 1967;**158**:1012–9.

Byrne-Bailey KG, Wrighton KC, Melnyk RA *et al.* Complete genome sequence of the electricity-producing 'Thermincola potens' strain JR. *J Bacteriol* 2010;**192**:4078–9.

Caccavo F, Lonergan DJ, Lovley DR *et al.* Geobacter sulfurreducens sp. nov., a hydrogen-oxidizing and acetate-oxidizing dissimilatory metal-reducing microorganism. *Appl Environ Microb* 1994;**60**:3752–9.

Cao X, Huang X, Liang P *et al.* A new method for water desalination using microbial desalination cells. *Environ Sci Technol* 2009;**43**:7148–52.

Carbajosa S, Malki M, Caillard R *et al.* Electrochemical growth of *Acidithiobacillus ferrooxidans* on a graphite electrode for obtaining a biocathode for direct electrocatalytic reduction of oxygen. *Biosens Bioelectron* 2010;**26**:877–80.

Carlson HK, Iavarone AT, Gorur A *et al.* Surface multiheme *c*-type cytochromes from *Thermincola potens* and implications for respiratory metal reduction by Gram-positive bacteria. *P Natl Acad Sci USA* 2012;**109**:1702–7.

Carmona-Martinez AA, Pierra M, Trably E *et al.* High current density via direct electron transfer by the halophilic anode respiring bacterium *Geoalkalibacter subterraneus*. *Phys Chem Chem Phys* 2013;**15**:19699–707.

Carver SM, Vuoriranta P, Tuovinen OH. A thermophilic microbial fuel cell design. *J Power Sources* 2011;**196**:3757–60.

Catal T, Bermek H, Liu H. Removal of selenite from wastewater using microbial fuel cells. *Biotechnol Lett* 2009;**31**:1211–6.

Catal T, Kavanagh P, O'Flaherty V *et al.* Generation of electricity in microbial fuel cells at sub-ambient temperatures. *J Power Sources* 2011;**196**:2676–81.

Cavicchioli R, Thomas T. Extremophiles. In: Lederberg J (ed). *Encyclopedia of Microbiology*, **Vol. 2**. San Diego: Academic Press, 2000, 317–37.

Cheng S, Xing D, Call DF *et al.* Direct biological conversion of electrical current into methane by electromethanogenesis. *Environ Sci Technol* 2009;**43**:3953–8.

Cheng SA, Xing DF, Logan BE. Electricity generation of single-chamber microbial fuel cells at low temperatures. *Biosens Bioelectron* 2011;**26**:1913–7.

Choe EK, Son EJ, Lee BS *et al.* NF process for the recovery of caustic soda and concentration of disodium terephthalate from alkaline wastewater from polyester fabrics. *Desalination* 2005;**186**:29–37.

Choi Y, Jung E, Park H *et al.* Construction of microbial fuel cells using thermophilic microorganisms, *Bacillus licheniformis* and *Bacillus thermoglucosidasius*. *B Korean Chem Soc* 2004;**25**: 813–8.

Clauwaert P, Rabaey K, Aelterman P *et al.* Biological denitrification in microbial fuel cells. *Environ Sci Technol* 2007;**41**:3354–60.

Cowan DA, Makhalanyane TP, Dennis PG *et al.* Microbial ecology and biogeochemistry of continental Antarctic soils. *Front Microbiol* 2014;**5**:154.

De Maayer P, Anderson D, Cary C *et al.* Some like it cold: understanding the survival strategies of psychrophiles. *EMBO Rep* 2014;**15**:508–17.

Dick GJ, Anantharaman K, Baker BJ *et al.* The microbiology of deep-sea hydrothermal vent plumes: ecological and biogeographic linkages to seafloor and water column habitats. *Front Microbiol* 2013;**4**:124.

Dopson M. Physiological adaptations and biotechnological applications of acidophiles. In: Anitori R (ed). *Extremophiles:*

Microbiology and Biotechnology. Norwich: Horizon Scientific Press, 2012, 265–94.

Dopson M, Holmes DS. Metal resistance in acidophilic microorganisms and its significance for biotechnologies. *Appl Microbiol Biot* 2014;**98**:8133–44.

Dopson M, Johnson DB. Biodiversity, metabolism and applications of acidophilic sulfur- metabolizing micro-organisms. *Environ Microbiol* 2012;**14**:2620–31.

Dopson M, Ossandon F, Lövgren L *et al.* Metal resistance or tolerance? Acidophiles confront high metal loads via both abiotic and biotic mechanisms. *Front Microbiol* 2014;**5**:157.

Erable B, Etcheverry L, Bergel A. Increased power from a two-chamber microbial fuel cell with a low-pH air-cathode compartment. *Electrochem Comm* 2009;**11**:619–22.

Fan YZ, Hu HQ, Liu H. Sustainable power generation in microbial fuel cells using bicarbonate buffer and proton transfer mechanisms. *Environ Sci Technol* 2007;**41**:8154–8.

Fu Q, Kobayashi H, Kawaguchi H *et al.* A thermophilic Gram-negative nitrate-reducing bacterium, *Calditerrivibrio nitroreducens*, exhibiting electricity generation capability. *Environ Sci Technol* 2013a;**47**:12583–90.

Fu Q, Kobayashi H, Kawaguchi H *et al.* Electrochemical and phylogenetic analyses of current-generating microorganisms in a thermophilic microbial fuel cell. *J Biosci Bioengin* 2013b;**115**:268–71.

Fu Q, Kobayashi H, Kuramochi Y *et al.* Bioelectrochemical analyses of a thermophilic biocathode catalyzing sustainable hydrogen production. *Int J Hydrogen Energ* 2013c;**38**: 15638–45.

Garcia-Munoz J, Amils R, Fernandez VM *et al.* Electricity generation by microorganisms in the sediment-water interface of an extreme acidic microcosm. *Int Microbiol* 2011;**14**: 73–81.

Gutierrez O, Park D, Sharma KR *et al.* Effects of long-term pH elevation on the sulfate-reducing and methanogenic activities of anaerobic sewer biofilms. *Water Res* 2009;**43**:2549–57.

Ha PT, Lee TK, Rittmann BE *et al.* Treatment of alcohol distillery wastewater using a Bacteroidetes-dominant thermophilic microbial fuel cell. *Environ Sci Technol* 2012;**46**:3022–30.

Hamelers HVM, ter Heijne A, Stein N *et al.* Butler-Volmer-Monod model for describing bio-anode polarization curves. *Bioresour Technol* 2011;**102**:381–7.

Harnisch F, Schröder U. Selectivity versus mobility: separation of anode and cathode in microbial bioelectrochemical systems. *ChemSusChem* 2009;**2**:921–6.

Harnisch F, Schröder U, Scholz F. The suitability of monopolar and bipolar ion exchange membranes as separators for biological fuel cells. *Environ Sci Technol* 2008;**42**:1740–6.

Hicks DB, Liu J, Fujisawa M *et al.* F_1F_0-ATP synthases of alkaliphilic bacteria: lessons from their adaptations. *BBA - Bioenergetics* 2010;**1797**:1362–77.

Hoehler TM, Jorgensen BB. Microbial life under extreme energy limitation. *Nat Rev Microbiol* 2013;**11**:83–94.

Holmes DE, Bond DR, O'Neil RA *et al.* Microbial communities associated with electrodes harvesting electricity from a variety of aquatic sediments. *Microb Ecol* 2004a;**48**:178–90.

Holmes DE, Nicoll JS, Bond DR *et al.* Potential role of a novel psychrotolerant member of the family Geobacteraceae, *Geopsychrobacter electrodiphilus* gen. nov., sp. nov., in electricity production by a marine sediment fuel cell. *Appl Environ Microb* 2004b;**70**:6023–30.

Huang JX, Sun BL, Zhang XB. Electricity generation at high ionic strength in microbial fuel cell by a newly isolated *Shewanella marisflavi* EP1. *Appl Microbiol Biot* 2010;**85**:1141–9.

Hussain A, Mehta P, Raghavan V *et al.* The performance of a thermophilic microbial fuel cell fed with synthesis gas. *Enzyme Microb Tech* 2012;**51**:163–70.

Jadhav GS, Ghangrekar MM. Performance of microbial fuel cell subjected to variation in pH, temperature, external load and substrate concentration. *Bioresource Technol* 2009;**100**: 717–23.

Jameson E, Rowe OF, Hallberg KB *et al.* Sulfidogenesis and selective precipitation of metals at low pH mediated by *Acidithiobacillus* spp. and acidophilic sulfate-reducing bacteria. *Hydrometallurgy* 2010;**104**:488–93.

Jong BC, Kim BH, Chang IS *et al.* Enrichment, performance, and microbial diversity of a thermophilic mediatorless microbial fuel cell. *Environ Sci Technol* 2006;**40**:6449–54.

Kang KH, Jang JK, Pham TH *et al.* A microbial fuel cell with improved cathode reaction as a low biochemical oxygen demand sensor. *Biotechnol Lett* 2003;**25**:1357–61.

Kawahara H. Cryoprotectants and ice-binding proteins. In: Margesin R, Schinner F, Marx JC *et al.* (eds). *Psychrophiles: From Biodiversity to Biotechnology*. Berlin: Springer, 2008, 229–46.

Ketep SF, Bergel A, Bertrand M *et al.* Lowering the applied potential during successive scratching/re-inoculation improves the performance of microbial anodes for microbial fuel cells. *Bioresource Technol* 2013;**127**:448–55.

Kim BH, Chang IS, Gil GC *et al.* Novel BOD (biological oxygen demand) sensor using mediator-less microbial fuel cell. *Biotechnol Lett* 2003;**25**:541–5.

Kim H, Kim B, Kim J *et al.* Electricity generation and microbial community in microbial fuel cell using low-pH distillery wastewater at different external resistances. *J Biotechnol* 2014;**186**:175–80.

Kim JR, Dec J, Bruns MA *et al.* Removal of odors from Swine wastewater by using microbial fuel cells. *Appl Environ Microb* 2008;**74**:2540–3.

Kim Y, Logan BE. Simultaneous removal of organic matter and salt ions from saline wastewater in bioelectrochemical systems. *Desalination* 2013;**308**:115–21.

Kong D, Liang B, Lee DJ *et al.* Effect of temperature switchover on the degradation of antibiotic chloramphenicol by biocathode bioelectrochemical system. *J Environ Sci* 2014;**26**:1689–97.

Kuntke P, Sleutels T, Saakes M *et al.* Hydrogen production and ammonium recovery from urine by a Microbial Electrolysis Cell. *Int J Hydrogen Energ* 2014;**39**:4771–8.

Kuntke P, Smiech KM, Bruning H *et al.* Ammonium recovery and energy production from urine by a microbial fuel cell. *Water Res* 2012;**46**:2627–36.

Larrosa-Guerrero A, Scott K, Head IM *et al.* Effect of temperature on the performance of microbial fuel cells. *Fuel* 2010;**89**: 3985–94.

Lefebvre O, Moletta R. Treatment of organic pollution in industrial saline wastewater: a literature review. *Water Res* 2006;**40**:3671–82.

Lefebvre O, Tan Z, Kharkwal S *et al.* Effect of increasing anodic NaCl concentration on microbial fuel cell performance. *Bioresource Technol* 2012;**112**:336–40.

Lewin A, Wentzel A, Valla S. Metagenomics of microbial life in extreme temperature environments. *Curr Opin Biotechnol* 2013;**24**:516–25.

Li XM, Cheng KY, Selvam A *et al.* Bioelectricity production from acidic food waste leachate using microbial fuel cells: effect of microbial inocula. *Process Biochem* 2013;**48**:283–8.

Li XM, Cheng KY, Wong JW. Bioelectricity production from food waste leachate using microbial fuel cells: effect of NaCl and pH. *Bioresource Technol* 2013;**149**:452–8.

Liang F, Xiao Y, Zhao F. Effect of pH on sulfate removal from wastewater using a bioelectrochemical system. *Chem Eng J* 2013;**218**:147–53.

Liu H, Cheng S, Huang L *et al.* Scale-up of membrane-free single-chamber microbial fuel cells. *J Power Sources* 2008;**179**:274–9.

Liu H, Grot S, Logan BE. Electrochemically assisted microbial production of hydrogen from acetate. *Environ Sci Technol* 2005;**39**:4317–20.

Liu L, Tsyganova O, Lee D-J *et al.* Anodic biofilm in single-chamber microbial fuel cells cultivated under different temperatures. *Int J Hydrogen Energ* 2012;**37**:15792–800.

Liu M, Yuan Y, Zhang LX *et al.* Bioelectricity generation by a Gram-positive *Corynebacterium* sp strain MFC03 under alkaline condition in microbial fuel cells. *Bioresource Technol* 2010;**101**:1807–11.

Liu Y, Wang Z, Liu J *et al.* A trans-outer membrane porin-cytochrome protein complex for extracellular electron transfer by *Geobacter sulfurreducens* PCA. *Environ Microbiol Rep* 2014;**6**:776–85.

Logan BE, Call D, Cheng S *et al.* Microbial electrolysis cells for high yield hydrogen gas production from organic matter. *Environ Sci Technol* 2008;**42**:8630–40.

Logan BE, Elimelech M. Membrane-based processes for sustainable power generation using water. *Nature* 2012;**488**:313–9.

Logan BE, Hamelers B, Rozendal R *et al.* Microbial fuel cells: methodology and technology. *Environ Sci Technol* 2006;**40**:5181–92.

Logan BE, Rabaey K. Conversion of wastes into bioelectricity and chemicals by using microbial electrochemical technologies. *Science* 2012;**337**:686–90.

Lovley DR, Malvankar NS. Seeing is believing: novel imaging techniques help clarify microbial nanowire structure and function. *Environ Microbiol* 2015;**17**:2209–15.

Lu L, Ren NQ, Zhao X *et al.* Hydrogen production, methanogen inhibition and microbial community structures in psychrophilic single-chamber microbial electrolysis cells. *Energy Environ Sci* 2011;**4**:1329–36.

Lu L, Xing DF, Ren NQ *et al.* Syntrophic interactions drive the hydrogen production from glucose at low temperature in microbial electrolysis cells. *Bioresource Technol* 2012;**124**:68–76.

Luef B, Frischkorn KR, Wrighton KC *et al.* Diverse uncultivated ultra-small bacterial cells in groundwater. *Nat Commun* 2015;**6**:6372.

Luo H, Xu P, Roane TM *et al.* Microbial desalination cells for improved performance in wastewater treatment, electricity production, and desalination. *Bioresource Technol* 2012;**105**:60–6.

Ma C, Zhuang L, Zhou SG *et al.* Alkaline extracellular reduction: isolation and characterization of an alkaliphilic and halotolerant bacterium, *Bacillus pseudofirmus* MC02. *J Appl Microbiol* 2012;**112**:883–91.

Malki M, Casado S, Lopez MF *et al.* Physicochemical characterization of *Acidiphilium* sp. biofilms. *ChemPhysChem* 2013;**14**:1237–44.

Malki M, De Lacey AL, Rodriguez N *et al.* Preferential use of an anode as an electron acceptor by an acidophilic bacterium in the presence of oxygen. *Appl Environ Microb* 2008;**74**:4472–6.

Malvankar NS, Vargas M, Nevin K *et al.* Structural basis for metallic-like conductivity in microbial nanowires. *MBio* 2015;**6**:e00084.

Marshall CW, May HD. Electrochemical evidence of direct electrode reduction by a thermophilic Gram-positive bacterium, *Thermincola ferriacetica*. *Energy Environ Sci* 2009;**2**:699–705.

Mathis B, Marshall C, Milliken C *et al.* Electricity generation by thermophilic microorganisms from marine sediment. *Appl Microbiol Biot* 2008;**78**:147–55.

Miceli JF, Parameswaran P, Kang DW *et al.* Enrichment and analysis of anode-respiring bacteria from diverse anaerobic inocula. *Environ Sci Technol* 2012;**46**:10349–55.

Michie IS, Kim JR, Dinsdale RM *et al.* The influence of psychrophilic and mesophilic start-up temperature on microbial fuel cell system performance. *Energy Environ Sci* 2011;**4**:1011–9.

Miller LG, Oremland RS. Electricity generation by anaerobic bacteria and anoxic sediments from hypersaline soda lakes. *Extremophiles* 2008;**12**:837–48.

Modin O, Fukushi K, Rabaey K *et al.* Redistribution of wastewater alkalinity with a microbial fuel cell to support nitrification of reject water. *Water Res* 2011;**45**:2691–9.

Moon H, Chang IS, Jang JK *et al.* On-line monitoring of low biochemical oxygen demand through continuous operation of a mediator-less microbial fuel cell. *J Microbiol Biotechn* 2005;**15**:192–6.

Morozkina EV, Slutskaya ES, Fedorova TV *et al.* Extremophilic microorganisms: biochemical adaptation and biotechnological application. *Appl Biochem Micro* 2010;**46**:1–14.

Mu Y, Rabaey K, Rozendal RA *et al.* Decolorization of azo dyes in bioelectrochemical systems. *Environ Sci Technol* 2009;**43**:5137–43.

Nevin KP, Hensley SA, Franks AE *et al.* Electrosynthesis of organic compounds from carbon dioxide is catalyzed by a diversity of acetogenic microorganisms. *Appl Environ Microb* 2011;**77**:2882–6.

Oren A. Life at high salt concentrations, intracellular KCl concentrations, and acidic proteomes. *Front Microbiol* 2013;**4**:315.

Osorio H, Mangold S, Denis Y *et al.* Anaerobic sulfur metabolism coupled to dissimilatory iron reduction in the extremophile *Acidithiobacillus ferrooxidans*. *Appl Environ Microb* 2013;**79**:2172–81.

Pant D, Van Bogaert G, Diels L *et al.* A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production. *Bioresource Technol* 2010;**101**:1533–43.

Parameswaran P, Bry T, Popat SC *et al.* Kinetic, electrochemical, and microscopic characterization of the thermophilic, anode-respiring bacterium *Thermincola ferriacetica*. *Environ Sci Technol* 2013;**47**:4934–40.

Paul VG, Minteer SD, Treu BL *et al.* Ability of a haloalkaliphilic bacterium isolated from Soap Lake, Washington to generate electricity at pH 11.0 and 7% salinity. *Environ Technol* 2014;**35**:1003–11.

Phung NT, Lee J, Kang KH *et al.* Analysis of microbial diversity in oligotrophic microbial fuel cells using 16S rDNA sequences. *FEMS Microbiol Lett* 2004;**233**:77–82.

Pierra M, Carmona-Martínez AA, Trably E *et al.* Specific and efficient electrochemical selection of *Geoalkalibacter subterraneus* and *Desulfuromonas acetoxidans* in high current-producing biofilms. *Bioelectrochemistry* 2015;**106**(Pt A):221–5.

Pikuta EV, Hoover RB, Tang J. Microbial extremophiles at the limits of life. *Crit Rev Microbiol* 2007;**33**:183–209.

Pitluck S, Sikorski J, Zeytun A *et al.* Complete genome sequence of *Calditerrivibrio nitroreducens* type strain (Yu37-1^T). *Stand Genomic Sci* 2011;**4**:54–62.

Pocaznoi D, Erable B, Etcheverry L *et al.* Towards an engineering-oriented strategy for building microbial anodes for microbial fuel cells. *Phys Chem Chem Phys* 2012;**14**:13332–43.

Puig S, Serra M, Coma M *et al.* Effect of pH on nutrient dynamics and electricity production using microbial fuel cells. *Bioresource Technol* 2010;**101**:9594–9.

Rabaey K, Rozendal RA. Microbial electrosynthesis - revisiting the electrical route for microbial production. *Nat Rev Microbiol* 2010;**8**:706–16.

Rao AG, Reddy TSK, Prakash SS *et al.* pH regulation of alkaline wastewater with carbon dioxide: a case study of treatment of brewery wastewater in UASB reactor coupled with absorber. *Bioresource Technol* 2007;**98**:2131–6.

Reguera G, McCarthy KD, Mehta T *et al.* Extracellular electron transfer via microbial nanowires. *Nature* 2005;**435**: 1098–101.

Reimers CE, Girguis P, Stecher HA *et al.* Microbial fuel cell energy from an ocean cold seep. *Geobiology* 2006;**4**:123–36.

Rivkina EM, Friedmann EI, McKay CP *et al.* Metabolic activity of permafrost bacteria below the freezing point. *Appl Environ Microb* 2000;**66**:3230–3.

Rousseau R, Dominguez-Benetton X, Délia M-L *et al.* Microbial bioanodes with high salinity tolerance for microbial fuel cells and microbial electrolysis cells. *Electrochem Comm* 2013;**33**: 1–4.

Rousseau R, Santaella C, Achouak W *et al.* Correlation of the electrochemical kinetics of high-salinity-tolerant bioanodes with the structure and microbial composition of the biofilm. *ChemElectroChem* 2014;**1**:1966–75.

Rozendal RA, Hamelers HVM, Buisman CJN. Effects of membrane cation transport on pH and Microbial Fuel Cell performance. *Environ Sci Technol* 2006;**40**:5206–11.

Rozendal RA, Hamelers HVM, Euverink GJW *et al.* Principle and perspectives of hydrogen production through biocatalyzed electrolysis. *Int J Hydrogen Energ* 2006;**31**:1632–40.

Rozendal RA, Hamelers HVM, Rabaey K *et al.* Towards practical implementation of bioelectrochemical wastewater treatment. *Trends Biotechnol* 2008a;**26**:450–9.

Rozendal RA, Leone E, Keller J *et al.* Efficient hydrogen peroxide generation from organic matter in a bioelectrochemical system. *Electrochem Comm* 2009;**11**:1752–5.

Rozendal RA, Sleutels TH, Hamelers HV *et al.* Effect of the type of ion exchange membrane on performance, ion transport, and pH in biocatalyzed electrolysis of wastewater. *Water Sci Technol* 2008b;**57**:1757–62.

SanMartin-Uriz P, Gomez MJ, Arcas A *et al.* Draft genome sequence of the electricigen *Acidiphilium* sp. strain PM (DSM 24941). *J Bacteriol* 2011;**193**:5585–6.

Sarethy IP, Saxena Y, Kapoor A *et al.* Alkaliphilic bacteria: applications in industrial biotechnology. *J Ind Microbiol Biot* 2011;**38**:769–90.

Sathish-Kumar K, Solorza-Feria O, Tapia-Ramirez J *et al.* Electrochemical and chemical enrichment methods of a sodic-saline inoculum for microbial fuel cells. *Int J Hydrogen Energ* 2013;**38**:12600–9.

Sathish-Kumar K, Solorza-Feria O, Vazquez-Huerta G *et al.* Electrical stress-directed evolution of biocatalysts community sampled from a sodic-saline soil for microbial fuel cells. *J New Mat Electr Sys* 2012;**15**:181–6.

Sato T, Atomi H. Novel metabolic pathways in Archaea. *Curr Opin Microbiol* 2011;**14**:307–14.

Shantaram A, Beyenal H, Raajan R *et al.* Wireless sensors powered by microbial fuel cells. *Environ Sci Technol* 2005;**39**: 5037–42.

Sleutels TH, Hamelers HV, Buisman CJ. Reduction of pH buffer requirement in bioelectrochemical systems. *Environ Sci Technol* 2010;**44**:8259–63.

Sleutels THJA, Heijne AT, Buisman CJN *et al.* Steady-state performance and chemical efficiency of Microbial Electrolysis Cells. *Int J Hydrogen Energ* 2013;**38**:7201–8.

Sleutels THJA, Ter Heijne A, Buisman CJN *et al.* Bioelectrochemical systems: an outlook for practical applications. *ChemSusChem* 2012;**5**:1012–9.

Slonczewski JL, Fujisawa M, Dopson M *et al.* Cytoplasmic pH measurement and homeostasis in bacteria and archaea. *Adv Microb Physiol* 2009;**55**:1–79.

Steinbusch KJJ, Hamelers HVM, Plugge CM *et al.* Biological formation of caproate and caprylate from acetate: fuel and chemical production from low grade biomass. *Energy Environ Sci* 2011;**4**:216–24.

Strik DP, Timmers RA, Helder M *et al.* Microbial solar cells: applying photosynthetic and electrochemically active organisms. *Trends Biotechnol* 2011;**29**:41–9.

Sulonen ML, Kokko ME, Lakaniemi AM *et al.* Electricity generation from tetrathionate in microbial fuel cells by acidophiles. *J Hazard Mater* 2015;**284**:182–9.

Takai K, Nakamura K, Toki T *et al.* Cell proliferation at 122°C and isotopically heavy CH_4 production by a hyperthermophilic methanogen under high-pressure cultivation. *P Nat Acad Sci USA* 2008;**105**:10949–54.

Tapia JM, Munoz JA, Gonzalez F *et al.* Interrelation between cells and extracellular polymeric substances (EPS) from *Acidiphilium 3.2Sup(5)* on carbon surfaces. *Adv Mat Res* 2009;**71–73**:287–90.

Ter Heijne A, Hamelers HV, Buisman CJ. Microbial fuel cell operation with continuous biological ferrous iron oxidation of the catholyte. *Environ Sci Technol* 2007;**41**:4130–4.

Ter Heijne A, Hamelers HV, De Wilde V *et al.* A bipolar membrane combined with ferric iron reduction as an efficient cathode system in microbial fuel cells. *Environ Sci Technol* 2006;**40**:5200–5.

Ter Heijne A, Liu F, Weijden R *et al.* Copper recovery combined with electricity production in a microbial fuel cell. *Environ Sci Technol* 2010;**44**:4376–81.

Tiago I, Pires C, Mendes V *et al. Bacillus foraminis* sp. nov., isolated from a non-saline alkaline groundwater. *Int J Syst Evol Micr* 2006;**56**:2571–4.

van de Vossenberg JLCM, Driessen AJM, Zillig W *et al.* Bioenergetics and cytoplasmic membrane stability of the extremely acidophilic, thermophilic archaeon *Picrophilus oshimae. Extremophiles* 1998;**2**:67–74.

van Eerten-Jansen MCAA, Ter Heijne A, Grootscholten TIM *et al.* Bioelectrochemical production of caproate and caprylate from acetate by mixed cultures. *SusChemEngin* 2013;**1**: 513–8.

Venkateswaran K, Moser DP, Dollhopf ME *et al.* Polyphasic taxonomy of the genus *Shewanella* and description of *Shewanella oneidensis* sp. nov. *Int J Syst Bacteriol* 1999;**49**:705–24.

Virdis B, Rabaey K, Rozendal RA *et al.* Simultaneous nitrification, denitrification and carbon removal in microbial fuel cells. *Water Res* 2010;**44**:2970–80.

Virdis B, Rabaey K, Yuan Z *et al.* Microbial fuel cells for simultaneous carbon and nitrogen removal. *Water Res* 2008;**42**: 3013–24.

Wang Y, Guo WQ, Xing DF *et al.* Hydrogen production using biocathode single-chamber microbial electrolysis cells fed by molasses wastewater at low temperature. *Int J Hydrogen Energ* 2014;**39**:19369–75.

Wrighton KC, Agbo P, Warnecke F *et al.* A novel ecological role of the Firmicutes identified in thermophilic microbial fuel cells. *ISME J* 2008;**2**:1146–56.

Wrighton KC, Thrash JC, Melnyk RA *et al*. Evidence for direct electron transfer by a Gram-positive bacterium isolated from a microbial fuel cell. *Appl Environ Microb* 2011;**77**: 7633–9.

Wu CY, Zhuang L, Zhou SG *et al. Corynebacterium humireducens* sp. nov., an alkaliphilic, humic acid-reducing bacterium isolated from a microbial fuel cell. *Int J Syst Evol Bacteriol* 2011;**61**:882–7.

Xu L, Liu W, Wu Y *et al*. Trehalose enhancing microbial electrolysis cell for hydrogen generation in low temperature (0°C). *Bioresource Technol* 2014;**166**:458–63.

Yong YC, Cai Z, Yu YY *et al*. Increase of riboflavin biosynthesis underlies enhancement of extracellular electron transfer of *Shewanella* in alkaline microbial fuel cells. *Bioresource Technol* 2013;**130**:763–8.

Yoon JH, Yeo SH, Kim IG *et al. Shewanella marisflavi* sp. nov. and *Shewanella aquimarina* sp. nov., slightly halophilic organisms isolated from sea water of the Yellow Sea in Korea. *Int J Syst Evol Mic* 2004;**54**:2347–52.

Yuan Y, Zhao B, Zhou SG *et al*. Electrocatalytic activity of anodic biofilm responses to pH changes in microbial fuel cells. *Bioresource Technol* 2011;**102**:6887–91.

Zhang L, Li C, Ding L *et al*. Influences of initial pH on performance and anodic microbes of fed-batch microbial fuel cells. *J Chem Technol Biotechnol* 2011a;**86**:1226–32.

Zhang L, Shen J, Wang L *et al*. Stable operation of microbial fuel cells at low temperatures (5–10°C) with light exposure and its anodic microbial analysis. *Bioproc Biosyst Eng* 2014;**37**:819–27.

Zhang TT, Zhang LX, Su WT *et al*. The direct electrocatalysis of phenazine-1-carboxylic acid excreted by *Pseudomonas alcaliphila* under alkaline condition in microbial fuel cells. *Bioresource Technol* 2011b;**102**:7099–102.

Zheng Y, Xiao Y, Yang Z-H *et al*. The bacterial communities of bioelectrochemical systems associated with the sulfate removal under different pHs. *Process Biochem* 2014;**49**:1345–51.

Zhuang L, Zhou SG, Li YT *et al*. Enhanced performance of air-cathode two-chamber microbial fuel cells with high-pH anode and low-pH cathode. *Bioresource Technol* 2010;**101**:3514–9.