Minireview

100 years of microbial electricity production: three concepts for the future

Jan B. A. Arends and Willy Verstraete*
Ghent University, Faculty of Bioscience Engineering, Coupure links 653, 9000 Gent, Belgium.

Summary

Bioelectrochemical systems (BES) have been explored according to three main concepts: to produce energy from organic substrates, to generate products and to provide specific environmental services. In this work, by using an engineering approach, biological conversion rates are calculated for BES resp. anaerobic digestion. These rates are compared with currents produced by chemical batteries and chemical fuel cells in order to position BES in the ‘energy’-market. To evaluate the potential of generating various products, the biochemistry behind the biological conversion rates is examined in relation to terminal electron transfer molecules. By comparing kinetics rather than thermodynamics, more insight is gained in the biological bottlenecks that hamper a BES. The short-term future for BES research and its possible application is situated in smart niches in sustainable environmental development, i.e. in processes where no large currents or investment cost intensive reactors are needed to obtain the desired results. Some specific examples are identified.

The BES concepts

Microbes are able to anaerobically produce an electrical current in the anode of bioelectrochemical systems (BES). When the current is harvested and used, the system is also called a microbial fuel cell (MFC) (Logan et al., 2006). When the biologically produced current is used to drive a reaction in the cathode and some extra energy is supplied by means of a power source to enhance this reaction, the system is referred to as a microbial electrolysis cell (MEC) (Logan et al., 2006). This concept has also been labelled BEAMR (bioelectrochemically assisted microbiological reactor) (Ditzig et al., 2007). Recently, the term MXC was coined meaning that the research focussed on a topic that can be of interest to either a MFC or a MEC (Parameswaran et al., 2011). In this work the term BES will be used but the focus will be on microbial processes on the anode. Processes in enzymatic fuel cells are not addressed here. Microbes living in the anode compartment can generate a current by means of anaerobic respiration with a solid material, the electrode in the anode. The electrons are transferred through an external circuit to the cathode, where the reduction of a final electron acceptor takes place. The reduction of the final electron acceptor can either be a biologically or a chemically catalysed reaction. In the 1910s, this phenomenon of microbial induced electrode reduction was discovered by Potter with his research on Escherichia coli cultures (Potter, 1911). Cohen and later Davis and Yarbrough constructed the first true MFC (Cohen, 1931; Davis and Yarbrough, 1962). During the 1960s, the phenomena of microbial respiration with a solid electron acceptor resp. donor were further studied in the context of biological corrosion (Lewis, 1966). The use of electron transport mediators was studied as a means to enhance power output (Davis and Yarbrough, 1962; Allen and Bennett, 1993). Since then interest in electrogenic respiration in reactor systems slowed. During the middle of the 1990s BES have received again more attention. This new interest was due to the potential use of BES for clean, sustainable and renewable energy production combined with the potential of a new wastewater treatment system. Even more and more potential applications have been established up till now. Three different concepts can now be defined among which all these processes can be divided (Fig. 1). Some processes can be placed in one or more concepts but one has to keep in mind whether other competitive processes are able to outperform a BES or not as will be discussed later. Power output (P in W or J s⁻¹), following Joule’s law, is defined as the amount of electrons produced per unit time (I in A or C s⁻¹) times the energy level of these electrons (E in V). Up till now, researchers have defined several resistances, also called voltage drops, to high power output that impair large scale
application of this technology. These resistances are based on losses in energy (V) of the electrons liberated (Clauwaert et al., 2008; Pham et al., 2009a). The losses in energy are based on the thermodynamics of the reactions involved. For this study the focus will be on sustained current production and biological limits of electron transfer rates. This means that particularly the kinetics of microbial respiration with solid electron acceptors in the anode will be examined. Pham and colleagues (2006) have argued that not generic but rather niche applications are the way to go for the BES technology. In this work this reasoning will be extended by examining electron transfer rates, meaning microbial respiration rates, of the microorganisms involved. These rates will be benchmarked in relation to existing energy production processes or energy carriers such as anaerobic digestion (AD) in terms of biological electron transfer rates and current density per m³ biological reactor volume. Chemical fuel cells (CFC) and batteries will be a benchmark in terms of current density. Subsequently, metabolic conversion rates and limitations to current production will be explored in relation to rates obtained during electron transfer in anaerobic digestion. Finally, several niches for future research are presented.

**BES relative to chemical batteries and chemical fuel cells as a benchmark for energy density**

To have a general benchmark for where the field of BES research stands in terms of power production, a comparison can be made with conventional energy carriers such as batteries and chemical fuel cells. In terms of biological substrate conversion rates and current generating potential, the best option is to compare a BES with anaerobic digestion (section 3). In terms of current and power density batteries and other types of fuel cells are candidates for
comparison. Normal household batteries, due to their chemical nature, can attain a very high power and current density up to 90 kW m$^{-3}$ (Table 1 and references therein). This is mainly due to their close electrode spacing and highly conductive electrolytes. Chemical fuel cells have been developed to form a large group of varying reactor types. However in terms of configuration i.e. anode, separator and cathode, they are comparable to BES. CFC have a wide range of operating conditions in varying temperatures (−25°C till +800°C), pressures and also substrates (various gasses and small alcohols). Current and power densities of CFC are usually reported per m² of electrode surface whereas these values in BES are reported per membrane, true electrode or projected electrode area. Current and power densities for BES are also reported per m³ total or net anode, cathode or reactor volume. This makes comparison with BES a bit more challenging but on the whole, 10–100 kW m$^{-3}$ (Table 1) is a reasonable estimate for CFC. Notwithstanding the advances that have been made in these technologies, a major drawback of both technologies is that batteries and CFC are not a sustainable technology yet. Batteries and CFC generally use primary, non-renewable energy sources. Indeed hydrogen and electricity for (re)charging are not yet readily available, thus need to be created from other sources (2 unit operations before current is obtained; hydrogen production followed by current generation). Whereas BES generally use waste streams that are most often readily available (1 unit operation; wastewater is directly converted into current). The electrode materials for CFC usually require noble metals which can be scarce, moreover, these materials are prone to fouling thus the fuels need to be processed before use. This can also be said of BES, especially considering cathodic reactions, but here biological alternatives are available. Last but not least, hydrogen gas used in CFC is difficult to store and transport. Yet, overall it is clear that conventional batteries and chemical fuel cells outrank by far the biological systems in terms of energy output per unit volume.

### BES relative to anaerobic digestion as a benchmark in terms of conversion rates and efficiencies

The performance of BES in terms of economic viability has usually been compared with that of anaerobic digestion of (low strength) wastewaters. This is done because the same feeds (liquid biomass (waste) streams) are applicable for both systems and the same type of output (electrical energy) can be generated in both systems. The incoming biomass is first hydrolysed, fermented and finally transformed by microorganisms into a final product (Verstraete et al., 1996; Angenent et al., 2004; Appels et al., 2008). The final product from an AD process is biogas (CH$_4$ and CO$_2$). The biogas is subsequently converted in a
combined heat and power module (CHP). Biogas can also be converted into hydrogen gas by means of steam methane reforming. The heat obtained from the CHP is usually returned to the digester which is operated at mesophilic or thermophilic temperatures. The electrical power can be put to other use (Pham et al., 2006). In a BES, an electrical current is directly generated by the microbes at the anode electrode.

Two distinct differences between AD and BES can be observed. The first is that biomass processing by AD is perfectly capable of dealing with suspended and particulate organic materials. In BES however, particulate matter is difficult to process. Most systems nowadays are operated with three dimensional (3D) electrode materials, to support the current producing microorganisms, such as granules, felts and meshes of carbon, graphite and (or in combination with) noble metals (Logan et al., 2006; 2007; Dumas et al., 2008; Aelterman et al., 2008a; Sleutels et al., 2009a). The use of these materials combined with suspended particles can cause clogging of the system which poses a serious threat towards sustained operation. The second difference is that using a BES, one has the option to produce direct electricity or various energy carriers such as methane (Cheng et al., 2009; Clauwaert and Verstraete, 2009) and hydrogen (Rozendal et al., 2006) or short-chain fatty acids (Nevin et al., 2010) and alcohols (Steinbusch et al., 2010). Besides these energy carriers, BES can be used for production of various other compounds (Rozendal et al., 2009; Rabaey et al., 2010) and even desalinated water (Cao et al., 2009).

Power aspects

During anaerobic digestion it is generally accepted that 1 kg COD can be converted to 4.16 kWh or 12.6 \times 10^6 \text{ C} at a rate of 1 kg of COD per m$^3_{\text{reactor}}$ per hour (Pham et al., 2006). On average a yield of about 1 kWh of usable energy can be obtained in the form of electricity. The other 3 kWh are used for operating the mesophilic or thermophilic digester in an economic fashion or are lost during the conversion of the biogas to electricity (Pham et al., 2006). For BES to become competitive with anaerobic digestion as a means of waste water treatment, the rates of conversion of substrates consequently needs to be up to 1 kg of COD per m$^3_{\text{anode}}$ per hour. For a BES to be competitive with anaerobic digestion as a means of bio energy production a power density around 1 kW per m$^3_{\text{anode}}$ volume needs to be realized. For this work, the focus is on the anode, although we realize that the reaction in the cathode is most of the time the limiting factor to increase current production. Although it is a simplification to compare various, as yet still at the 1–10 l scale level, BES with m$^3$ scale anaerobic digesters, the comparison helps to assess the R&D priorities and the practical potentials of the respective systems. Therefore the main unit of comparison will by output per m$^3$ of reactor volume.

Energetic losses

Various internal resistances have been described that decrease the effective working voltage of BES. These resistances or voltage drops, limit the energy that can be gained from a reaction and consequently decrease the thermodynamic efficiency of the system. The overall theoretical voltage is limited by the bacterial and/or chemical reactions that are taking place at both electrodes. The theoretical potential of the reactions at the individual electrodes and the overall cell voltage can be calculated by applying the Nernst equation (Logan et al., 2006). Two methods for determining the total internal resistance of a BES can be described. (i) The current interrupt method is a rough method which can give a quick impression of the total internal resistance (Logan et al., 2006). (ii) Impedance spectroscopy gives the researcher the tools for a more sophisticated determination and interpretation of the internal resistances present in a fuel cell (He et al., 2006; Borole et al., 2010). The overall internal resistance of a BES can be broken down into partial resistances. These resistances will be shortly discussed here in terms of the resulting voltage drop.

The overpotential ($\eta$) is related to the electrochemistry at the electrodes. The overpotential at the electrode can be described with the Tafel equation when concentration polarization is no issue (Fregua et al., 2007; Clauwaert et al., 2008). Included in the measured activation overpotential is also the energy needed for bacterial maintenance in case of a bacterial catalyst (Sleutels et al., 2009b). Overpotentials arise due to the surface electrochemistry of the electrodes and their coating. Depending on the measurement procedure, some researchers also include charge and mass transfer to and from the electrode (biofilm) in this parameter, although this does not truly reflect the overpotential.

The ohmic voltage drop is experienced when a current is produced and charge moves through a conductor. These resistances in the reactor system are due to (i) ionic resistance and (ii) charge transport resistance. In a two or more compartment BES, these individual resistances need to be determined for each compartment and each membrane.

Ionic resistance is the resistance against charge transfer in the electrolyte and relates to the conductivity of the liquid in the anode and the cathode compartment. Liquids treated in AD and BES usually have a conductivity in the order of 1–10 mS cm$^{-1}$, combined with a diffusion distance in the order of 0–5 cm from the electrode to the
membrane results in an order of magnitude for this type of voltage drop of about 0–20 mV (Sleutels et al., 2009b).

Charge transport resistance occurs in two or more chambered systems where the compartments are separated by cation exchange membranes (CEM), proton exchange membranes (PEM), anion exchange membranes (AEM), several types of cloths, polymer filters (Biffinger et al., 2007; Zhang et al., 2009) or bipolar membranes (Harnisch et al., 2008). The drawback of these separators is that a resistance to charge transport is introduced within the BES and that a pH gradient will develop more easily (see below). The voltage drop across the membrane/separator cannot be calculated from the intrinsic properties of the material but needs to be determined experimentally (Ter Heijne et al., 2006; Harnisch et al., 2008; Sleutels et al., 2009a). A membrane can have a good transport number for protons or hydroxyl ions but the concentration of protons is at most 10^{-6} mol l^{-1} and of hydroxyl ions at most 10^{-4} mol l^{-1}, whereas other ions such as K^{+}, Na^{+}, Mn^{2+}, NH_{4}^{+}, Cl^{-}, PO_{4}^{3-}, NO_{3}^{-}, SO_{4}^{2-}, etc. are present at concentrations of 10^{-4} mol l^{-1} or higher in the solution and are thus more likely to facilitate charge transport across the membrane. The consequence is that charge balancing is warranted but other issues such as an increase in the pH difference, decreasing the equilibrium voltage of the BES (see below) can arise. The transport of these other ions might even be beneficial for product formation (Cao et al., 2009; Rabaey et al., 2010). The extent of this loss depends on the specific situation under study.

A pH gradient develops between the both electrode compartments due to the reactions taking place at the electrodes combined with the slow exchange of protons and hydroxyl ions between the anode and the cathode surfaces. This gives rise to a voltage loss which is a thermodynamic parameter that alters the equilibrium potentials at the electrodes. This voltage loss can be calculated by the Nernst equation and the equation for the cell potential and is approximately 59 mV per pH unit depending on the operating temperature. A pH difference of 2 units is easily occurred in between the two electrodes of a BES, which leads to a pH voltage drop of 118 mV. The causes of this voltage loss can be related to flow regimes in the specific compartments, buffer capacity, boundary layer exchange and transport across the membrane. Good results in lowering this voltage drop by applying extra compartments or using advanced liquid recirculation schemes have been obtained (Clauwaert et al., 2009; Sleutels et al., 2010). However, care should be taken that only a low amount of COD is left in the anode effluent before transferring it to the cathode (Zhang et al., 2010a).

Transport loss is another major resistance. Transfer processes from bulk to boundary layer (electroactive biofilm) make up the largest component of the overall internal resistance at higher currents. This means that the reactants cannot move to or from the electrodes or microorganisms as fast as the reaction is occurring. Thus an accumulation of products and a depletion of substrates can be witnessed at the reaction interface. The extent of this loss needs to be determined for each operating condition and design separately. With a polarization curve the current at which this loss becomes dominant can be determined (Logan et al., 2006).

Whereas the above-described resistances are due to the thermodynamics and kinetics of the whole system, here the microbial level will be considered in more detail. Kinetics in BES research are interpreted in terms of substrate conversion rates, which can also be considered currents. Below, biological conversion rates in AD and BES will be examined on a m^3 reactor basis to make a comparison on the microbiological level.

Substrate conversion rates

Although methanogenesis does not involve membrane-bound electron transfer for all its electrons, all the electrons do have to pass through the membrane. This is either in the form of COD (chemical oxygen demand) as substrate and methane and carbon dioxide as products. Therefore, a true electron flux is present during methanogenic activity.

A default figure considered by engineers under stable operating conditions for substrate conversion rates is that 1 kg VSS_{biomass} can convert 2 kg COD per day. Anaerobic digesters can be operated at organic loading rates of 5–25 kg COD per m^3 per day (Pham et al., 2006). These rates are obtained by suspended microorganisms, either fully in suspension or in granules. The electron donors and acceptors are also in the liquid phase, either in solution or in a particulate form. From these values a ‘current’ per square metre of biological surface area can be calculated. Considering that 1 kg VSS_{biomass} contains approximately 10^{15} cells and an average cell diameter of 2 μm, it can be calculated that 1 kg VSS_{biomass} contains 12.6 × 10^{7} m^2 biological outer membrane. Through this membrane 2 kg of COD are passing during 1 day. This results in a current density of 22.3 mA m^{-2} biological membrane, i.e. the total flux of electrons per m^2 biological membrane during removal of COD (Fig. 2).

In the anode of a BES, electron transfer has to occur across the microbial cell membrane to the solid electron acceptor. Considering the same metabolic conversion rate as in AD, i.e. 280 A kg VSS_{biomass}^{-1} (2 kg COD per kg VSS_{biomass} per day) in a BES and a biologically relevant electrode area of 100 m^2 m^{-3} with a biofilm thickness of 10 μm and density of 20 kg VSS_{biomass} m^{-3}, a current of 0.056 A m^{-2} electrode surface or 5.6 A m^{-3} anode com-
part can be reached (Fig. 2) Cusick and co-workers actually reached a comparable output of 7.4 A m\(^{-3}\) in the first pilot scale MEC (Cusick et al., 2011). This calculated current density of 5.6 A m\(^{-3}\) is 625 times smaller than the current density obtained in AD (i.e. 3500 A m\(^{-3}\); Tables 2 and 3). Several improvements to the above calculation can be made. First, several researchers have observed anode biofilm thicknesses of 50–80 \(\mu\)m in experiments with low electrode surface to reactor volume ratios (Lee et al., 2009; McLean et al., 2010; Nevin et al., 2010). It is not clear how such a thick biofilm can be supplied with sufficient substrates and drained in terms of metabolites. Especially proton transport from the biofilm to the bulk liquid is a limiting factor (Torres et al., 2008). Second, higher metabolic conversion rates than assumed here have been reported, up till 22.3 kg COD per kg VSS\(_{\text{biomass}}\) per day (Lee et al., 2009; McLean et al., 2010; Nevin et al., 2010). It stems to reason that an attached electrogenic microorganism can amp up its specific metabolic rate once it is effectively connected to an electrode. Thirdly, higher electrode surface areas per anode volume have been reported, such as carbon or graphite felt with an actual surface area of 4.5 \(\times\) 10\(^4\) to 5.5 \(\times\) 10\(^4\) m\(^2\) m\(^{-3}\) (Alfa-Aesar, London, UK and National Electrical Carbon Products BV., Hoorn, The Netherlands). For graphite granules surface areas of 10\(^4\) m\(^2\) m\(^{-3}\) (Freguia et al., 2008) up till 10\(^6\) m\(^2\) m\(^{-3}\) (Mersen, Wemmel, Belgium) can be found. These surface areas are determined through nitrogen absorption thus it remains to be seen which fraction of this surface is biologically relevant. For the following a surface area of 1000 m\(^2\) m\(^{-3}\) will be considered. As a fourth consideration, biofilm densities on an anode can vary, even up to 50 kg VSS\(_{\text{biomass}}\) m\(^{-3}\) (Lee et al., 2009). Taking all these considerations into account it can be seen that all these factors, when multiplied, are needed to reach a performance that comes close to AD when the aim is conversion of organic matter per m\(^3\) of reactor volume (Table 3). Comparing these data with Table 2 it can be seen that state of the art results are now up to 595 A m\(^{-3}\) under sustained operation. This is only 17% of the maximum AD metabolic rate obtained and was achieved in a L-scale BES reactor.

**Dedicated electrogenic metabolism**

In the anode compartment of a BES, the microorganisms responsible for the final electron transfer step need to touch the electrode to transfer their electrons. This contacting can be done in two ways, direct and indirect (Stams et al., 2006; Schröder, 2007; Torres et al., 2010). Direct electron transfer (DET) indicates that the microorganisms use their various terminal cytochromes or reductases of their electron transport chain to transfer electrons to the electrode by means of physical contact. For electron transfer to occur through physical contact, a maximal distance of approximately 15 Å is allowed. This is the distance that electrons are able to move between haem groups (Leys and Scrutton, 2004; Paquete and Louro, 2010). Direct electron transfer also includes electron transfer by means of the type 4 pili of various electrogenic species as these pili are cell appendages attached to the main body (Reguera et al., 2005; Shi et al., 2009; Leang et al., 2010; McLean et al., 2010). The exact mechanism(s) of pili mediated electron transfer still needs to be elucidated. Most work has been done on the pili of *Geobacter sulfurreducens*. For the latter it was shown that OmcS (an outermembrane C-type cytochrome) lined the pili but could not be responsible for electron conductance along the pili as the distance...
Table 2. State-of-the-art BES results.

| Current output | Maximum during polarization measurements | Reactor description |
|----------------|------------------------------------------|---------------------|
|                | Maximum during sustainable operation     |                     |
|                | Electrode surface/anode volume (m$^2$.m$^{-3}$) | Anode volume (l)    |
|                | Anode area/anode volume (m$^2$.m$^{-3}$)   | Anode material      |
|                | Cathode reaction                          | Reference           |
|                | Projected (mA.m$^{-3}$)                  | Real (mA.m$^{-3}$)  |
|                | Projected (mA.m$^{-3}$)                  | Real (mA.m$^{-3}$)  |
| 333            | 2 000                                    | 6.55 $^b$           | 51 000                          | 0.0012                          | 167 Uncoated low density graphite | Pt-catalysed O$_2$ reduction | Biffinger et al. (2007) |
| 447            | 3 800                                    | 3.96                | 541                             | 4 600                            | 4.8                            | 112 813                         | 0.007                          | 118 Graphite cloth          | FeCN or O$_2$ (non-limiting) | Nevin et al. (2008) |
|                |                                          |                     | 0.00535                         | 420                              | 0.00012                         | 46 400                          | 0.016                          | 78.5 Carbon felt            | Pt-catalysed O$_2$ reduction | Borole et al. (2010) |
| 446            | 8 920                                    | 0.89                | 422                             | 8 440                            | 0.9                             | 497 250                         | 0.128                          | 50 Granular graphite         | CH$_4$ production (MEC)    | Claauwaert and Verstraete (2009) |
| 1178           | 17 700                                   |                      | 253                             | 3 800                            |                                  | 1178                            | 167 FeCN (non-limiting)        |                                  | Aelterman et al. (2008a) |
| 1464           | 16 400                                   |                      | 81                              | 20 630                           | 226                             | 357                             | 0.292                          | 4 Granular graphite          | H$_2$ production (MEC)      | Sleutels et al. (2009a) |
|                |                                          |                      | 63                              | 1.7                              |                                  | 3 700                           | 0.350                          | 50 Granular graphite          | H$_2$O$_2$ production (MEC) | Rozendal et al. (2009) |
| 26.32          | 4 670                                    | 520                  | 6.73                            | 1 200                            | 130                             | 51                              | 0.52                           | 5.63 Reticulated vitreous carbon | FeCN (not limiting)        | He et al. (2006) |
| 49             | 4 430                                    |                      | 41                              | 3 710                            | 6                               | 6 800                           | 0.94                           | 66.7 Granular graphite        | FeCN (not limiting)         | Aelterman et al. (2006) |
| 1015           | 30 450                                   |                      | 595                             | 17 850                           |                                  | 1015                            | 1.02                           | 167 Graphite felt            | NaOH production (MEC)       | Baeyens et al. (2010) |
| 20             | 450                                      |                      | 16.7                            | 380                              |                                  | 20                              | 4.9                            | 44$^d$ Granular graphite      | Biological O$_2$ reduction, pH 2 | Zhang et al. (2010a) |
| 0.14           | 2 800                                    | 930                  | 26.85                           | 1 150                            | 0.06                             | 442 000                         | 6.48                           | 87.5 Granular graphite        | Biological O$_2$ reduction, pH 4 | Claauwaert et al. (2009) |

Data are recalculated to anode dimensions from data available in the respective papers and their references. For all systems, projected means perpendicular view to the membrane, for stacked systems only 1 membrane area is used for the calculation. For stacked systems, total anode volume and total electrode area are considered. For all systems, recirculation volume is not taken into account.

$^a$  True electrode surface.
$^b$  No number means not mentioned in the paper or not possible to recalculate.
$^c$  Average of four different materials: graphite felt, carbon felt, graphite wool and graphite granules.
$^d$  Tubular design, all others are a flat plate design.
Table 3. Current densities in the anode of a BES compared with high rate AD.

| Output                | Compared with AD (%) (based on current density) | Reference                        |
|-----------------------|--------------------------------------------------|-----------------------------------|
|                       | g COD g VSS\textsubscript{dom}^{-1} day\textsuperscript{-1} | A g VSS\textsubscript{dom}^{-1}   |
| Electrode surface/anode volume (m\textsuperscript{2} m\textsuperscript{-3}) | Biofilm thickness (μm) | Biofilm density (kg VSS m\textsuperscript{-3}) | A m\textsuperscript{-a} | A m\textsuperscript{-b} | Wm\textsuperscript{-c} |
| AD performance        | 2                                                                 | 0.28                             | –                      | –                      | 12.5                    | –                      | 3500                  | 400 | 100 | This text; Pham \textit{et al.} (2006) |
| AD values             | 1                                                                 | 2                                | 0.28                   | 100                    | 20                      | 0.06                   | 5.58                  | 2.79 | 0.16 | This text                                    |
| + increased biofilm thickness | 2                                                                 | 0.28                             | 100                    | 66                     | 20                      | 0.37                   | 36.85                 | 18.43 | 1.05 | Lee \textit{et al.} (2009) McLean \textit{et al.} (2010) |
| a\textsuperscript{3}  | + increased metabolic rate                        | 6.9                              | 0.96                   | 100                    | 10                      | 20                     | 0.19                   | 19.26        | 9.63 | 0.55 | Aelterman \textit{et al.} (2008b)          |
| b                     |                                                                 | 22.3                             | 3.11                   | 100                    | 10                      | 20                     | 0.62                   | 62.26        | 31.13 | 1.78 | Lee \textit{et al.} (2009)                  |
| + increased electrode surface | 4                                                                 | 2                                | 0.28                   | 1000                   | 10                      | 20                     | 0.06                   | 55.84        | 27.92 | 1.60 | This text                                    |
| + increased biofilm density | 5                                                                 | 2                                | 0.28                   | 100                    | 10                      | 50                     | 0.14                   | 13.96        | 6.98  | 0.40 | Lee \textit{et al.} (2009)                  |
| +2 & 3\textsuperscript{3} | 22.3                                           | 3.11                             | 100                    | 66                     | 20                      | 4.11                   | 410                   | 205        | 12   |                                              |
| +2 & 4                | 2                                                                 | 0.28                             | 100                    | 10                     | 20                      | 0.06                   | 55                     | 27         | 2    |                                              |
| +2 & 5                | 2                                                                 | 0.28                             | 100                    | 66                     | 50                      | 0.92                   | 92                     | 46         | 3    |                                              |
| +3 & 4                | 22.3                                           | 3.11                             | 1000                   | 10                     | 20                      | 0.62                   | 622                    | 311        | 18   |                                              |
| +3 & 5                | 22.3                                           | 3.11                             | 100                    | 10                     | 50                      | 1.56                   | 155                    | 77         | 5    |                                              |
| +4 & 5                | 2                                                                 | 0.28                             | 1000                   | 10                     | 50                      | 139                    | 139                    | 69         | 4    |                                              |
| +2 & 3 & 4            | 22.3                                           | 3.11                             | 1000                   | 66                     | 20                      | 4                      | 410                    | 205        | 117  |                                              |
| +2 & 3 & 5            | 22.3                                           | 3.11                             | 100                    | 66                     | 50                      | 10                     | 1027                   | 513        | 29   |                                              |
| +2 & 4 & 5            | 2                                                                 | 0.28                             | 1000                   | 66                     | 50                      | 1                      | 921                    | 460        | 26   |                                              |
| +3 & 4 & 5            | 22.3                                           | 3.11                             | 1000                   | 10                     | 50                      | 1.6                    | 1556                   | 778        | 45   |                                              |
| +2 & 3 & 4 & 5        | 22.3                                           | 3.11                             | 1000                   | 66                     | 50                      | 10                     | 10272                  | 5136       | 294  |                                              |
| +2 & 3 & 4 & 5        | 22.3                                           | 3.11                             | 1000                   | 66                     | 50                      | 10                     | 10272                  | 5136       | 294  |                                              |

Improvements in current density from literature and combinations thereof are also shown. One has to keep in mind that most improvements were attained in ml or l scale anodes.

\textbf{a.} Current per electrode surface.

\textbf{b.} Per anode volume.

\textbf{c.} Based on a cell voltage of 0.5 V.

\textbf{d.} \textit{a} = highest reported mixed culture rate, \textit{b} = highest reported pure culture rate.

\textbf{e.} For these calculations the value of \textit{3b} is used.

\textemdash = not applicable to AD.
between two individual cytochromes was too large for inter cytochrome electron transfer (Leang et al., 2010).

The second mechanism of electron transfer is indirect electron transfer (iDET) meaning that electrons are transferred to an introduced (Park and Zeikus, 2000) or indigenously produced electron shuttle (Mehta et al., 2006; Wang et al., 2007; Marsili et al., 2008a). The reduced shuttle is subsequently transported (by any means) to the anodic electrode where it can deposit the acquired electrons and become re-oxidized again. Mediated electron transfer can also entail syntrophic interactions where reducing equivalents are transferred from cell to cell and where the receiving cell performs the final electron transfer step at the electrode (Fregua et al., 2008). Excretion of cytochromes that form a wire in the EPS-matrix (EPS = extracellular polymeric substances) of the biofilm has also been reported (Lower et al., 2009), this is also considered iDET.

Most work on elucidating the microbial metabolism under electrode or solid material respiring conditions has been done on *Shewanella* and *Geobacter* species. These organisms are also called dissimilatory metal respiring (DMR) organisms. Both species have distinct pathways of routing electrons to their final electron acceptor (Shi et al., 2009; Bird et al., 2011). Taking a close look at the enzymatic machinery involved and the electron routing in *G. sulfurreducens*, it can be seen that electrons are shuttled from the electron donating substrate towards the quinone pool in the cell membrane (Richardson, 2000; Shi et al., 2009; Bird et al., 2011). In this process, one proton is transported into the periplasmic space of the cell to generate a proton motive force (pmf). This pmf is subsequently used for energy generation by means of a membrane-bound ATPase, i.e. oxidative phosphorylation. From the quinone pool the electrons are transferred through a cascade of cytochromes in the periplasm and outermembrane towards the final electron acceptor (i.e. the electrode) (Richardson, 2000; Shi et al., 2009).

As the electrons are transported from the inner cell membrane at the level of the quinone pool, only 1 proton is moved out of the cell to generate the pmf. For ATP generation during metabolism with soluble electron acceptors, 1 electron can generate a pmf of 3 protons and subsequently 1 ATP can be formed. This means that 3 electrons are needed under current generating conditions to form 1 ATP. Given the same metabolic rates *G. sulfurreducens* can only trap 1/3 of the energy during electrode respiration compared with respiration with soluble electron acceptors (Mahadevan et al., 2006; 2011). From an energy harvesting perspective this is beneficial as less energy is spent on microbial processes and more energy is being transferred to the final electron acceptor, the electrode in BES.

For *Shewanella oneidensis* MR-1 (which does not use oxidative phosphorylation but instead gains its energy from substrate level phosphorylation; Hunt et al., 2010) extensive studies on cell to electrode electron transfer mechanisms have revealed that MtrC and OmcA (outermembrane decaheam C-type cytochromes) are responsible for final electron transfer to an oxidized shuttle molecule or a solid metal oxide (Shi et al., 2009; Bird et al., 2011). Although it is recognized that *Shewanella* spp. mostly make use of iDET pathways and do not occur abundantly in anode biofilms inoculated with mixed environmental samples, here an example calculation is presented which can possibly be translated to other electrogenic microorganisms when data become available. These data were gathered from experiments on anode biofilms of *S. oneidensis* MR-1. Lower and co-workers have shown that MtrC and OmcA can occupy 8–34% of the cell surface (4–7 \times 10^{15} \text{cytochromes m}^{-2} \text{cell surface}; cytochrome diameter 5–8 nm) (Lower et al., 2009). Thus *S. oneidensis* can have a cytochrome loading on their cell walls of 0.08–0.34 m^{2} m^{-2} (specific surface area) or 1.4 \times 10^{4} to 2.5 \times 10^{4} cytochromes per cell. Combining these numbers with electrode surface areas, biofilm densities (Table 3) and current densities (Table 2), a current per cell can be calculated in the range of 10^{-5} to 10^{2} \text{fA per cell}. This value can be compared with the current per cell of a microbial community during AD, which can be calculated as 2.8 \times 10^{2} \text{fA per cell} (data in Fig. 2).

Comparing the calculated current of *S. oneidensis* MR-1 with measured values of current per cell by Mclean and co-workers, the same values were obtained i.e. up to 200 fA per cell (McLean et al., 2010). Relating the current per cell to the cytochrome loading of a cell, a maximum electron transfer rate of 500 s^{-1} is obtained. This is in the same order of magnitude as the electron transfer rates reported for MtrC to electrodes i.e. 100–276 s^{-1} (Hartshorne et al., 2007) but larger than the reported numbers for electron transfer from MtrC and OmcA to haematite 0.26 s^{-1} and 0.11 s^{-1} respectively (Shi et al., 2009). From these results, it can be seen that cytochromes in a living biofilm on an anode can reach the same performance as measured during tests on isolated cytochromes and cell-free extracts. This indicates that a maximum performance of electron transfer in anodes with *S. oneidensis* is reached.

Major achievements have been made to elucidate the electron transfer pathway to electrodes and other solid materials for *Geobacter* spp., a more relevant organism in terms of DET to anodes. OmcZ, OmcS and, to a lesser extent, OmcB are implicated, based on deletion mutant experiments and SEM localization, to be the important C-type cytochromes for *Geobacter* spp. (Marsili et al., 2008b; Nevin et al., 2009; Richter et al.,...
2009; Inoue et al., 2010; Qian et al., 2011). PilA, the structural protein of the type 4 pili, is also an important factor. Till thus far it is not yet known whether PilA has the capacity to conduct electrons (Richter et al., 2009). It is hypothesized that the pili are a scaffold for OmcS and OmcZ (Leang et al., 2010; Inoue et al., 2011). To perform the same exercise as above for *Geobacter* spp. (i.e. calculating the electron transfer rate of individual cytochromes based on measured current densities of electrogenic biofilms and cytochrome distributions in the cell wall/biofilm and compare these to electron transfer rates of individual cytochromes) detailed kinetic data on the essential cytochromes are needed as well as cytochrome distributions on cell walls and electrodes. These data are only partially available yet but when they become available, they can provide information on possible bottlenecks in the electron transport chain of this important organism.

Researchers have already engineered a strain of *G. sulfurreducens* to increase its metabolic conversion rates without extra growth by adding an ATP drain to the cell. This resulted in higher metabolic rates and lower rates without extra growth by adding an ATP drain to the cell. This resulted in increased respiration rates, electron routing and transfer to the electrode are essential steps to look into and thus offer interesting targets for metabolic engineering. A possible route for metabolic engineering can thus be an increased number of cytochromes or other electron transfer molecules in the bacterial cell wall and periplasmic space. Another option is to pinpoint the bottlenecks for fast electron transfer within and between cytochromes and possibly increase these rates. Clearly, such metabolically engineered species will have to be, in terms of reactor configuration, operated under very well defined (most probably axenic) conditions.

**Engineering the system**

The key factor in terms of BES is to direct microbial metabolism in such a way that it generates an electrical current (rather than power), which permits highly valued biochemical conversions. Indeed, in case the electrons are withdrawn resp. deposited in a way that selective biochemistry is involved, there is a better chance of attaining sufficient added value per unit reactor invested (Fig. 1).

Several practical applications for BES have already been described that do not require high rate processes. An example is the sensor-powering sediment system as described by various researchers (Tender et al., 2002; Donovan et al., 2008). These systems rely on the slow flux of organic matter into the sediment and by definition cannot be high rate. Slow rate systems also include the plant or rhizodeposition powered plant-MFCs (De Schamphelaere et al., 2008; Kaku et al., 2008). These systems have a low current density of ~ 50 mA m⁻² (De Schamphelaere et al., 2008) but, due to their scale, can have a reasonable current for low power applications, which nevertheless can constitute a unique application in case no other alternatives are possible.

The decontamination of polluted soils and sediments also offers a niche perspective for the use of BES. In common practice, the biological clean-up of these soils is allowed to take decades. The main issue with these processes is the slow metabolic flux of the remediating organisms. This is usually due to a lack of suitable electron donor or acceptor to complete the decontaminating reaction (Guimarães et al., 2010). Electrodes, possibly inoculated with capable micro-organisms, can provide an extra electron donor or acceptor and thus expanding the metabolic opportunities for the (indigenous) microbial population. Several researchers have already shown that depolution reactions are feasible at an electrode of a BES. Pham and co-workers have shown that the chlorinated pollutant 1,2-DCA can be removed at a rate of 2.9 g m⁻² electrode surface per day at a flow rate of 0.09 l d⁻¹ in a reactor type BES anode previously fed with acetate. This was accomplished without the formation of any toxic by-products (Pham et al., 2009b). It was also shown that chlorinated and iodated organic pollutants could be removed at the cathode of a MEC by using bio-palladium as a catalyst (Hennebel et al., 2010). During these transformations no harmful intermediates were detected. Aromatic hydrocarbons such as toluene and benzene can also be used as a substrate for anode metabolism in a BES (Zhang et al., 2010b). The latter authors showed a removal rate of 2.3 mg toluene per kg sediment per day and 1.7 mg benzene per kg sediment per day after five successive additions of the compound to sediments equipped with an anode. Nitrobenzene is another compound that was successfully used as a substrate in a BES (Li et al., 2010). These reactors can be implemented as a flow through mesh anode in an aquifer as proposed by Zhu and colleagues (2009). The practical feasibility of this design should be demonstrated. Overall, not only the active depolution of soils and sediments but also the monitoring of these processes constitutes a potential niche of application for BES systems (Williams et al., 2010).

Recently, some indications of the synergy of a BES with AD have been described. Instead of going into competition with AD as a COD removal or bioenergy production system, researchers have shown that a BES is able to stabilize AD performance and enhance biogas production.
For these configurations the main contribution of the BES seems to be that the potential is controlled or certain critical enzymatic reactions are favoured above others (Sasaki et al., 2010; Weld and Singh, 2011). This territory has just been touched upon but warrants vigorous exploration of its possibilities.

Similar as for the stabilization of biogas production, the control of redox process is a proper niche for the unique capabilities of a BES. An example of a possible application is mitigation of the release of the greenhouse gas CH₄. This greenhouse gas is produced under specific redox conditions. By adding electrodes (possibly inoculated with an active microbial community) the release of these gasses can possibly be prevented. This research track is opposite to enhancing anaerobic digestion but also finds its application in completely different settings such as settling lagoons, waste dumps (i.e. leachate), wetlands (constructed or natural) and possibly cultivated land such as rice paddies.

Another example is the mitigation of H₂S release from sewers as already proposed by Zhang and co-workers (Zhang et al., 2008). No full-scale developments for these processes have been reported yet. Finally, in terms of the bio-based economy, BES can contribute to a range of services such as a sustainable system for CO₂ sequestration (Clauwaert and Verstraete, 2009), organic (Nevin et al., 2010) and H₂ (Ditzig et al., 2007) production, which need to be further explored.

Conclusions

In this work BES research has been positioned in three different concepts, i.e. the energy, product and sustainability concept. Positioning was based on metabolic rates that are obtained during anaerobic processes and the influence of having a solid electron acceptor involved in the reaction. When undertaking a new research project, one should realize what the biological limits of the project are. This work has provided a set of reference values and a conceptual framework for future BES-research.

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