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Minireview

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

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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 Yarborough 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 Bennetto, 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

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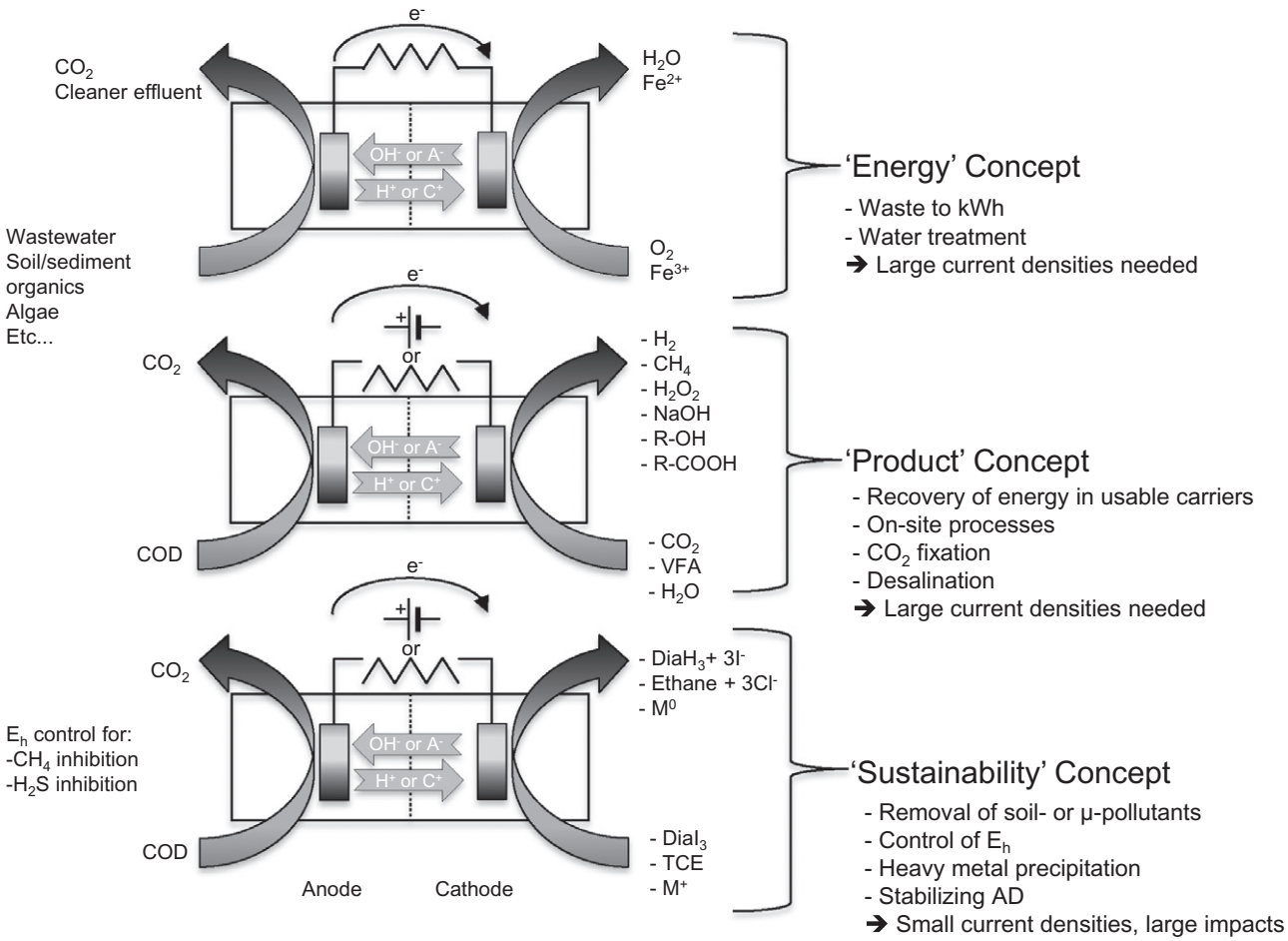

Fig. 1. Three concepts for positioning a BES. Some process can be placed in another concept than depicted here. For instance, metal precipitation can also be considered in the product concept. However, there it deals with large volumes and loading rates whereas in the sustainability concept heavy metals are removed in function of decontamination. The same accounts for remote sediment systems for sensor powering. These also fit in a sustainable concept as no expandable batteries and expensive exchange operations are needed. A^- = anion. C^+ = cation. M^+ = oxidized metal. M^0 = zero valent metal. $DiaI_3$ = diatrizoate, medical contrast medium. $DiaH_3$ = de-iodated medical contrast medium. TCE= tri-chloroethylene.

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 micro-organisms 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 m^3 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 m^2 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 m^3 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.

Table 1. Comparison of various (bio)-electricity producers with respect to energy density and conversion rates.

Size	Reaction	Electrolyte	Weight (g)	Volume (ml)	Operating time (h)	Operating potential (V)	Power density (kW m^{-3})	Power density (W Kg^{-1})	COD equivalent (kg COD m^{-3} day^{-1})	Reference
Conventional batteries	Zn/MnO_2	KOH	24	8	Limited	1.3	30	10	173[a]	Duracell[b]
	Li-ion		45	17	Limited	3.6	90	35	520[a]	Panasonic
Chemical fuel cells	H2 or reform gas/O2 or air	Polymer membrane	–	–	Continuous	–	140	120	810[c]	Sundmacher (2010)
Anaerobic digestion	COD to kW_{el} and kW_{heat}	–	–	500–1000[d]	Continuous	–	4	–	25	Pham *et al.* (2006)
BES anode	COD to kW_{el}	Waste water/ conductive membrane	–	1–500	Continuous	0.3–0.7	0.1	–	2.5	This text

Data are indicative and represent order of magnitude.
a. Based on the notion that 1 kg COD ~ 4.16 kWh.
b. Data from product specifications of the respective companies. Available online on the company website (http://www1.duracell.com/oem/Pdf/new/MX1500_US_UL.pdf). Accessed December 2010.
c. This is an indicative sample for the polymer exchange fuel cell. More information can be found in Conte and colleagues (2009) and Sundmacher (2010).
d. AD reactor volume in m^3.
–, not applicable.

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 12.6×10^6 C at a rate of 1 kg of COD per $m^3_{reactor}$ 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_{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_{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 (η) 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 (Freguia *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 12.6×10^3 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-

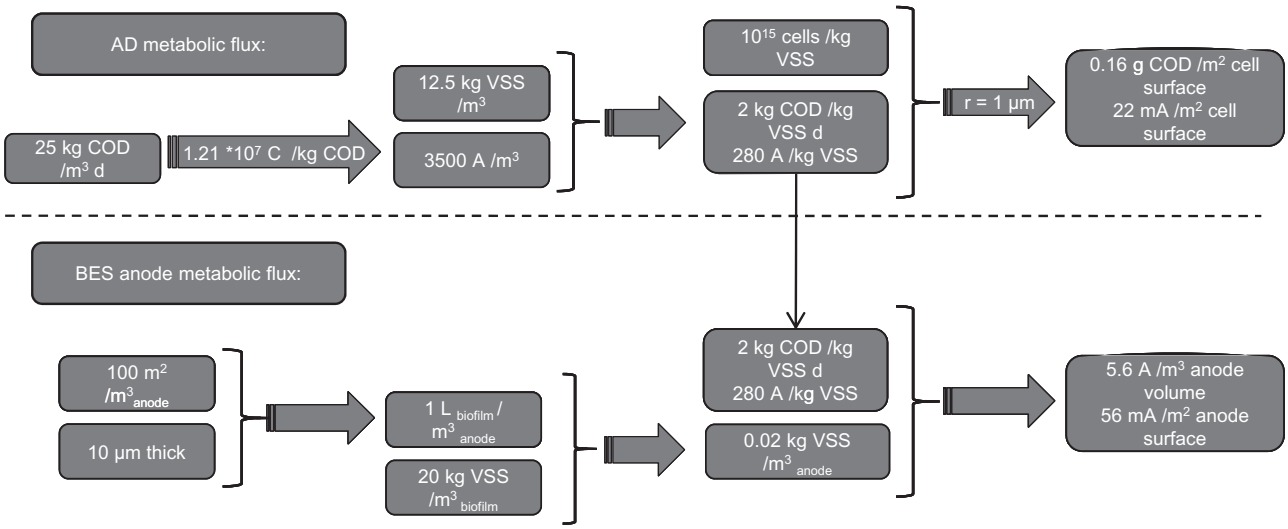

Fig. 2. Calculation scheme for metabolic rates in AD and BES based on AD default values. This scheme is used to calculate the values in Table 3. C = Coulomb. VSS = volatile suspended solids. COD = chemical oxygen demand. r = radius. d = day.

partment 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 µm 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 $VSS_{biomass}$ per day (Lee *et al*., 2009). 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 4.5×10^4 to 5.5×10^4 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 $VSS_{biomass}$ 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						Reactor description					
Maximum during polarization measurements			Maximum during sustainable operation								
($A\ m^{-3}$)	Projected ($mA\ m^{-2}$)	Real ($mA\ m^{-2}$)	($A\ m^{-3}$)	Projected ($mA\ m^{-2}$)	Real ($mA\ m^{-2}$)	Electrode[a] surface/anode volume ($m^2\ m^{-3}$)	Anode volume (l)	Membrane area/anode volume ($m^2\ m^{-3}$)	Anode material	Cathode reaction	Reference
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)	Clauwaert and Verstraete (2009)
1178	17 700		253	3 800			0.156	67	[c]	FeCN (non-limiting)	Aelterman *et al.* (2008a)
1464	16 400						0.280	90	1 mm thick graphite felt	H_2 production (MEC)	Sleutels *et al.* (2009a)
81	20 630	226				357	0.292	4	Granular graphite		Ditzig *et al.* (2007)
			292	5 840			0.340	50	Granular graphite	H_2O_2 production (MEC)	Rozendal *et al.* (2009)
63		1.7				3 700	0.350		Granular graphite	Non-catalysed O_2 reduction	Freguia *et al.* (2007)
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	7	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			1.02	167	Graphite felt	NaOH production (MEC)	Rabaey *et al.* (2010)
20	450		16.7	380			4.9	44[d]	Granular graphite	Biological O_2 reduction, pH 2	Zhang *et al.* (2010a)
			26.85	1 150	0.06	442 000	6.48	87.5	Granular graphite	Biological O_2 reduction	Clauwaert *et al.* (2009)
0.14	2 800	930				150	10	200	Mixed metal oxide coated titanium	Pure O_2 reduction, pH 4	Dekker *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.

			Specific substrate conversion rate					Output				
			g COD g $VSS_{biomass}^{-1}$ day^{-1}	A g $VSS_{biomass}^{-1}$	Electrode surface/anode volume (m^2 m^{-3})	Biofilm thickness (μm)	Biofilm density (kg VSS m^{-3})	A m^{-2} [a]	A m^{-3} [b]	Wm^{-3} [c]	Compared with AD (%) (based on current density)	Reference
		AD performance	2	0.28	–	–	12.5	–	3500	400	100	This text; Pham *et al.* (2006)
1		AD values	2	0.28	100	10	20	0.06	5.58	2.79	0.16	This text
2		+ increased biofilm thickness	2	0.28	100	66	20	0.37	36.85	18.43	1.05	Lee *et al.* (2009) McLean *et al.* (2010)
3	a[d]	+ increased metabolic rate	6.9	0.96	100	10	20	0.19	19.26	9.63	0.55	Aelterman *et al.* (2008b)
	b		22.3	3.11	100	10	20	0.62	62.26	31.13	1.78	Lee *et al.* (2009)
4		+ increased electrode surface	2	0.28	1000	10	20	0.06	55.84	27.92	1.60	This text
5		+ increased biofilm density	2	0.28	100	10	50	0.14	13.96	6.98	0.40	Lee *et al.* (2009)
		+2 & 3[e]	22.3	3.11	100	66	20	4.11	410	205	12	
		+2 & 4	2	0.28	1000	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	4109	2054	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	

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.

a. Current per electrode surface.

b. Per anode volume.

c. Based on a cell voltage of 0.5 V.

d. a = highest reported mixed culture rate, b = highest reported pure culture rate.

e. For these calculations the value of 3b is used.

–, 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 (Freguia *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 ($4–7 \times 10^{15}$ cytochromes m^{-2} 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 1.4×10^4 to 2.5×10^4 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 10^3 fA per cell. This value can be compared with the current per cell of a microbial community during AD, which can be calculated as 2.8×10^2 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 biomass yield but did not result in enhanced current generation on electrodes (Izallalen *et al*., 2008). This result combined with a calculation of cytochrome electron transfer rates, as exemplified for *S. oneidensis* above, indicates that despite increased respiration rates, also 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 Schamphelaire *et al*., 2008; Kaku *et al*., 2008). These systems have a low current density of ~ 50 mA m^{-2} (De Schamphelaire *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 CH_4. 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 H_2S 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 different services such as a sustainable system for CO_2 sequestration (Clauwaert and Verstraete, 2009), organic (Nevin *et al.*, 2010) and H_2 (Ditzig *et al.*, 2007) production, which need to be further explored.

Outlook

Current state of the art and the rate of development in power output and energy production warrants a thorough rethinking of the applied value and niches for BES systems in practice. Therefore, it is argued that the energy concept is beyond the reach of current possibilities. Notwithstanding the great advances that have been made in the past and will be made in the coming decades in BES and extracellular electron transport research, the current research should be guided along two distinct paths. On the one hand there is the need for more fundamental knowledge. This knowledge can be gained by an in-depth study of the mechanisms and catalysts involved and warrants the use of small (ml) scale and defined reactor setups. The findings of this research track cannot be one-to-one translated and extrapolated to an application. The second track has to further the knowledge on the application of a BES as a reactor system. The focus should not be primarily on energy nor on current density, as shown in this text. Indeed, the rates obtained by the micro-organisms are low in comparison with current (bio)energy production processes such as anaerobic digestion or other electrochemical conversion processes. Therefore, bulk products are not a viable option; the focus should be on special added value applications combining very novel microbial processes with clever application niches. The future field of application for BES at the current state of art is situated in the sustainability concept. Bioelectrochemical systems should be developed and integrated in sustainable green technology i.e., that not necessarily produces hard currency but renders less tangible benefits in terms of improved environmental quality.

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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