



ELSEVIER

Available online at [www.sciencedirect.com](http://www.sciencedirect.com)

# Metabolic and practical considerations on microbial electrosynthesis

Korneel Rabaey<sup>1</sup>, Peter Girguis<sup>2</sup> and Lars K Nielsen<sup>3</sup>

The production of biofuels and biochemicals is highly electron intensive. To divert fermentative and respiratory pathways to the product of interest, additional electrons (i.e. reducing power) are often needed. Meanwhile, the past decade has seen the breakthrough of sustainable electricity sources such as solar and wind. Microbial electrosynthesis (MES) is at the nexus of both, as it uses electrical energy as source of reducing power for microorganisms. This review addresses the key opportunities and challenges for MES. While exciting as a concept, MES needs to overcome many biological, electrochemical, logistical and economic challenges. Particularly the latter is critical, as on a 'per electron basis' MES does not yet appear to deliver a substantial benefit relative to existing approaches.

## Addresses

<sup>1</sup> Advanced Water Management Centre, The University of Queensland, Brisbane, Queensland 4072, Australia

<sup>2</sup> Biological Labs, Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA, USA

<sup>3</sup> Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Brisbane, Queensland 4072, Australia

Corresponding author: Rabaey, Korneel ([k.rabaey@uq.edu.au](mailto:k.rabaey@uq.edu.au))

**Current Opinion in Biotechnology** 2011, **22**:1–7

This review comes from a themed issue on  
Energy biotechnology  
Edited by Peter Duerre and Tom Richard

0958-1669/\$ – see front matter  
© 2011 Elsevier Ltd. All rights reserved.

DOI [10.1016/j.copbio.2011.01.010](https://doi.org/10.1016/j.copbio.2011.01.010)

## Introduction

The market for biochemicals is rapidly growing and diversifying. Increasingly, microbially derived products are replacing pharmaceuticals, polymers, as well as fuels and other commodities. Interestingly, most of these products are electron dense, which implies that microbially mediated production relies on the provision and consumption of reducing power. In this context, microbes are typically provided with an energy-rich feedstock, such as sugar, that is oxidized (or 'burned') to generate the necessary reducing equivalents for the biosynthesis of the target product. For high value, lower-yield products such as pharmaceuticals, the substrate consumption represents only a fraction of the margin, whereas for medium and low value products substrate consumption constitutes a major

fraction of the production cost. For example, butanol production from glucose requires the consumption of ~3 kg glucose per kg butanol produced [1]. This high glucose requirement poses an economic constraint as the production of fermentable substrate requires arable land, water, and nutrients. Those latter requirements in particular are presently fuelling the debate on the sustainability of biofuels and biochemicals [2].

Recent years have been a watershed for research in microbial bioelectrochemical processes; whole microorganisms are used to catalyze oxidation and/or reduction reactions at electrodes [3]. The best known examples of these are microbial fuel cells, which harness electrical current from microbes by providing an electrode as the oxidant to degrade the organic substrate [4,5]. Within this bioelectrochemical conversion context, microbial electrosynthesis (MES) has recently emerged as an alternative option to provide reducing/oxidizing power for biochemical production via electricity [6,7]. MES concerns the use of microbial cells as biocatalysts for synthesis reactions in electrochemical cells. Hence, electrical current can be supplied to or extracted from microorganisms, with the objective of stimulating and sustaining biochemical production. Examples of reductive processes are the production of acetate from carbon dioxide (CO<sub>2</sub>) [6], fumarate to succinate conversion [8] and increased glutamate yield from glucose fermentation [9,10]. An example of an oxidative process is the conversion of glycerol to ethanol [11].

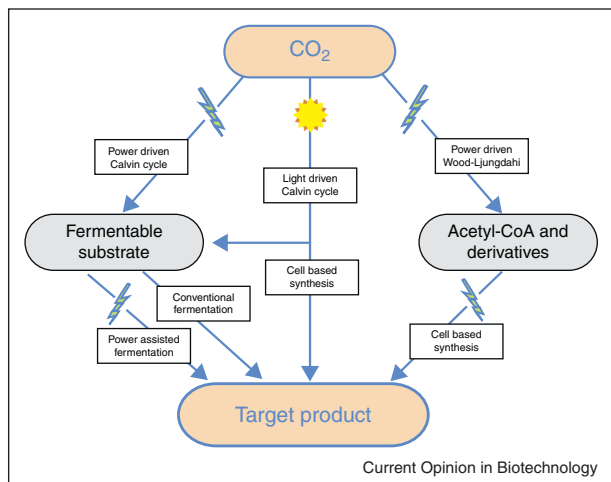
MES thus relies on electrical current as driver. Interestingly, the growing movement toward power production from solar, wind or wave energy (as opposed to petroleum, coal or gas) has led to renewed interest in energy storage or conversion technologies to solve the transport and storage issues associated with electrical energy. MES can contribute to addressing these issues by allowing on site conversion of electrical energy (current) to chemical energy (a fuel). However, MES is in its infancy, and as yet there has been no comparison between MES with existing approaches in terms of metabolic impact, energy/substrate requirement and anticipated cost/benefits. For the purpose of this study, we will focus on reductive conversions.

## Options and assumptions

Figure 1 depicts the different bioproduction approaches relevant to this context. Primary production uses sunlight and CO<sub>2</sub> for the synthesis of target products, or fermentable substrate used in industry. Electrical energy can

## 2 Energy biotechnology

Figure 1



Overview of the different routes toward bioproduction, indicating the required inputs of solar energy or electrical energy (as indirect solar derivative). There are several pathways involving MES, highlighting the potential to integrate existing approaches with this novel approach.

potentially be used to drive CO<sub>2</sub> fixation as a means to directly produce the target compound, or lead to the formation of acetyl-CoA and its derivatives for further synthesis. One could speculate that, instead of the Wood-Ljungdahl pathway (which would produce acetyl-CoA), the Calvin-Benson-Bassham cycle (which yields triose phosphates) can be driven on electrical current, leading to the formation of fermentable substrate from electricity and CO<sub>2</sub>. This fermentable substrate could then further be used for bioproduction purposes. Lastly, the fermentation itself can be complemented by electrical current [9], as a means to provide reducing equivalents to the cell. This can be considered as a hybrid metabolism when effective charge transfer occurs toward the cell. Notably, it remains to be seen whether this net charge is effectively exchanged with the microbial metabolism, or whether the more reduced/oxidized environment created by the electrode causes a change in the microbial pathways without effective electron transfer.

Comparing the different pathways for bioproduction requires many assumptions. We have summarized the major assumptions in Table 1, as a guideline to our qualitative assessments. The theoretically achievable bioproduction densities for MES (product-carbon per hectare per annum) appear excessive at first glance. However, it is crucial to point out that photovoltaic panels are relatively efficient in capturing solar energy [12], and that a first study producing acetate from CO<sub>2</sub> has indicated high electron yields [6]. Other factors such as CO<sub>2</sub> and nutrient supply are likely to become limiting before these theoretical values are achieved.

Assumptions regarding the theoretical production rates as well as expected substrate requirements for MES, conventional fermentations and algal bioproduction systems (c\$ refers to dollar cent)	Fermentations – aerobic		Fermentations – anaerobic		Current driven lithoautotrophy – aerobic		Current driven lithoautotrophy – anaerobic		Algal production	
	Glucose (c\$0.6)	Glucose (c\$0.15)	Glucose (c\$0.6)	Glucose (c\$0.15)	CO <sub>2</sub> (\$0)	Electricity (c\$0.16)	CO <sub>2</sub> (\$0)	Electricity (c\$0.16)	CO <sub>2</sub> (\$0)	Water (c\$ 0)
Carbon source (cost c\$/mol C) <sup>a</sup>										
Electron donor (cost c\$/mol C) <sup>a</sup>	0.57		0.14		0.13		0.015		0.04–0.10	
Growth yield (C-mol/C-mol) <sup>b</sup>	<3 tonnes C		<3 tonnes C		<1121 tonnes C		<1121 tonnes C		<25 tonnes C	
Maximal production density per hectare <sup>c</sup>	(as glucose)		(as glucose)		(as butanol)		(as butanol)		(as biodiesel)	

<sup>a</sup> Assumptions: cost of fermentable substrate c\$20 per kilogram and at 24 electrons per glucose molecule; Electrical power delivered at 1 V and 0.06 \$/kWh.  
<sup>b</sup> Data from Heijnen [28\*] and Lee *et al.* [29].  
<sup>c</sup> Assumes 100% conversion of raw materials to product. For glucose, annual average production per hectare from sugarcane is assumed. For lithoautotrophy, case calculations assume photovoltaic electricity productions at 500 W m<sup>-2</sup> averaged irradiation and 20% efficiency of the photovoltaic panel. The bioelectrochemical system is assumed to have a 1 V cell voltage. For algal production, assumed annual production 50 tonnes biomass dry weight per hectare [30], of which less than 50% biodiesel-C.

**From the electrode to the cell**

Before assessing how microorganisms deal with electrical current, it is important to discuss how electrons may be transported from the electrode to the cell. There are two approaches, that is direct and indirect. Direct electron transfer, in which electrons move between the cell and the electrode via direct contact, relies on the existence of a biofilm or at least a single cell layer on the electrode surface [13]. While biofilms have been observed on cathodes reducing nitrate [14], in the context of bioproduction only pre-grown (using e.g. acetate as electron donor) biofilms have been obtained thus far [6,15]. Key advantages of direct electron transfer are the possibility for direct catalysis and the retention of the biocatalyst inside the cathode compartment. Key disadvantages of a biofilm are internal and external diffusion limitations. Internal diffusion limits substrate (CO<sub>2</sub>, substrate organics) and product (OH<sup>-</sup>, biochemical) movement within the biofilm. Notably in the case of products such as butanol, which become inhibitory at higher concentrations, diffusion limitations may decrease the production rate. External diffusion relates to the need to bring substrates into and products out of the biofilm, which is typically a function of the biofilm surface area.

The second approach, indirect electron transfer, occurs among planktonic cells, which acquire reducing power via soluble or miscible shuttles [8], or capacitive particles [16]. Product inhibition due to diffusion limitation is less likely to occur. Indeed, known fermenters could be provided with reducing power via an electrochemical bypass unit. This would arguably be the least challenging scenario in terms of engineering. The disadvantage of such an approach may be the limited biomass retention in continuous production, and the possible diffusion limitations

associated with bringing reducing power via the shuttles/particles toward the microorganisms and into the cell. Where cell-associated products are formed (storage polymers, lipids) the use of planktonic cells poses an additional harvesting advantage. Clearly, the viability of any approach will strongly depend on the target product and operational mode, and will in turn profoundly impact on how the microorganisms are chosen or engineered.

**MES operational choices and challenges**

**Fully autotrophic bioproduction**

This option was recently demonstrated by Nevin *et al.* [6] and involves direct electricity-driven CO<sub>2</sub> capture via an autotrophic metabolism. Key challenges include the large electron requirement for CO<sub>2</sub> reduction, the supply of CO<sub>2</sub> (without the introduction of other potentially problematic gasses such as sulfide from exhaust flues) and the supply of cheap electrical current. Also, thus far the production rates and concentrations were very low compared to industrial practice [6]. We suggest that MES will need to deliver at comparable rates and concentrations to be competitive. The key advantages are an apparent complete independence of arable land and the considerable carbon sequestration resulting from a fully CO<sub>2</sub> based bioproduction (if some fraction of the biomass is eventually sequestered). A possible example of such a production process is the production of butanol with a homoacetogenic organism [17], using electrical current instead of hydrogen.

**Partial or fully heterotrophic bioproduction**

The most common route described thus far involves modifying fermentation outcomes [9,18] or providing reduction of organic substrates [8]. Key challenges include providing the substrate to the microorganisms

**Table 2**

**A comparison between CO<sub>2</sub> and substrate organics**

CO <sub>2</sub>	Substrate organics
+ Available in excess in the atmosphere, seawater and in solid minerals	- Availability depends on the location and may vary depending on the size of plant or supply
- Low atmospheric concentrations hamper CO <sub>2</sub> flux into solution per unit land surface	+ High solubility of most substrate organics facilitates dosing
+ CO <sub>2</sub> supply to reactor medium may provide buffering capacity	+/- Depending on the substrate, the resulting pH upon addition may be unfavorable for bioproduction (e.g. butyrate)
- High number of electrons required for product formation as CO <sub>2</sub> is fully oxidized	+ The substrate is already partially reduced (containing considerable electrons), hence limited electrons needed for bioproduction
- Autotrophic growth and fixation requires energy investment by cell to activate, for example Wood-Ljungdahl pathway	+ Heterotrophic growth can be achieved on substrate organics
+ Complete or nearly complete independence of arable land	+/- May require arable land in case high quality substrate is required
- Nutrient requirement for biocatalyst growth	- Nutrient requirement for biocatalyst growth
+ CO <sub>2</sub> removed from atmosphere provides positive impact on greenhouse gas budget (depending on electricity source and net sequestration)	+ Waste derived organics have a negative value hence processing delivers a net benefit
+ CO <sub>2</sub> uptake by the cell does not require energy investment	+/- Depending on the substrate, energy may be required for transport, phosphorylation or activation of the substrate

#### 4 Energy biotechnology

(which might require sterilization), ensuring sufficient specificity of the production pathway relative to existing conversions, and minimizing cell growth. For example, Steinbusch *et al.* [19] used a mixed population to achieve fatty acid reduction to the corresponding alcohols. Overall, the product yields were low due to the formation of side products. This approach still requires production of the substrate on arable or marginal land, or recovery of organics from, for example, wastewater. The key advantages are the lower electron requirement for bioproduction (relative to CO<sub>2</sub> reduction), facilitated the growth of the biocatalysts and in many cases use of existing infrastructure (with minor modification). Table 2 summarizes the aspects related to bioproduction starting from CO<sub>2</sub> or substrate organics.

#### Temporal heterotrophy and autotrophy

There are several aspects that make alternating heterotrophic/autotrophic growth/production phases attractive. It has thus far been challenging to culture microorganisms autotrophically using electrical current, and earlier studies have found significant improvement of performance in the presence of some organics [20,21]. On the other hand, continuous supply of organic (fermentable) substrate leads to higher growth yields, hence more loss of carbon (Table 1). Therefore, a strategy where rapid, heterotrophic growth is followed by an autotrophic production phase can be beneficial. Outside the pure culture context, microbial populations can be driven to biopolymer production by alternating feast/famine periods [22], and a similar model can be envisaged here.

#### Comparing apples and oranges: electricity versus organic substrate as electron donor

In view of the limited data available on MES, quantitative comparisons between electricity and organic substrate are not realistic today. Therefore, the discussion below and Table 3 provide an intellectual consideration toward the key benefits and challenges of MES.

#### Routing the electrons inside the cell

Providing reducing power via organic substrate or via electrical current appears vastly different both from a metabolic and an operational standpoint. Organic electron donors allow the simultaneous production of NADH and NADPH, as well as ATP via well-known biochemical pathways [23]. Electrical current supply may pose issues with respect to ATP and NADPH formation, and the issue depends on whether the metabolism is lithoautotrophic or heterotrophic. For a lithoautotrophic approach, similarities with hydrogen-driven bioproduction exist, however there are two important remarks:

- (i) Electricity represents only electrons, and hence these electrons need to be compensated by an influx of cations, preferably protons. Limitations in supply of

**Table 3**

**A comparison between glucose and electricity as source of reducing power**

	Production	Transport	Storage	CO <sub>2</sub> balance	Dosing	Cost	Energy level	Metabolic consequence
Glucose	Crop growth on arable land, competes with food	As a solid via conventional transport	As a solid, safe	Consumption releases CO <sub>2</sub> , however this is recent CO <sub>2</sub>	Dissolved into reactor medium	\$0.4 per mol electrons	-0.45 V vs SHE	Heterotrophic growth, production of ATP, NADH and NADPH
Electricity	Produced anywhere (sustainable and conventional)	Power lines	Difficult to store	If sustainably produced no CO <sub>2</sub> impact	Via electrode surface, direct or mediated	\$0.25 per mol electrons	As chosen by operator	Autotrophic growth, production of ATP and NADH



protons [24] can thus lead to localized high pH and loss of proton motive force.

- (ii) The potentials at which cathodes can operate are sometimes outside the realistic range for hydrogen production [21]. The latter means that the cathode potential is thus high, that electron transfer via hydrogen and hydrogenases appears unlikely.

Heterotrophic approaches assisted by electrical current imply that an existing fermentation is redirected by the supply of reducing power. In the past, redox mediators such as methyl viologen and neutral red have been used to redirect fermentations [18]. While this and other physiological studies highlighted that such mediators can reduce ferredoxin, it is as yet unknown whether the mediators work along this pathway. Moreover, we presently assume that via this route only NADH is generated, while for biosynthesis NADPH is required. The production of the latter requires an active transhydrogenase. As yet, the pathways for electron transfer are unknown, and further research will need to demonstrate whether NADPH can be produced using electrical current in a similar manner as, for example, homoacetogenic respiration.

#### Would electricity-driven bioproduction be effective?

Both from an economic and an environmental standpoint, efficient electron management is of primary concern. On an electron basis, electricity does not appear to deliver an economic benefit over organic substrate (Table 1). A theoretical consideration on electron balances, based on the assumption that electrical current is supplied as electrons, can be found in the [supplementary information](#). This assessment establishes that the cost in terms of electrons to make an additional unit of product is the same whether done as part of mixed metabolism or lithoautotrophic metabolism. Indeed, a glucose (or other hexose) fermentation captures 24 electrons in reduced products, and releases excess carbon as CO<sub>2</sub>. Going from CO<sub>2</sub> to the product will cost the same whether CO<sub>2</sub> is from excess glucose carbon or a separate CO<sub>2</sub> source. A mixed metabolism is basically a superimposition of heterotrophic and lithoautotrophic metabolism.

For longer chain products such as diesel precursors (fatty acids), it appears unlikely that their production will benefit from mixed metabolism, as little excess energy is available to recapture the CO<sub>2</sub>. We suggest that the most important advantage of bioproduction via a mixed metabolism (relative to a fully lithoautotrophic metabolism) is that it can be done in a way that is more energetically feasible. While the energetics of the chemiosmotic mechanism that make lithoautotrophic acetate production from CO<sub>2</sub> and H<sub>2</sub> feasible is not well understood, it appears likely that limited energy will be available to drive bioproduction. Acetate production is

achieved as the ATP used in capturing CO<sub>2</sub> is regenerated in the hydrolysis of the CoA ester. If the acetyl-CoA is used for further synthesis, more ATP needs to be generated elsewhere. This implies that an anaerobic, lithoautotrophic production will likely lead to a mixture of product outputs as well as, for example acetate production for ATP generation. Considering the high-energy requirement for bioproduction, this implies that substantial amounts of acetate may be produced for a modest amount of desired end product.

In principle, the energy issue could be addressed by moving to aerobic metabolism or using an alternative external electron acceptor. Such an approach may have several incompatibilities with the present, anaerobic approach. Firstly, to our knowledge it is incompatible with the Wood–Ljungdahl metabolism (in which key enzymes are strictly anaerobic) [25]. A solution to this would be to drive production via the Calvin–Benson cycle or a reductive tricarboxylic acid cycle-based CO<sub>2</sub> fixation, if these were feasible. A second incompatibility relates to redox mediators, which are generally easily oxidized by oxygen and thus lose their effectiveness.

There are, however, some distinct biological advantages of MES. Where full autotrophic growth is taken as a basis, the growth yields are typically low. In many cases, MES may rely on biofilms, where the catalyst retention time is thus high, potentially leading to a high production capacity per unit active biomass and modest carbon, nitrogen, and phosphorus requirements for growth. The disadvantage of this approach is the considerable electron requirement to reduce CO<sub>2</sub>. Mixed metabolisms appear more attractive from this perspective, as the electron requirement is strongly reduced for the MES component (this excludes the initial electron requirement for producing, e.g. fermentable substrate). Ultimately, the success of MES will depend on how effective NAD(P)H can be generated using electrons, and how amenable the microorganism is to performing a hybrid metabolism. Potentially, a higher product yield per unit supplied organic carbon can be achieved, thereby limiting the required organic substrate for NAD(P)H generation. Critical in this will be the electron requirement for ATP formation, leading to side processes and thus undesired product outcomes.

#### Independence of land use

Perhaps the major advantage of MES is associated with geographical location. MES allows on site use of (renewable) electricity for bioproduction, and as such is independent of the availability of arable land. Moreover, the theoretically achievable production densities (Table 1) are immense, further restricting the possible land impact of MES-based bioproduction. Also for electricity-driven fermentations advantages exist, as the use of electrical current as electron sources limits the requirement for

## 6 Energy biotechnology

fermentable substrate, and hence limits the required land for its production. From the electrical current perspective, on site synthesis limits the need for transport and storage of the electricity

### Non-microbial challenges

While achieving carbon efficient growth of the biocatalyst and high product specificity are primary challenges, MES research needs to overcome many hurdles in other disciplines as well. To date, research on electrode materials (surface structure and chemistry) is in its infancy. Not only is the interaction with the microorganism important, but also the electrode material must be scalable and cost effective. This relates further to reactor sizing and the need for current collection. Existing pilot work on microbial fuel cells is starting to deliver key information on the larger scale operation of bioelectrochemical systems [26]. Finally, the reactor engineering aspect will lead to the most crucial assessment, of whether MES-based bioproduction is economically viable and sustainable.

### Conclusions

An existing life cycle analysis has shown considerable benefits for the use of bioelectrochemical systems for product formation, starting from wastewater as anode driver [27]. While there will be fewer environmental benefits if the production process is not linked to wastewater treatment, aspects such as decreased land usage and CO<sub>2</sub> fixation also need to be taken into account. Even if carboxylic acids are the only derivatives of a MES process, there are considerable opportunities for further processing these precursors to more attractive end products. Recently, the approach to produce fatty acids as intermediates for further downstream processing was termed 'the carboxylate platform' by Agler *et al.* [31\*\*].

To be practical, however, MES will need to overcome tremendous microbial and technical hurdles. At present, economic cost appears by far the key constraint for MES. Not only is there a cost associated with sustainable electricity production (see earlier calculations), but the bioelectrochemical reactors themselves are also cost intensive due to the need for electrode materials, current collectors, membranes, etc. However, the overall perspective of electricity-driven bioproduction at any location, at high density today appears a sufficient driver to further explore the economic and environmental viabilities of MES.

### Acknowledgements

KR is supported by the Australian Research Council (DP0879245) and a UQ Foundation Research Excellence Award. PG is supported by the US National Science Foundation (grants MCB-0702504 and OCE-0426109).

### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.copbio.2011.01.010.

### References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Jones DT, Woods DR: **Acetone–butanol fermentation revisited.** *Microbiol Rev* 1986, **50**:484-524.
  2. Clarens AF, Resurreccion EP, White MA, Colosi LM: **Environmental life cycle comparison of algae to other bioenergy feedstocks.** *Environ Sci Technol* 2010, **44**:1813-1819.
  3. Rabaey K, Rodriguez J, Blackall LL, Keller J, Gross P, Batstone D, Verstraete W, Nealsen KH: **Microbial ecology meets electrochemistry: electricity-driven and driving communities.** *ISME J* 2007, **1**:9-18.
  4. Logan B, Aelterman P, Hamelers B, Rozendal R, Schroder U, Keller J, Freguia S, Verstraete W, Rabaey K: **Microbial fuel cells: methodology and technology.** *Environ Sci Technol* 2006, **40**:5181-5192.
- Seminal review article describing the principles and state-of-the-art in microbial fuel cells.
5. Rabaey K, Verstraete W: **Microbial fuel cells: novel biotechnology for energy generation.** *Trends Biotechnol* 2005, **23**:291-298.
  6. Nevin KP, Woodard TL, Franks AE, Summers ZM, Lovley DR:
    - **Microbial electrosynthesis: feeding microbes electricity to convert carbon dioxide and water to multicarbon extracellular organic compounds.** *mBio* 2010, **1**: e00103-00110; doi:00110.01128/mBio.00103-00110.

First demonstration of electricity-driven bioproduction starting from CO<sub>2</sub>; in this case acetate was the key end product.
  7. Rabaey K, Rozendal RA: **Microbial electrosynthesis – revisiting the electrical route for microbial production.** *Nat Rev Microbiol* 2010, **8**:706-716.
- Recent review article outlining the principles and applications of microbial electrosynthesis.
8. Park DH, Laivenieks M, Guettler MV, Jain MK, Zeikus JG: **Microbial utilization of electrically reduced neutral red as the sole electron donor for growth and metabolite production.** *Appl Environ Microbiol* 1999, **65**:2912-2917.
  9. Hongo M, Iwahara M: **Electrochemical studies on fermentation.**
    - **1. Application of electro-energizing method to L-glutamic acid fermentation.** *Agric Biol Chem* 1979, **43**:2075-2081.

First study to use electrical current to modify a fermentation, in this case of glucose to L-glutamic acid. A redox mediator was added to facilitate the electron transfer.
  10. Hongo M, Iwahara M: **Electrochemical studies on fermentation.**
    - **2. Determination of electro-energizing conditions for L-glutamic acid fermentation.** *Agric Biol Chem* 1979, **43**:2083-2086.
  11. Flynn JM, Ross DE, Hunt KA, Bond DR, Gralnick JA: **Enabling unbalanced fermentations by using engineered electrode-interfaced bacteria.** *mBio* 2010, **1**: e00190-00110–e00190-00117.
- Highly innovative paper in which *Shewanella* is engineered to stoichiometrically convert glycerol to ethanol, by diverting excess reducing equivalents to an anode.
12. Ginley D, Green MA, Collins R: **Solar energy conversion toward 1 Terawatt.** *MRS Bull* 2008, **33**:355-372.
  13. Bond DR, Lovley DR: **Electricity production by *Geobacter sulfurreducens* attached to electrodes.** *Appl Environ Microbiol* 2003, **69**:1548-1555.
  14. Virdis B, Read ST, Rabaey K, Rozendal RA, Yuan Z, Keller J: **Biofilm stratification during simultaneous nitrification and denitrification (SND) at a biocathode.** *Biores Technol* 2010, **102**:334-341.
  15. Gregory KB, Bond DR, Lovley DR: **Graphite electrodes as electron donors for anaerobic respiration.** *Environ Microbiol* 2004, **6**:596-604.
- First manuscript to establish electron transfer from a cathode to a microorganism without the addition of mediators or the production of hydrogen.

16. Mayer MJJ, Buisman CJN, Hamelers HVM, Strik DPBTB: **Device and method for performing a biologically catalyzed electrochemical reaction**. 2009 WO/2009/131452.
17. Köpke M, Held C, Huker S, Liesegang H, Wiezer A, Wollherr A, Ehrenreich A, Liebl W, Gottschalk G, Dürre P: ***Clostridium ljungdahlii* represents a microbial production platform based on syngas**. *Proc Natl Acad Sci U S A* 2010, **107**:13087-13092.
- A homoacetogen was engineered to produce butanol starting from syngas. This opens an array of possible bioproduction pathways based on hydrogen as electron donor.
18. Kim TS, Kim BH: **Electron flow shift in *Clostridium acetobutylicum* by electrochemically introduced reducing equivalent**. *Biotechnol Lett* 1988, **10**:123-128.
19. Steinbusch KJJ, Hamelers HVM, Schaap JD, Kampman C, Buisman CJN: **Bioelectrochemical ethanol production through mediated acetate reduction by mixed cultures**. *Environ Sci Technol* 2010, **44**:513-517.
20. Freguia S, Rabaey K, Yuan ZG, Keller J: **Sequential anode-cathode configuration improves cathodic oxygen reduction and effluent quality of microbial fuel cells**. *Water Res* 2008, **42**:1387-1396.
21. Rabaey K, Read ST, Clauwaert P, Freguia S, Bond PL, Blackall LL, Keller J: **Cathodic oxygen reduction catalyzed by bacteria in microbial fuel cells**. *ISME J* 2008, **2**:519-527.
22. Kleerebezem R, van Loosdrecht MCM: **Mixed culture biotechnology for bioenergy production**. *Curr Opin Biotechnol* 2007, **18**:207-212.
23. Madigan MT, Martinko J, Parker J: *Brock Biology of Microorganisms*. Prentice Hall; 2000.
24. Rozendal RA, Hamelers HVM, Buisman CJN: **Effects of membrane cation transport on pH and microbial fuel cell performance**. *Environ Sci Technol* 2006, **40**:5206-5211.
25. Ragsdale SW, Pierce E: **Acetogenesis and the Wood-Ljungdahl pathway of CO<sub>2</sub> fixation**. *Biochim Biophys Acta* 2008, **1784**:1873-1898.
26. Logan BE: **Scaling up microbial fuel cells and other bioelectrochemical systems**. *Appl Microbiol Biotechnol* 2010, **85**:1665-1671.
27. Foley JM, Rozendal RA, Hertle CK, Lant PA, Rabaey K: **Life cycle assessment of high-rate anaerobic treatment, microbial fuel cells, and microbial electrolysis cells**. *Environ Sci Technol* 2010, **44**:3629-3637.
28. Heijnen JJ: **Bioenergetics of microbial growth**. In *Bioprocess Technology: Fermentation, Biocatalysis and Bioseparation*. Edited by Flickinger MC, Drew SW. John Wiley & Sons, Inc; 1999:267-291. Seminal book chapter describing reaction thermodynamics and derived calculations such as cell yield.
29. Lee YK, Tan HM, Hew CS: **The effect of growth temperature on the bioenergetics of photosynthetic algal cultures**. *Biotechnol Bioeng* 1985, **27**:555-561.
30. De Schampelaire L, Verstraete W: **Revival of the biological sunlight-to-biogas energy conversion system**. *Biotechnol Bioeng* 2009, **103**:296-304.
31. Agler MT, Wrenn BA, Zinder SH, Angenent LT: **Waste to bioproduct conversion with undefined mixed cultures: the carboxylate platform**. *Trends Biotechnol* 2011, **29**:70-78. Recent review article that depicts the emerging 'carboxylate' platform that uses microbial populations to degrade biomass to carboxylic acids, and subsequently use these intermediates to achieve bioproduction.