Microbial electrocatalysis to guide biofuel and biochemical bioprocessing

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> “…a promising and bright, but challenging, future is ahead of scientists in the field of microbial electrocatalysis.”

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Microbial electrocatalysis

During the last 10 years, research on bioelectrochemical systems with alive microbes on anodes or cathodes (electrodes) has blossomed. We refer to microbial activity on electrodes as microbial electrocatalysis, especially when no artificial mediators are added to the cultures. Initially, researchers focused on converting organic materials in sediments or wastewater into spontaneous electric power production with microbial fuel cells. Researchers shaped an open culture of mixed microbial consortia to utilize the anode as a solid-state electron acceptor with, for example, a platinum-coated cathode for abiotic oxygen reduction. Over the years, vast leaps in current densities were made and recently a value of approximately 300 A/m^2 was reported with 3D electrodes [1]. Others successfully used microbes as biocatalysts at the cathode to circumvent platinum. Still, the low value of electric power and the high investment and operating costs has prevented a sustainable economic model [2]. The low value of electric power can also be turned around as an advantage by using a power source to manipulate electrode potentials and to enforce higher current densities (rates). This has already resulted in microbial electrolysis cells with acetate production at the cathode via microbial electrosynthesis from CO_2 [3]. Others are exploring the use of electrodes to manage surpluses or shortages of reducing equivalents in anaerobic fermentations (guiding fermentation) [4]. This article will unveil the possible uses of electrodes in biotechnology applications.

▪ With artificial mediators

Almost all research on bioelectrochemical systems that was performed before 2003 was with the addition of artificial mediators, such as methylene blue to the anode chamber and neutral red to the cathode chamber, to microbial cultures. The advantage of adding these mediators is that a relatively high current density can be achieved, but with the disadvantages that they add considerably to operating costs and that they may be toxic resulting in hazardous waste generation. Three examples of research projects with mediators at the cathode resulted in two papers and one patent from these reseachers: Park and Zeikus, who used neutral red to reduce fumurate to succinate [5]; Shin [101], who used methyl viologen to reduce CO_2 to acetate in a similar

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manner as described for microbial electrosynthesis without mediators [3]; and Steinbusch *et al.*, who used methyl viologen to reduce acetate to ethanol [6]. Two of these studies also reported that product efficiencies were enhanced through the addition of electrons as reducing equivalents by reducing the production of byproducts.

▪ Without artificial mediators

The popularity of research in this area during the last 10 years came after the finding that microbes, such as *Geobacter sulfurreducens*, deliver electrons to anodes directly and without the requirement of artificial mediators [7]. The circumvention of artificial mediators made inroads to treat organic wastewater in anode chambers with the simultaneous production of electric power. In addition, another finding with *Geobacter* sp. was made in that the reverse process of uptaking electrons at the cathode also occurred without mediators [8]. Many other discoveries in this quickly expanding field of microbial electrocatalysis were made, resulting in a research focus on bioelectrochemical systems without adding artificial mediators. However, circumvention of artificial mediators cannot be completely ruled out before natural electron transfer mechanisms achieve sufficient power densities, especially at the cathode.

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▪ Metabolic engineering

While many previously studied applications of bioelectrochemical systems employed open cultures of mixed microbial consortia, more and more studies use pure cultures of microbes with the aim to generate specific biofuels and biochemicals. The additional advantage of using pure cultures is the possibility of applying metabolic engineering and synthetic biology to directly influence biocatalysis. With metabolic engineering, biochemical and bioenergetics pathways can be streamlined to the desired bioproduction platform in naturally electrode-active bacteria, such as *Shewanella* sp. [9]. With synthetic biology, newly ‘created’ biocatalytic pathways of interest can be established in naturally electrode-active bacteria, and in the biotechnology-chassis bacterium *Escherichia coli* after incorporating the capacity to interact with electrodes [10]. Fast advances in physiological analytics (‘omics’ techniques) and molecular biology tools will greatly benefit this new research area.

▪ Biotechnology with electrodes

Anaerobic biocatalysis has two considerable advantages over aerobic biocatalysis for full-scale applications because it avoids the need for sufficient oxygen supply, which is a chemical, technical and economical challenge; it also limits extensive biomass growth, which in turn increases product yields. However, anaerobic bioprocesses are very sensitive to cofactor recycling and redox balancing. The efficiency of anaerobic biocatalysis towards specific product formation depends on a well-balanced removal or supply of reducing equivalents. Importantly, solid-state electrodes can provide a stable and sustainable solution. Anodes can serve as unlimited terminal electron acceptor for the discharge of surplus microbial reducing equivalents, while cathodes can provide lacking reducing equivalents for biochemical processes. Thus, the strength of the electron acceptor or donor can be fine tuned through the applied electrode potential, while the resulting electrode current can be used as an *in situ* measurement for the biocatalytic performance.

> “…the biggest challenge will be to increase the microbial reaction rates, which are very sluggish at this point.”

▪ Anode as an electron sink to guide fermentations & microbial electrooxidation

The use of anodes to discharge surplus reducing equivalents with the goal to balance fermentation reactions has already been demonstrated in a research publication with genetically modified *Shewanella* sp. [10]. During conventional fermentation, the efficiency of oxidation of glycerol (a waste product in the biodiesel production) to ethanol is very low because a sustainable redox balance can only be maintained with numerous byproducts production to get rid of reducing equivalents resulting from the more reduced state of glycerol than of ethanol and CO_2. The researchers were able to balance this fermentation without generating many byproducts by carefully guiding the fermentation in the host *Shewanella oneidensis* through the addition of functional genes and by knocking out a gene in the acetate production pathway. This strain at the anode was able to considerably improve the stoichiometric conversion of glycerol into ethanol [10]. Similarly, microbes may also be able to catalyze simple oxidative transformation reactions (microbial electrooxidation) with the anode as electron acceptor. However, examples for this nonfermentative oxidation pathway are not yet known.

▪ Cathode for microbial electrosynthesis

The use of electrodes in biotechnology was found to be attractive in a recent publication that described the microbial conversion of CO_2 and reducing equivalents from the cathode into acetate (microbial electrosynthesis) with the homoacetogen *Sporomusa ovata* [3]. While rates were very low (1 mmol acetate in 6 days), the efficiency was very good (~86%), promising a new route for efficient biosynthesis. In a follow-up study, various other homoacetogenic Clostridia were identified for the electrosynthesis process [11] and, currently, genetic engineering is employed to engineer these bacteria towards better rates and increased product spectra [12]. These reports have inspired microbial electrosynthesis projects in many research groups throughout the world [13,14]. The possibilities of using microbial synthetic reactions to reduce CO_2 and store surplus renewable electricity are their strong motivations for improving production rates. Rabaey and Rozendal reviewed the various promising scenarios for microbial electrosynthesis [15]. However, any significant step forward to an application of this technology will require intensive fundamental investigations into the cathodic microbial physiology, energy availability for growth and the mechanisms of extracellular electron transfer, because very little is known about the underlying mechanisms at this point in time [16].

Cathode for microbial electroreduction

A possibly slightly easier process to realize than electrosynthesis from CO_2 is sustaining simple reductive transformation reactions (microbial electroreduction). Hereby, one or two electrons are transferred onto a specific substrate through microbial electrocatalysis. In the simplest case, this could be the biocathodic production of molecular hydrogen from protons, which was shown before [17], or dechlorination [18]. However, economically and scientifically more interesting are chemically complicated reductive transformations, or stereoselective reduction. The possibilities in fine chemical or pharmaceutical synthesis are endless and synthetic biology likely will be employed to equip microbes with the necessary reaction pathways.

Is economic scale-up possible?

Economic scale-up will largely depend on the specific microbial electrocatalytic process. An application for electricity storage or fuel production will have completely different boundaries compared with an application within an industrial fermentation or a fine chemical transformation process. The three key challenges for scale-up are material costs, efficient reactor design and microbial reaction rates. In the past, extensive research efforts have been put into electrode material and design studies with significant progress. In the end, however, economic viability will depend on value product generation at the microbial cathode, or usefulness in guiding fermentations with a microbial anode. Economic scale-up is already in reach for fine chemical transformations, where a 100-l scale may be of technical interest. Still, the biggest challenge will be to increase the microbial reaction rates, which are very sluggish at this point. This will require a deep investigation into the cathodic microbial physiology and will require modification of microbial pathways through the use of advanced molecular tools. Thus, a promising and bright, but challenging, future is ahead of scientists in the field of microbial electrocatalysis. At this point, a judgment on economical feasibility would be unjustified.

Financial & competing interests disclosure

L Angenent is on the board of directors of Electrochaea, LLC (MO, USA). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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