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Current Opinion in Biotechnology

Engineering microbial electrocatalysis for chemical and fuel production

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In many biotechnological areas, metabolic engineering and synthetic biology have become core technologies for biocatalyst development. Microbial electrocatalysis for biochemical and fuel production is still in its infancy and reactions rates and the product spectrum are currently very low. Therefore, molecular engineering strategies will be crucial for the advancement and realization of many new bioproduction routes using electroactive microorganisms. The complex and unresolved biochemistry and physiology of extracellular electron transfer and the lack of molecular tools for these new non-model hosts for genetic engineering constitute the major challenges for this effort. This review is providing an insight into the current status, challenges and promising approaches of pathway engineering for microbial electrocatalysis.

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Current Opinion in Biotechnology 2014, **29**:93–98

This review comes from a themed issue on **Cell and pathway engineering**

Edited by **Tina Lütke-Eversloh** and **Keith EJ Tyo**

0958-1669/$ – see front matter,

http://dx.doi.org/10.1016/j.copbio.2014.03.003

Introduction

In microbial bioelectrochemical systems (BES), whole cell microbial biocatalysts act on electrodes to catalyze electrochemical oxidations (at an anode) or reductions (at a cathode). Initially, microorganisms were mainly studied and employed for anodic oxidation of organic substances with the purpose of converting stored chemical energy into electrical energy. These systems are called microbial fuel cells [1–3]. In recent years, the focus has shifted to microbial reductive processes at the cathode. Here, microorganisms can catalyze electrochemical reactions like proton reduction to molecular hydrogen [4,5] or the reduction of carbon dioxide to organics like methane [6,7] or acetate [8•,9••]. Thereby, the bioelectrochemical reduction of CO_2 to acetate has been termed microbial electrosynthesis, which holds strong promises for a new concept for biofuel generation [10•,11]. In this process, the central intermediate acetyl-CoA is a versatile building block for a range of chemicals and potential biofuels like ethanol, n-butanol or longer chain fatty acids and alcohols. Metabolic engineering and synthetic biology approaches are expected to open up these new biochemical electrofuel production pathways. However, these new microbial electrocatalytic reactions are not limited to CO_2 as a substrate or to purely reductive reactions. They can also be utilized to perform difficult redox reactions, for example, biochemical or pharmaceutical synthesis that rely on regioselectivity or stereoselectivity of the desired reactions.

There are two major challenges facing this new concept at this point. On the one hand electrocatalytic activity of the studied microorganisms, especially the rate of electron uptake (for reductions), is currently much too low for technical applications. On the other hand, advances in metabolic engineering and synthetic biology for non-model organisms are urgently required to implement the desired biochemical pathways in the respective electroactive microorganisms. Once these molecular challenges will be solved, further engineering hurdles for technical applications will move into the focus. The last section of this article will briefly highlight those future challenges.

Microbial biocatalysts and potential processes

The most well studied electroactive microorganisms to this point are *Geobacter sulfurreducens* and *Shewanella oneidensis*, both of which have been mainly studied for bioelectricity applications. *G. sulfurreducens* is a true specialist for extracellular respiration (acetate oxidation with an anode as terminal electron acceptor) and employs specific redox enzymes in its cell envelop (mostly *c*-type cytochromes) to transfer electrons outside the cell [12–14]. Here, the electrons can directly reduce an extracellular electron acceptor or be further transferred to such over a distance via conductive pili protein nanowires. The mechanism of conductivity of these protein pili is currently under scientific debate [15–18]. *S. oneidensis*, on the other hand, is more versatile and less specialized in its electroactivity [19]. It is a facultative anaerobic organism, but in the absence of oxygen it can perform a facilitated fermentation of lactate to acetate in presence of an electrode for discharge of surplus electrons. *S. oneidensis* also employs a series of *c*-type cytochromes to transfer electrons across the cell envelop [20]. From there, the electrons can be transferred to extracellular electron

acceptors directly or mediated via self-synthesized flavin-type redox shuttles [21,22].

Both organisms also show activity for electron consumption at the cathode [23,24], for example, *G. sulfurreducens* can reduce fumarate to succinate with a cathode as electron donor. But here the biochemical pathways and the energetics of electron uptake are much less understood. Methanogens and homoacetogens are also highly interesting for reductive processes at the cathode for their ability to reduce carbon dioxide to methane with reducing equivalents from a cathode instead of hydrogen [6,7], and to reduce carbon dioxide to acetate, respectively [8•,9••].

While in the past decade the main focus in BES microbiology research was on investigating electroactive physiology and screening for new electroactive microorganisms, recent advances are fueling a transition to modifying and optimizing electroactive microbes for target applications. This trend was especially driven by the emergence of the concept of microbial electrosynthesis from carbon dioxide and is now expanded to a great variety of potential redox bioconversions both at the cathode (reductions) or at the anode (oxidations) [25,26•]. However, current levels of cathodic productivity in microbial electrosynthesis are very low. The best of the homoacetogenic organisms known for electrosynthesis, *Sporomusa ovata*, was reported to produce about 150 μmoles acetate per day from CO_2 with a cathode at −400 mV versus SHE (standard hydrogen electrode) as sole electron donor [9••] at a good coulombic efficiency of 85% (ratio of electrons stored in acetate over electrons consumed from the cathode). This fairly low performance (conversion rate) is still about 10 times higher than the activity of *Clostridium ljungdahlii* — the most intensively studied microorganism for electrosynthesis [8•]. This means that for any reasonable application of these organisms as biocatalysts in bioelectrochemical productions, a drastic improvement of the electron transfer rate is prerequisite.

C. ljungdahlii attracts significant research interest for advancing microbial electrosynthesis, because its genome is sequenced and genetic engineering efforts for this organism are currently advancing for its applications in autotrophic synthesis gas fermentations of potential fuels and biochemicals [27••,28•]. The investigation and clarification of the electron uptake physiology, which is currently completely unknown, is one important task of current research. With the low rates of cathodic physiology, it is for example very difficult to determine if cells are growing in this situation. Currently, it is mainly assumed that they are not growing but long-term viable, which would present an important advantage for biocatalytic applications. First engineering tools are currently being developed for *C. ljungdahlii* (see below) to pave the way for physiological investigations, but progress is fairly slow and existing hypotheses for electron uptake mechanisms could not be resolved over the past years [29•]. Another possibility to enhance the rate of cathode to microbe electron transfer, which is currently explored, would be to generate molecular hydrogen at more reduced cathode potentials, which can then mediate electron transfer to the biocatalytic organism. This would also allow utilizing the entire reactor volume for bioproduction, instead of being limited to the two-dimentional electrode. Additionally, the search for naturally more efficient biocatalysts for cathodic bioconversions has just begun and future will show, if we can identify specialists for extracellular electron uptake similar to the high efficiency of *Geobacter* species for anodic electron discharge.

Thus, it is important for real progress in the field of microbial electrocatalysis, that — for both anodic oxidations and cathodic reductions — we always analyze and optimize both ends of the reaction: electron transfer to or from microorganisms *and* the target bioconversion.

Challenges to (synthetic) pathway engineering

The challenge of tailoring an electroactive biocatalyst can be approached by two different strategies (Figure 1). The first strategy includes the transfer of pathways involved in the electroactivity from one organism to an already established biocatalyst. This requires extensive knowledge of the protein functions and the corresponding pathways, which are involved in electron donation to an anode or uptake from a cathode, respectively, including their regulation. These functional proteins have subsequently to be transferred and adapted in order to be functional in a heterologous biocatalytic host. For example, although, there is basic insight in the extracellular transfer of electrons to an anode for *G. sulfurreducens* and *S. oneidensis*, it is still difficult to define the minimum set of reductases that are required to reproduce extracellular electron transfer. First attempts have been made to tailor *Escherichia coli* in this direction by installing parts of the extracellular electron transport chain from *S. oneidensis* (genes *mtrCAB*) with promising initial results [30•]. Although the engineered *E. coli* showed some activity toward Fe(III) reduction, this hypothetical minimal set of genes for extracellular electron transfer did not enable electroactivity in *E. coli*. Thus, other, so far unknown, components are required to successfully recreate extracellular electron transfer. On the other hand, little to nothing is known about the uptake of electrons from a cathode. Since electroactivity is a complex phenomenon it will prove rather difficult in the short term to engineer a functioning heterologous electroactive biocatalyst.

The second strategy addresses the engineering of an electroactive organism by integrating established or novel biocatalytic pathways into it. This strategy has the advantage that the biocatalytic pathways, with respect to enzyme functions and regulation, are in general much better understood than the electron transfer pathways via