Bioelectrocatalysis

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Self-Assembling Enzyme Networks—A New Path towards Multistep Bioelectrocatalytic Systems

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Why are bioelectrochemical fuel cells so fascinating? Lying at the interface between microbiology, biochemistry, and electrochemistry, they enable biological systems such as living bacterial cells, their organelles, and their isolated redox enzymes to serve as electrocatalysts for the oxidation of compounds for which no chemical electrocatalyst exists. The bioelectrochemical energy conversion makes it possible to exploit waste compounds, and it usually takes place at ambient temperature and pressure and at neutral pH—features that are in line with the concepts of sustainability.

According to the nature of the biocatalyst used, the two dominating classes of biofuel cells are enzymatic and microbial fuel cells (Figure 1).[1] Microbial fuel cells exploit redox enzymes in living microorganisms. Here, the enzymes are surrounded by the cell membrane, which serves as a protecting shell (great longevity!) but also sets a barrier to diffusion and limits overall turnover. In enzymatic fuel cells redox enzymes are used in their isolated forms, directly applied in the electrochemical system. Without the protective cell membrane the enzymes are much more exposed to degradation (thus usually low longevity), yet high surface concentrations and the absence of a diffusion barrier allow great turnover numbers and thus high electrocatalytic currents. The great advantage of using isolated enzymes as electrocatalysts is their reaction specificity which prevents the cross reactions that usually affect conventional catalysts. Based on this specificity even the physical separation of anode and cathode compartmenst—as generally required in fuel cells—may become unnecessary, allowing simplification and miniaturization of these devices.[2]

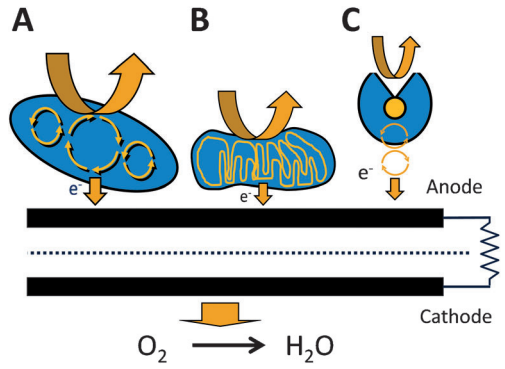

Figure 1. Principle of biofuel cells based on A) microorganisms, B) cell organelles, and C) redox enzymes (relative size not true to scale).

Yet the enzyme specificity also causes a severe limitation: single redox enzymes are not able to perform a multistep reaction, such as the complete oxidation of an organic substrate. Since conventional enzyme electrodes are based on single enzymes—chosen for a certain organic substrate—they can tap only a fraction of the chemical energy of the fuel. For example, an alcohol dehydrogenase anode can access only two out of six electrons available for the complete oxidation of methanol. The situation is even more pronounced in the case of glucose. Glucose liberates 24 electrons upon complete oxidation. In glucose oxidase based fuel cells—the most often used enzyme fuel cell type, glucose is oxidized only to gluconic acid. The reaction represents a two-electron oxidation, leaving 92% of the electrons unexploited—a severe limitation for the energy efficiency of enzymatic fuel cells.

To overcome this limitation and to improve the energetic yield of enzymatic fuel cells, the use of an enzyme cascade that catalyzes a series of consecutive redox steps is the only option. For example, an enzyme cascade for the oxidation of methanol should consist of an alcohol dehydrogenase (catalyzing the oxidation of methanol to formaldehyde), an aldehyde dehydrogenase (transforming formaldehyde to formic acid), and formate dehydrogenase for the final oxidation step leading to the liberation of CO_2. The simplest approach is to cast a mixture of the respective enzymes onto an electrode surface (of course, in combination with potentially required mediators and co-factors). This procedure already considerably improves the energetic yield of the substrate oxidation.[3] So far, however, the performance of such electrodes is limited by the rate of diffusion of the intermediate products between the randomly distributed individual enzymes and by diffusion losses of the intermediates from the electrode.

The use of enzymes in whole cell organelles like mitochondria (Figure 1B) may be a solution;[4] yet the introduction of the diffusional barrier of the organelle's cell membrane and the restricted enzyme density may limit the overall

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electrode performance. To improve this situation, the use of metabolon structures as anode catalysts has been proposed recently.[5] Within these metabolons, enzymes of a metabolic chain catalyzing consecutive reaction steps are closely attached. Thus, the intermediate enzyme products are transferred directly from one enzyme to the other, avoiding long-range diffusion. This concept is very promising, though the electronic wiring of the metabolon to the electrode has to be improved, and the preparation of the metabolons from natural mitochondria lysates may restrict the tailoring of the enzyme combinations.

A new, very elegant, and efficient approach has now been proposed by the team led by S. Banta.[6] The researchers used protein engineering to modify three enzymes: alcohol dehydrogenase (ADH), aldehyde dehydrogenase (ALDH), and formate dehydrogenase (FDH) for self-assembly. For this purpose they used α-helical leucine zipper domains[7] that allowed the enzymes to connect through coiled–coil interactions. The resulting hydrogel represents a synthetic metabolic pathway for the complete oxidation of methanol (Figure 2). The consecutive reaction steps are efficiently

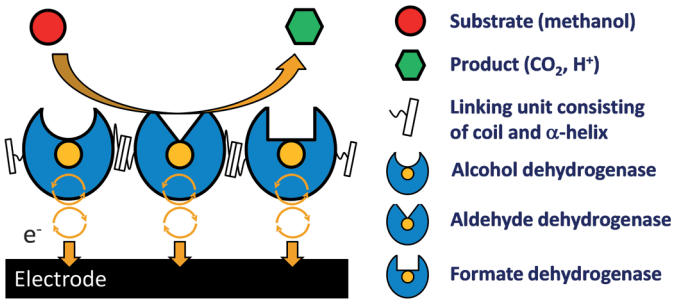

Figure 2. Schematic drawing of a self-assembled metabolic path for the complete oxidation of formate (based on Ref. [6]).

connected, allowing the performance of the system (in terms of maximum power and current density) to increase almost linearly from the single ADH to the combination ADH-ALDH to the complete ADH-ALDH-FDH hydrogel. This indicates an almost loss-free and complete methanol oxidation process. Finally, the electrochemical performance of the presented methanol biobattery is impressive. With a maximum current density of 26 mA cm^{-2} the biofuel cell clearly outperforms previous systems.

Quite surely, much work remains. For example, the stability of the hydrogel in an aqueous environment needs to be improved to allow a continuous substrate supply. Typical problems of enzymatic fuel cells, such as a generally low longevity and the dependence on soluble cofactors like NADH, have to be tackled. In the case of enzyme-cascade electrodes the longevity issue is of greatest importance since the deactivation of a single enzyme in a cascade may lead to complete failure of the electrode.

For any multistep bio(electro)catalytic reaction—especially for heterogeneous reactions—the fast and direct transfer of intermediates into the next reaction step is of greatest importance to maximize reaction yield and efficiency. Thus, the importance of the work by Banta et al,[6] in combination with previous work by other groups (for example, Ref. [7]), goes far beyond the field of biofuel cells. New applications and processes that require consecutive bio(electro)catalytic reactions—like complex bioelectrochemical syntheses—may thus become possible.

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