

Enzymatic Biofuel Cells Using NAD-dependent Dehydrogenases and Bilirubin Oxidase as Electrocatalysts

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Increasing attention has been recently paid to biofuel cells, which are devices for converting chemical energy into electrical energy by means of enzymatic oxidations of biological reductants such as H₂, NAD(P)H, alcohols and carbohydrates at anodes and enzymatic reductions of O₂ at cathodes.¹ The advantages of biofuel cells are ease in handling, simplicity of cell structure and flexibility in size (which allow miniaturization and bio-implantable type), mild conditions for operation, and minimized pollution in scrapping.

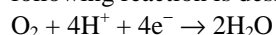
Anode compartment

Nicotinamide adenine dinucleotide (phosphate) (NAD(P)⁺) is an important coenzyme participating in various biochemical redox reactions, and NAD-dependent enzymes constitute the largest group of redox enzymes. Therefore, NAD(P)-dependent dehydrogenases in biofuel cells allow the use of various fuels such as saccharides, H₂ and alcohols. Because the direct electrochemical oxidation of NAD(P)H requires large overpotential, organic or inorganic compounds may be used as non-enzymatic catalysts. We believe more efficient catalytic systems would be mediated bioelectrocatalysis consisting of diaphorase and suitable mediators. Because of the low electron-accepting substrate selectivity of diaphorase, several kinds of metal complexes, flavines, quinones, and viologens have been utilized as mediators. The formal potential of mediator (E°_M) and the rate constant between diaphorase and mediator (k_M) are important factors. The more negative in E°_M and the larger in k_M , the better the mediator is. Considering the fact that k_M increases exponentially with E°_M and reaches the diffusion-controlled limiting value at increased E°_M , vitamin K₃ (VK₃, -0.2 V vs. Ag|AgCl) is one of the most promising mediators with more negative E°_M among the mediators with diffusion-controlled value of k_M ($\geq 10^8 \text{ M}^{-1} \text{ s}^{-1}$).² However, VK₃ has serious drawbacks in O₂ sensitive property of the reduced form and difficulty in the immobilization on electrode surfaces.

We have designed and synthesized a redox polymer containing Os (-0.15 V vs. Ag|AgCl) as an efficient mediator of diaphorase-catalyzed electrochemical oxidation of NADH and as a support to immobilize enzyme(s) on electrode surfaces.³ In this presentation, we demonstrate the bio-electrochemical oxidation of glucose electrons), ethanol (4 electrons), and pyruvate (4 electrons) utilizing NAD-dependent dehydrogenase and diaphorase. Both enzymes and NAD(P)⁺ are assembled in the polymer matrix built up on the electrode surface.

Cathode compartment

In the cathode of biofuel cells (bio-cathode), the following reaction is desired.



Metal catalysts would be difficult to reduce dioxygen (O₂) to water near neutral pH with low overpotential. We focused our attention to bilirubin oxidase (BOD; EC 1.3.3.5, from *Myrothecium verrucaria*) containing four copper atoms per molecule as a bio-cathode catalyst for

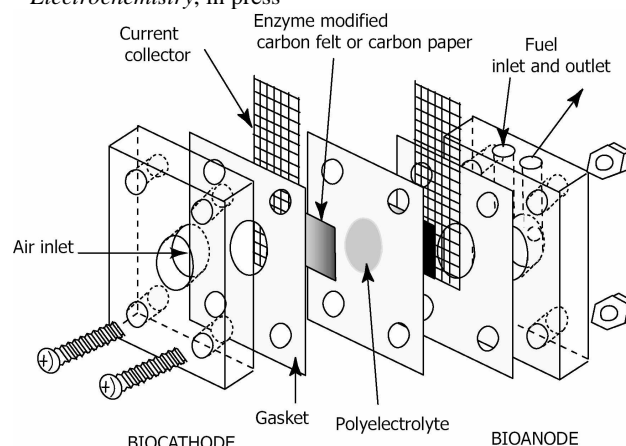
the 4e⁻-reduction of O₂. The electron-donating substrate specificity of BOD is extremely low and a variety of electroactive organic compounds and metal complexes function as electron shuttling mediator.⁴ We have already realized a diffusion-controlled O₂ reduction at a BOD and cyano-metal complexes (such as [Fe(CN)₆]^{3-/4-}, [W(CN)₈]^{3-/4-}, [Os(CN)₆]^{3-/4-}, and [Mo(CN)₈]^{3-/4-}) modified electrode. BOD and mediator were electrostatically entrapped on the electrode surface with the aid of cationic polymer(s).⁵ On the other hand, it has been found that adsorbed BOD exhibits a catalytic wave of O₂ reduction at carbon electrodes without any ET mediator.⁶ The current-potential curves were interpreted by considering the enzyme catalytic constant, surface electron transfer kinetics, surface concentration of BOD, and the formal potential of BOD. The electrochemical behavior was affected by the functional groups and/or nano-structural properties of carbon electrode surface. In this presentation, we prepared gas diffusion biocathode modified with BOD by the procedures described above.

Cell construction

The selection of electrode materials is essential to construct the fuel cell. It is possible to increase the (apparent) current density several times by increasing the microscopic surface area and the amount of immobilized enzymes. In this sense, mesoporous (pore size is ca. 2 – 50 nm) carbon and carbon felt are promising material. The heterogeneous electron-transferring rate also depends on the electrode materials. The low electrode kinetics causes the potential drops. The introduction of suitable functional groups and selection of hydrophobic or hydrophilic property are considered according to the characteristics of redox species. The cell diagram is illustrated in Scheme 1.

References

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Scheme 1. Illustration of a biofuel cell diagram.