

## Using yeast and fungi to produce electricity - Towards a self-regenerating enzymatic biofuel cell cathode

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Biofuel cells (BFCs) directly transform chemical energy into electricity for as long as fuel and oxidant are supplied. To catalyze the electrode reaction in biofuel cells, for instance biochemical pathways of complete microorganisms or enzymatic biocatalysts can be used [1]. The aim of our research is to improve the long-term stability of efficient, but currently short-lived enzymatic biofuel cell electrodes [2]. We aim to continually supply catalytically-active enzymes at the electrode using living microorganisms that grow in an electrode-integrated micro-bioreactor.

In the present work, we demonstrate the feasibility of using the crude culture supernatant of the fungus *Trametes versicolor* and the recombinant yeast *Yarrowia lipolytica* [3] to supply the biocatalyst laccase to a biofuel cell cathode. Both *T. versicolor* and *Y. lipolytica* were grown in a synthetic deficient (SD) medium. At approximately the highest enzyme activity, which was 3.6 U/ml for *T. versicolor* and 0.02 U/ml for *Y. lipolytica*, culture supernatant was transferred into a biofuel cell cathode compartment [4]. To record the loadcurve, current was incrementally increased (steps of 5.6  $\mu\text{A}/(\text{cm}^2 \cdot \text{h})$ ) and the cathode potential was measured against a saturated calomel electrode (SCE). At a cathode potential of 0.4 V vs. SCE, we obtained a current density of 134  $\mu\text{A}/\text{cm}^2$  for *T. versicolor*. The same enzyme activity of commercial *T. versicolor* laccase (Sigma) in SD medium yielded a current density of only 75  $\mu\text{A}/\text{cm}^2$  and in citrate buffer a current density of 87  $\mu\text{A}/\text{cm}^2$ . For *Y. lipolytica*, a current density of 4  $\mu\text{A}/\text{cm}^2$  was measured. The same amount of purified laccase from *Y. lipolytica* in SD medium and in citrate buffer resulted in a current density of 8  $\mu\text{A}/\text{cm}^2$  and 12  $\mu\text{A}/\text{cm}^2$  respectively.

Our results are a first step towards constructing a self-regenerating enzymatic biofuel cell with extended lifetime. Furthermore, we have shown that the choice of microorganism, has an influence on the obtained current density, because it has a large influence on the composition of the culture supernatant as well as on laccase activity. Important topics for future work will be the clarification of the secreted byproducts and the integration of the laccase-producing microorganisms in the electrode compartment.

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[4] A. Klope et al., Biosens. Bioelectron. (2010), pp. 2559-2565