A simple mediator-less enzymatic biofuel cell based on unpurified fungus culture supernatant

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We present for the first time the use of crude culture supernatant from enzyme-secreting microorganisms to supply biocatalysts to the electrodes of a mediator-less enzymatic biofuel cell. By using crude culture supernatant, we overcome the need for time consuming and expensive enzyme purification. Suitable enzymes that are secreted by fungi are laccase for cathodic oxygen reduction and cellobiose dehydrogenase (CDH) for anodic lactose oxidation. Both enzymes are capable of direct electron transfer (DET) and thus do not require a mediator, which can also be a cost factor. The cathode was supplied with culture supernatant of the fungus *Trametes versicolor*, grown in modified SC medium, and containing2.20 Uml^{-1} secreted laccase. The anode was supplied with culture supernatant of the fungus *Phanerochaete chrysosporium* grown in modified SC medium and ATM medium, yielding CDH activities of 0.07 Uml^{-1} and 0.05 Uml^{-1} respectively. Furthermore, 30 mM ß-lactose was added to the anode. The two compartments were separated with a proton exchange membrane. To record the fuel cell polarization curve, the load current density was incrementally increased (steps of 2.2 $\mu Acm^{-2}h^{-1}$) and the potential was measured against a saturated calomel electrode (SCE). By using supernatant of *P. chrysosoprium* at the anode, we achieved a power density of 2.7 ± 0.7 μWcm^{-2} when the fungus was grown in SC medium and of 4.9 ± 0.3 μWcm^{-2} when it was grown in ATM medium. No significant power could be generated in control experiments using the culture medium alone, clearly demonstrating the catalytic activity of the supernatants for biofuel cell operation. In comparison, power densities of 1.9 μWcm^{-2} [1] and 5 μWcm^{-2} [2] have been reported in literature for BFCs based on purified laccase and CDH. Polarization curves of anode and cathode potentials indicate that the anode limits the power density. In summary, our results show that even with unpurified enzymes, we can achieve a power output which is in the same range as biofuel cells using purified enzymes. This opens the opportunity for simple, low-cost enzymatic biofuel cells. Future work will focus on enhancing the anode performance. Furthermore, we want to explore whether the fuel cell lifetime can be extended by resupplying fresh enzymes to the electrodes. The feasibility of this approach has already been demonstrated for the laccase cathode using a crude culture supernatant of *T. versicolor* [3].

[1] Stoica et al., *Fuel Cells* 9 (2009) 53
[2] Coman et al., *Phys.Chem.Chem.Phys.* 10 (2008) 6093
[3] Sané et al. *Proc. 63rd Annual Meeting of the ISE* (2012) 40