

CHARACTERIZATION OF GLUCOSE DEHYDROGENASE IMMOBILIZED ON ACRYLIC BEADS

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To be presented to the Department of Energy
as final research paper on
December 6, 2000

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* Research supported by the ED Industrial Sector B Total Program of the Office of Energy Efficiency and Renewable Energy, U.S. Department of Energy under contract DE-AC05-00OR22725 with UT-Battelle, LLC.

Abstract

If a fuel cell can be constructed that uses hydrogen produced by an enzymatic pathway that oxidizes carbohydrates, its use as an alternative fuel is possible. Glucose dehydrogenase (GDH) oxidizes glucose to gluconic acid. The reducing equivalents generated are used by hydrogenase to generate molecular hydrogen. In order to construct an enzymatic fuel cell, these enzymes need to be immobilized. Different polyacrylamide materials with azlactone functionality were provided by the 3M Corporation and used in this investigation to immobilize GDH from *Thermosplasma acidophilum*. It was found that the properties of the polymer backbone effected the yield of active immobilized enzyme. The best yields of 50+% were achieved when the coupling reaction was carried out in the presence of a competitive binding reagent (BSA) on the 60:10:30 beads (10% vinyl-dimethyl azlactone). The use of different quenching solutions, which change the microenvironment of the immobilized GDH, altered the optimal pH of the immobilized enzyme. However, the immobilized enzyme did lose activity over time. The majority of activity was lost after 65h in all conditions tested to date with the 60:10:30 beads. This reduction in activity is most likely due to continued amide bond formation between the azlactone bead and the GDH rather than the GDH being released from the bead. Another sample of beads (55:5:40) containing 5% vinyl-dimethyl azlactone was tested. The preliminary data indicate that the yields were comparable with the 60:10:30 beads and the immobilized enzyme is stable.