Immobilization of active hydrogenase enzymes by encapsulation in polymeric porous gels.

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It has been demonstrated previously that various enzymes can be immobilized in silica-oxide gel matrices (Sol-Gel) and remain fully active. In fact, in many instances the enzyme stability and long term durability or half-life of certain enzymes is increased. The ability to encapsulation enzymes has tremendous promise in for both basic science and biotechnological applications. In some cases, immobilization of enzymes can allow for the stabilization of intermediates that are very short lived in solution. For biotechnology the encapsulation can result in generating durable heterogenous catalyst for potential industrial applications.

We have been able demonstrate the encapsulation of several examples of purified active hydrogenases in tetramethyl ortho silicate gels. In our preliminary work, we have shown that a high percentage of the overall hydrogenase both hydrogen oxidation and proton reduction activity is retained when these enzymes are embedded in these porous silica oxide polymeric gels. The activity of encapsulated hydrogenases from *Clostridium pasterianum*, *Lamprobacter modestogalophilus*, and *Thiocapsa roseopersicina* can be immobilized with an apparent activity at least 65-70% of that of the enzyme in solution measured in the reaction of hydrogen evolution. Encapsulated hydrogenases show some enhanced stability under storage and increased temperature. We have shown that the immobilized NiFe hydrogenase from *L. modestogalophilus* retains 85% of its hydrogen producing activity over a ten day period when stored at room temperature under nitrogen atmosphere. We are only in the initial stage of these studies and there are many factors that can be explored for optimization of hydrogen producing capabilities of these materials. The demonstration that hydrogenase enzymes can be immobilized in an active form, however, represents a major step in addressing the practicality of utilizing hydrogenases in solid phase hydrogen producing materials by heterogenous catalysis.