Enzymatic Conversion of Carbon Dioxide to Methanol: Enhanced Methanol Production in Silica Sol−Gel Matrices

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Strategies for effective conversion of atmospheric CO_2 to methanol offer promising new technologies not only for recycling of the greenhouse gas but also for an efficient production of fuel alternatives.[1] Partial hydrogenation of carbon dioxide has been accomplished by means of heterogeneous catalysis,[2] electrocatalysis,[3] and photocatalysis.[4] Oxide-based catalysts are predominantly used for industrial fixation of carbon dioxide.[2−4] A unique approach in this direction involves the use of enzymes as catalysts for conversion of carbon dioxide to methanol.[5] The use of enzymes is particularly appealing since it provides a facile low-temperature route for generation of methanol directly from gaseous carbon dioxide.

Herein, we report an enzymatically coupled sequential reduction of carbon dioxide to methanol by using a series of reactions catalyzed by three different dehydrogenases. Overall, the process involves an initial reduction of CO_2 to formate catalyzed by formate dehydrogenase ($F_{ate}DH$), followed by reduction of formate to formaldehyde by formaldehyde dehydrogenase ($F_{ald}DH$), and finally formaldehyde is reduced to methanol by alcohol dehydrogenase (ADH). In this process, reduced nicotinamide adenine dinucleotide (NADH) acts as a terminal electron donor for each dehydrogenase-catalyzed reduction. The overall reaction process is shown in Scheme 1.

Our strategy for CO_2 reduction takes advantage of the fact that dehydrogenases can effectively catalyze the reverse reactions (i.e. reduction) in the presence of suitable electron donors.[5,6] The ability of the dehydrogenases to catalyze the reverse reactions in the presence of an excess of NADH is well-established. Additionally, since the process involves a sequential reaction of in situ generated substrates with three different enzymes, it was expected that confinement of the system in a porous matrix would result in an enhanced probability of primary reaction events due to an overall increase in local concentration of reactants within the nanopores of the sol−gel processed glasses. The silica sol−gels have been shown to be effective matrices for stability and reactivity of different proteins, enzymes, and other biosystems with retention of biological reactivity upon encapsulation.[7] Indeed, when the enzymes are encapsulated in the porous silica sol−gel matrix, it is found that the yield of methanol production is substantially increased as compared to that in solution media.

The reaction was studied in the solution phase by using an enzyme stock solution that was comprised of 10 mg/mL of each enzyme dissolved in 0.1 M phosphate buffer at pH 7. The reaction mixtures used in this study were prepared by adding 1.0 mL of the enzyme stock solution to 1.0 mL of NADH solution in a polystyrene cuvette such that the final concentration of NADH varied from 0.025 to 0.1 M. The cuvette was covered with Parafilm, and gaseous CO_2 was then bubbled through this solution for 3 h using a small nozzle with an approximate outlet diameter of 0.5 mm through a hole made in the Parafilm. The extended time for bubbling of CO_2 ensured that reaction was allowed to go to completion and equilibrium was established. The Parafilm cover was used to prevent extensive loss of methanol produced due to evaporation.

Quantitative measurement of methanol was carried out by using gas chromatography (GC). A calibration curve was established for aqueous methanolic solutions with known concentrations of methanol ranging from 0.001 to 0.05 M. To evaluate the concentration of methanol produced as a result of the enzyme-catalyzed reaction, 1.0 μL of the final reaction solution was used for GC measurements. The concentration of methanol was calculated by using peak areas for the characteristic methanol band in the chromatogram.

The sol−gel encapsulated samples were prepared by using the biocompatible synthesis method previously reported in the literature.[7a] Tetramethoxysilane (TMOS) was used as precursor for making the silica sol−gel. The initial sol was prepared by mixing 3.82 g of TMOS, 0.85 g of water, and 0.055 g of 0.04 M HCl. The mixture was then sonicated for 20 min to form sol. The gels were prepared by adding 1.0 mL of the enzyme stock solution to 1.0 mL of the sol in a polystyrene cuvette. Typical gelation times are on the order of 10−30 s. The cuvette was then covered with Parafilm and gel was allowed to age at 4 °C for 24 h. After the initial aging process, the gels shrink and can be removed from the cuvette. The aged gel was then transferred to a beaker containing 250 mL of 0.1 M phosphate buffer at pH 7 and placed in refrigerator at 4 °C for 24 h. The gel was then transferred to another 250 mL beaker containing fresh 0.1 M phosphate buffer at pH 7 and was placed in the refrigerator for another 24 h. This step was repeated once more, for a total of 72 h of soaking in the buffer bath, to ensure complete removal of methanol generated due to hydrolysis of TMOS during the sol−gel process.[8]

After the initial equilibration, the gel was transferred to a standard polystyrene cuvette followed by addition of 1.0 mL of NADH solution (the final concentration of NADH varied from 0.025 to 0.1 M). To allow the NADH to diffuse into the gel, the sample containing the gel and the NADH solution was left undisturbed for 48 h. To this mixture, CO_2 was then bubbled for 3 h for production of methanol. The concentration of methanol produced was determined using GC by taking a 1.0 μL aliquot of the solution.

The results for methanol production in solution and the sol−gel system are shown in Figure 1. The amount of each enzyme

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(8) GC of the external solution prior to addition of NADH and CO_2 was used to ascertain that there was no residual methanol present in the system.

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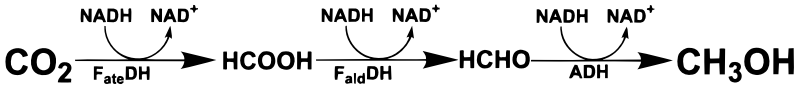
Scheme 1



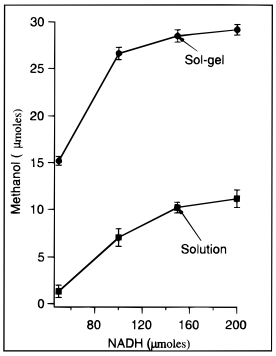


Figure 1. Plot of methanol produced as a function of terminal electron donor (NADH) present in solution and sol−gel matrix.

Table 1. Relative Comparison of Methanol Production in Solution and Sol−Gel

	solution			sol−gel		
NAD (μmol)	MeOH (μmol)	MeOH/ NADH	% yield[a]	MeOH (μmol)	MeOH/ NADH	% yield[a]
50	1.3 ± 0.7	0.02	7.8	15.2 ± 0.4	0.30	91.2
100	7.0 ± 0.9	0.07	21.0	26.6 ± 0.6	0.26	79.8
150	10.2 ± 0.6	0.07	20.4	28.5 ± 0.7	0.19	57.0
200	11.2 ± 0.9	0.05	16.8	29.2 ± 0.6	0.15	43.8

[a] % yield = [moles of MeOH/{0.33(moles of NADH)}] × 100.

in solution and sol−gels is 5 mg, and the amount of methanol generated with respect to variation of NADH enables monitoring of the overall efficiency of the reaction. The moles of methanol produced are plotted as a function of the moles of the terminal electron donor (NADH). Since NADH serves as a limiting reagent in the overall reaction, it provides a relative measure of the efficiency of the reaction and the yield of the methanol production. As can be seen from Scheme 1, 3 mol of NADH are consumed per mol of methanol produced. As such, for 100% yield, the moles of methanol produced should be 1/3 of the NADH added. Table 1 compares the relative yield of methanol per mole of NADH added. The overall yield of the reaction in solution is very low. However, in sol−gels, the production of methanol is substantially enhanced.

The enhanced production of methanol is due to enzymatic reactions and not due to probable hydrolysis of residual methoxides present in the TMOS sol−gels. This is confirmed by control experiments performed with plain TMOS sol−gels. The sol−gel processed glasses without the encapsulated enzymes do not generate any methanol under identical conditions of treatment with carbon dioxide. Furthermore, it is found in order to generate methanol all four species (i.e. F_{ate}DH, F_{ald}DH, ADH, and NADH) must be present. This was established by preparing several sol−gels with systematic exclusion of one or more of the four components. It is observed that sol−gels prepared without any of the four components fail to show any production of methanol.[9] Thus, from the results on control experiments, it can be concluded that the enhanced production of methanol is an intrinsic feature of the nanoconfined reaction system brought about by subtle influence of the sol−gel matrix.

The results obtained in this study indicate that confinement of the multienzyme system in the nanopores of silica sol−gels alters the reaction thermodynamics and final equilibrium of the reaction. The yield of the reaction is very low in the solution phase (10−20%). However, for the same concentration of NADH, it is seen that in the sol−gel, the production of methanol is significantly enhanced as compared to solution with yields ranging from 40 to 90% indicating that the overall equilibrium is shifted more toward the products. It is important to note that the overall yield of the reaction is lowered at higher concentration of NADH presumably due to an increased tendency of the system to undergo the reverse reaction (i.e. conversion of methanol to carbon dioxide).

Although at present a detailed understanding of the reaction kinetics of the system remains to be fully evaluated, the enhancement of methanol production in sol−gel can be tentatively attributed to confinement and matrix effects.[10] The immobilized system in the nanopores of a sol−gel matrix is characterized by limited pore volume. As such an increased local concentration of enzymes and reactants is likely to prevail in the porous structure of silica sol−gel.[10] As a result, the overall efficiency of enzyme reactions is enhanced such that the final equilibrium is shifted more toward the product. The overall reaction involves conversion of a gas to a liquid, and such a shift in equilibrium toward products is most likely consistent with reduction in available volume as a result of confinement.

In summary, the primary feasibility of enzyme catalysis for efficient conversion of carbon dioxide to methanol is reported.[11] A sequential reduction of carbon dioxide by three different dehydrogenases encapsulated in sol−gel matrix results in enhanced yields for generation of methanol. The efficient production of methanol provides a facile pathway not only for on-site generation of methanol from readily available resources but also for potential applications related to energy technology and environmental fixation of carbon dioxide.

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(9) TMOS sol−gels containing the following dopants were studied: (a) NADH; (b) ADH + NADH; (c) F_{ate}DH + NADH; (d) F_{ate}DH + ADH; (e) F_{ate}DH + F_{ald}DH + ADH; (f) F_{ald}DH + ADH + NADH; (g) F_{ate}DH + ADH + NADH; and (h) F_{ate}DH + F_{ald}DH + NADH.

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(11) The sol−gel processed glasses retain full activity for months if they are stored at 4 °C in pH 7 phosphate buffer. However, drying leads to rapid loss of activity and the gels become inactive after a week.