ENZYMATIC CONVERSION OF CARBON DIOXIDE TO METHANOL BY DEHYDROGENASES ENCAPSULATED IN SOL-GEL MATRIX

Zhongyi JIANG , Hong WU , Songwei XU , Shufang HUANG

State Key Lab. of C1 Chemistry and Chemical Technolgy, School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, China

Introduction

Due to the abundance of CO_2 as the major greenhouse and the most oxidized form of carbon, the efficient utilization of CO_2 has aroused much interest from fundamental research to application application.

Herein, we report a novel and promising approach to convert carbon dioxide into methanol through consecutive reduction catalyzed by three different dehydrogenases. The whole process consists of three steps: reduction of CO_2 to formate catalyzed by formate dehydrogenase ($F_{ate}DH$), reduction of formate to formaldehyde by formaldehyde dehydrogenase ($F_{ald}DH$), and reduction of formaldehyde to methanol by alcohol dehydrogenase (ADH). Reduced nicotinamide adenine dinucleotide (NADH) acts as a terminal electron donor for each dehydrogenase-catalyzed reduction.

It is now well established that a wide variety of enzymes retain their characteristic reactivities and chemical functions when they are confined within the pores of the silica sol-gel derived matrix. The porosity of sol-gel glasses allows small molecules and ions to diffuse into the matrix while the enzymes remain physically trapped in the pores, and thus resulting in an enhanced probability of reaction due to an increase in local concentration of substrates within the nanopores. Our experiments demonstrated that when the above-mentioned three enzymes are encapsulated in the silica sol-gel matrix, the yield of methanol is considerably increased as compared to that in solution phase.

Experimental

The reaction was studied in the solution phase by using an enzyme stock solution that was comprised of 7mg/mL of $F_{ate}DH$, 2mg/mL of $F_{ald}DH$, 2mg/mL of ADH dissolved in 0.1M phosphate buffer at pH7. Add 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 0.1M . The cuvette was covered with Parafilm to prevent the evaporation loss of methanol produced, and CO_2 was then bubbled through the solution for 8 h to ensure that the reaction equilibrium was established.

The concentration of methanol was determined by gas chromatography. A calibration curve was established for methanol aqueous solutions with known concentration of methanol ranging from 0.001 to 0.005M . To evaluate the concentration of methanol produced as a result of the enzyme-catalyzed reaction. 1.0μL 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 as follows: Tetraethoxysilane (TEOS) was used as precursor for making the silica sol-gel. The initial sol was prepared by mixing 2.60g of TEOS , 1.10g of 3%(v/v) of HCl solution . The mixture was then vigorously mixed for 10 min to form sol. The gel were prepared by adding 1.0 mL of the stock enzyme solution to 1.0mL of the sol in a polystyrene cuvette. Typical gelation times are on the order of 10-30s. The cuvette was then covered with Parafilm and gel was allowed to age at 4℃ for 24 h. The aged gel was then put into a dialysis membrane using 250 mL of 0.1M phosphate buffer at pH7 as dialysis solution , and placed in refrigerator at 4℃ with frequent change of 0.1M phosphate buffer at pH7 The dialysis lasted 24-48 h to completely remove ethanol generated in the sol-gel process. 0.1 mL of NADH solution diffused into the gel by dialysis in the similar manner (the final concentration of NADH varied from 0.025 to 0.1M), the sample containing the gel and the NADH solution was left undisturbed for 48h. To this mixture , CO_2 was then bubbled for 8h for production of methanol. The concentration of methanol produced was determined using GC by taking a 1.0μL aliquot of the solution.

Results and Discussion

Table 1 shows the preliminary methanol yields in solution and sol-gel matrix. The overall yield of the reaction in solution is near to 100%. In sol-gels, the production yield of methanol is substantially decreased due to the distribution effect, stereo hindrance, diffusion resistance.

Table1 Representative methanol yields in solution and sol-gel matrix

	Reaction Temperature ℃	NADH（μmol）	Methanol yield（%）
Solution	25	100	98.0
	37	100	100
	37	150	94.8
Solution	25	100	30.0
	37	100	91.9
	37	150	42.2

It is also found that all four species (i.e. $F_{ate}DH$, F_{ald} DH, ADH,NADH) must be present to generate methanol.

The introduction of chemical modification to the enzymes and the creation of mesoporous structure of gel by biomimetic synsthesis is under investigation.

To sum up, the feasibility of enzymatic conversion of carbon dioxide to methanol is tentatively explored. The consecutive reduction of carbon dioxide by three different dehydrogenases encapsulated in sol-gel matrix results in enhanced yields for generation of methanol. This will open up a new avenue not only for on-site production of methanol from cheap raw material but also for efficient fixation of the greenhouse carbon dioxide.

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