

A New Biochemical Way for Conversion of CO₂ to Methanol *via* Dehydrogenases Encapsulated in SiO₂ Matrix

Hong WU¹, Zhong Yi JIANG^{1,2*}, Song Wei XU¹, Shu Fang HUANG¹

¹School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072

²State Key Laboratory of C1 Chemistry and Chemical Technology, Tianjin University, Tianjin 300072

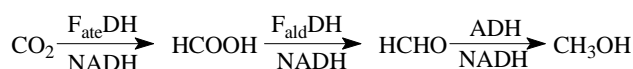
Abstract: CO₂ is converted to methanol through an enzymatic approach using formate dehydrogenase (F_{ate}DH), formaldehyde dehydrogenase (F_{ald}DH) and alcohol dehydrogenase (ADH) co-encapsulated in silica gel prepared by modified sol-gel process as catalysts, TEOS as precursor, NADH as an electron donor. The highest yield of methanol was up to 92.1% under 37°C, pH7.0 and 0.3Mpa.

Keywords: Dehydrogenases, sol-gel, carbon dioxide, methanol.

The efficient utilization of CO₂ has attracted considerable attention from fundamental research to industrial application due to the abundance of CO₂ as the major greenhouse gas and the most oxidized form of carbon. Conversion of CO₂ to methanol is considered to be a most useful approach because of the significant value of methanol as a raw material and clean fuel. Heterogeneous catalysis, electrocatalysis, and photocatalysis have been predominantly used presently for this purpose¹⁻⁴. These methods need either high temperature and high pressure or additional electric or luminous energy, and both the selectivity and yields are low.

Herein, we report a novel and promising approach to convert CO₂ into methanol through consecutive reduction catalyzed by three different dehydrogenases encapsulated in silica gel matrices prepared by modified sol-gel process. The whole enzymatic reaction consists of three steps^{5,6}: reduction of CO₂ 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 (**Scheme 1**).

Scheme 1



*E-mail: zhyjiang@tju.edu.cn

Preparation of silica gel encapsulated enzymes

Tetraethylorthosilicate (TEOS) was used as precursor for making the silica sol-gel. The initial sol was prepared by mixing 1.94 g TEOS, 1.10 g of 3 % (v/v) of HCl solution. The mixture was then vigorously mixed for 10 min to form sol. The gel was prepared by adding 1.0 mL of the stock enzyme solution which comprised of 7.0 mg F_{ate}DH, 2.0 mg F_{ald}DH, 2.0 mg ADH, to 1.0 mL of the sol in a polystyrene cuvette. Gelation times are on the order of 50-60s. The cuvette was then covered with Parafilm and gel was allowed to age at 4°C for 48 h.

Enzymatic reactions

The aged gels were put into a dialysis membrane using 250 mL of 0.1 mol/L phosphate buffer at pH7.0 as dialysis solution, and placed in refrigerator at 4°C with frequent change of 0.1 mol/L phosphate buffer at pH7.0. The dialysis lasted 24 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 0.1 mol/L), the sample containing the gel and the NADH solution was left undisturbed for 48 h. To this mixture, CO₂ was then bubbled for 8 h for production of methanol. The concentration of methanol was determined by gas chromatography (HP 6890 Gas Chromatographer, USA).

The yield of methanol was calculated based on the NADH amount:

Yield of methanol (%) = $3 \times \text{moles of methanol product} / \text{moles of NADH initial amount} \times 100\%$

From the preliminary experimental results, the feasibility of enzymatic conversion of carbon dioxide to methanol has been successfully demonstrated. It was found that F_{ate}DH, F_{ald}DH, ADH and NADH must be present together to generate methanol. Typical experimental results are summarized in **Table 1**.

Table 1 Typical experimental results of conversions of CO₂ to methanol catalyzed by dehydrogenases

No.	Temperature (°C)	pH	NADH amount (μmol)	Pressure (Mpa)	Yield (%)
0	37	7.0	100	0.3	98.1 (free form)
1	25	7.0	100	0.3	30.0
2	37	7.0	100	0.3	92.1
3	25	7.0	150	0.5	27.0
4	37	7.0	150	0.3	42.2
5	37	7.5	100	0.3	66.9
6	37	8.0	100	0.3	49.5

The highest yield of methanol using the gel encapsulated dehydrogenases as catalysts reached 92.1 %. Under the same reaction conditions, the yield of methanol using dehydrogenases in the free form as catalysts was 98.1 %. The decrease in the

activity of immobilized enzymes was mainly due to the minor conformation change of enzyme and the additional diffusion hindrance of substrate and product.

Each dehydrogenase had its own optimum reaction temperature. The optimum temperature were 37°C for F_{ate}DH and F_{ald}DH, while the optimum temperature for ADH was 25°C. Our experiments were carried out at 25°C and 37°C respectively in order to find the optimum temperature for the three enzymes co-immobilized in the same gel. It showed that the better reaction temperature is 37°C.

Compared the methanol yields at different pH values, we found the highest methanol yield was obtained at pH7.0 (No.2, 5, 6). According to the tentative analysis of reaction mechanism of dehydrogenase, the reduction reaction using NADH as electron donor needed the existence of proton. The lower pH was more favorable for the protonation and thus more favorable for the increase of methanol yields.

Conclusions

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 TEOS sol-gel matrix results in high 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.

Acknowledgment

The financial support from the National Natural Science Foundation of China (No.20176039) was greatly acknowledged.

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Received 6 June, 2002