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Thermodynamic Feasibility of Enzymatic Reduction of Carbon Dioxide to Methanol

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Abstract Production of valuable chemicals from CO_2 is highly desired for the purpose of controlling CO_2 emission. Toward that, enzymatic reduction of CO_2 for the production of methanol appeared to be especially promising. That has been achieved by reversing the biological metabolic reaction pathways. However, hitherto, there has been little discussion on the thermodynamic feasibility of reversing such biological pathways. The reported yields of methanol have been generally very low under regular reaction conditions preferred by naturally evolved enzymes. The current work examines the sequential enzymatic conversion of CO_2 into methanol from a thermodynamic point of view with a focus on factors that control the reaction equilibrium. Our analysis showed that the enzymatic conversion of carbon dioxide is highly sensitive to the pH value of the reaction solution and, by conducting the reactions at low pHs (such as pH 6 or 5) and ionic strength, it is possible to shift the biological methanol metabolic reaction equilibrium constants significantly (by a factor of several orders of magnitude) to favor the synthesis of methanol.

Keywords Carbon dioxide · Enzymatic biocatalysis · Reduction · Sequestration · Methanol · Thermodynamics

Introduction

Despite the tremendous efforts that have been made so far, controlling of CO_2 emission remains as the single most challenging topic for both social and scientific communities in

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achieving sustainable environment's well-being. One strategy is to sequester CO_2 at its emission sources and bury it underground or in the ocean [1]. Since 1990, a variety of chemical [2–4], electrochemical [5], and biological [6–9] methodologies have been examined for CO_2 sequestration [10]. One profitable way, however, is to produce valuable chemicals even fuels from CO_2.

Catalytic reduction of CO_2 with H_2 to produce methanol is one reaction that has long been demonstrated. Thermodynamically, this is not a favored reaction under ambient conditions with a positive standard Gibbs free energy change of reaction ($\Delta_r G^o$=0.84 kcal/mol). Nevertheless, the reaction has been shown feasible with catalysts such as Cu–Al/Zn/B [11], Mn–Cu/ZnO/ZrO_2 [12], Cu/Ga_2O_3/ZrO_2 [13], Cu/B_2O_3/ZrO_2 [13], and Cu–ZnO/ZrO_2 [14] operated at elevated temperatures in the range of 150 to 300 °C and high pressures ranging from 3 to 14 MPa. The high pressures and temperatures are needed primarily for the activation of the catalysts. In addition, most of the metallic catalysts require high-purity feedstocks to maintain their activities. All these factors make the thermochemical reduction of carbon dioxide an expensive route to practice in industry.

Alternatively, biocatalysts have also been shown capable of catalyzing the reduction of CO_2 at ambient conditions. Certain microorganisms can reduce CO_2 into valuable organic compunds via several steps of reactions usually with formate dehydrogenases as the leading enzyme effecting the initial reduction of CO_2. For microrganisms, such reactions are simply the reversed biological metabolic pathway reactions. Formate dehydrodenases can be either metalloenzymes that realize the reduction of CO_2 with the chemical energy of redox species such as methyl viologen [15, 16] or NAD(P)-dependent enzymes which utilize the reduction energy of the cofactors [17]. Further conversion of the resulted formate into other chemicalls are generally achieved with cofactor-dependent enzymes, and one biotransformation route that has been studied vigorously in recent years is the production of methanol with NAD-effected sequential enzymatic reactions [18–22]. Such biocatalytic reduction of CO_2 are attractive in that they require mild reaction conditions and afford efficient processing as they can make use of low-purity reactants and tolerate many impurities (such as sulfur compounds which are common in flue gasses) that are toxic to chemical catalysts. Nevertheless, the reported reaction rates and equilibrium yields are generally low and, therefore, are not suited for large-scale CO_2 sequestration. For knietic considerations, there is a need in intensifying the biocatalysts for faster and more efficient conversion of CO_2. That requires both discovery of new enzymes and engineering of the reaction systems for improved catalytic efficiency. That is the current focus of the recent research in this area. On the other hand, theoretical equilibrium limitations of the biotransformation reactions have generally been ignored. To our understanding, this is equally, if not more, important a limiting factor as reaction kinetics in advancing the biocatalytic conversion of CO_2. The aim of this work is to examine the thermodynamic limitations of the enzymatic reduction of CO_2 and thus to explore the feasibilities of achieving efficient CO_2 sequestration and utilization via biocatalytic transformations.

Model of Reaction

The enzymatic reduction of CO_2 requires an electron donor, which can be a coenzyme such as nicotinamide adenine dinucleotide (NADH) or a synthetic chemical like methyl viologen, depending on the specificity of the enzymes. The NADH-mediated

reduction of CO_2 for production of methanol can be described as a multistep reaction process:

$$CO_2 + NADH + H^+ \xrightarrow{F_{ate}DH} HCOOH + NAD^+, \quad (1)$$

$$HCOOH + NADH + H^+ \xrightarrow{F_{ald}DH} CH_2O + NAD^+ + H_2O, \quad (2)$$

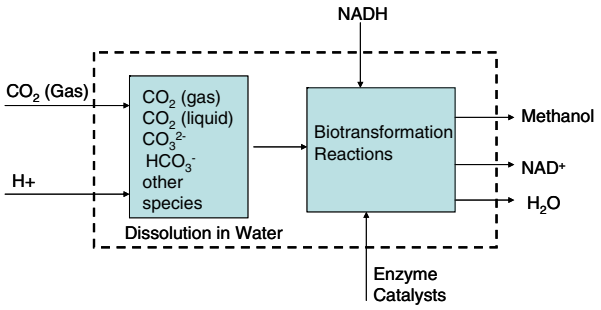
$$CH_2O + NADH + H^+ \xrightarrow{ADH} CH_3OH + NAD^+. \quad (3)$$

The actual reaction process is indeed more complicated than what is depicted through the above three reactions, as the solvation of CO_2 involes the formation of several species including CO_3^{2-}, HCO_3^-, and H_2CO_3 whose thermodynamic equilibrium and concentration distribution are subject to the effects of pH and other physicochemical properties of the solution. The enzyme may actually be able to take all of these species as substrates for methanol synthesis other than the free state CO_2 only. To simplify the analysis, herein, we summarize all the processes into an overall one-step reaction by concentrating on the initial and final chemical species conerned in this transformation. This black box approach is depicted in Scheme 1 of which details within the boundaries of the dashed lines are not considered, while focus is placed on the input and output of the process. By adapting such an approach, the overall biotransformation with methanol as the ending product can be summarized as:

$$CO_2 + 3NADH + 3H^+ \xrightarrow{3\ enz.\ sys.} CH_3OH + 3NAD^+ + H_2O. \quad (4)$$

The estimated value of the Gibbs free energy change for the overall reaction 4 varied greatly when different reference states for Gibbs energy of formation ($\Delta_f G_i^o$) were applied (Table 1). According to Alberty's method, which takes $\Delta_f G_i^o$ of proton as zero since the reference state was defined as zero inonic strength [23], $\Delta_r G^o$ is −22.58 kcal/mol for reaction 4. That is very different from the calculation using the group contribution method [24], which gives a value of 8.16 kcal/mol. Calculations using a recently published reference [25] on the group contribution method gives more positive value of 14.7 kcal/mol. The results from the two calculations of the group contribution method differ mainly in the value the Gibbs free energy of formation of water (Table 1). We believe that the latter is more reliable as it agrees much better with values from other references.

Apparently, the divergency of $\Delta_r G^o$ for reaction 4 shown here between the group contribution method and Alberty's treatment is a result of the different definitions of the reference state. The group contribution method defines a reference state as 25 °C and pH 7 with the $\Delta_f G_i^o$ of



Scheme 1 Carbon dioxide dissolution and transformation processes for enzymatic synthesis of methanol. A black box approach (indicated by the boundaries of *dashed lines*) focusing on the inputs and outputs of the process was applied in the current work

Table 1 Gibbs free energy change of reaction for the synthesis of methanol from carbon dioxide calculated using different reference values.

Ref.	$\Delta_f G^o$ (kcal/mol)		
	[23]	[24]	[25]
CH_3OH	−41.9	−45.0	−45.1
H_2O	−56.7	−56.6	−51.0
NADH	5.41	3.70	3.32
NAD^+	0	0	0
H^+	0	−9.49	−9.49
CO_2	−92.25	−92.3[a]	−92.3[a]
$\Delta_r G^o$ (kcal/mol)	−22.6	8.16	14.7

[a] Value was not available from the original references, but was determined using the group contribution method through calculations performed in Professor Linda Broadbelt's laboratory [25] at Northwestern University

proton as −9.49 kcal/mol, which is different from Alberty's reference state. When the contribution of pH is included into the Alberty's data system, as to be discussed in the following, we see that the results from the two different methods indeed agree well with each other.

Results and Dicussion

pH and ionic strength (I) are important parameters for biological systems to regulate and control enzyme activities. They also affect the thermodynamic activities of the chemical species involved in the reactions and thus impact the reaction equilibria. The combination of these two factors allows the biological metabolic pathways to undergo directions as needed. At low ionic strengths with I ranging from 0.05 to 0.25 M, where most of the biological reactions take place, activity coefficients of chemical species can be correlated to I through the Debye–Hückel theory. The Debye–Hückel theory can be further extended to also include the effect of pH [23]. Accordingly, the pH-adjusted and I-adjusted enthalpy ($\Delta_f H_i'^o$) and Gibbs free energy of formation ($\Delta_f G_i'^o$) of a chemical species can be expressed as:

$$\Delta_f H_i'^o(I) = \Delta_f H_i^o(I=0) + \mathrm{RT}^2\left(\partial\alpha/\partial T\right)_P \frac{z_i^2 - N_\mathrm{H}(i) I^{1/2}}{1 + BI^{1/2}}, \tag{5}$$

$$\Delta_f G_i'^o(\mathrm{pH}, I) = \Delta_f G_i^o(I=0) - \mathrm{RT}\left[N_\mathrm{H}(i)\ln 10^{-\mathrm{pH}} - \alpha\frac{\left(z_i^2 - N_\mathrm{H}(i)\right) I^{1/2}}{1 + BI^{1/2}}\right] \tag{6}$$

where B is an empirical term and is assumed to be 1.6 $\mathrm{L/mol}^{1/2}$ for most common biochemical temperatures [23], z_i is the electronic charge of species i, $N_i(\mathrm{H}^+)$ the number of hydrogen atoms, R the gas constant, and α is a temperature-dependent Debye–Hückel constant. Clarke and Glew [26] calculated the terms $\mathrm{RT}\alpha$ and $\mathrm{RT}^2(\partial\alpha/\partial T)_P$ for aqueous solutions of 0 through 150 °C, and Alberty [23] further investigated these terms for biological reaction temperatures. Values of Debyde–Hückel constant and Debye–Hückel slopes in this study are calculated using the values fitted by Alberty [23]. The value of pH

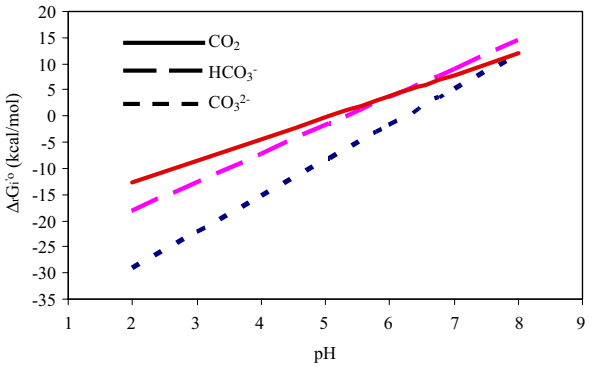


Fig. 1 pH dependency of $\Delta_r G_i'^{o}$ for methanol synthesis with different carbonaceous species as substrate (I=100 mM and T=25 °C)

for the extended Debye–Hückel theory to be applied for calculations in Eq. 6 is a value that combines the actual pH value along with an ionic strength term:

$$\mathrm{pH} = \mathrm{pH}_{\mathrm{measured}} - \frac{\alpha}{\ln(10)} \frac{I^{1/2}}{1 + BI^{1/2}} \tag{7}$$

where $\mathrm{pH}_{\mathrm{measured}}$ is the actual pH of the solution. For a solution at 25 °C with I=0.1 M, the Gibbs free energy change for reaction 4 was calculated to be 5.01 kcal/mol when the

Table 2 Theoretical prediction and experimental measurements of methanol production (experimental data were cited from references as indicated).

T (°C)	pH	[NADH] (mM)	$[\mathrm{MeOH}]^{(\mathrm{ref})}$ (mM)	$[\mathrm{MeOH}]^{\mathrm{pred.}}$ (mM)	Ref.
25	7	0.05	0.08	0.09	[20]
		0.1	0.011	0.015	
		0.2	0.015	0.028	
		0.5	0.021	0.059	
25	7	50	1.3[a]	2.3	[19]
		100	7.0[a]	3.0	
		150	10[a]	5.5	
		200	11[a]	6.8	
		50	15[a]	2.3	[21]
		100	27[a]	4.0	
		150	29[a]	5.5	
		200	29[a]	6.8	
37	7		0.31[b]	0.14	[22]
20	7		0.28[b]	0.18	
25	7		0.30[b]	0.17	
37	7	0.94	0.24[b]	0.14	
37	6		0.31[b]	0.30	
37	7.5		0.27[b]	0.048	
37	8		0.25[b]	0.012	

[a] Reactions were carried out at room temperature with I=225 mM

[b] Experiments were carried out at 0.5 MPa with I=50 mM

adjusted values of Gibbs free energy of formation ($\Delta_f G_i'^o$) were applied based on Alberty's method. That is consistent with the calculations from the group contribution method for the same conditions. Apparently, the reaction equilibrium in a biological environment is controlled to thermodynamically favor the oxidation instead of synthesis of methanol. However, the negative value of $\Delta_r G^o$ at Alberty's reference state for the same reaction also implies that the reaction can be reversed to favor the reduction of carbon dioxide to methanol by manipulating the pH and I of the reaction media. In the following discussions, which are based on calculated results from Alberty's method, we demonstrate such possibilities with more details.

One question regarding the enzymatic reduction of CO_2 that remains vague is whether CO_2 or its hydrated derivatives are transformed directly into methanol via the enzymatic reactions. The equilibria among CO_2, carbonic acid (H_2CO_3), bicarbonate (HCO_3^-), and carbonate (CO_3^{2-}) in water have been well understood. Even though it has been reported that HCO_3^- and CO_3^{2-} had been supplied as substrates, there is currently a lack of knowledge as to which species is directly involved in the biotransformations, since all the species exist simultaneously in the solution. We examined this issue by following the calculation of the Gibbs free energy of reaction. Figure 1 illustrates the pH dependency of the $\Delta_r G_i'^o$ for methanol synthesis for biotransformations with different carbonaceous species as the direct substrate. Interestingly, the $\Delta_r G_i'^o$ values are about the same for all of them when the pH is around 7, indicating that the availability of substrate determines its chances for reaction if the enzyme shows no structural preference. However, $\Delta_r G_i'^o$ for CO_3^{2-} deviates from the others significantly as pH value decreases, thus suggesting that CO_3^{2-} is the preferred substrate at acidic conditions.

We further examined the equilibrium methanol concentrations predicted by using the calculated constants of the reaction in comparison with values gleaned from references reported previously (Table 2). CO_3^{2-} was taken as the substrate for these calculations.

Table 3 Changes of enthalpy ($\Delta_r H_i' o$) and Gibbs free energy ($\Delta_r G_i'^o$) for methanol synthetic reaction at different conditions (unit of enthalpy and Gibbs free energy: kilocalories per mole).

I (mM)	pH	$\Delta_r H_i'^o$ T=4 °C	$\Delta_r G_i'^o$	$\Delta_r H_i'^o$ T=25 °C	$\Delta_r G_i'^o$	$\Delta_r H_i'^o$ T=37 °C	$\Delta_r G_i'^o$
50	2	−11.44	−28.39	−11.69	−29.66	−11.86	−30.38
	4		−15.19		−16.02		−16.19
	6		−3.02		−2.38		−2.00
	7		3.32		4.44		5.10
	8		9.66		11.26		12.19
100	2	−11.66	−27.88	−11.98	−29.09	−12.20	−29.78
	4		−15.19		−15.45		−15.59
	6		−2.51		−1.81		−1.40
	7		3.83		5.01		5.70
	8		10.17		11.83		12.79
225	2	−11.94	−27.20	−12.36	−28.34	−12.64	−28.98
	4		−14.52		−14.70		−14.79
	6		−1.84		−1.06		−0.60
	7		4.50		5.76		6.49
	8		10.84		12.58		13.59

Overall, the theoretical prediction generally agreed well with most of the reported yields of methanol, considering that most of the studies were conducted not under strictly controlled conditions for equilibrium evaluations. A systematic study for better agreement may be realized by using more sophisticated models and better controlled experiments. Nevertheless, the current results indicate a good reliability of the theoretical predictions.

With the equilibrium concentration of methanol in the order of micromolars, which means 3,000,000 t of water is needed for the production of 1 t of methanol in a batch reactor (for a methanol concentration of 100 μM), reduction of CO_2 at large scales is unrealistic. Theoretically, the reaction equilibrium can be shifted by controlling the pH, I, and temperature of the reaction media. Table 3 shows the results of such evaluations. As shown in Table 3, increasing pH and ionic strength will generally increase the $\Delta_r G_i'^o$ value of the reaction, but pH appeared to be a much more sensitive factor. Temperature demonstrated the least yet very interesting impact. For pH values below 6, $\Delta_r G_i'^o$ values decreased as T increases; however, $\Delta_r G_i'^o$ values increased with increase in T for pH values above 6. Overall, it shows that it is possible to shift the $\Delta_r G^o$ of the reaction from 5.10 kcal/mol at regular biological conditions to values as low as −30 kcal/mol, pointing to an unlimited equilibrium concentration of methanol. Even with a very mild change of pH from 7 to 6, it is possible to increase the equilibrium concentration of methnol by 10^5 times, reaching concentrations in the order of molars instead of micromolars.

Conclusions

There have been many successful examples in shifting the thermodynamics of enzymatic reactions for the syntheses of valuable chemicals by manipulating the reaction media. One vigorously studied area is the use of nonaqueous solutions for esterification reactions with hydrolases [27]. The current analysis showed that the enzymatic reduction of carbon dioxide is highly sensitive to the pH value of the reaction solution, and it is possible to shift the biological metabolic reactions to favor the synthesis of methanol by conducting the reactions at low pHs and ionic strength with elevated temperatures. However, such favorable conditions may not be easy to reach with currently available biocatalysts, as native enzymes catalyzing such reactions tend to be denatured and inactivated at acidic and elavated temperature conditions. Nevertheless, this study verified the feasibility of biocatalytic reduction of carbon dioxide, although enzyme engineering is proably needed to produce biocatalysts that can function well under the preferred conditions to capitalize the power of the biochemical reduction of carbon dioxide in the future.

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