[Review Paper]

Hydrogen and Methanol Formation Utilizing Bioprocesses

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Increases in the atmospheric levels of carbon dioxide and methane are the main causes of global warming, so measures to reduce the emissions of both gases are essential. A process converting carbon dioxide and methane into methanol was investigated combining three systems as follows, photolysis of water with solar energy, conversion of carbon dioxide and hydrogen into methane, and conversion of methane into methanol. Photolysis of water with solar energy and conversion of methane into methanol with methanotroph are described. Two different systems were attempted to achieve the photoinduced cleavage of water: photoinduced hydrogen evolution combined with Photosystem I and hydrogenase, and the combination of oxygen evolution and photoinduced hydrogen evolution systems. The oxygen evolution system contains grana and an electron carrier such as NAD and NADP. Photoinduced hydrogen evolution systems contain an electron donor, a photosensitizer, an electron carrier and a catalyst such as hydrogenase. Methane was converted into methanol by methane-oxidizing bacteria, methanotrophs which utilize methane monooxygenase to catalyze hydroxylation of methane to methanol. Using methanotrophs for methanol production, accumulation of methanol was not detected, because the methanol is subsequently oxidized by methanol dehydrogenase. The cell suspension of methanotrophs was treated by cyclopropanol as a selective inhibitor of methanol dehydrogenase, leading to extracellular methanol accumulation. The conditions of methane hydroxylation were optimized and semicontinuous methanol synthesis was achieved.

Keywords

Methanol formation, Hydrogen, Bioprocess, Methane oxidation

1. Introduction

Increases in the atmospheric levels of carbon dioxide and methane are the main causes of global warming, so measures to reduce the emissions of both gases are essential. One possibility is the development of processes for converting carbon dioxide and methane into methanol as shown in **Fig. 1**, which is a combination of three systems, the photolysis of water with solar energy, the conversion of carbon dioxide and hydrogen into methane, and the conversion of methane into methanol. This process has the multiple advantages of producing hydrogen gas as an alternate source of fuel, converting the global warming agent carbon dioxide into methane, and production of methanol, an economically useful industrial solvent, by the partial oxidation of methane.

Development of an industrial process for the photolysis of water would have a great impact on industrial technology and might start a new trend in the production of the alternate energy source hydrogen, the combustion of which produces water. Therefore, recycling of water would provide almost unlimited energy without causing environmental pollution. Such a process

would require a supply of reducing power, which could be provided by effective photoinduced charge separation, one of the most promising approaches to the use of solar energy. Utilization of solar energy is remarkably expanding, so application to reducing power may help to solve serious environmental problems.

The international agenda requires not only the creation of new renewable energy resources, but also solutions for serious global environmental problems. The methanogenesis process offers a method to control the both of carbon dioxide and methane emissions resulting from the burning of fossil fuels and fermentation of

Fig. 1 Processes for Converting Carbon Dioxide and Methane into Methanol

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Fig. 2 Photoinduced Cleavage of Water

organic compounds, and would also yield a useful product of economic importance, methanol.

This review describes the possible approaches to systems for methanol formation from carbon dioxide and methane.

2. Photolysis of Water with Solar Energy

Systems for the photoinduced cleavage of water can be divided into the hydrogen evolution system (Fig. 2(a)) and the oxygen evolution system (Fig. 2(b)), and both types have been investigated to establish effective photoinduced cleavage of water.

2.1. Photoinduced Hydrogen Evolution Combining Plant Photosystems and Hydrogenase

The photolysis system of green plants can be briefly summarized as splitting water under irradiation by sunlight (Fig. 2(a)). Green plants perform electronic oxidation with the aid of light energy and transport the electrons into the Calvin cycle through an electron transfer protein, ferredoxin (Fd). Hydrogenase removes the electrons from ferredoxin, so the electron flow results in the formation of hydrogen through the reduction of protons.

Photoinduced hydrogen evolution has been observed during the irradiation of a system containing chloroplasts, Fd, and hydrogenase. The amount of hydrogen evolved was about 10 mmol per mg-chloroplast. A total of 188 μmol of hydrogen was evolved after 2 h irradiation¹⁾. However, the hydrogen evolution rate decreases with irradiation time in any system, so continuous hydrogen evolution is difficult to obtain for a long time. The cause of the decrease is the simultaneous evolution of oxygen, which is a strong inhibitor of hydrogenase. Therefore, the oxygen must be removed to obtain hydrogen over a long period, for example by the addition of glucose and glucose oxidase, or a

hydrogenase could be developed which is unaffected by oxygen. Hydrogenase from *Clostridium pasteurianum* is more stable towards oxygen if fixed on glass beads²⁾. To avoid enzyme deactivation by oxygen, a flow system of compressed carrier gas (either helium or nitrogen) was used to continuously purge the reaction cuvettes and electrolysis cell³⁾. Simultaneous photoproduction of hydrogen and oxygen in the marine green algae. *Chlamydomonas* species (clone f-9), for example, has a steady state rate of hydrogen and oxygen production during irradiation with a stoichiometric ratio close to 2 : 1. The photolysis system is the most efficient molecular device for conversion of solar energy into chemical energy, but the light energy is exclusively stored in carbohydrates, so the photoconversion efficiency for hydrogen production is low^{4),5)}.

In aerobic phototrophic organisms, Photosystem II (PSII) captures sunlight and splits water into molecular oxygen, protons, and mobilized electrons. These electrons are further photoexcited by sunlight at Photosystem I (PSI), and are transferred from PSI to the electron carrier protein Fd. The photosynthetic reducing potential mediated by Fd is separated into at least two pathways, carbon dioxide fixation and hydrogen production. In carbon dioxide fixation, Fd passes the reducing potential to Fd-NADP⁺ reductase (FNR) to generate NADPH for the carbohydrate production through the Calvin cycle^{6),7)}. In hydrogen production, the reducing potential is passed to the enzyme hydrogenase for proton reduction^{8),9)}. The carbon dioxide fixation pathway is preferred to hydrogen production^{4),5)}.

A hardwired protein complex consisting of PSI and hydrogenase (hydrogenase/PSI complex) has recently been shown to act as a direct light-to-hydrogen conversion system. The directed electron transfer in the hydrogenase/PSI complex is simple and is expected to ensure a high quantum yield for hydrogen production.

Arrow indicates the pathway of electrons.

Fig. 3 Structure of Photosystem I and Hydrogenase

PSI, a multisubunit complex, is embedded in the thylakoid membrane¹⁰. The peripheral subunits, PsaC, PsaD, and PsaE form the Fd-binding site, where the photoexcited electrons are transferred from the terminal iron-sulfur cluster (Fb) to Fd (Fig. 3)¹¹⁻¹³. Hydrogenases are heterodimeric enzymes consisting of a large subunit containing the Ni-Fe active site and a small subunit accommodating three Fe-S clusters for intramolecular electron transfer^{14,15}. The distal Fe-S cluster located at the C terminus of the small subunit may serve as a junction for electron-donating or electron-accepting components^{14,15}. PsaE is fused to the C terminus of the small subunit of hydrogenase from *Ralstonia eutropha* (designated Hyd-PsaE)¹⁶. Hyd-PsaE exhibited *in vitro* self-assembly with PsaE-free PSI (designated as PSI*), and production of hydrogen from the Hyd-PsaE/PSI* complex was observed *in vitro* under light irradiation (0.58 $\mu\text{mol H}_2$ /mg chlorophyll/h).

2.2. Photoinduced Hydrogen Evolution with Viologen-linked Zinc Porphyrins

Oxygen and hydrogen evolution systems can be combined as shown in Fig. 2(b). The system contains grana, an electron carrier such as NAD and NADP, and hydrogenase.

Photoinduced hydrogen evolution systems containing an electron donor, a photosensitizer, an electron carrier and a catalyst have been studied extensively.

Fig. 4 Structure of Viologen Linked Porphyrins

Metalloporphyrins have been widely used as photosensitizers, and methylviologen is a common electron carrier. Recently, viologen-linked water-soluble zinc porphyrins ($\text{Zn-P}(\text{C}_n\text{V})_4$) were synthesized, which combine both photosensitizer and electron carrier in the same molecule, and have been applied to photoinduced hydrogen evolution. $\text{Zn-P}(\text{C}_n\text{V})_4$ with different methylene chain lengths ($n=2-5$) between porphyrin and viologen were synthesized¹⁷ and applied to photoinduced hydrogen evolution in a system containing electron donor- $\text{Zn-P}(\text{C}_n\text{V})_4$ -hydrogenase under steady state irradiation. The structure of the $\text{Zn-P}(\text{C}_n\text{V})_4$ is shown in Fig. 4. These compounds were synthesized as follows. The starting material, zinc meso-tetra(4-pyridyl) porphyrin (Zn-TpyP), was synthesized according to the literature^{18,19}. Zn-TpyP was then quaternized with excess α, ω -dibromoalkane at 130°C . The quaternized porphyrin and 100-200 fold molar excess of *N*-methyl-4,4'-bipyridyl iodide were refluxed in methanol, and the precipitate filtered and washed with methanol. Steady state irradiation used a 200 W tungsten lamp with wavelengths <390 nm removed by a L-39 filter.

Photoinduced hydrogen evolution was carried out with $\text{Zn-P}(\text{C}_n\text{V})_4$ with $n=2-5$ under steady state irradiation at 30°C . A sample solution containing the reduced form of NADP (NADPH), $\text{Zn-P}(\text{C}_n\text{V})_4$, and hydrogenase was deaerated by repeated freeze-pump-thaw cycles. Irradiation of the sample solution generated hydrogen with the time dependence as shown in Fig. 5. Clearly all $\text{Zn-P}(\text{C}_n\text{V})_4$ ($n=2-5$) can act as substrates of the enzyme hydrogenase, and as an electron carrier in the same molecule. The charge-separated species formed in the intramolecular electron transfer from porphyrin to viologen is so stable that the electron can move from the reduced viologen to the catalyst

hydrogenase. The hydrogen evolution rate strongly depends on the methylene chain length (n) of $\text{ZnP}(\text{C}_n\text{V})_4$. Within a certain range ($n = 2-4$), the hydrogen evolution rate decreases with increasing chain length. However, a higher hydrogen evolution rate was observed in the case of $\text{ZnP}(\text{C}_5\text{V})_4$. If the chain length is long enough, the conformational change of the molecule may allow the porphyrin ring to approach the viologen, so that the electron may transfer directly from the porphyrin ring to the viologen.

2.3. Application of Hydrophobic Porphyrin to Photoinduced Hydrogen Evolution in Water

Photoinduced intramolecular electron transfer occurs from the photoexcited porphyrin to viologen, so that effective hydrogen evolution is observed in viologen-linked porphyrin. The porphyrin moiety of these compounds is water-soluble, but this does not facilitate the electron transfer to viologen. The electron transfer mainly occurs via the photoexcited triplet state in the viologen linked water-soluble porphyrin, with a low probability of an electron transfer via the photoexcited singlet state, because the hydrophilic sphere is responsible for the loss of reorganization energy. Therefore, application of hydrophobic porphyrin is very important to achieve high efficiency in the photoinduced hydro-

gen reaction in water.

Hydrophobic porphyrin, triphenyl-1-pyridyl porphyrin, was used for the preparation of bis-viologen linked porphyrin²⁰. The structures of bis-viologen linked porphyrins are shown in Fig. 6. The second viologen of the bis-viologen moiety includes the $-\text{NH}_3^+$ group at the terminal of the structure. Therefore, the bis-viologen linked porphyrin is amphiphatic and shows a hydrophilicity gradient from the porphyrin moiety to the second viologen in the same molecule, that is, the porphyrin is hydrophobic, the first viologen is slight hydrophilic, and the second viologen is strong hydrophilic. The purpose of the hydrophilicity gradient is: (i) using hydrophobic porphyrin provides a highly efficient electron transfer to the linking viologen, and (ii) hydrogenase is strongly hydrophilic, so an electrostatic attraction between the anionic protein and the second cationic viologen ($-\text{NH}_3^+$ group) would facilitate the electron transfer from the reduced viologen to the hydrogenase.

Photoreduction of bis-viologen linked porphyrin under steady state irradiation was carried out at 30°C. The light source was a 100 W metal halide lamp with a cut-off filter for wavelengths less than 370 nm. The solution for photoreduction contained triethanolamine ($1.0 \text{ mol} \cdot \text{dm}^{-3}$) and bis-viologen linked porphyrin ($5.0 \mu\text{mol} \cdot \text{dm}^{-3}$) in $25 \text{ mmol} \cdot \text{dm}^{-3}$ Tris-HCl (pH 7.4), and oxygen was removed from the solution.

Photoinduced hydrogen evolution with bis-viologen linked porphyrin was carried out at 30°C. The light source used the same conditions as for photoreduction. The solution for photoinduced hydrogen evolution contained triethanolamine ($1.0 \text{ mol} \cdot \text{dm}^{-3}$), bis-viologen linked porphyrin ($5.0 \mu\text{mol} \cdot \text{dm}^{-3}$), and hydrogenase ($32 \mu\text{mol} \cdot \text{dm}^{-3}$) in $25 \text{ mmol} \cdot \text{dm}^{-3}$ Tris-HCl (pH 7.4), and oxygen was removed from the solution. The amount of evolved hydrogen was analyzed by gas chromatograph. Purification of hydrogenase used the literature procedure²¹.

Bis-viologen linked porphyrin was synthesized and the amphiphatic porphyrin analyzed by UV-vis spectrometry. Figure 7 shows the absorption spectra of the porphyrin. The absorption spectra of triphenyl-1-pyridyl porphyrin (non viologen binding) in DMF

Fig. 5 Photoinduced Hydrogen Evolution with Viologen Linked Porphyrins

Fig. 6 Structure of Bis-viologen Linked Porphyrin

Solid line is absorption spectrum of triphenyl-1-pyridyl porphyrin (non viologen binding) in DMF. Dashed line is absorption spectrum of bis-viologen linked porphyrin in DMF. Dotted line is absorption spectrum of bis-viologen linked porphyrin in $25 \text{ mmol} \cdot \text{dm}^{-3}$ Tris-HCl buffer.

Fig. 7 Absorption Spectra of Bis-viologen Linked Porphyrin and Triphenyl-1-pyridyl Porphyrin

and bis-viologen linked porphyrin in DMF are shown. The Soret band of porphyrin is in the same position as that of bis-viologen linked porphyrin (417 nm). This result shows that the porphyrin ring (π -electron orbital) is free from the electrostatic effect, that is, no aggregation between porphyrins with π -electron occurs in the case of bis-viologen linked porphyrin. The baseline spectrum increases for low wavelengths in bis-viologen linked porphyrin, indicating that bis-viologen linked porphyrins forms micelles. The spectrum of bis-viologen linked porphyrin in Tris-HCl buffer (pH 7.4) is shown. Triphenyl-1-pyridyl porphyrin (non viologen binding) does not dissolve in H_2O and Tris-HCl buffer (pH 7.4), but bis-viologen linked to porphyrin was dissolved. In addition, the increase in baseline is large compared with the spectrum in DMF, showing that the tendency to form micelles is stronger in hydrophilic solvents. The absorption spectra indicate that the bis-viologen linked porphyrin is amphipathic and shows a hydrophilicity gradient in one molecule. **Figure 8** shows the absorption spectra of reduced bis-viologen linked porphyrin under steady state irradiation (photoreduction) for 27 min. The baseline of the spectra refers to the sample solution containing triethanolamine ($1.0 \text{ mol} \cdot \text{dm}^{-3}$) and bis-viologen linked porphyrin ($5.0 \text{ } \mu\text{mol} \cdot \text{dm}^{-3}$) in $25 \text{ mmol} \cdot \text{dm}^{-3}$ Tris-HCl (pH 7.4), so no absorption is obtained at 0 min. Increased absorbance at 600 nm caused by reduced viologen was observed, indicating that the reduced viologen moiety accumulated during irradiation.

The reduced bis-viologen spectra in the region from

Fig. 8 Photoreduction of Bis-viologen Linked Porphyrin under Steady State Irradiation

500 nm to 700 nm are the same shape as viologens, such as methyl viologen. Most bis-viologen or bis-viologen linked porphyrins in the reduced form have a different spectral shape from reduced methyl viologen, and in the presence of aggregation the peak wavelength is 550 nm (dimer of dication form and monocation form), because the two reduced viologens are aggregated²²). The bis-viologen used in this study is amphipathic, so the reduced bis-viologen moiety did not aggregate. Therefore, the hydrophilicity gradient was still maintained during photoreduction.

The peak position of the Soret band of porphyrin (417 nm) does not change but decrease in intensity was observed, suggesting that reduced viologen is not strongly hydrophilic compared with oxidized viologen, so the hydrophilicity gradient was modified to slightly hydrophobic. However, the peak position of the Soret band does not change and aggregation of the bioviologen moiety did not occur. Therefore, the micelles were maintained in solution during photoreduction.

The time dependence of photoinduced hydrogen evolution with bis-viologen linked porphyrin and hydrogenase is shown in **Fig. 9**. The amount of evolved hydrogen increased according to irradiation time. The hydrophilicity decreases if the bis-viologen moiety is reduced, so that the rate of hydrogen evolution slowed gradually during irradiation. However, degradation of the porphyrin is not observed, showing that the bis-viologen linked porphyrin is stable under strong irradiation. Hydrophobic porphyrin is more stable to light than water-soluble porphyrin, but hydrophobic porphyrin is difficult to use for a variety of photoinduced elec-

Fig. 9 Photoinduced Hydrogen Evolution with Bis-viologen Linked Porphyrin and Hydrogenase

tron transfer reactions in aqueous solution. In this study, hydrophobic porphyrin was investigated for photoinduced hydrogen evolution in aqueous solution. This important result gives new insight into the design of photosensitizers for hydrogen evolution systems in aqueous solution.

3. Conversion of Carbon Dioxide into Methane and Methane into Methanol with *Methanotrophs*

Methanogens can produce methane by the reduction of carbon dioxide in the presence of hydrogen. Among the *Methanobacterium*, *thermoautotrophicum* is one of the most effective methane-forming bacterium species. Methane production using *methanogens* has been well studied and is already utilized in anaerobic sewage treatment. Here, methane hydroxylation using *methanotrophs* is described.

3.1. Methanol Production Using *Methanotrophs*

Methane-oxidizing bacteria (*Methanotrophs*) have the unique ability to utilize methane as the sole carbon source. *Methanotrophs* are aerobic bacteria that have metabolic pathways to oxidize methane to carbon dioxide (Fig. 10)^{23,24}. *Methanotrophs* utilize molecular oxygen to convert methane to methanol in the first step of the metabolic pathway catalyzed by a unique monooxygenase, methane monooxygenase (MMO), according to the following equation.

MMO has been purified from some *methanotrophs* and the enzymatic properties have been studied. However, the purified MMO is not stable and suitable for long-term use in methane hydroxylation. The bacterium is more stable and suitable for long-term use. However, no accumulation of methanol was detected using *methanotroph* for methanol production, because the methanol is subsequently oxidized by methanol dehydrogenase (MDH) in the same bacterium and finally completely oxidized to carbon dioxide. Therefore, MDH activity must be inhibited to utilize *methanotrophs* for methanol production. A high concentration of phos-

MMO: Methane monooxygenase, MDH: Methanol dehydrogenase, FADH: Formaldehyde dehydrogenase, FDH: Formate dehydrogenase, PQQ: Pyrroloquinoline quinine.

Fig. 10 Pathway of Methanol Metabolism in *Methanotrophs*

phate ions ($>80 \text{ mmol} \cdot \text{dm}^{-3}$) selectively inhibited MDH activity in the cells of *M. trichosporium* NCIB 11131²⁵, and a high concentration of phosphate buffer, pH 7.0, inhibited MDH activity in *M. trichosporium* OB3b. However, methanol accumulation decreased with increasing concentration of phosphate buffer, pH 7.0. These results show that a high concentration of phosphate ions inhibited both MDH activity and MMO activity in the cells. Methanol dehydrogenase contains pyrroloquinoline quinone (PQQ) as a cofactor. Therefore, cyclopropanol can be used to inhibit methanol dehydrogenase, because cyclopropanol reacts selectively with PQQ by the ring opening of cyclopropanol²⁶. *Methanotrophs* treated with cyclopropanol can be used as a methanol production catalyst. A cell suspension of *M. trichosporium* OB3b treated with cyclopropanol results in extracellular methanol accumulation²⁷.

3.2. Optimization of Methanol Biosynthesis

Methanol accumulation was achieved by using cells of *M. trichosporium* OB3b treated with cyclopropanol. However, the reaction ceased after approximately 3 h with a final methanol concentration below $2.96 \text{ mmol} \cdot \text{dm}^{-3} (\text{g dry cell})^{-1}$. The methanol production efficiency (ratio of methanol produced per methane consumption) was 2.90%. To optimize methanol accumulation by *M. trichosporium* OB3b, the effects of cell density, reaction temperature, concentration of sodium formate, concentration of phosphate buffer, pH 7.0, and concentration of cyclopropanol were examined²⁸. The optimum cell density for methanol accumulation by *M. trichosporium* OB3b was $34.6 \text{ mg dry cell dm}^{-3}$. Higher cell density provided no further increase in methanol accumulation. The optimum reaction temperature for methanol accumulation by *M. trichosporium* OB3b was 25°C . Higher temperatures resulted in decreased methanol accumulation, possibly caused by instability of the enzyme MMO. Methanol accumula-

Fig. 11 Effects of Cyclopropanol on MMO Activity and MDH Activity

tion increased with higher concentration of sodium formate, but did not increase above $14.3 \text{ mmol} \cdot \text{dm}^{-3}$ sodium formate.

Figure 11 shows the effects of cyclopropanol concentration on MMO activity and MDH activity measured by propene epoxidation and methanol consumption, respectively. MDH activity decreased with increasing cyclopropanol concentration. At a concentration of cyclopropanol of $6.18 \mu\text{mol} \cdot \text{dm}^{-3}$ in the reaction mixture, MDH activity decreased by 79.3%, whereas the loss of MMO activity was only 12%. The maximum methanol accumulation occurred with $0.67 \mu\text{mol} \cdot \text{dm}^{-3}$ cyclopropanol in the reaction mixture, with losses of MMO activity and MDH activity in *M. trichosporium* OB3b of 5.2 and 49.8%, respectively.

Methanol synthesis with these optimized reaction conditions continued for 100 h and produced methanol was $152 \text{ mmol} \cdot \text{dm}^{-3} (\text{g dry cell})^{-1}$, which is 51-fold higher than that of the conventional conditions (**Fig. 12**). In addition, the methanol production yield per consumed methane, which was consumed by the culture and methanol synthesis reaction, was 60.5% (compared to 2.9% under conventional conditions). Such selection of the culture conditions and reaction conditions maintained the methane hydroxylation activity in the cell for a long time and dramatically increased the efficiency of methanol production.

3.3. Semicontinuous Methanol Biosynthesis

Methanol synthesis using methanotroph cells treated with cyclopropanol stopped when the methanol concentration reached about $5 \text{ mmol} \cdot \text{dm}^{-3}$ under the optimized conditions. If excess substrates such as methane, di-

Fig. 12 Time Course of Methanol Production by *M. trichosporium* OB3b

oxygen and sodium formate were introduced, no further accumulation of methanol was observed. MMO activity in the *methanotroph* remained after incubation for 100 h. The MMO activity decreased to about 50% when $5 \text{ mmol} \cdot \text{dm}^{-3}$ methanol was present in the reaction mixture. Therefore, MMO was inhibited by the methanol product. Methanol must be removed from the reaction mixture to produce methanol continuously.

A semicontinuous methanol synthesis was developed to remove the methanol produced by *M. trichosporium* OB3b was carried out as follows²⁹). A standard 0.05 dm^{-3} capacity ultrafiltration cell was used as a semicontinuous stirred reactor. The sample solution containing cell suspension treated with cyclopropanol and sodium formate in phosphate buffer was introduced into the ultrafiltration cell attached to an ultrafilter. Methanol production was carried out in the ultrafiltration cell with methane at 30°C . After incubation for 90 min, the reaction mixture was filtrated by nitrogen pressure, leading to separation of the methanol from the cell suspension. The above procedure was repeated several times. **Figure 13** shows the changes in methanol concentration with time in the reaction mixture. Methanol synthesis was repeated five times for 6 h at a stationary rate of $3.17 \text{ mol} \cdot \text{dm}^{-3} \cdot \text{h}^{-1} (\text{g dry cell})^{-1}$ for 6 h. Thus, semicontinuous methanol biosynthesis could be achieved by removing the methanol produced.

3.4. Continuous Methanol Production Using Thermophilic Methanotrophs

The inhibition of MMO by methanol is a significant problem in any system for methanol production. To improve the methanol production system using *methanotrophs*, a new system for continual methanol production was proposed. Methanol production in the gas phase is a desirable method for methanol synthesis using *methanotrophs*. **Figure 14** shows a system for

Fig. 13 Semicontinuous Methanol Synthesis with *M. trichosporium* OB3b

Fig. 14 Proposed System of Methanol Production in the Gas Phase

methanol production in the gas phase at over the boiling point of methanol, so methanol product can be removed from the reaction mixture in gaseous form. Therefore, methanol does not accumulate and MMO is not inhibited by methanol. In addition, the methanol is easily collected from the reaction mixture using this system. The gas phase reaction will require thermophilic *methanotrophs* that preserve MMO activity at over the boiling point of methanol. Methanol production using thermophilic *methanotrophs* has not been possible because no species of thermophilic *methanotroph* has been isolated which can grow at over 64°C. Recently, the thermophilic *methanotroph* *Methylothermus thermalis* was described which can

grow at 67°C³⁰⁾. The development of a continuous methanol synthesis using this thermophilic *methanotroph* can be expected.

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要 旨

生物工学的手法による水素製造とメタンの部分酸化によるメタノール合成

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大気中における二酸化炭素やメタンの増加に伴う地球温暖化が懸念されており、二酸化炭素やメタンを固定化する技術の開発が求められている。その一つとして、生物工学的手法を利用して生産した水素を用いた二酸化炭素およびメタンからのメタノール生産プロセスがある。このプロセスは太陽エネルギーを用いる水の分解、水素を還元剤とする二酸化炭素からのメタン生産、メタンの部分酸化によるメタノールの生産からなる。このうち、本稿では、太陽エネルギーを用いる水の分解、およびメタンの部分酸化によるメタノールの生産プロセスの研究について述べる。太陽エネルギーを用いた水の分解では、光合成反

応中心とヒドロゲナーゼを用いて水素と酸素を生産するシステムと、光増感剤とヒドロゲナーゼを用いた光水素発生反応と植物のグラナを利用した還元剤のリサイクルを組み合わせたシステムの二つについて検討した。また、メタンの部分酸化によるメタノール生産では、酵素メタンモノオキシゲナーゼを有するメタン資化細菌を利用したメタノール生産法について検討した。メタノール酸化酵素をシクロプロパノールにより不可逆的に阻害することにより、メタン資化細菌を用いてメタノールを蓄積することが可能となった。さらに、メタノール生産の条件や半回分法によるメタノール合成を行った。

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