J. Microbiol. Biotechnol. (2016), 26(4), 717–724
http://dx.doi.org/10.4014/jmb.1601.01013

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Biological Methanol Production by a Type II Methanotroph *Methylocystis bryophila* S

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Received: January 8, 2016
Revised: January 30, 2016
Accepted: January 31, 2016

First published online
February 3, 2016

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S upplementary data for this paper are available on-line only at http://jmb.or.kr.

pISSN 1017-7825, eISSN 1738-8872

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Methane (CH_4) is the most abundant component in natural gas. To reduce its harmful environmental effect as a greenhouse gas, CH_4 can be utilized as a low-cost feed for the synthesis of methanol by methanotrophs. In this study, several methanotrophs were examined for their ability to produce methanol from CH_4; including *Methylocella silvestris*, *Methylocystis bryophila*, *Methyloferula stellata*, and *Methylomonas methanica*. Among these methanotrophs, *M. bryophila* exhibited the highest methanol production. The optimum process parameters aided in significant enhancement of methanol production up to 4.63 mM. Maximum methanol production was observed at pH 6.8, 30°C, 175 rpm, 100 mM phosphate buffer, 50 mM $MgCl_2$ as a methanol dehydrogenase inhibitor, 50% CH_4 concentration, 24 h of incubation, and 9 mg of dry cell mass ml^{-1} inoculum load, respectively. Optimization of the process parameters, screening of methanol dehydrogenase inhibitors, and supplementation with formate resulted in significant improvements in methanol production using *M. bryophila*. This report suggests, for the first time, the potential of using *M. bryophila* for industrial methanol production from CH_4.

Keywords: Biogas, greenhouse gases, methane, methanol production, *Methylocystis bryophila*

Introduction

Methane (CH_4) is currently receiving great attention owing to its severe impact on the environment as a greenhouse gas (GHG). CH_4 is a primary component of biogas (produced as a result of anaerobic digestion), including natural gas, shale gas, and landfill gases [1, 4, 6, 8]. Therefore, there is an urgent need to reduce the harmful environmental effects due to the release of CH_4. It is possible to capture CH_4 and transform it into useful products for sustainable development. Recent studies have suggested that biogas could be a potential feed for the synthesis of biofuels and industrially relevant organic chemicals [4, 35, 42]. Thus, the adoption of various alternative processes is encouraged to permit efficient utilization of CH_4 for the synthesis of valuable products, such as methanol. Compared with CH_4, methanol can be stored and transported more safely [6]. Methanol can be synthesized industrially from CH_4 via traditional chemical engineering approaches, or through the judicious use of microorganisms [34, 39]. Biological conversion of methanol from CH_4 has many industrial advantages over the traditional methods, including lower energy consumption, higher conversion efficiency, higher selectivity, and lower equipment costs [6, 24, 28, 37].

Biotransformation of CH_4 into methanol is carried out naturally by a group of microorganisms known as methanotrophs. Methanotrophs belong to the Proteobacteria group of prokaryotes. They can be found in diverse habitats, including soils, peat bogs, wetlands, sediments, lakes, fresh waters, and marine waters [1, 6, 23, 37]. Methanotrophs

play a key role in the natural carbon cycle and in the metabolism of CH_4 for the synthesis of biomass, releasing carbon dioxide (CO_2) as a final oxidation product [4, 23]. Methane monooxygenase (MMO) is the enzyme involved in the single-step conversion of CH_4 into methanol. Subsequently, methanol is metabolized to formaldehyde by methanol dehydrogenase (MDH), which is converted in turn to formate by formaldehyde dehydrogenase. Finally, formate is metabolized by formate dehydrogenase, releasing CO_2.

Methanotrophs can possess two types of MMOs: particulate (pMMO: associated with membranes) and soluble (sMMO). Methanotrophs are broadly classified into three types (I, II, and X) on the basis of the type of MMOs they contain. Type I methanotrophs produce only pMMO. They assimilate CH_4 through the ribulose monophosphate cycle, and include *Methylobacter*, *Methylomicrobium*, *Methylocaldum*, *Methylococcus*, *Methyloglobulus*, *Methylohalobius*, *Methylomarinum*, *Methylomonas*, *Methylosphaera*, *Methylosoma*, *Methylosarcina*, *Methylothermus*, and *Methylovolum*. Type II methanotrophs produce both pMMO and sMMO. They can assimilate CH_4 through the serine cycle, and include *Methylosinus*, *Methylocapsa*, *Methylocella*, *Methylocystis*, and *Methyloferula*. Type X methanotrophs possess certain properties of both Types I and II. *Methylococcus capsulatus* is an example of a Type X methantroph [1, 4, 8, 36].

Previous studies have examined the bioconversion of CH_4 to methanol in certain methanotrophic strains, including *Methylocaldum* sp. [35], *Methylococcus capsulatus* [7], *Methylosinus trichosporium* [9, 21, 22, 41], and *Methylosinus sporium* [33, 42]. In addition, various MDH inhibitors such as phosphate buffer, ethylenediaminetetraacetic acid (EDTA), sodium chloride (NaCl), and ammonium chloride (NH_4Cl) have been used to enhance methanol production. Methanol accumulates upon inhibition of its metabolism by MDH [3, 7]. In light of these studies, we sought to explore the feasibility and efficiency of methanol production using a range of different methanotrophs. In this study, we screened one Type I and three Type II methanotrophic species. The Type I species we tested was *Methylomonas methanica*. The Type II species tested were *Methylocella silvestris, Methylocystis bryophila,* and *Methyloferula stellata*. Optimization of the process parameters, screening of MDH inhibitors, and supplementation with formate resulted in significant improvements in methanol production using *M. bryophila*. Our results suggest that *M. bryophila*, a Type II methanotroph, may have potential to be developed for industrial methanol production.

Materials and Methods

Bacterial Strains and Growth Conditions

Methylocella silvestris DSM 15510, *Methylocystis bryophila* DSM 21852, *Methyloferula stellata* DSM 22108, and *Methylomonas methanica* DSM 25384 were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ). Cells were grown on nitrate mineral salt (NMS) medium containing 0.26 g/l KH_2PO_4, 0.716 g/l $Na_2HPO_4{\cdot}12H_2O$, 1.0 g/l KNO_3, 0.20 g/l $CaCl_2$, 1.0 g/l $MgSO_4{\cdot}7H_2O$, 0.38 g/l Fe-EDTA, and 0.026 g/l $Na_2MO_4{\cdot}2H_2O$. Trace element solution (1 ml) was added to the medium. The trace element solution contained 0.4 g/l $ZnSO_4{\cdot}7H_2O$, 0.015 g/l H_3BO_3, 0.05 g/l $CoCl_2{\cdot}6H_2O$, 0.25 g/l Na_2-EDTA, 0.02 g/l $MnCl_2{\cdot}4H_2O$, and 0.01 g/l $NiCl_2{\cdot}6H_2O$. The pH of the medium was adjusted to 7.0 using 1 M H_2SO_4 and 1 M NaOH. Millipore water (18 MΩ) was used in all the reagent preparations and for each measurement. All chemical reagents were of analytical grade and were purchased from Sigma-Aldrich (USA). Pure CH_4 (99.995%) was purchased (NK Co., Busan, Korea). Cells were cultivated in a 1 L flask (Duran-Schott, Germany) with an air-tight screw cap (Suba seal) containing 200 ml of NMS under an atmosphere containing 20% of CH_4. Cultures were incubated at 30°C on a rotary shaker (Lab Champion IS-971R, USA) at 200 rpm for 5 days. During cultivation, CH_4 was added every alternate day, to maintain its concentration at 20%. Cell growth was measured by determining the optical density (OD) at 595 nm with a UV/Vis spectrophotometer (Jenway Scientific, UK) [2, 13]. At the completion of incubation, cells were harvested by centrifugation (Gyrozen 1580 MGR, South Korea) at 11,200 ×*g* for 15 min at 4°C [5, 16, 25], and then washed twice with sodium phosphate buffer (20 mM, pH 7.0). Harvested cells were stored at 4°C until use [15]. The dry cell mass (DCM) was calculated after incubation for 48 h at 70°C. The specific growth rate (μ) of *M. bryophila* was determined using the method described previously [35]. Strains were maintained by subculturing them every 3−4 weeks, and they were stored at 4°C on NMS agar plates.

Methanol Production

The experiments assessing methanol production were carried out using 120 ml serum bottles (Sigma-Aldrich, USA) containing a total of 20 ml of the reaction volume, under batch culture conditions. The reaction mixture contained 10 μM of iron (II) sulfate (Fe^{2+}) solution, 5 μM of copper (II) sulfate (Cu^{2+}) solution, and free cells from 3 mg of DCM ml^{-1} as an inoculums in sodium phosphate buffer. Pure CH_4 was added to the head space volume until it completely displaced the air. The reaction mixture was incubated for 120 h at 30°C, with shaking at 150 rpm. All the values presented here are based on three different experiments. All our data have been subjected to standard deviation analysis.

Assessing the Effect of the Copper Ions on Growth and Methanol Production

To assess the effect of Cu^{2+} metal ion concentrations on growth and methanol production, cells batches were initially cultured in growth media (NMS) containing copper ions in the range of 1−10 μM. Subsequently, the respective batches of cells were placed in 20 mM phosphate buffer (pH 7.0) and used in the methanol

production experiments. In this set of copper-dependent experiments, only a portion of the headspace air volume (30%) was replaced with pure CH_4. These cultures were incubated for 24 h at 30°C with an agitation rate of 150 rpm.

Optimization of Process Parameters

Methanol production was evaluated using buffered solutions covering a range of pH values (5.0−8.5). The reaction mixture was incubated for 24 h at 150 rpm, with 30% CH_4 in the headspace as a feed. To evaluate the effect of incubation temperature and agitation rate on methanol production, the reaction mixture was incubated at optimal pH 6.8 for 24 h at different temperatures (25°C–40°C) and at different agitation rates (50–250 rpm).

Assessing the Effect of MDH Inhibitors and Na-Formate on Methanol Production

Phosphate buffer has been reported to inhibit MDH, leading to an accumulation of methanol. Initially, the effects of phosphate buffers (concentrations ranging 25–125 mM, pH 6.8) were tested under optimum conditions. For further enhancement of methanol production, incubations of 24 h using 30% CH_4 were performed that screened other MDH inhibitors, in combination with phosphate buffer (100 mM, pH 6.8). These other chemicals that we tested were $MgCl_2$ (5–75 mM), NaCl (10–100 mM), NH_4Cl (5–75 mM), and EDTA (0.05–1.5 mM). A possible supportive role of Na-formate (concentration range 25–150 mM) for cofactor (NADH) regeneration during methanol production was also evaluated.

MDH Activity Measurements

MDH activity was measured spectrophotometrically by monitoring the change in absorbance at 600 nm induced by phenazine methosulfate-mediated reduction of 2,6-dichlorophenol-indophenol (DCPIP) [14]. The assay was performed in a 1 ml reaction volume containing $CaCl_2$ (10 mM), NH_4Cl (45 mM), phosphate buffer (0.3 M, pH 7.5), the whole cell supernatant (5 mg of DCM), DCPIP (0.13 μM), and phenazine methosulfate (3.3 μM).

Effect of Methane Concentration and the Inoculum Load on Methanol Production

The methanol production profile was evaluated at different concentrations of CH_4 (10–50% in the headspace) for up to 120 h of incubation under optimum conditions (pH 6.8, 30ºC, and 175 rpm). The effect of altering the inoculum load was investigated by adding DCM in the ranges of 1.5–9.0 mg/ml to the reaction mixture under optimum conditions, containing 30% of CH_4 as a feed.

Analytical Methods

Methanol concentration was analyzed using an alcohol oxidase assay (Sigma-Aldrich), instead of the $KMnO_4$ method described previously [40]. Methanol concentration was also analyzed using a gas chromatography (GC) system (Agilent 7890A) equipped with an HP-5 column (Agilent 19091J-413) connected to an FID detector. Helium was used as a carrier gas along with H_2 at a makeup flow of 25 ml/min and air (300 ml/min). The oven temperature was initially maintained at 35°C for 5 min. Following this, the temperature was raised at the rate of 5°C/min to 150°C, and subsequently at a rate of 20°C/min to 250°C. Injector and detector temperatures were set at 220°C and 250°C, respectively.

Results

Screening of Methanotrophs for Methanol Production

One Type I methanotroph (*Methylomonas methanica*) and three Type II methanotrophs (*Methylocella silvestris*, *Methylocystis bryophila*, and *Methyloferula stellata*) were screened for their methanol production potential in NMS medium. Methanol production was observed in the range of 0.01−1.00 mM (Table S1). Among these strains, *M. bryophila* gave the highest methanol production, so it was selected for further production optimization.

Influence of Copper Ions on Growth and Methanol Production

The growth of *M. bryophila* was significantly influenced by the presence of Cu^{2+} metal ions, in the concentration range of 1−10 μM in the NMS growth media (Table 1). Growth rates (μ) between 0.021 and 0.031 h^{-1} were observed. When Cu^{2+} was excluded from the medium as a control, a rather low μ value of 0.017 h^{-1} was obtained, and the methanol production reached 1.00 mM. On the other hand, methanol production was always higher in the presence of Cu^{2+}. Both maximum μ (0.31 h^{-1}) and methanol production (1.18 mM) were observed at 2.5 μM of Cu^{2+}.

Effect of Process Parameters on Methanol Production

The process parameters (pH, incubation temperature, and agitation rate) were optimized for methanol production (Fig. 1). At different pH values, the methanol production was observed in the range of 0.29−1.22 mM (Fig. 1A). In the pH range between 6.5 and 8.5, methanol production was steadily in the range of 1.01−1.22 mM, whereas acidic pH values of 5.0, 5.5, and 6.0 resulted in lower methanol

Table 1. Effect of copper ions on methanol production and growth of *M. bryophila*.

Cu (μM)	Specific growth rate (h^{-1})	Methanol production (mM)
0	0.017 ± 0.07	1.00 ± 0.06
1	0.026 ± 0.15	1.09 ± 0.07
2.5	0.031 ± 0.18	1.18 ± 0.08
5	0.027 ± 0.16	1.12 ± 0.07
10	0.021 ± 0.15	1.04 ± 0.07

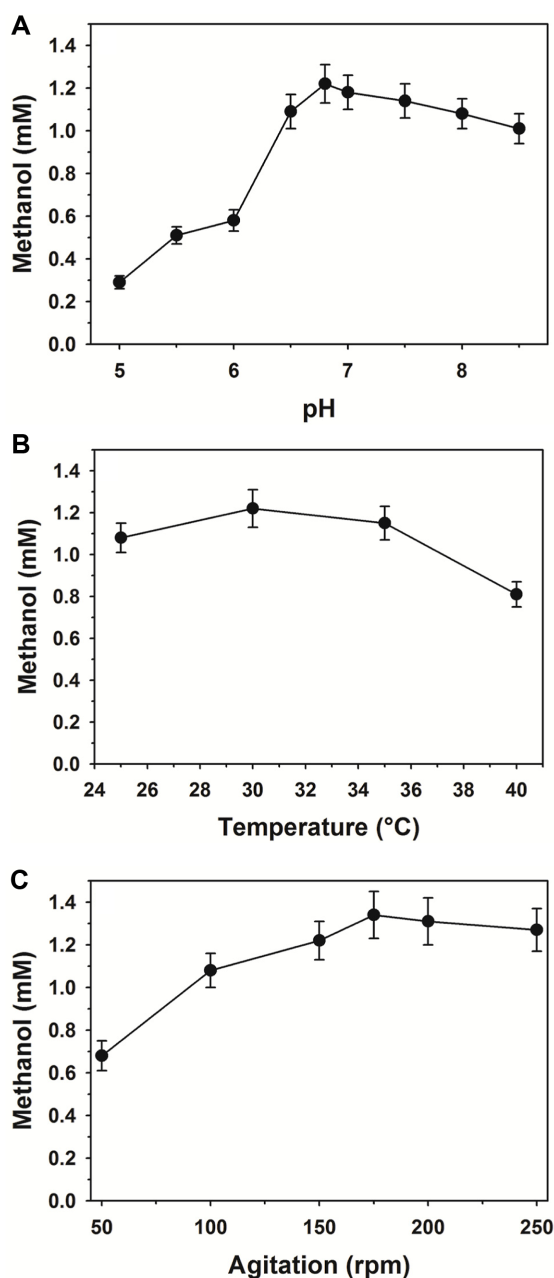

Fig. 1. Effect of process parameters (pH (**A**), temperature (**B**), and agitation rate (**C**)) on methanol production by *M. bryophila*.
Under batch culture experiments, methanol production was performed in a serum bottle (120 ml) with a total working volume of 20 ml, containing 30% CH_4 in the headspace as feed. The reaction mixture consisted of Cu^{2+} (5 μM), Fe^{2+} (10 μM), and 3 mg of DCM ml^{-1} (inoculum) in phosphate buffer (20 mM), incubated for 24 h. Each value represents the mean of triplicate measurements that varied from the mean by no more than 10%.

production. The optimum temperature for methanol production was 30°C, producing a maximum concentration of 1.22 mM (Fig. 1B). As agitation rate increased from 50 rpm to 175 rpm, an increase in methanol production was observed (0.68 mM to 1.34 mM, Fig. 1C). At higher agitation rates up to 250 rpm, methanol production was quite stable, with a value of 1.27 mM. Overall, the maximum methanol production (1.34 mM) was observed at pH 6.8 after 24 h incubation at 30°C and 175 rpm.

Table 2. Effect of phosphate buffer concentration and MDH inhibition on methanol production.

Phosphate buffer (mM)	Methanol production (mM)	Relative MDH inhibition (%)[a]
25	1.39 ± 0.12	3.2 ± 0.07
50	1.50 ± 0.14	9.8 ± 0.10
75	1.64 ± 0.15	15.5 ± 0.14
100	1.88 ± 0.16	19.2 ± 0.16
125	1.76 ± 0.16	21.9 ± 0.17

[a]MDH activity in 20 mM phosphate buffer was considered as 100%, with methanol production of 1.22 ± 0.09 mM.

Effect of MDH Inhibitors

Inhibition of MDH is a primary route to reduce the further metabolism of methanol. Initially, the effect of phosphate buffer concentration was evaluated at pH 6.8 (Table 2). As the phosphate buffer concentration increased, up to 100 mM, a significant improvement in methanol production was observed, from 1.34 to 1.88 mM. MDH activity assays demonstrated that this enhanced production in the presence of 100 mM phosphate buffer was directly related to the relative inhibition of MDH activity by 19.2% (80.8% activity remained). Furthermore, an increase in the buffer concentration to 125 mM resulted in higher relative MDH inhibition of 21.9%, but a lower methanol production of 1.76 mM. Thereafter, other MDH inhibitors were screened to enhance methanol production, in combination with phosphate buffer 100 mM (Table S2). The maximum methanol production of 2.21, 2.05, 1.96, and 2.04 mM was observed at optimum inhibitor concentration of 50 mM $MgCl_2$, 50 mM NaCl, 10 mM NH_4Cl, and 0.1 mM EDTA, respectively. The optimum concentration of these inhibitors, in combination with phosphate buffer (100 mM), resulted in higher MDH inhibition of 34.2%, 31.8%, 26.2%, and 48.4%, respectively (Fig. 2). Overall, these other MDH inhibitors, in combination with phosphate buffer, greatly enhanced methanol production.

Effect of Na-Formate

Supplementation of the production media with Na-formate resulted in an enhancement of methanol production (Fig. 3).

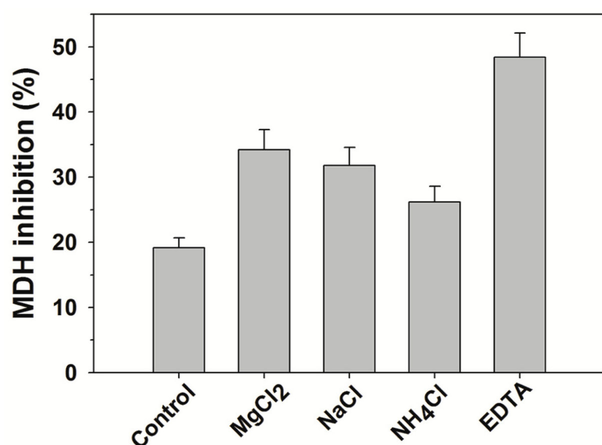

Fig. 2. Relative MDH inhibition (%) by different inhibitors in phosphate buffer.

The 100% activity was defined as the initial MDH activity in 20 mM phosphate buffer (pH 6.8). The relative decrease in MDH activity was measured at optimum inhibitor concentrations of $MgCl_2$ (50 mM), NaCl (50 mM), NH_4Cl (10 mM), and EDTA (0.1 mM), in combination with 100 mM of phosphate buffer. Each value represents the mean of triplicate measurements that varied from the mean by no more than 10%.

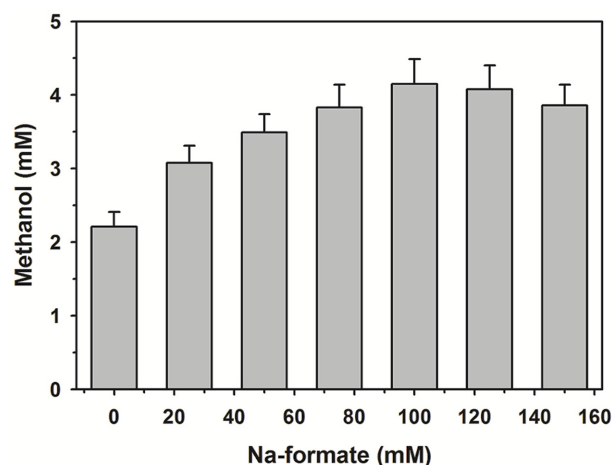

Fig. 3. Effect of Na-formate on methanol production.

Methanol production was evaluated at different Na-formate concentrations in the range of 20−120 mM, in phosphate buffer (100 mM, pH 6.8) containing $MgCl_2$ (50 mM) as an MDH inhibitor and 30% of CH_4 as a feed, incubated for 24 h. Each value represents the mean of triplicate measurements that varied from the mean by no more than 10%.

An increase in methanol production from 2.21 to 4.15 mM was obtained by increasing the Na-formate concentration to 100 mM, which was the optimal concentration for methanol production. Further increase of Na-formate concentration to 150 mM resulted in lower methanol production (3.86 mM).

Effect of Methane Concentration

By altering the feed concentration of CH_4 in the headspace of the culture vessel, significant enhancements in methanol production could be produced. These enhancements were only seen during the first 6 to 24 h of the incubation period. Methanol production decreased thereafter, up to the 120 h limit of our incubation periods (Fig. 4). Methanol production increased with increasing feed concentrations of CH_4, from 10% to 50%. The greatest proportionate increase in methanol production occurred when CH_4 was increased from 10% to 30% under optimum conditions; the production increased from 2.22 mM to 4.15 mM. Further increasing the CH_4 concentration to 50% only resulted in a slightly higher methanol production of 4.24 mM.

Effect of Inoculum Load

The effect on methanol production of different *M. bryophila* inoculum loads was evaluated (Fig. 5). An increase in the inoculum loading from 1.5 to 9.0 mg of DCM ml^{-1} increased the methanol production from 3.04 mM to 4.53 mM. Here, higher inoculum loads (6 and 9 mg of DCM ml^{-1}) resulted in a more rapid methanol production, compared with lower inoculum loads (1.5 and 3.0 mg of DCM ml^{-1}).

Discussion

The suitability of various alternative energy sources,

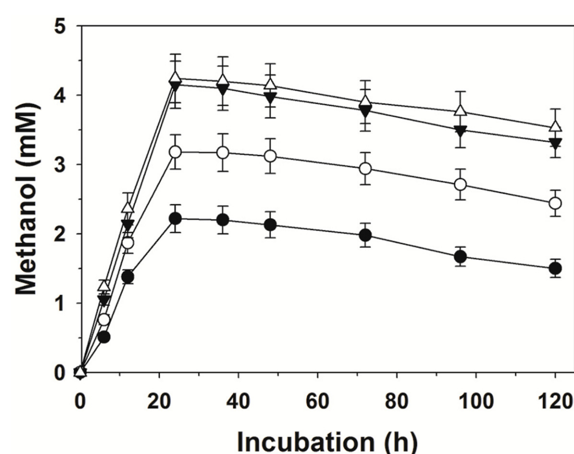

Fig. 4. Effect of CH_4 concentration on methanol production.

Methanol production was performed in phosphate buffer (100 mM, pH 6.8) containing $MgCl_2$ (50 mM) as an MDH inhibitor, and incubated up to 120 h in the presence of different feed CH_4 concentrations: ● (10%), ○ (20%), ▼ (30%), and △ (50%). Each value represents the mean of triplicate measurements that varied from the mean by no more than 10%.

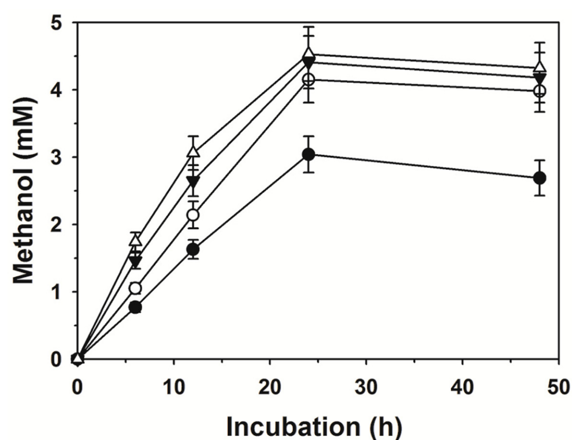

Fig. 5. Effect of inoculum on methanol production.
Methanol production was performed in phosphate buffer (100 mM, pH 6.8) containing $MgCl_2$ (50 mM) as an MDH inhibitor, incubated up to 48 h with different inoculum loads (mg of DCM ml^{-1}): ● (1.5), ○ (3.0), ▼ (6.0), and △ (9.0). Each value represents the mean of triplicate measurements that varied from the mean by no more than 10%.

such as alcohols, hydrogen gas, and CH_4, has been widely evaluated [3, 10−12, 17, 18, 20, 26, 27, 29, 30, 32, 34, 43]. Unlike hydrogen gas, CH_4 has received significant attention owing to its harmful environmental effects as a GHG. To minimize the adverse impact of CH_4, it has been suggested that it can be diverted for the production of methanol, which is a starting material for the synthesis of valuable organic compounds. Methanotrophs can use CH_4 to produce methanol efficiently [4, 34, 37]. In spite of this, only a handful of methanotrophs, such as *Methylosinus trichosporium* and *Methylosinus sporium* strains, have been characterized and shown experimentally to be able to produce methanol from CH_4 [9, 33, 42]. In this study, we evaluated methanol production by both Type I and Type II methanotrophs. We tested the Type I methantroph *Methylomonas methanica*, and the Type II methanotrophs *Methylocella silvestris, Methylocystis bryophila,* and *Methyloferula stellata*. Among these strains, *M. bryophila* produced the most methanol. To the best of our knowledge, ours is the first study to demonstrate the production of methanol from CH_4 using *M. bryophila*.

The growth of methanotrophs is highly influenced by metal ions such as Cu^{2+} in the NMS growth medium [22, 38]. The maximum μ of *M. bryophila* was enhanced about 1.8-fold in the presence of 2.5 μM Cu^{2+}, compared with a μ of 0.017 h^{-1} in the absence of any Cu^{2+}. During methanol production, process parameters such as pH, temperature, and agitation rate were critical for the well-studied strains *M. tricosporium* [3, 9] and *Methylosinus sporium* [33, 42]. The optimum process parameters, resulting in maximum methanol production, for *M. bryophila* were observed at pH 6.8, 30°C, and 175 rpm. Inhibition of MDH can enhance methanol production by reducing methanol consumption by the CH_4 metabolic pathway. A 1.4-fold increase in methanol production was observed at a concentration of 100 mM of phosphate buffer, compared with the control (phosphate buffer, 20 mM). Here, phosphate buffer (100 mM) resulted in a higher MDH inhibition of 19.2%, compared with the 9.3% inhibition reported for *Methylosinus trichosporium* OB3b [9]. Supplementation of another MDH inhibitor, $MgCl_2$ (50 mM), in combination with phosphate buffer (100 mM) gave a maximum methanol production of 2.21 mM. Interestingly, greater inhibition of MDH activity did not result in higher methanol production. Similar results were reported for pure *Methylosinus trichosporium* OB3b [9], and mixed cultures of *Methylosinus sporium* NCIMB 11126, *Methylosinus trichosporium* OB3b, and *Methylococcus capsulatus* Bath [7]. Here, higher MDH inhibition might be not suitable, due to the requirement for cofactor regeneration during higher MMO activity [21]. On the other hand, a significant enhancement (1.9-fold) in methanol production was observed by *M. bryophila* when 100 mM Na-formate was supplied in the production mixture, using 30% of CH_4 as feed. According to previous studies, the enhanced production seen upon addition of Na-formate in the Type II methanotroph *Methylosinus trichosporium* NCIB1113 is primarily due to the high regeneration of the NADH cofactor [19, 21]. We speculate that a similar mechanism is at work for *M. bryophila* in our study.

In previous studies, differing feed CH_4 concentrations resulted in variable methanol yields, depending on the type of methanotrophic strains, and on optimum production conditions [3, 28, 31, 33]. Here, *M. bryophila* produced methanol efficiently during an incubation period of 24 h, in contrast to the longer incubations times of 27, 40, and 48 h reported for *Methylosinus sporium* KCTC 22312 [42], *Methylosinus trichosporium* OB3b [3], and *Methylocaldum* sp. 14B [35], respectively. The decreasing methanol production in *M. bryophila* thereafter, up to 120 h of incubation, might be due to the incomplete inhibition of MDH activity, which allowed further metabolism of methanol. The effect of inoculum loads on methanol production was quite variable in *Methylosinus sporium* and *Methylosinus trichosporium* strains [3, 33]. A significant increase in methanol production (1.5-fold) was observed in *M. bryophila* upon increasing the cell biomass in the inoculum loads from 1.5 to 9.0 mg of DCM ml^{-1}. Inoculum loads needed for enhanced methanol

production were reported as being much higher (105 mg of DCM ml^{-1}) for *Methylosinus sporium* B2121, with 0.35 mM of maximum methanol production [31]. Here, *M. bryophila* had almost a 13-fold higher methanol production than *Methylosinus sporium* B2121, and did so at much lower inoculum of 9.0 mg of DCM mL^{-1} using pure CH_4. Moreover, our work has resulted in much higher methanol production than the maximum production of 0.71 mM and 0.02 mM reported for *Methylosinus sporium* KCTC 22312 [42] and *Methylosinus trichosporium* IMV 3011 [41] grown on synthetic biogas feed.

In conclusion, biological methanol production by methanotrophs seems a suitable and environmentally friendly approach to reduce GHGs such as CH_4. *M. bryophila*, a Type II methanotroph, produced methanol in much higher concentration than other previous reports, for example using *Methylosinus sporium* strains and *Methylosinus trichosporium* IMV 3011. This is the first report of methanol production from CH_4 using *M. bryophila*. Our results demonstrate that *M. bryophila* can be used as a potential methanol producer. Furthermore, methanol production using biogas, originating from anaerobic digestion, may be a promising low-cost approach.

Acknowledgments

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (NRF-2013R1A1A2012159 and NRF-2013R1A1A2007561). This work was also supported by the 2015 KU Brain Pool Fellowship of Konkuk University.

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