Improvement in methanol production by regulating the composition of synthetic gas mixture and raw biogas

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Improvement in methanol production by regulating the composition of synthetic gas mixture and raw biogas

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HIGHLIGHTS

- Utilization of raw biogas as a low cost feed originated from anaerobic digestion is evaluated.
- The regulation of gas composition significantly improves methanol yield from raw biogas.
- Supplementation of H_2 into raw biogas increases methanol production up to 3.5-fold.
- Whole cell immobilization of *Methylosinus sporium* results in higher methanol production.

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ABSTRACT

Raw biogas can be an alternative feedstock to pure methane (CH_4) for methanol production. In this investigation, we evaluated the methanol production potential of *Methylosinus sporium* from raw biogas originated from an anaerobic digester. Furthermore, the roles of different gases in methanol production were investigated using synthetic gas mixtures of CH_4, carbon dioxide (CO_2), and hydrogen (H_2). Maximum methanol production was 5.13, 4.35, 6.28, 7.16, 0.38, and 0.36 mM from raw biogas, CH_4:CO_2, CH_4:H_2, CH_4:CO_2:H_2, CO_2, and CO_2:H_2, respectively. Supplementation of H_2 into raw biogas increased methanol production up to 3.5-fold. Additionally, covalent immobilization of *M. sporium* on chitosan resulted in higher methanol production from raw biogas. This study provides a suitable approach to improve methanol production using low cost raw biogas as a feed containing high concentrations of H_2S (0.13%). To our knowledge, this is the first report on methanol production from raw biogas, using immobilized cells of methanotrophs.

1. Introduction

Primary dependence on the fossil fuels as energy sources has received major environmental concern over the past few decades in addition to their limited natural reserves. Therefore, the feasibility of various alternative energy sources including hydrogen (H_2), methane (CH_4), and methanol is widely evaluated (Fei et al., 2014; Hwang et al., 2014; Patel et al., 2014a, 2015, 2016a,b; Pierie et al., 2015). Additionally, CH_4 and carbon dioxide (CO_2) also play a key role in the environmental impact, as potential greenhouse gases (GHGs). However, transformation of these gases into useful energy products is urgently needed to overcome the problems associated with both alternative fuels and GHGs for sustainable development (Shamsul et al., 2014; Strong et al., 2015; Patel et al., 2016b). Methanol production as an alternative energy source from GHGs seems a feasible process (Ganesh, 2014; Ge et al., 2014). Nevertheless, conversion of CH_4 into useful chemicals is challenging (Lunsford, 2000). Different approaches include studies on methanol production, but an environmental friendly process seems more attractive and has received considerable attention (Hwang et al., 2014; Shamsul et al., 2014; Trop et al., 2014; Strong et al., 2015). Biological production of methanol is recognized as a cost effective process, which can be efficiently carried out at ambient conditions as compared to the chemical processes (Duan et al., 2011; Andersson et al., 2014; Yoo et al., 2015; Mardina et al., 2016; Patel et al., 2016b). Methanotrophs are known for the utilization of both CH_4 and CO_2 as carbon sources for the production of methanol through a complex metabolic

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pathway involving different enzymes such as methane monooxygenases (MMOs), methanol dehydrogenase (MDH), and formaldehyde and formate dehydrogenases (Xin et al., 2004a; Fei et al., 2014; Kalyuzhnaya et al., 2015; Sigdel et al., 2015). Methanotrophs are mostly aerobic in nature and classified as Gram negative (Proteobacteria). They are abundant in naturally diverse environmental habitats (Murrell, 1992; Op den Camp et al., 2009). MMOs are broadly classified into three groups: i) type I [particulate MMO (pMMO)], ii) type II [pMMO and soluble MMO (sMMO)], and iii) type X (possess common properties, to some extent, of both type I and II). Expression of MMOs is highly influenced by concentrations of metals in the growth medium, including copper and iron (Mardina et al., 2016; Patel et al., 2016a,b). MMOs are the primary enzymes involved in the conversion of CH_4 to methanol, whereas MDH and formaldehyde- and formate dehydrogenases are required for the conversion of CO_2 to methanol (Xin et al., 2007). However, methanol production using methanotrophs is still challenging because of difficulty of accumulating methanol in the presence of the remainder of the CH_4 oxidation pathway, and the need of reducing equivalents for MMO activity (Yoo et al., 2015; Patel et al., 2016b).

The utilization of pure methane to produce methanol is widely studied and considered a costly process (Mehta et al., 1991; Kim et al., 2010; Pen et al., 2014; Hwang et al., 2015; Yoo et al., 2015). Very few reports are available on the combined utilization of CH_4 and CO_2 gases for methanol production (Fei et al., 2014; Xin et al., 2004a; Yoo et al., 2015; Sheets et al., 2016). Thus, low cost feed such as biogas can be utilized for better economic and environmental benefits. In biogas, CH_4 exists in the mixture with CO_2 and H_2 in addition to traces of NH_3 and H_2S originating from the methanogenic anaerobic digester (Yoo et al., 2015). CH_4 and CO_2 in biogas may compete with each other during the metabolism for methanol production (Xin et al., 2004a). In addition, a significant influence of H_2 can be expected on methanol production (Mountfort et al., 1990; Patel et al., 2016b). The effective role of these gas mixtures is not evaluated extensively during the biosynthesis of methanol compared to that of pure CH_4 (Mehta et al., 1991; Duan et al., 2011; Hwang et al., 2015). Therefore, the feasibility of utilizing the synthetic gas mixture or biogases as low cost feed will be a suitable approach. Nevertheless, no report is available on the role of the actual anaerobic mixture of gases in methanol production. Previously, a mixed response of CO_2 and H_2 was observed during methanol production as well as of CH_4 in separate studies (Mountfort et al., 1990; Xin et al., 2004a,b; Yoo et al., 2015). In this study, we evaluated the potential use of raw biogas obtained from the methanogenic anaerobic digester as a low cost feed for methanol production by *Methylosinus sporium*. Furthermore, the role of different gas combinations as synthetic gas mixtures, including CH_4, CO_2, and H_2, was determined, to improve methanol production and efficient utilization of raw biogas. These results demonstrate a suitable approach for the efficient utilization of raw biogas originated from the waste treatment plant. Finally, indirect utilization of biowaste materials for methanol production led to improved process economy and waste management, which acts as an advantage by significantly reducing GHGs.

2. Materials and methods

2.1. Organism and growth conditions

M. sporium DSMZ 17706 (German Collection of Microorganisms and Cell Cultures) was grown on nitrate mineral salt (NMS) medium containing (g/L) KH_2PO_4 (0.26), $Na_2HPO_4{\cdot}12H_2O$ (0.716), KNO_3 (1.0), $CaCl_2$ (0.20), $MgSO_4{\cdot}7H_2O$ (1.0), Fe-EDTA (0.38), and $Na_2MO_4{\cdot}2H_2O$ (0.026). A trace element solution (1 mL) was added, containing (g/L) $ZnSO_4{\cdot}7H_2O$ (0.40), H_3BO_3 (0.015), $CoCl_2{\cdot}6H_2O$ (0.050), Na_2-EDTA (0.250), $MnCl_2{\cdot}4H_2O$ (0.020), and $NiCl_2{\cdot}6H_2O$ (0.010). Medium pH was adjusted to 7.0 using 1 M H_2SO_4 or 1 M NaOH. The strain was maintained by sub-culturing, and the possibility of contaminants was checked on an R2 agar plate as described previously (Patel et al., 2016a). Cultivation of cells was performed in a 1 L flask (Duran-Schott, Germany) with an air tight screw cap (Suba seal) containing 200 ml of NMS under an atmosphere of CH_4 (30%) and incubated at 30 °C on a rotary shaker (Lab Companion IS-971R, USA) at 200 rpm for 7 days. During this cultivation, 30% of CH_4 was added on each alternate day of incubation. Cell growth was measured by determination of optical density (O.D.) at 600 nm using a UV/Vis spectrophotometer (Patel et al., 2016c). Full grown cells were harvested by centrifugation (Gyrozen 1580 MGR, South Korea) at 10,000 rpm for 15 min at 4 °C and washed twice with phosphate buffer (20 mM, pH 7.0). These cells were stored at 4 °C for further use. Dry cell mass (DCM) was calculated after incubation for 48 h at 70 °C. Millipore water (18 MΩ cm) was used in all of the reagent preparations and measurements. All chemicals used were of analytical grade and purchased from commercial sources. All pure gases used in this study were obtained from NK Co. Ltd., Busan, South Korea. Raw biogas was obtained from an anaerobic digester (Seoul, South Korea).

2.2. Methanol production

Batch culture experiments were performed in a 120-mL serum bottle (Sigma-Aldrich, USA). A total of 20 mL reaction volume was carried out for the production of methanol in phosphate buffer (100 mM) containing 20 mM of $MgCl_2$, 10 μM of Fe(II), 5 μM of Cu(II), and free cells (3 mg of DCM·mL^{-1}) as an inoculum. The different percentage of pure, synthetic, or raw biogas was filled with replacement of head space air and incubated at 30 °C with shaking at 150 rpm for up to 96 h. The conversion yield (%) of raw biogas or synthetic gas mixture to methanol was determined by dividing the moles of methanol produced by the moles of CH_4 consumed in the feed.

2.2.1. Raw biogas

A raw mixed biogas originated from the municipal waste treatment anaerobic digester plant was obtained (Seoul, South Korea). Different dilutions of the raw biogas mixture were performed to maintain the CH_4 concentration in the range of 10–50% as a feed for methanol production over a 96 h period of incubation.

2.2.2. Synthetic gas mixture

To evaluate the specific effect of different gases on methanol production, different combinations of synthetic mixed gases were prepared from the pure gases including: (i) CH_4 and CO_2; (ii) CH_4 and H_2; and (iii) CH_4, CO_2, and H_2. A fixed concentration of CH_4 (20%) in different ratios was used as a feed. In these mixed gases, CO_2 and H_2 concentrations were 5–30 and 1–15%, respectively.

2.2.3. Pure CO_2 and gas mixture (CO_2 and H_2)

The biotransformation potential of *M. sporium* from pure CO_2 to methanol was evaluated in the concentration range of 5–40% under optimum conditions and up to 60 h of incubation. In addition, the influence of MDH inhibitors on phosphate buffer and $MgCl_2$ were analyzed during methanol production from pure CO_2 (10%). Furthermore, the effect of H_2 (1–10%) supplementation during methanol production from CO_2 (10%) was evaluated in different ratios.

2.2.4. Effect of the inoculum

To increase methanol production from raw biogas (20% of CH_4) or a synthetic gas mixture (20:10:10 of CH_4:CO_2:H_2), the DCM in the range of 1.5–18 mg·mL^{-1} of reaction mixture was evaluated under optimum conditions.

2.2.5. Effect of the H_2 supplementation into raw biogas

An influence of H_2 (10%) supplementation in raw biogas was evaluated to improve the methanol at inoculums of 3 mg of DCM·mL^{-1} over a period up to 96 h of incubation.

2.3. Methanol production by immobilized whole cells

M. sporium whole cells immobilized on Amberlites (XAD-2, XAD-4, and XAD-7HP) and Duolite A-7 were used for methanol production from raw biogas containing 20% of CH_4 as a feed for incubation up to 120 h as described previously (Patel et al., 2014b, 2016b,c).

2.4. Analytical methods

Methanol concentration was analyzed via enzymatic oxidation of methanol by alcohol oxidase (Sigma-Aldrich), as described previously (Patel et al., 2016a,b). In addition, methanol concentration was analyzed using a gas chromatography (GC) system (Agilent 7890A) equipped with an HP-5 column (Agilent 19091J-413) connected with 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, and then 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 and 250 °C, respectively. The residual gas composition (CH_4, CO_2, and H_2) was analyzed using a GC system (Agilent 7890A) equipped with a Carboxen 1010 Plot fused silica capillary column (Supelco, Bellefonte, PA) connected with thermal conductivity detector. N_2 was used as a carrier gas. The temperatures of oven, injector, and detector were maintained at 65, 200, and 200 °C, respectively. Each value represents the mean of three sets of experiments and varies from the mean by not more than 15%. Scanning elecron microscopy (SEM) images of *M. sporium* cells immobilized on Chitosan were analyzed using Field Emission SEM (JEOL, Japan) as described previously (Patel et al., 2016b).

3. Results and discussion

3.1. Methanol production

3.1.1. From raw biogas

The raw biogas obtained from the anaerobic digester (Phygen Co. Ltd.) was composed of CH_4 (62.3%), CO_2 (36.7%), and H_2S (0.13%). It was used as feed in different concentrations of CH_4 in the range of 10–50% (dilution with air). The methanol production was observed in the range of 0.51–4.62 mM (Fig. 1). Initially, an increase in methanol production (4.62 mM) with a conversion yield of 28.9% was observed up to 20% of CH_4 as raw biogas; thereafter, the methanol production significantly decreased to 1.38 mM at a CH_4 concentration of 50%. Here, higher concentration of raw biogas may have inhibitory effects on methanol production. The maximum methanol production occurred at 48 h of incubation. Compared to pure CH_4, raw biogas resulted in a shift in maximum methanol production at 24–48 h (Patel et al., 2016b). This might be due to the differential response of *M. sporium* towards pure CH_4 and mixed feed. Furthermore, a decrease in methanol production was observed up to 96 h of incubation. The observed results suggest that the association of CO_2 and inhibitory gas H_2S plays a key role in the methanol yields. The direct use of raw biogas will be a cheap alternative to pure CH_4 as primary feed. The yield of methanol production from raw biogas is significantly higher than the previous report of methanol production by *M. sporium* (KCTC 22312) from a synthetic simulation gas mixture of CH_4 and CO_2 with maximum methanol concentration of 0.71 mM (Yoo et al., 2015). Previously, methanol production by *M. sporium* was not reported from raw biogas. In addition, low concentration (0.05%) of H_2S significantly inhibited the oxidation of CH_4 by *Methylomicrobium album* ATCC 33003 (type I), *Methylocaldum* sp. 14B (type I), and *Methylocystis* sp. ATCC 49242 (type II) methanotrophs (Caceres et al., 2014; Sheets et al., 2016). Here, the fact that raw biogas containing a high concentration of H_2S (0.13%) was successfully used for methanol production suggests that raw biogas can be directly used as a feed instead of purified biogas.

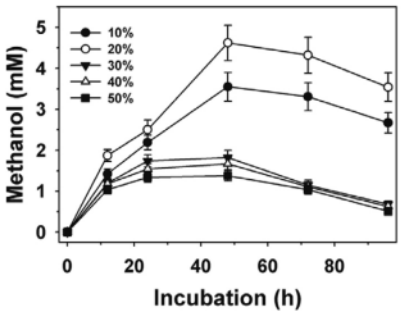

Fig. 1. Methanol production potential of *M. sporium* from raw biogas (CH_4%).

3.1.2. From synthetic gas mixture

A concentration of CH_4 greater than 20% in raw biogas is inhibitory for methanol production. Thus, to evaluate the effective utilization of raw biogas (high CH_4 concentration), we checked the individual and combined effects of CH_4 and CO_2 on methanol production along with H_2 using pure CH_4 as a control. For this purpose, we prepared different compositions of synthetic gases containing a mixture of CH_4, CO_2, and H_2 at the fixed concentration of CH_4 (20%).

3.1.2.1. Mixture of CH_4 and CO_2. The effective ratio of 2:1 was reported for the CH_4 to CO_2 in the methanogenic anaerobic digester (Yoo et al., 2015). The different combinations of CH_4 and CO_2 in the ratios of 4:1, 2:1, 4:3, 1:1, and 2:3 were evaluated. In all cases, methanol production increased up to 48 h, then decreased at 96 h of incubation. Methanol production was observed in the range of 2.97–4.35 mM (Fig. 2a). Here, optimum incubation was observed at 48 h compared with 24 h for pure CH_4. Maximum methanol production of 4.35 mM was observed at a ratio of 4:1 with a conversion yield of 53.6%. This yield was substantially higher than the yield with pure CH_4 (20%) as a control (3.86 mM), and a considerably more stable methanol production was observed over a period of 92 h. However, a further increase in the ratio of CH_4:CO_2 (up to 2:3) in the mixed gas, resulted in lower methanol production. These results suggest that a higher ratio (CH_4:CO_2) is required to increase methanol production. Thus, a suitable ratio of 4:1 (CH_4:CO_2) is necessary to improve methanol production. At a higher ratio of 2:1 (CH_4:CO_2), methanol yield (4.11 mM) was comparable to that of pure CH_4 (4.09 mM). In contrast, CH_4 concentration above 20% in raw biogas at the ratio of 2:1 (CH_4:CO_2) resulted in significantly lower methanol production of 1.82, 1.67, and 1.38 mM at 30, 40, and 50% of CH_4, respectively (Fig. 1). Here, lower methanol production might be associated with increasing inhibitory gases H_2S and NH_3 present in the raw biogas. The maximum

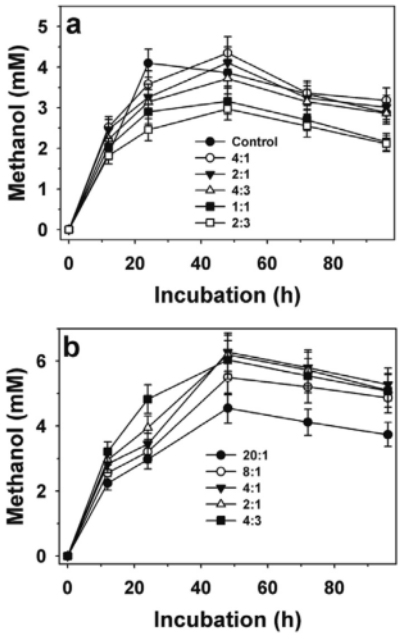

Fig. 2. Methanol production from synthetic gas mixtures of CH_4:CO_2 (a) and CH_4:H_2 (b).

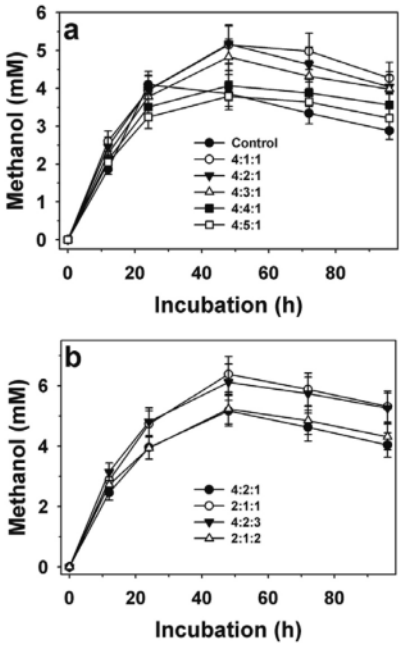

Fig. 3. Methanol production from synthetic gas mixture (CH_4:CO_2:H_2) at different ratios of CO_2 (a) and H_2 (b).

methanol production from synthetic gas mixture of CH_4 and CO_2 (4:1) is about 6-fold higher than 0.71 mM previously reported for CH_4 and CO_2 (1:1) by *M. sporium* KCTC 22312 (Yoo et al., 2015). However, *Methylosinus trichosporium* IMV 3011 resulted in significantly lower methanol yields of 0.02 mM at a ratio of 3:4 (Xin et al., 2004a). In contrast, *M. trichosporium* IMV 3011 did not produce methanol from pure CH_4 (30%) under similar conditions.

3.1.2.2. Mixture of CH_4 and H_2. As the content of H_2 in methanogenic anaerobic biogas is significantly low, the effect of H_2 concentration in the range of 1–15% was evaluated on methanol production along with fixed 20% of CH_4 (Fig. 2b). A gas mixture in a ratio (CH_4:H_2) of 20:1, 8:1, 4:1, 2:1, and 4:3 resulted in significant improvement in methanol production, compared to that of pure CH_4. Methanol production in the range of 4.55–6.28 mM was observed. Here, the presence of H_2 resulted in 1.7-fold higher methanol at 12 h of incubation compared to that of pure CH_4 (4.10 mM). The maximum methanol production of 6.28 mM was observed at a ratio of 4:1 (CH_4:H_2) with a conversion yield of 60.4%. Further increase in the CH_4:H_2 ratio in synthetic gas mixture did not result in higher methanol yields. Methanol production of 6.04 mM was obtained at a ratio of 4:3 (CH_4:H_2) in the synthetic gas mixture. Here, we observed a 53.2% increase in methanol production at the ratio of 4:1 (CH_4:H_2). A positive influence of H_2 on methanol production might be due to its role as an electron source to a pyridine nucleotide-linked hydrogenase reaction or depletion of reducing power, as described previously during the different alkanes oxidation by *M. trichosporium* OB3b (Mountfort et al., 1990).

3.1.2.3. Mixture of CH_4, CO_2, and H_2. We observed that the high concentration of CO_2 in the raw biogas is not suitable for methanol production. Thus, we evaluated the influence of both CO_2 and H_2 concentration in the synthetic gas mixture (CH_4:CO_2:H_2) on methanol production (Fig. 3). A suitable combination of H_2 and CO_2 with CH_4 results in higher methanol production in comparison with that of either pure CH_4 or a mixture of CH_4 and CO_2. Here, maximum methanol production was 5.17 mM at a CH_4:CO_2:H_2 ratio of 4:2:1 (Fig. 3a). Furthermore, an increase in CO_2 up to a ratio of 4:5:1 resulted in 3.79 mM of methanol production. In contrast, an increase in the ratio of CH_4:CO_2:H_2 from 4:2:1 to 2:1:1 significantly improved methanol production from 5.17 to 6.38 mM (Fig. 3b). Thereafter the yield was reduced to 5.23 mM at a ratio of 2:1:2 (CH_4:CO_2:H_2). These results for methanol production using a synthetic mixture (CH_4:CO_2:H_2) suggest that the appropriate ratio of these gases is crucial for high methanol production from the biogas. Here, a maximum methanol production of 6.38 mM from synthetic gas mixture (CH_4:CO_2:H_2) was observed at a ratio of 2:1:1 with a conversion yield of 63.7%. Methanol production by *M. sporium* has not been reported from raw biogas obtained from a methanogenic anaerobic digester.

3.1.3. From CO_2 and gas mixture (CO_2:H_2)

To check the methanol production abilities of *M. sporium* from pure CO_2, its concentration in the range of 5–40% was used as a feed. As CO_2 concentration was increased in the feed, methanol production increased during incubation up to 24 h (Fig. 4a). Maximum methanol production was in the range of 0.15–0.33 mM at an incubation of 48 h; thereafter, it significantly declined up to 60 h. The maximum methanol production of 0.33 mM was observed at 30% CO_2. In comparison with methanol production by *M. trichosporium* IMV 3011 from pure CO_2, *M. sporium* exhibited 16.5-fold higher methanol yields at a similar inoculum of 3 mg DCM·mL^{-1} (Xin et al., 2004a). These results suggest that CO_2 also contributes to methanol production from either a synthetic gas mixture or raw biogas mixture. However, 0.33 mM of maximum methanol yield is significantly lower than that from pure CH_4 (4.10 mM). This can be directly co-related to the role of the MDH inhibitor during methanol production from mixed gas containing both CH_4 and CO_2. Thus, we evaluated the role of buffer

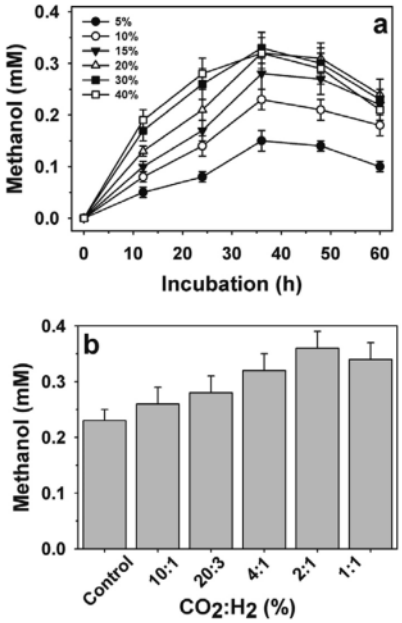

Fig. 4. Methanol production from pure CO_2 (a) and a gas mixture of CO_2:H_2 (b) as a feed.

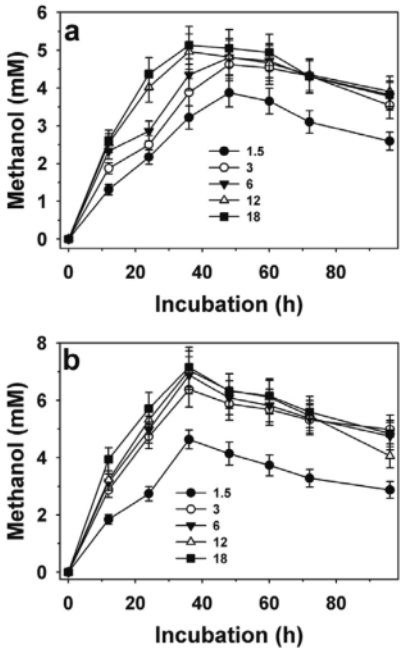

Fig. 5. Effect of inoculum on methanol production from raw biogas containing 20% of CH_4 (a) and a synthetic gas mixture of CH_4:CO_2:H_2 (b) as a feed.

and $MgCl_2$ on methanol production from 10% of pure CO_2 as a feed (Table A.1). In the absence of $MgCl_2$, an increase in methanol production was observed with a yield of 0.31 mM, compared to 0.23 mM at 20 mM $MgCl_2$ and 100 mM phosphate buffer. Furthermore, lowering of the buffer concentration from 100 to 20 mM resulted in an increase in methanol production from 0.23 to 0.38 mM. These results suggest that the presence of MDH inhibitors lowers methanol production by 65.2%. Overall, methanol production from CO_2 is significantly lower than that from CH_4. This result confirms that supplementation of H_2 is required for the improvement in methanol production from the mixed gas. To evaluate the influence of H_2 during the reduction of CO_2 into methanol, different concentrations of H_2 in the range of 1–10% were supplemented with 10% CO_2 as a primary feed (Fig. 4b). Methanol production increased significantly from 0.23 to 0.36 mM as the ratio was increased up to 2:1 (CO_2:H_2). Thereafter, it slightly declined to 0.34 mM at a higher ratio of 1:1 (CO_2:H_2). Therefore, supplementation with H_2 is necessary for the higher methanol production from the synthetic or raw biogas to maintain the concentration of CO_2 and H_2 in the suitable ratios.

3.2. Effect of the inoculum on methanol production

The cell mass concentration of the methanotrophs as a biocatalyst has quite a variable influence on methanol production (Senko et al., 2007; Duan et al., 2011). Thus, to improve methanol production from the raw biogas, different inoculums in the range of 1.5–18 mg of DCM·mL^{-1} of reaction mixture were evaluated (Fig. 5a). Here, an increase in methanol production was observed from 3.87 to 5.13 mM by increasing cell biomass from 1.5 to 18 mg of DCM·mL^{-1}. In contrast, much higher cell inoculum (105 mg of DCM·mL^{-1}) of *M. sporium* B21 resulted in significantly lower methanol production of 0.35 mM (Razumovsky et al., 2008). In addition, higher inoculums (12 and 18 mg of DCM·mL^{-1}) of *M. sporim* resulted in faster methanol production, compared to lower inoculums (3 mg of DCM·mL^{-1}) up to 32 h of incubation. A similar effect of inoculums was observed using synthetic gas mixture (CH_4:CO_2:H_2) in a ratio of 4:1:1 (Fig. 5b). Here, the maximum methanol production of 7.16 mM was higher than that from the raw biogas with a yield of 5.13 mM under similar CH_4 concentration (20%) in the feed.

3.3. Effect of the H_2 supplementation into raw biogas

H_2 (10%) was supplemented into raw biogas to increase methanol production at inoculums of 3 mg of DCM·mL^{-1} (Fig. 6a). Interestingly, a significant influence of H_2 supplementation on methanol production was observed at different concentrations of CH_4 (10–50%) in raw biogas at ratios of 1:1, 2:1, 3:1, 4:1, and 5:1, respectively. Here, maximum methanol production was 4.67, 6.68, 6.43, 5.21, and 4.12 mM, respectively, which was 1.3, 1.5, 3.5, 3.1, and 3.0-fold higher in methanol production. Maximum methanol conversion yield (42.5%) was observed at 10% CH_4 as a feed in the raw biogas. These results suggest that the lower methanol production from the raw biogas at high CH_4 concentration might be due to high concentration of either CO_2 or inhibitory gas H_2S. The ratio of these gases cannot be regulated by simple dilutions. Thus, for efficient methanol production we need to provide the suitable ratio of these gases CH_4, CO_2, and H_2 through supplementation into the raw biogas.

3.4. Methanol production by immobilized cells

Immobilization of methanotrophs to improve methanol production from pure CH_4 or synthetic gas mixture (CH_4:CO_2:H_2) has been previously studied (Mehta et al., 1991; Senko et al., 2007; Razumovsky et al., 2008; Mardina et al., 2016; Patel et al., 2016b). Nevertheless, no report is available on methanol production by immobilized methanotrophs from raw biogas. Here, the potential of different support materials including Amberlites (XAD-2, XAD-4, and XAD-7HP), Duolite A-7, and chitosan for

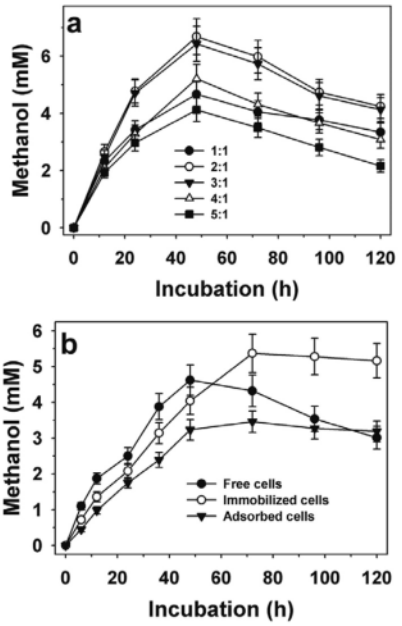

Fig. 6. Methanol production profiles. Effect of H_2 (10%) supplementation into raw biogas (a) and methanol production from raw biogas containing 20% of CH_4 by immobilized *M. sporium* (b).

methanol production from a raw biogas was evaluated. The methanol production efficiency of whole cells through adsorption and covalent immobilization were in the ranges of 52.7–69.9% and 55.6–87.6% after 48 h of incubation compared to that of free cells (100%), respectively (Table A.2). Among these different supports, chitosan-immobilized cells resulted in high methanol production from the raw biogas. Maximum methanol production yields by free and immobilized cells through adsorption and covalent immobilization were 4.62, 3.46, and 5.37 mM, respectively (Fig. 6b). Here, methanol production by covalently immobilized *M. sporium* was significantly higher than that produced by free cells. SEM images of the immobilized cells on Chitosan are presented in Fig. A.1. The observed methanol production yield from raw biogas by covalently immobilized *M. sporium* was approximately 7.6-fold higher than previously reported from a synthetic simulated biogas mixture ($CH_4 + CO_2$) with a maximum yield of 0.71 mM by *M. sporium* KCTC 22312 (Yoo et al., 2015). In addition, a significantly lower methanol production of 0.02 mM was reported for *M. trichosporium* IMV3011 (Xin et al., 2004b). In comparison with previous reports on methanol production by immobilized *M. sporium* from pure CH_4 (Table A.3), covalently immobilized *M. sporium* resulted in significantly higher methanol production from raw biogas. Whereas, *M. sporium* strains (B2119–B2123) immobilized through encapsulation in a polymer matrix resulted in methanol production in the range of 1.37–2.34 mM (Senko et al., 2007; Razumovsky et al., 2008).

In summary, *M. sporium* has the ability to utilize both CH_4 and CO_2 for methanol production. Compared to methanol production from pure CH_4 (4.10 mM), raw biogas resulted in higher methanol production of 4.62 mM at the same concentration of CH_4 (20%) as a feed. These results suggest that additional association of CO_2 and H_2 plays a key role in methanol production from the raw biogas. Therefore, synthetic gas mixtures (CH_4:CO_2, CH_4:H_2, and CH_4:CO_2:H_2) were prepared to evaluate their influence on methanol production. A higher methanol production was observed compared to that of pure CH_4 with maximum yields of 4.35, 6.28, and 6.38 mM at the ratios of 4:1 (CH_4:CO_2), 4:1 (CH_4:H_2), and 2:1:1 (CH_4:CO_2:H_2), respectively. Here, H_2 showed a significant influence on methanol production from either pure CH_4 and CO_2 or a mixture of these gases. Furthermore, an increase in methanol production was observed as the cell inoculum increased with maximum methanol production of 5.13 and 7.16 mM from raw biogas (20%, CH_4) and synthetic gas mixture CH_4:CO_2:H_2 (2:1:1), respectively. These results recommend that higher methanol can be obtained through either balancing the suitable ratio of 2:1:1 (CH_4: CO_2:H_2) or increasing the inoculums. Furthermore, supplementation of the H_2 in the raw biogas resulted in significant improvement up to 3.5-fold in methanol production. In addition, whole cell immobilization of *M. sporium* resulted in a 1.2-fold higher methanol production from raw biogas in comparison with free cells. Overall, the *M. sporium* seems to be a suitable candidate for methanol production from the raw biogas and synthetic gas mixtures.

4. Conclusions

Mostly, pure CH_4 is used for methanol production by methanotrophs. In this study, we evaluated the utilization of the raw biogas as a low cost feed originated from anaerobic digestion. The observed results suggest that the direct use of raw biogas is not suitable for methanol production, which is probably due to either a desired ratio of CH_4 and CO_2 gases or high concentration of H_2S as an inhibitory gas. Thus, low methanol yield could be significantly improved through the regulation of gas compositions. This study provides better support towards efficient utilization of either raw biogas or synthetic gas mixture.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2016.06.065.

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