Bioresource Technology 263 (2018) 25–32

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Methanol production from simulated biogas mixtures by co-immobilized *Methylomonas methanica* and *Methylocella tundrae*

Sanjay K.S. Patel, Virendra Kumar, Primata Mardina, Jinglin Li, Rowina Lestari, Vipin C. Kalia, Jung-Kul Lee*

Department of Chemical Engineering, Konkuk University, Seoul 05029, Republic of Korea

ARTICLE INFO

Keywords:
Co-culture
Immobilization
Methanol
Methylocella tundrae
Methylomonas methanica

ABSTRACT

In the present study, co-cultures of the methanotrophs *Methylocella tundrae*, *Methyloferula stellata*, and *Methylomonas methanica* were evaluated for improving methanol production with their application. Among the different combinations, the co-culture of *M. tundrae* and *M. methanica* increased methanol production to 4.87 mM using methane (CH_4) as feed. When simulated biogas mixtures were used as feed, the maximum methanol production was improved to 8.66, 8.45, and 9.65 mM by free and encapsulated co-cultures in 2% alginate and silica-gel, respectively. Under repeated batch conditions, free and immobilized co-cultures using alginate and silica-gel resulted in high cumulative production, up to 24.43, 35.95, and 47.35 mM, using simulated biohythane (CH_4 and hydrogen), respectively. This is the first report of methanol production from defined free and immobilized co-cultures using simulated biogas mixtures as feed.

1. Introduction

The greenhouse gas (GHG) nature of methane (CH_4), and its continuous increasing global emissions (774 Tg $year^{-1}$) through anthropogenic as well as natural processes, has had a great negative influence on the environment (Strong et al., 2015). Therefore, the utilization of CH_4 as a promising feedstock to produce value-added products may reduce these effects. CH_4 (113 trillion m^3) reserves as natural fuel sources and it has an energy potential of 2.0×10^{15} kWh (Ge et al., 2014). Additionally, generation of CH_4 through anaerobic digestion (AD) of lignocellulosic biomass has been demonstrated (Liu et al., 2016). The global warming potential of CH_4 is very high and approximately 25-fold greater than that of carbon dioxide (CO_2). Thus, utilization CH_4 has been recommended to reduce its negative environmental effects. Methanotrophs can biotransform CH_4 into value-added bioproducts such as biopolymers, methanol, and lipids (Fei et al., 2014; Ishikawa et al., 2017; Strong et al., 2016; Su et al., 2017). Recent studies suggested that the conversion of GHGs into liquid fuels such as methanol by methanotrophic strains is a more effective than chemical methods for their reduction, because of the environmental friendly nature, high conversion rates, selectivity, and low capital/energy costs of this method (Barzgar et al., 2017; Hur et al., 2017; Patel et al., 2018a; Strong et al., 2015). Additionally, GHG conversion can be broadly applied to synthesize industrially important chemicals such as formaldehyde and higher alcohols (Ge et al., 2014; Lee et al., 2016; Whitaker et al., 2015). Methane monooxygenase (MMO) enzymes [particulate (pMMO) and soluble (sMMO) forms] are involved in the oxidation of CH_4 to methanol by methanotrophs. Subsequently, methanol is oxidized to formaldehyde and then to formate, via methanol dehydrogenase (MDH) and formaldehyde dehydrogenase, respectively. Finally, CO_2 is produced through the oxidation of formate by formate dehydrogenase (Lawton and Rosenzweig, 2016; Li et al., 2018). sMMO requires the cofactor nicotinamide adenine dinucleotide (NADH) to oxidize CH_4 into methanol, whereas pMMO catalyzes NADH-independent oxidation of CH_4. Generally, lower methanol accumulation has been observed in methanotrophs because of its further oxidation by MDH (Han et al., 2013; Yoo et al., 2015). Therefore, to enhance methanol production, various MDH inhibitors including ammonium chloride, cyclopropanol, ethylenediaminetetraacetic acid, magnesium chloride ($MgCl_2$), phosphate buffer, and sodium chloride have been used (Ge et al., 2014; Han et al., 2013; Sheets et al., 2016). Because the production of methanol by sMMO is highly dependent on the effective

Abbreviations: AD, anaerobic digestion; CH_4, methane; CO_2, carbon dioxide; DCM, dry cell mass; DCPIP, 2,6-dichlorophenol-indophenol; FTIR, Fourier transform infrared; GC, gas chromatography; GHG, greenhouse gas; FE-SEM, field emission scanning electron microscopy; MDH, methanol dehydrogenase; NADH, nicotinamide adenine dinucleotide; NMS, nitrate mineral salt; pMMO, particulate methane monooxygenase; rpm, revolution per minute; sMMO, soluble methane monooxygenase; μM, micromolar; mg, milligram; mL, milliliter; mM, millimolar

* Corresponding author at: Department of Chemical Engineering, Konkuk University, 1 Hwayang-dong, Gwangjin-gu, Seoul 05029, Republic of Korea.
E-mail address: jkrhee@konkuk.ac.kr (J.-K. Lee).

https://doi.org/10.1016/j.biortech.2018.04.096
Received 10 March 2018; Received in revised form 21 April 2018; Accepted 23 April 2018
Available online 25 April 2018
0960-8524/

regeneration of NADH, partial inhibition of NADH and supplementation of formate has been suggested to increase methanol production (Ge et al., 2014).

Previously, the conversion of CH_4 into methanol using a methanotrophic consortium, including *Methylosinus sporium* NCIMB 11126, *Methylosinus trichosporium* OB3b, and *Methylococcus capsulatus* Bath, as a mixed culture inoculum developed by enriching landfill cover soil samples, was adopted to improve methanol production (Han et al., 2013). Similarly, a thermotolerant methanotrophic consortium of mixed culture was developed for methanol production through enrichment of the digestate in the AD system (Su et al., 2017). Here, the syntrophic behavior of strains resulted in high methanol production. Additionally, the use of pure culture methanotrophs is vulnerable to contamination by other organisms, has narrow ranges of physical stability, or is prone to inefficient utilization of raw feed as biogas mixtures contain inhibitory gases, which may lead to process failure during large-scale production. Therefore, the use of a defined methanotroph consortium, selective methanotroph co-culture, or association with another type of organism as an inoculum may improve process efficiency through better utilization of biogas, increase production, and reduce process variability, compared with the results achieved using an undefined methanotrophic consortium (Han et al., 2013; Hill et al., 2017; Su et al., 2017). The immobilizations strategies have been well demonstrated to improve the properties of biocatalysts (Jiang et al., 2016; Ling et al., 2016; Zhuang et al., 2017). The use of immobilized cells has also been suggested as an effective approach for enhancing the biotransformation efficiency because of their higher stability than free cells, including methanotrophs (Mehta et al., 1991; Patel et al., 2015; Senko et al., 2007; Sheets et al., 2017; Sun et al., 2018). However, no studies have examined methanol production from GHGs using immobilized, defined mixed culture or co-culture. In this study, the enhancement of methanol production using co-cultures of the methanotrophic strains *Methylocella tundrae*, *Methyloferula stellata*, and *Methylomonas methanica* was evaluated. Immobilization of co-culture by encapsulation using two different polymeric matrixes of alginate and silica-gel improved methanol production stability using simulated biogas (CH_4 and CO_2) and biohythane [CH_4 and hydrogen (H_2)] mixtures as a feed. Further, effective methanol production under repeated batch conditions was verified. The results showed that co-culture of *M. methanica* and *M. tundrae* produced more methanol than pure cultures and other combinations, i.e., co-cultures of two and three strains. These results suggest that immobilization of co-culture is a valuable approach for improving methanol production from simulated biogas and biohythane under repeated batch conditions. This is the first report of using immobilized, defined co-cultures of methanotrophs for methanol production using simulated biogas and biohythane as a feed.

2. Materials and methods

2.1. Materials

Methanotrophic strains *M. tundrae* (DSMZ 15673), *M. stellata* (DSM 22108), and *M. methanica* (DSM 25384) were purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). Pure CH_4, CO_2, and H_2 were purchased from NK Co. (Busan, Republic of Korea). Municipal waste treatment anaerobic digester (Seoul, South Korea) raw biogas procured from Phygen Co. Ltd. Glycerol, pluronic (P-123) tri-block polymer [poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol)], poly-ethyleneglycol, sodium-alginate, and tetraethylorthosilicate (TEOS) were procured from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Culture conditions and preparation of co-cultures

Strains were cultured in nitrate mineral salts medium, as reported previously (Patel et al., 2016a,b,c). These strains were grown in 1-L Erlenmeyer flasks (200 mL working volume) containing 20% of CH_4 feed and incubated for 5 days under shaking (150 rpm) at 30 °C. Cell growth was monitored, and cells were harvested by centrifugation as described previously (Mardina et al., 2016). The co-cultures of two (three sets) and three (one set) strain combinations were prepared by mixing individual strains in equal proportions, obtaining a final dry cell mass (DCM) concentration of 3.0 mg mL^{-1} reaction mixture.

2.3. Methanol production

Initially, the methanol production conditions under batch conditions were optimized for *M. stellata* and *M. methanica* using different concentrations of phosphate (20–120 mM, pH 7.0), $MgCl_2$ (5–60 mM), and formate (20–120 mM) with Fe^{2+} (10 μM), Cu^{2+} (5 μM), and 3.0 mg DCM mL^{-1} of cells as the inoculum at 30 °C and under 150 rpm shaking (Mardina et al., 2016; Patel et al., 2016d). The final reaction volume of 20 mL was prepared using pure or co-culture in serum bottles (120 mL) and CH_4 (30%) was used as feed, with replacement of an equal volume of headspace air as described previously (Patel et al., 2016d).

2.3.1. Effect of inoculum and feed concentration

The influence of the ratio of the strains (*M. tundrae*: *M. methanica*) at 1:3, 1:2, 1:1, 2:1, and 3:1 in the co-culture as inoculums with a fixed final inoculum of 3.0 mg DCM mL^{-1}, on methanol production using 30% CH_4 as feed, was examined after incubation for 24 h. Further, the effects of CH_4 concentration (10–50%) on methanol production during co-culture of *M. tundrae* and *M. methanica* incubated for up to 96 h were evaluated.

2.4. MDH and MMO activity

MDH activity was measured by phenazine methosulfate-mediated reduction of 2,6-dichlorophenol-indophenol (DCPIP) at a wavelength of 600 nm, as described previously (Patel et al., 2016d). Briefly, the 1mL reaction assay was evaluated using $CaCl_2$ (10 mM), NH_4Cl (45 mM), phosphate buffer (0.3 M, pH 7.5), cell supernatant (5.0 mg DCM), DCPIP (0.13 μM), and phenazine methosulfate (3.3 μM). Similarly, naphthalene oxidation was performed to evaluate sMMO activity using a 2mL reaction mixture containing 0.9 mL of naphthalene saturated solution, 1 mL of cell suspension (5.0 mg DCM), and 0.1 mL of 0.2% (w v^{-1}) of tetrazotized *o*-dianisidine at 530 nm, as described previously (Han et al., 2013).

2.5. Whole cell encapsulation

Co-immobilization of *M. methanica* and *M. tundrae* was performed by encapsulation of different sodium-alginate concentrations (1.0–3.0%) in cells loaded with 1.0 and 2.0 mg DCM mL^{-1} mixture, respectively, as reported previously (Mardina et al., 2016). Further, loosely bound cells from the Na-alginate beads were removed by washing twice with saline solution. The encapsulation of co-cultures through silica gel was accomplished using 20 mL of precursor solution (mixture of TEOS/P-123/H_2O/ethanol/HCl/glycerol in a molar ratio of 1.0:0.015:5.3:18.1:0.3:1.13, pH 5.0) and 40 mL of cells (3.0 mg DCM mL^{-1}, 40 mL), as described previously (Niu et al., 2013). Thereafter, loosely bound cells were separated by washing twice with distilled water followed by washing with buffer solution. These immobilized cells were stored at 4 °C.

2.6. Methanol production by immobilized co-cultures

The methanol production profile of free and immobilized co-cultures was assessed using 30% CH_4 with cell inoculums of 3.0 mg DCM mL^{-1} for 96 h with shaking at 150 rpm.