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Biological conversion of biogas to methanol using methanotrophs isolated from solid-state anaerobic digestate

Johnathon P. Sheets [a], Xumeng Ge [a], Yueh-Fen Li [b], Zhongtang Yu [b], Yebo Li [a,*]

[a] Department of Food, Agricultural and Biological Engineering, The Ohio State University/Ohio Agricultural Research and Development Center, 1680 Madison Ave., Wooster, OH 44691-4096, USA
[b] Department of Animal Sciences, The Ohio State University, Columbus, OH 43210, USA

HIGHLIGHTS

- Methanotrophs were isolated from solid-state anaerobic digestate.
- Both biogas and purified methane were suitable substrates for methanotrophs.
- Biogas from a commercial anaerobic digester was biologically converted to methanol.
- Several methanol dehydrogenase inhibitors promoted methanol production.
- Methanol production increased 4-fold to 22-fold using formate as an electron donor.

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ABSTRACT

The aim of this work was to isolate methanotrophs (methane oxidizing bacteria) that can directly convert biogas produced at a commercial anaerobic digestion (AD) facility to methanol. A methanotrophic bacterium was isolated from solid-state anaerobic digestate. The isolate had characteristics comparable to obligate methanotrophs from the genus *Methylocaldum*. This newly isolated methanotroph grew on biogas or purified CH_4 and successfully converted biogas from AD to methanol. Methanol production was achieved using several methanol dehydrogenase (MDH) inhibitors and formate as an electron donor. The isolate also produced methanol using phosphate with no electron donor or using formate with no MDH inhibitor. The maximum methanol concentration (0.43 ± 0.00 g L^{-1}) and 48-h CH_4 to methanol conversion ($25.5 \pm 1.1\%$) were achieved using biogas as substrate and a growth medium containing 50 mM phosphate and 80 mM formate.

1. Introduction

Biogas produced during anaerobic biodegradation of organic wastes contains methane (CH_4) as the major component (30%–70%) and it is an emerging renewable energy source. In the United States alone, it is estimated that over 650 billion ft^3 of biogas could be captured yearly from landfills and anaerobic digestion (AD) systems (USDA et al., 2014). This large source of energy could be used to power more than 3 million homes or generate enough compressed natural gas (CNG) to replace 2.5 billion gallons of gasoline (USDA et al., 2014; USDOE, 2014). However, biogas is difficult to store and transport because it is a gas at ambient conditions (Ge et al., 2014). This issue could be addressed by converting biogas to methanol, a valuable liquid chemical that can be used directly as fuel or further converted to other products such as olefins and gasoline. CH_4 can be thermo-chemically converted to methanol, but the process uses expensive metal catalysts and operates at high temperatures (200 °C–900 °C) and pressures (5–20 MPa) (Riaz et al., 2013). Besides, biogas contains carbon dioxide (CO_2, 30%–70%) and trace impurities such as hydrogen sulfide (H_2S, 0–2000 ppm) (Yang et al., 2014), while thermochemical technologies require a CH_4 source that is free of impurities (Riaz et al., 2013). Although biogas can be cleaned and upgraded to purified CH_4 (>97%), the purification processes are usually expensive (Yang et al., 2014). Biological conversion of biogas to methanol is an emerging, attractive approach as it may not require biogas purification and uses ambient conditions, reducing operational requirements and energy demands (Ge et al., 2014).

Methanotrophs are aerobic bacteria that can convert CH_4 to methanol using the enzyme methane monooxygenase (MMO) (Kalyuzhnaya et al., 2015). Under normal conditions,

* Corresponding author. Tel.: +1 330 263 3855.
E-mail address: li.851@osu.edu (Y. Li).

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methanotrophs further oxidize methanol to CO_2 via methanol dehydrogenase (MDH) and other enzymes in a sequential manner, generating both ATP and reducing power that can be used for other metabolic reactions (Kalyuzhnaya et al., 2015). While MDH inhibitors, such as phosphate and NaCl, have been used to increase methanol production by methanotrophs (Duan et al., 2011; Mehta et al., 1991), inhibition of methanol oxidation by MDH inhibitors decreases production of ATP and reducing power. Thus, another organic substrate, such as formate, is needed to maintain the metabolic activity of methanotrophs. Recently, electrochemical production of formic acid from CO_2 and H_2O has been achieved, which provides an opportunity to effectively reduce the cost of formate as an electron donor (Li et al., 2012; Reda et al., 2008). However, improvements in several aspects are still needed for biological conversion of biogas to methanol to reach industrial scale production. Currently, pure CH_4 is routinely used for growing methanotrophs. No studies have used methanotrophs to convert biogas from a commercial AD facility to methanol.

Isolation of methanotrophs that can directly use biogas will improve the feasibility of biological conversion of CH_4 to methanol. Methanotrophs have been isolated from a variety of sources such as soil, natural gas fields, and waste treatment facilities (Ge et al., 2014), and a few studies have isolated methanotrophs to produce methanol (Han et al., 2013). Methanotrophs have also been detected and isolated from commercial liquid AD (L-AD) systems that operate at total solids (TS) contents less than 15% (Corder et al., 1986; Rastogi et al., 2009). This indicates that methanotrophs can survive long-term anoxia and may use biogas as their sole source of carbon and energy (Ge et al., 2014). Compared to L-AD, solid-state AD (SS-AD) operates at TS contents greater than 15% (Sheets et al., 2015), and has higher porosity than L-AD, which may provide higher O_2 levels that support the growth of methanotrophs (Ahn et al., 2010). Potentially, methanotrophs isolated from SS-AD could be used to directly convert biogas to methanol. To date, no methanotrophs have been isolated from SS-AD or been used to convert biogas from a commercial AD facility to methanol. Additionally, no studies have examined the effects of MDH inhibitors and formate on the biological conversion of biogas to methanol by methanotrophs. To address these research gaps, this study aimed to: (1) isolate and characterize methanotrophs from an SS-AD system; (2) compare biogas and purified CH_4 as substrates for a methanotrophic isolate; and (3) determine the effects of MDH inhibitors and formate on biological conversion of biogas to methanol.

2. Methods

2.1. Isolation of methanotrophs

Eight digestate samples (5 g each) were collected from SS-AD reactors described in Sheets et al. (2015). The 1-L SS-AD reactors were fed switchgrass (feedstock (*F*)) and inoculated with anaerobically digested wastewater sludge (inoculum (*I*)) at an *F*/*I* ratio of 3. The SS-AD reactors were controlled at either 20% or 30% TS and incubated under mesophilic (36 ± 1 °C) conditions for 70 days. The 70-day CH_4 yield of the SS-AD reactors varied from 88 L kg^{-1} volatile solids (VS_{added}) (30% TS) to 113 L kg VS_{added}^{-1} (20% TS) (Sheets et al., 2015). The isolation procedure was a modified version of the protocols described in Bowman (2006) and Dedysh and Dunfield (2011). The SS-AD samples were inoculated into a nitrate mineral salts (NMS) medium (Bowman, 2006) in 250-mL flasks (45 ml NMS per flask). The NMS medium contained $MgSO_4{\cdot}7H_2O$ (1.0 g L^{-1}), KNO_3 (1.0 g L^{-1}), KH_2PO_4 (0.272 g L^{-1}), Na_2HPO_4 (0.284 g L^{-1}), $CaCl_2{\cdot}2H_2O$ (0.134 g L^{-1}), chelated Fe solution (0.2% (v/v)), and a trace element solution (0.05% (v/v)). The chelated Fe solution contained ferric (III) ammonium citrate (1.0 g L^{-1}), EDTA (2.0 g L^{-1}), and concentrated HCl (0.3% (v/v)) in deionized (DI) water. The trace element solution contained EDTA (500 mg L^{-1}), $FeSO_4{\cdot}7H_2O$ (200 mg L^{-1}), $ZnSO_4{\cdot}7H_2O$ (10 mg L^{-1}), $MnCl_2{\cdot}4H_2O$ (3.0 mg L^{-1}), H_3BO_3 (30 mg L^{-1}), $CoCl_2{\cdot}6H_2O$ (20 mg L^{-1}), $CaCl_2{\cdot}2H_2O$ (1.0 mg L^{-1}), $NiCl_2{\cdot}6H_2O$ (2.0 mg L^{-1}), and $Na_2MoO_4{\cdot}2H_2O$ (3.0 mg L^{-1}) in DI water. The initial pH of the NMS medium was 6.6–6.8. Each of the flasks was sealed with a rubber stopper and its headspace was filled with a filtered (0.2 μm) mixture of purified CH_4 (99% purity) and air at a CH_4: air ratio of 1:4 (v/v). The flasks were incubated at 37 °C with continuous shaking at 200 rpm. After three days, 5–10 ml sample of each enriched culture was transferred into a new flask that contained 40–45 mL of fresh NMS medium and a similar mixture of CH_4 and air. This process was repeated every 3 days. After 25 days, the enriched culture from each flask was spread on an NMS agar plate. The plates were then incubated at 37 °C in an anaerobic jar, with a headspace of CH_4 and air at a ratio of 1:4 (v/v), for one to two weeks. Individual colonies were picked and streaked on fresh NMS agar plates until single colonies (based on color, size, and shape) were observed. Fifteen isolates were obtained and each was transferred to 1 mL of NMS medium in a 15 mL sealed vial with a headspace containing filtered (0.2 μm) CH_4 and air at a ratio of 1:4. The vials were incubated at 37 °C with continuous shaking at 300 rpm until there was an observable increase in culture turbidity. Cell morphology was examined by light microscopy. Cultures were considered to be pure if they did not grow in NMS amended with 0.05% (w/v) glucose (Dedysh and Dunfield, 2011).

For each methanotrophic isolate, the full-length 16S rRNA gene was amplified and sequenced at the Plant–Microbe Genomics Facility at The Ohio State University, Columbus, Ohio, USA. The 16S rRNA gene sequences were analyzed and taxonomically classified using RDP Classifier (Wang et al., 2007) and BLAST against the NCBI RefSeq database. The 16S rRNA gene sequence of all 15 isolates shared >97% sequence similarity with strains of *Methylocaldum*, a genus containing obligate methanotrophs. One of the fastest growing isolates, henceforth referred to as *Methylocaldum* sp. 14B, was selected for further experiments. Strain 14B was isolated from the digestate of a mesophilic SS-AD reactor that was operated at 30% TS content and produced biogas composed of 50–60% (v/v) CH_4 and 1–3% (v/v) O_2 (Sheets et al., 2015).

2.2. Cultivation with different chemical and physical inputs

To test the ability of strain 14B to utilize various carbon sources, it was aerobically grown in 1 mL of liquid NMS medium supplemented with 0.1% (w/v) of methanol, formate, acetate, xylose, glucose, or citrate. Cultivation was conducted in 15 mL vials at 37 °C and 200 rpm. Growth was determined by the change in optical density (OD) at 600 nm and cell morphology was examined by light microscopy.

The effects of copper (Cu^{2+}), NaCl, nitrogen source, pH, and temperature on cell growth using CH_4 as the sole carbon source were evaluated by culturing strain 14B in 20 mL of NMS medium in 125 mL flasks with CH_4 and air (at 1:4 ratio, v/v) in the headspace. Copper was tested because it was shown to influence cell growth, especially for those methanotrophs that possess particulate methane monooxygenase (pMMO) (Kalyuzhnaya et al., 2015). The Cu^{2+} concentrations assessed were 0, 1.0, 5.0, and 10 μM of $CuCl_2$ supplemented in NMS. Liquid NMS medium containing 1 μM $CuCl_2$ was amended with 0, 2, 5, 10, or 20 g L^{-1} NaCl to determine the effects of different salt concentrations. Growth on ammonium was examined by replacing the KNO_3 in NMS medium (containing 1 μM $CuCl_2$) with 1.0 g L^{-1} ammonium sulfate ($(NH_4)_2SO_4$). The optimal growth pH was evaluated using NMS medium containing 1 μM $CuCl_2$ at pH 5.0–7.6. For tests to evaluate Cu^{2+}, NaCl, nitrogen