**Isolation of a methanotroph from a hydrogen sulfide-rich anaerobic digester for methanol
production from biogas**

Wenxian Zhang^{a,b,1}, Xumeng Ge^{a,1,*}, Yueh-Fen Li^c, Zhongtang Yu^c, 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. Engineering Research Center of Industrial Microbiology, Ministry of Education, College of
Life Sciences, Fujian Normal University, Fujian 350108, China

c. Department of Animal Sciences, The Ohio State University, Columbus, OH, 43210, USA

* Corresponding authors. Phone: +1 330 263 3855. E-mail: li.851@osu.edu (Y. Li);
ge.162@osu.edu (X. Ge)

¹ Authors contributed equally to this work.

Abstract

A methanotrophic bacterium, named SAD2, was isolated from a hydrogen sulfide (H₂S)-rich anaerobic digester. Based on its 16S rRNA gene sequence, SAD2 belongs to *Methylocaldum* sp. SAD2 grew stably on methane/air mixtures containing 500 and 1000 ppm of H₂S, and showed H₂S tolerance higher than those reported for *Methylomicrobium album* and *Methylocystis* sp.. Exposure to 1000 ppm or less H₂S during SAD2 cell preparation did not affect the subsequent methanol production, while the presence of 1000 ppm H₂S in the methanol production process caused a 44%-60% decrease in methanol concentration. When a biogas/air mixture containing about 500 ppm or less H₂S was used for methanol production by SAD2, 276-343 mg/L methanol was obtained with methane-to-methanol conversion efficiencies of 30%-34%. This new isolate may be used in conversion of biogas to methanol without scrubbing of H₂S.

Keywords: Anaerobic digestion; Biogas upgrading; Methanol; Methanotrophs; Hydrogen sulfide

1. Introduction

More than 300 million tons (dry weight) of waste biomass, including animal manure, food processing waste, and crop residues, are generated every year from agricultural and food systems in the U.S. [1]. This waste biomass is considered to be an important renewable feedstock for production of energy and valuable products, thus it can improve the sustainability of agricultural and food systems. Anaerobic digestion (AD) is a well-established and widely adopted dual-purpose technology for waste treatment and biogas generation [2]. Biogas mainly consists of methane and carbon dioxide (CO_2), with traces of other “contaminant” gases, such as hydrogen sulfide (H_2S) and ammonia (NH_3). Biogas has a low energy density, and is difficult and costly to store, transport, and distribute. As a result, biogas from small scale AD systems is frequently flared and wasted [3]. A plausible approach to utilize biogas is to convert it into methanol, which is a valuable precursor of fuels and chemicals and a promising energy carrier for long-term storage [4,5].

Compared to thermochemical processes that are commonly used for conversion of methane to methanol, biological conversions are attractive for their mild operating conditions [4]. In nature, there are methanotrophs (a diverse group of bacteria) that use monooxygenase (MMO) to oxidize methane to methanol, consuming two reducing equivalents in oxidizing one molecule of methane. Under normal conditions, methanol dehydrogenase (MDH), formaldehyde dehydrogenase (FaldH), and formate dehydrogenase (FDH) in methanotrophs further oxidize methanol to CO_2 , generating more reducing equivalents (six per molecule of methanol) for metabolic reactions. As a result, methanol production with methanotrophs is usually conducted by inhibiting the MDH with inhibitors, such as sodium chloride and phosphate, and providing external electron donors, mainly formate to sustain survival and metabolic activity of the

methanotrophs [4]. Recent progress on electro-synthesis of formate from CO₂ and H₂O has shown an economically feasible way to supply formate as an electron donor [6,7]. However, there are still several foreseeable challenges for methanol production from biogas with methanotrophs.

One of the major challenges is the inhibition of methanotrophs by H₂S present in all biogas [4]. The contents of H₂S in biogas often exceed 500 ppm, especially in biogas produced from protein-rich feedstocks, such as animal manure [8–10] and fishery wastes [11]. A methane/air mixture containing 500 ppm H₂S showed a substantial inhibitory effect on cell growth of two commonly studied methanotrophs, resulting in a 37%-47% reduction in microbial biomass [12]. CH₄ oxidation capacity of *Methylocystis* sp. isolated from landfill cover soil was significantly inhibited (>40%) by 200 ppm H₂S [13]. Although H₂S can be removed from biogas using different methods, the operating cost for biogas cleaning with H₂S removal can be up to \$0.38 per N m³ of CH₄ [5], or over \$0.63 per gallon of methanol produced, which is about 50% of the methanol price. Therefore, methanotrophs with improved H₂S tolerance are desirable for cell cultivation and methanol production from raw biogas.

Methanotrophs have been isolated from different sources with CH₄ emissions under dramatically different environments [4]. Sources under extreme conditions, such as high temperature and low pH, are generally selected for isolating strains that are tolerant to the environmental stress. For example, thermophilic methanotrophs have been isolated from hot springs [14–17]. AD systems that generate biogas with high levels of H₂S can be a source for isolating H₂S adapted and tolerant methanotrophs [4]. So far, there have been very few studies on isolation and characterization of methanotrophs from AD systems [18,19]. Recently, a methanotrophic bacterium was isolated from the digestate of solid-state AD (SS-AD) of

switchgrass [18]. However, switchgrass usually results in biogas with low H₂S contents due to the low sulfur content (about 0.3-0.7 g/kg) of this feedstock [20].

The objectives of this study were to isolate a methanotrophic bacterium with high H₂S-tolerance from a H₂S-rich anaerobic digester, and evaluate its performance in cell growth and methanol production using raw biogas. Methanotrophs were isolated from digestate of SS-AD of expired dog food, a high-protein feedstock. One methanotrophic strain tolerant to high levels of H₂S was isolated, identified, and characterized with gene sequencing and morphological and physiological tests. The effect of H₂S on this isolate was evaluated with respect to cell growth, methane and oxygen consumption, CO₂ generation, and methanol production.

2. Materials and methods

2.1. Isolation of H₂S-tolerant methanotrophs

Expired dog food provided by quasar energy group (Wooster, OH, USA) and effluent collected from a mesophilic liquid anaerobic digester (KB BioEnergy, Akron, OH, USA) were used as feedstock and inoculum, respectively. SS-AD was conducted in 1-L reactors at 37°C with a total solids content of 20% (adjusted with deionized water) and a feedstock to inoculum (F/I) ratio of 3 (based on volatile solids). Biogas was collected in a 5-L gas bag (CEL Scientific Tedlar gas bag, Santa Fe Springs, CA, USA) attached to the outlet of each reactor. The volume of biogas produced and its composition were determined every 1-2 days.

Five grams (wet weight) of digestate collected from the SS-AD reactors were inoculated into 20 ml of nitrate mineral salts (NMS) medium in a 125-mL flask (in duplicate) [18]. The NMS medium contained MgSO₄·7H₂O (1.0 g L⁻¹), KNO₃ (1.0 g L⁻¹), KH₂PO₄ (0.272 g L⁻¹), Na₂HPO₄ (0.284 g L⁻¹), CaCl₂·2H₂O (0.134 g L⁻¹), chelated Fe solution (0.2%, v/v), and 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 water. The trace element solution contained EDTA (500 mg L⁻¹), FeSO₄·7H₂O (200 mg L⁻¹), ZnSO₄·7H₂O (10 mg L⁻¹), MnCl₂·4H₂O (3.0 mg L⁻¹), H₃BO₃ (30 mg L⁻¹), CoCl₂·6H₂O (20 mg L⁻¹), CaCl₂·2H₂O (1.0 mg L⁻¹), NiCl₂·6H₂O (2.0 mg L⁻¹), and Na₂MoO₄·2H₂O (3.0 mg L⁻¹) in deionized water. The pH of the NMS medium was about 6.7.

The enrichment and isolation procedures were based on methods described by Sheets et al. [18]. Each flask was sealed with a rubber stopper and its headspace was filled with a gas mixture containing 20% (v/v) CH₄ (99% purity purchased from Praxair[®], Danbury, CT, USA) and 80% (v/v) air. The flasks were incubated at 37°C and 150 rpm for enrichment of methanotrophs. Every 5 days, 5 mL of the culture was successively transferred to 50 mL of fresh NMS medium in a 250-mL flask which was then filled with the CH₄/air (1:4, v/v) mixture and incubated under the same conditions. After 5 successive transfers, samples from the enriched culture were spread on NMS agar plates. The plates were then incubated at 37°C in an airtight jar containing a CH₄/air (1:4, v/v) mixture in the headspace until colonies were observed (in 5 days). Individual colonies were picked and streaked on fresh NMS agar plates which were then incubated in the airtight jar under the same conditions until single colonies were observed.

Isolates from single colonies were cultured in 1 mL of NMS medium in 15-mL sealed culture tubes with a headspace containing a CH₄/air (1:4, v/v) mixture. The tubes were incubated at 37°C and shaken at 150 rpm until cell growth was observed as indicated by turbidity, e.g. optical density (OD) at 600 nm (OD_{600nm}) of 0.1 or higher. H₂S tolerance of the methanotrophic isolates was preliminarily tested by culturing each isolate under the same conditions but at

different H₂S concentrations (1000, 3000, and 5000 ppm) in the headspace. Isolates that tolerated high levels of H₂S were selected for further characterization.

2.2. Identification and characterization of H₂S-tolerant methanotrophic isolate

The full-length 16S rRNA gene of one H₂S-tolerant isolate was amplified and sequenced at the Plant–Microbe Genomics Facility at The Ohio State University, Columbus, Ohio, USA. The 16S rRNA gene sequence was taxonomically classified using RDP Classifier [21] and BLAST against the NCBI RefSeq database. Cell morphology and motility were examined by light microscopy. Gram staining was performed according to Cleenwerck et al. [22]. The heat tolerance was assessed according to Hoefman et al. [23]. The effect of temperature on cell growth was evaluated by cultivating the isolate in 20 mL of NMS medium in a 125-mL flask with a CH₄/air (1:4, v/v) mixture in the headspace at different temperature levels (20°C, 25°C, 30°C, 37°C and 45°C) with shaking at 150 rpm. The effect of pH on cell growth of SAD2 was evaluated at 37°C and 150 rpm using NMS media with different pH levels (5.0, 5.5, 6.0, 6.8, 7.5, 8.0, 8.5, and 9.0).

2.3. Cell growth under different levels of H₂S using pure methane as a carbon source

The methanotrophic isolate was inoculated into 50 mL of NMS medium in 250-mL flasks to reach an initial OD_{600nm} of 0.05. Each flask was connected to a 500-mL Tedlar gas bag, and the headspace of the flask and Tedlar gas bag was filled with a CH₄/air (1:4, v/v) mixture with different levels of H₂S (0, 500, 1000, 3000, and 5000 ppm). The total gas volume was 700 mL. The flasks were incubated at 37°C with shaking at 150 rpm for 108 hours. Cell density (OD_{600nm}) and gas composition (CH₄, O₂, N₂, and CO₂) were monitored twice a day. Three replicates were

used for each H₂S level. The cells prepared at different H₂S levels in the headspace were used to convert methane or biogas to methanol to evaluate how pre-exposure to H₂S affects methane-to-methanol conversion.

2.4. Methanol production under different levels of H₂S using pure methane as a carbon source

The experimental design is shown in Table 1. Briefly, methanotrophic isolate cells grown with a CH₄/air (1:4, v/v) mixture with 0, 500, and 1000 ppm of H₂S (as described in Section 2.3) were harvested by centrifugation at 8,000 g for 10 min (cells cultured at 3000 and 5000 ppm of H₂S were not used, since they did not grow at these H₂S levels). The harvested cells were resuspended in fresh NMS medium with 5 µM CuCl₂ and 100 mM sodium formate to reach an OD_{600nm} of 0.6. Each cell suspension (1 mL) was transferred to a 15-mL sealed culture tube, which was then filled with a CH₄/air (1:4, v/v) mixture containing different levels of H₂S (0, 500, and 1000 ppm) in the headspace. The tubes were incubated at 37°C in a shaking incubator at 150 rpm. After 48 hours, the cell suspensions were filtered (0.2 µm) and the filtrates were subjected to methanol analysis using gas chromatography (GC). Three replicates were used for each H₂S level.

2.5. Methanol production under different levels of H₂S using biogas as a carbon source

Biogas (70.6% CH₄, 28.6% CO₂, 0.5% N₂, and 0.3% O₂) was collected from a commercial scale digester (quasar energy group, Wooster, OH, USA) which was fed with food waste. The H₂S content in the biogas was lower than 50 ppm because H₂S control technologies were used in the AD facility [24]. Cell suspensions of the methanotrophic isolate were prepared as described

in Section 2.3 (under 0 ppm H₂S). Each cell suspension (50 mL) was transferred to a 250-mL flask, which was connected to a 500-mL Tedlar gas bag. The headspace of the flask and Tedlar gas bag was filled with biogas/air (1:2, v/v) with or without addition of 500 ppm H₂S to reach a total gas volume of 700 mL. The final H₂S levels were about 516 or 16 ppm. The flasks were then incubated at 37°C with shaking at 150 rpm for 48 h. Methanol concentration and gas composition (CH₄, O₂, N₂, and CO₂) were monitored twice a day. At the end of the incubation, samples of cell suspension were filtered (0.2 µm) and the filtrates were subjected to GC analysis for methanol. CH₄ to methanol conversion efficiency (%) was determined by dividing the moles of methanol produced by the moles of CH₄ consumed. Three replicates were used for each H₂S level.

2.6. Analytical methods

Optical density (OD_{600nm}) of the cell suspension was measured with an Eon® microplate spectrophotometer (Biotek, Winooski, VT, USA). The specific growth rate was calculated as $[\ln(OD_t/OD_0)]/t$, where OD₀ and OD_t are OD_{600nm} at the beginning and the end of the exponential growth phase, respectively, and t is the duration of the exponential growth phase.

Gas composition (CH₄, CO₂, N₂, and O₂) was analyzed using a gas chromatograph (Agilent, HP 6890, Wilmington, DE, USA), which was equipped with a 30 m × 0.53 mm × 10 µm Rt®-Alumina Bond/KCl deactivation column and a thermal conductivity detector. Helium gas was used as the carrier gas with a flow rate of 5.2 mL/min. Temperatures of the injector and detector were set at 150°C, and 200°C, respectively. The temperature of the column oven was initially set at 40°C for 4 min and later increased to 60°C at 20°C/minute and held at 60°C for 5 min.

Hydrogen sulfide content was determined according to Cline [25] with slight modifications. Briefly, 30 mL of gas sample was slowly bubbled into 2 mL of 2% zinc acetate in a sealed and vacuumed test tube that was upside down. The zinc acetate solution (0.5 mL) was mixed with 2.6 mL of de-ionized water, 0.5 mL of 20 mM N,N-dimethyl-p-phenylenediamine dihydrochloride (DPDH, in 7.2 M HCl), and 0.4 mL of 30 mM FeCl₃ (in 1.2M HCl). The mixture was incubated at room temperature for 20 min before measuring the absorbance at 670 nm with a spectrophotometer (Biotek, Winooski, VT, USA).

Methanol concentration in the filtrate samples was analyzed using a gas chromatograph (Shimadzu, 2010PLUS, Columbia, MD, USA), which was equipped with a Stabilwax polar phase column (30 m × 0.32 mm × 0.5 µm) and flame ionization detector. The temperatures of both the injector and the detector were set at 250°C, while the temperature of the column oven was initially set at 50°C and gradually increased to 80°C at a rate of 5.0°C min⁻¹. Helium was used as the carrier gas with a total flow rate of 24.8 mL min⁻¹ and a split ratio of 15.

2.7. Statistical analysis

Analysis of variance (ANOVA, $\alpha = 0.05$) was conducted using Minitab (Version 16, Minitab, Inc., State College, PA, USA) for assessing statistical significance.

3. Results and discussion

3.1. Isolation and characterization of a H₂S-tolerant methanotrophic strain SAD2

During 20-day SS-AD of expired dog food, the H₂S content in the biogas increased sharply to about 1000, 2000, 3000, and 4000 ppm in 1, 3, 5, and 8 days, respectively. CH₄ content also increased rapidly to 17% on day-1 and to over 60% on day-12. The CH₄ and H₂S contents

reached steady-state levels of about 63% on day-12 and 3900 ppm on day-7, respectively. Therefore, the digestate was considered suitable for isolating methanotrophic strains that are tolerant to H₂S. One methanotrophic isolate that grew with a CH₄/air mixture containing 1000 ppm of H₂S was obtained.

The V1 to V3 region of the 16S rRNA of the H₂S-tolerant isolate was 98% identical to that of *Methylocaldum gracile* VKM 14L (454/462), and thus the isolate was named as *Methylocaldum gracile* SAD2. PCR product of expected size was obtained using the *pmoA*-specific primers but not the *mmoX*-specific primers (Table 2), suggesting that strain SAD2 probably carries the *pmoA* gene (encoding the sub-unit of particulate methane monooxygenase, pMMO) gene, but not the *mmoX* gene (encoding the sub-unit of soluble methane monooxygenase, sMMO) and strain SAD2 likely uses pMMO, rather than sMMO, for methane oxidation to methanol.

Strain SAD2 cells were stained Gram-negative and motile short rods or cocci, which are typical cell morphologies of *Methylocaldum* species (Table 2). Unlike the reported *Methylocaldum* species, which form brown colonies and broth cultures, strain SAD2 formed white colonies and white broth cultures (Table 2). Besides, strain SAD2 had a broader pH range (6.0-8.0) for growth than *Methylocaldum* sp. 14B, which was recently isolated from solid-state anaerobic digestate (with switchgrass as a feedstock) [18] (Table 2). Although optimal pH for growth was 6.8-7.5 for strain SAD2, its growth at pH 8.0 was decreased by only less than 23% compared to that at the optimal pH (OD_{600nm} of 0.7-0.8 in 48 h). The temperature range in which strain SAD2 grew was 30°C-37°C, indicating that it is a mesophilic strain. However, strain SAD2 grew much slower at 30°C than that at 37°C, and the cell density at 30°C was only 35% of that at 37°C (OD_{600nm} of 0.7-0.8 in 48 h). No significant growth was observed at 20°C, 25°C, or

45°C. In addition, strain SAD2 appeared to be more sensitive to high temperature than other *Methylocaldum* species (Table 2).

3.2. Effect of H₂S on growth of strain SAD2

In the absence of H₂S, SAD2 grew normally and reached the stationary stage in 84 h (Figure 1), which was longer than that (72 h) of *Methylocalsum* sp. 14B [18]. The specific growth rate (0.05 h⁻¹) of strain SAD2 in the absence of H₂S was also lower than that of strain 14B (0.06 h⁻¹) [18], but comparable to those (0.02-1.0 h⁻¹) of *Methylosinus trichosporium* OB3b, a well-studied methanotroph for CH₄ conversion to methanol [26–28].

Cell growth of strain SAD2 was inhibited by H₂S at 500 ppm or higher (Figure 1). Complete inhibition was observed at H₂S contents of 3000 and 5000 ppm, with no or minimal cell growth, CO₂ generation, and O₂ and CH₄ consumption during 108 h of cultivation (Figure 1). Strain SAD2 still grew in the presence of 500 and 1000 ppm H₂S, though its specific growth rate was decreased to about 0.03 h⁻¹, indicating that strain SAD2 can be cultivated with H₂S contents of up to 1000 ppm in the gas phase.

Tolerance of strain SAD2 to H₂S was compared to that of two methanotroph strains reported in the literature (Table 3). The addition of 500 ppm H₂S resulted in a 39% and 47% reduction in biomass production of *Methylomicrobium album* ATCC 33003 and *Methylocystis* sp. ATCC 49242, respectively [12], but only caused a 17% decrease in biomass production of strain SAD2 (Table 3). At 1000 ppm H₂S, less reduction in biomass production was also observed for SAD2 (35%) than for *Methylomicrobium album* ATCC 33003 and *Methylocystis* sp. ATCC 49242 (49% and 85%, respectively) [12] (Table 3). Therefore, strain SAD2 showed greater H₂S tolerance than the two ATCC strains. *Methylomicrobium album* ATCC 33003 and *Methylocystis*

sp. ATCC 49242 were isolated from freshwater mud and aquifer (in southern California), where high levels of H₂S are not likely to occur [29,30]. In contrast, strain SAD2 was isolated from H₂S-rich AD systems where strains may have adapted to the H₂S inhibition. This could be the reason why strain SAD2 can tolerate higher H₂S than other two species.

3.3. Effect of H₂S on methanol production by strain SAD2 using pure CH₄

Overall, previous exposure to H₂S during growth of strain SAD2 had no significant ($p > 0.05$) effect on subsequent methanol production from pure methane (Figure 2). This indicates that cell preparation can be conducted under a wide range of H₂S levels (0-1000ppm) in terms of methanol production. The presence of H₂S at 500 ppm had no significant ($p > 0.05$) effect on methanol production by the cell suspension from pure CH₄, but numerically ($p > 0.05$) decreased methanol concentration, from 300-326 mg/L to 273-284 mg/L (Figure 2). The methanol concentration obtained by strain SAD2 in the presence of 500 ppm H₂S falls within the range of those (170-1,120 mg/L) obtained by other strains in the absence of H₂S [4,18]. In the presence of 1000 ppm H₂S, however, methanol production was significantly ($p < 0.05$) decreased (Figure 2). Based on these results, strain SAD2 can be grown on a CH₄/air mixture containing H₂S up to 1000 ppm, and it can convert methane to methanol in the presence of H₂S up to 500 ppm, which is above the H₂S level in the biogas from most anaerobic digesters. It should be noted that air needs to be mixed into the biogas to provide O₂ for methanotrophs to respire, resulting in dilution of H₂S present in biogas. Thus, strain SAD2 may be used in microbial conversion of biogas to methanol.

3.4. Effect of H₂S on methanol production by strain SAD2 using biogas

As shown in Figure 3, methanol concentration increased over time (Figure 3). When no H_2S was added to the gas phase, methanol concentration increased rapidly within 24 h, and started to level off at 36 h. A maximum methanol concentration of 343 mg/L was achieved at 48 h (Figure 3a), which is comparable to that obtained using pure methane (Figure 2). When 500 ppm H_2S was added to the gas phase, methanol production by strain SAD2 using biogas was lower ($p < 0.05$), with a maximum methanol concentration of 276 mg/L at 36 h (Figure 3), which is similar to that achieved with a CH_4/air (1:4, v/v) mixture containing 500 ppm of H_2S (Figure 2). CH_4 content decreased concomitantly with increasing methanol concentration during the incubation, and addition of 500 ppm H_2S decreased the methane consumption (Figure 3). The addition of 500 ppm H_2S also decreased CH_4 -to-methanol conversion efficiency (from 34% to 30%). Until now, microbial conversion of CH_4 to methanol in the presence of H_2S has not been reported. Strain SAD2 is the first methanotrophic bacterium that can convert raw biogas to methanol, and the H_2S level can be up to at least 500 ppm. As mentioned in the introduction, additional cost for H_2S removal can offset the benefit of methanol production by 50%. By using H_2S -tolerant methanotrophic bacteria, the cost for H_2S removal can be saved with only slight decrease in methanol production, e.g. 12% for strain SAD2 (Figure 3). Therefore, isolation of robust methanotrophic bacteria from H_2S -rich environments is economically feasible to address the issue of inhibition by H_2S (or other compounds) when raw biogas is used for methanol production. As shown in Table 4, the CH_4 -to-methanol conversion efficiency (30%) obtained by SAD2 using biogas/air (1:2) with addition of 500 ppm H_2S falls within the range of those (27-80%) achieved by other methanotrophs in the absence of H_2S or with a minimal amount of H_2S [4,18]. It should be noted that the CH_4 -to-methanol conversion efficiency can possibly be further improved through selection of MDH inhibitors as reported by Han et al. [31].

4. Conclusions

A new methanotrophic isolate, *Methylocaldum gracile* SAD2, was isolated, and it can grow on biogas in the presence of 1000 ppm H₂S in the headspace. Strain SAD2 showed greater H₂S tolerance than two commonly studied methanotrophic species, *Methylomicrobium album* and *Methylocystis* sp. ATCC 49242. Strain SAD2 was shown to convert biogas containing H₂S of up to 500 ppm to methanol at a methane-to-methanol efficiency comparable to other methanotrophic strains in the absence of H₂S. Therefore, strain SAD2 may be used in microbial conversion of raw biogas to methanol.

Acknowledgements

This material is based upon work that is supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, under award number 2012-10008-20302. The authors would like to thank Mary Wicks (Department of Food, Agricultural and Biological Engineering, OSU) for reading through the manuscript and providing useful suggestions. The authors also thank Dr. Fuqing Xu for her valuable comments.

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Figure 3 Effect of H₂S on methanol production and CH₄ consumption by SAD2 using biogas

Table 1 Experimental design for methanol production with SAD2

No.	Initial H ₂ S levels (ppm)	
	Cell preparation	Methanol production
1	0	0
2	500	0
3	1000	0
4	0	500
5	500	500
6	1000	500
7	0	1000
8	500	1000
9	1000	1000

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Table 2 Comparison of characteristics of strain SAD2 to those of other reported *Methylocaldum* species

Strains	Strain SAD2	<i>Methylocaldum</i> . sp. 14B	<i>M. gracile</i> VKM 14L	<i>M. tepidum</i> LK6	<i>M. szegediense</i> OR2
pMMO	+	+	+	+	+
sMMO	-	-	-	-	-
Gram staining	-	ND	ND	ND	ND
Motility	+	+	+	+	+
Cell shape	Rod/coccus	Coccus/pleomorphic	Thin rod/coccus	Rod/pleomorphic	Rod/pleomorphic
Colony color	White	Brown	Brown	Brown	Brown
Color in broth culture	White	Brown	Brown	Light brown	Dark brown
Growth pH	6.0-8.0	6.0-7.6	ND	ND	ND
Growth temperature (°C)	30-37	37-42	20-47	30-47	37-62
Heat tolerance	-	ND	-	-	+
Isolation source	Digestate from AD of dog food	Digestate from AD of switchgrass	Activated sludge	Agricultural soil	Hot spring
Reference	This study	[18]	[14]	[14]	[14]

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Table 3 Comparison of H₂S tolerance between strain SAD2 and two ATCC strains

Strains	<i>Methylomicrobium album</i> ATCC 33003	<i>Methylocystis</i> sp. ATCC 49242	Strain SAD2
Inhibition on biomass production (%)*			
Initial H ₂ S of 500 ppm	39	47	17 ± 4
Initial H ₂ S of 1000 ppm	49	85	35 ± 9
Reference	[12]	[12]	This study

* Relative to control that was not exposed to H₂S.

Table 4 Comparison of methane to methanol conversion efficiency between different methanotrophs

Species	Mixture of gases (v/v)	H ₂ S in head space (ppm)	CH ₄ to methanol conversion efficiency (%)	Reference
<i>Consortium</i>	Biogas:air = 4:6 ^a	0	43-80 ^e	[4]
<i>Methylosinus trichosporium</i>	CH ₄ :O ₂ = 1:1	0	27	[4]
<i>Methylosinus trichosporium</i>	CH ₄ :Air = 1:1.6	0	61	[4]
<i>Methylosinus trichosporium</i>	CH ₄ :Air = 1:1	0	>60	[4]
Strain 14B	Biogas:Air = 1:3.1 ^b	<12 ^c	26	[18]
Strain SAD2	Biogas:Air = 1:2 ^b	<17 ^c	34	This study
Strain SAD2	Biogas:Air = 1:2 ^b	~517 ^d	30	This study

a: Artificial biogas; b: Biogas contained a small amount (<50ppm) of H₂S; c: H₂S concentration was calculated based on the biogas to air ratio; d: Besides the H₂S from the biogas, additional 500ppm H₂S was supplied to the headspace; e: Methane to methanol conversion efficiency varied with different types of MDH inhibitor.

(a)

(b)

(c)

(d)

Figure 1 Effect of H₂S on cell growth (a), CO₂ generation (b), and consumption of O₂ (c) and CH₄ (d) during strain SAD2 cultivation

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478 Figure 2 Effect of H₂S on methanol production by strain SAD2 using pure CH₄. Means with
479 different letters are significantly different ($p < 0.05$).
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Figure 3 Effect of H_2S on methanol production and CH_4 consumption by SAD2 using biogas