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Biological conversion of H_2 and CO_2 into CH_4 at room temperature and atmospheric pressure: a promising approach for reducing CO_2 emissions and increasing biomethane production

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An inoculum of methane-fermented active sludge was used to enrich hydrogenotrophic methanogens, by culturing using a gas mixture of H_2, CO_2 and N_2 as the substrate. Culturing was performed at room temperature and atmospheric pressure and the types and changes of methanogenic archaea in the active sludge before and after the tests were characterized. The results showed that the highest yield of CH_4 obtained was 258 mL from 3L of gas mixture, representing 51.6% of the theoretical maximum. The archaeal population present in the inoculum at the start of the experiment was dominated (84%) by the obligate acetotrophic *Methanosaeta*, which was reduced to 64% at the end of the experiment. In contrast, *Methanosarcina*, which is capable of using H_2 and CO_2, doubled from 8% in the original inoculum to 16% at the end. These experiments demonstrate that cultivation by gas cycling can realize bioconversion of H_2 and CO_2 into CH_4.

Key words: Hydrogenotrophic methanogenic archaea, H_2, CO_2, CH_4, Bioconversion, Gas cycle

INTRODUCTION

During the production of biogas, the gases H_2 and CO_2 can be used by hydrogenotrophic methanogenic archaea to form methane (CH_4) [1]. This metabolic pathway contributes up to 28% of the CH_4 generation in a biogas fermentation system [2]. A large amount of research has been conducted on the bioconversion of CO_2 into CH_4 with the aim of reducing CO_2 emissions [3-7]. For instance, the study of Koide *et al*. indicated that CH_4 may be produced while injecting CO_2 into the ground through artificial simulation of the natural carbon cycle, which would partially solve the problem of greenhouse gas emissions by means of carbon capture and storage [8].

Biogas contains approximately 60% of CH_4 and 35% of CO_2 [9]; the relatively high content of the latter lowers the efficiency of the biogas as energy carrier and is a considerable waste of the carbon source that could, at least potentially, be used by the microorganisms to produce methane. The efficiency of the bioconversion of CO_2 into CH_4 *via* the net reaction $4H_2+CO_2 \rightarrow CH_4+2H_2O$ [10, 11] could be greatly enhanced if the remaining CO_2 could also be converted.

Methanogenic prokaryotes can be classified into acetotrophic species that metabolize acetate as a substrate [12], hydrogenotrophic species that use the substrates H_2 and CO_2 [13], and methylotrophic species using methanoic acid, methanol and methylamine as substrates [14]. Tracer tests have indicated that acetotrophic methanogenic archaea account for more than 70% of the methanogenic archaea typically present in a biogas fermentation system [15]. With the necessary measures taken, growth of hydrogenotrophic methanogens can be promoted, resulting in higher efficiency performance. To this purpose, a biogas fermentation system was designed for operation at normal temperature and pressure, to promote the metabolism and growth of hydrogenotrophic methanogens by addition of excess H_2 and CO_2. The efficiency of methane production was studied and the microbial populations at play were characterized.

EXPERIMENTAL

Inoculum and metabolic substrates

A mixture of anaerogenic and anaerobic active sludge was used as the inoculum for the described experiments, which has been cultivated for a long time in our laboratory. The sludge parameters are 6.83% total solid (TS) content and 59.97% volatile solid (VS) content, with a pH of 7.5. The metabolic substrates used were high purity H_2 and CO_2, while high purity N_2 was provided as the nitrogen source for the microorganisms. No other carbon source than CO_2 was added.

Test setup

Figure 1 shows a schematic drawing of the test setup, which consisted of a fermentation reactor made of plexiglass (dimensions: content 3.18 L, height-to-diameter ratio 5:1), a wet-seal gas holder made of plexiglass (effective volume 4.08 L), a gas circulation pump (YZ2515X, Chuangrui Pump, Co., Ltd., China) with a flow rate ranging from 0 to 250

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mL/min, PVC gas circulating tubes and gas input cylinders for H_2, CO_2 and N_2.

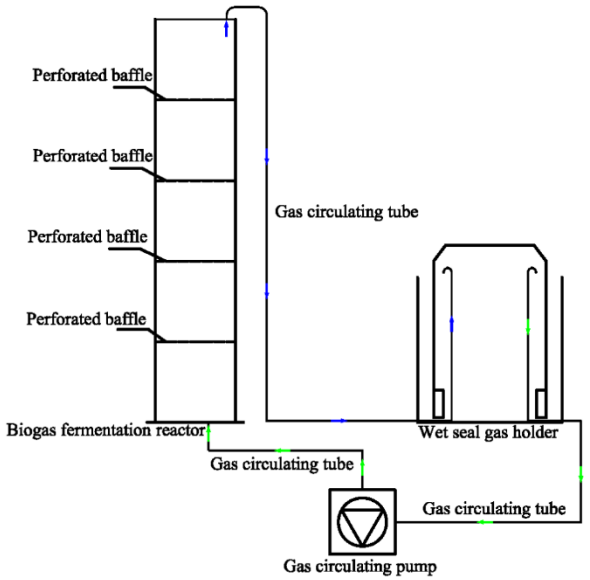

Fig. 1. Schematic diagram of the test setup for enrichment and cultivation of hydrogenotrophic methanogenic archaea

Test method

At the start of the experiment, the fermentation reactor was filled with 1.5 L of inoculum (approximately half of the reactor volume) and then filled up with liquid bioslurry from our laboratory. A gas mixture with volume ratio H_2:CO_2:N_2=4:1:1 was used in accordance with the stoichiometry of the reaction equation ($4H_2+CO_2 \rightarrow CH_4+2H_2O$). Approximately 3 L of gas (containing 2 L of H_2, 0.5L of CO_2 and 0.5 L of N_2) was added through the wet-seal gas holder till the floating bell raised into the lock position. The gas mixture was then pumped into the biogas fermentation reactor by the circulating pump at a flow rate of 12.5 mL/min and returned to the gas holder after passage through the sludge bed. Gas was continuously recirculated at room temperature and normal pressure, while the floating bell continuously sank during the reaction process. When the floating bell reached final position, the test series was considered completed. After composition analysis of the gas left inside the floating bell, the gas holder was emptied and cleaned for the next set of experiments. The inoculum was not replaced for each novel test. The tests included two stages: experimental start-up stage and test operation stage.

Test method

The pH of the sludge was monitored with a digital pH meter (PHS-3C, Hongyi Instruments China) [16]. The gas composition was determined by gas chromatography (GC-6890A, Lunan Apparatus, China) [17]. TS and VS contents were determined using a gravimetric method after drying at 110 °C and 560 °C, respectively [18]. The types and variations in methanogenic archaea were monitored by 16S rDNA sequencing, for which a DNA extraction kit (MO BIO Laboratories, USA) [19] was used to extract the total DNA of samples while the 16S rDNA amplification (515F and 806R primers) [20, 21] and sequencing was outsourced.

RESULTS AND DISCUSSION

Test start-up stage

The test start-up stage was needed to eliminate any disturbing factors for the subsequent analysis. For instance, water inside the reactor may initially absorb CO_2 (1L water has the capacity to absorb 1L CO_2 at normal temperature and pressure [22]) or any residual CH_4 present in the inoculum could be at first released. The start-up stage lasted 10 days, during which CO_2 continuously entered the reactor, until the gas volume was stabilized and the floating bell remained in position indicating that water and CO_2 in the reactor were saturated and at equilibrium. During this period, the gas composition was analyzed which showed that during the start-up stage 2.2 mL of CH_4 were produced, while it was obvious that this CH_4 has not been liberated from the inoculum since the inoculum did not contain any metabolic substrates for biogas fermenting microorganisms. Thus, it was concluded that after 10 days the system was stable and ready for further tests.

Test operation stage

During the test operation stage, five subsequent tests were performed (each test lasted 5 days) to cultivate hydrogenotrophic methanogenic archaea, while the CH_4 yields were recorded. The results are shown in Figure 2.

As can be seen, CH_4 was produced in all 5 tests, proving that methane can be produced from CO_2 and H_2 by microorganisms as a result of adding external H_2 and CO_2 into the system. During the second, best-performing test, 258 mL of CH_4 was produced, which is 51.6% of the theoretical maximum. The efficiencies of the other tests varied from 27.7% to 37.0%.

Methanogenic archaea community and change analysis

The methane produced in the test setup was most likely produced by hydrogenotrophic methanogenic archaea, while variation in the yield could have been

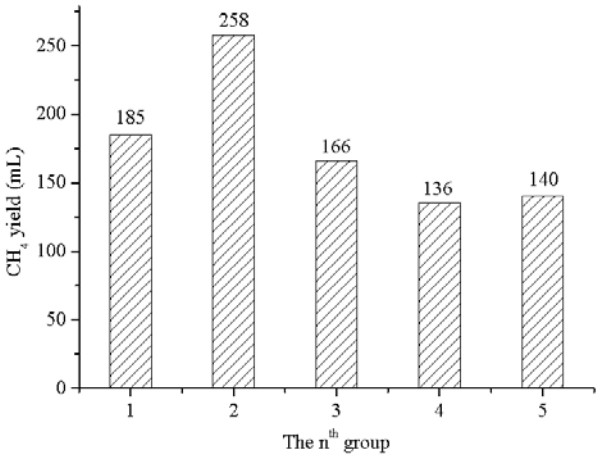

Fig. 1. CH_4 final yield during the test operation stage

result of shifts in the populations. In order to characterize the key players, total DNA was extracted from the inoculated sludge prior to the start of the experiment, and again from the active sludge after completion of the last test. This DNA was used for 16S rDNA amplification and sequencing to analyze the archaeal populations. The results are summarized in Table 1.

As can be seen from Table 1 the obligate acetotrophic *Methanosaeta* [23] comprised a large fraction of the initial inoculum (as determined by 16S sequencing) but their number was significantly reduced during the tests, probably because no acetic substrates were provided. Nevertheless, this genus still represented 64% of all genera detected at the end of the experiment.

Table 1. Contents and changes in methanogenic archaea in populations

Methanogenic archaea	Percentage present	
	At start	After tests
Methanosaeta	84	64
Methanosarcina	8	16
Methanocorpusculum	0.6	0.7
Methanoculleus	0.5	0.9
Methanospirillum	0.4	1
Methanolinea	ND	0.7
Methanoplanus	ND	0.1
Methanofollis	ND	0.2
Methanobacterium	0.9	1

Notes: ND means no DNA detected.

Methanosarcina was the next most abundant species, which can utilize both acetic substrates, as well as H_2 and CO_2 [24]. The latter capacity allowed their increase during the experiment, resulting in a doubling of their number. The hydrogenotrophic *Methanocorpusculum* [25], *Methanoculleus* [26], *Methanospirillum* [27] and *Methanobacterium* [28] species significantly increased during the tests, though their number was relatively low in comparison to *Methanosarcina*. It is worth noting that other hydrogenotrophic methanogens such as *Methanolinea* [29], *Methanoplanus* [30] and *Methanofollis* [31] species could not be detected in the inoculum due to their originally low abundance, though after the test they were present in detectable numbers.

The results reported here can be advantageous for initiatives to reduce CO_2 emission, produce a renewable energy carrier (biological synthesis of CH_4), and increase potential economic benefits of such initiatives by improved efficiency. The biological conversion of H_2 into the relatively safer energy carrier CH_4 may be carried out using CO_2 which is typically present at levels of 35% in biogas. This would increase the CH_4 content of the biogas, leading to an improved energy content of the biogas.

CONCLUSIONS

(1) Methane was produced from circulating gas in all tests performed, with the highest yield reaching 51.6% of the theoretical maximum. (2) During the experiment, the microbial composition of the sludge changed, from an initial dominance of obligate acetic *Methanosaeta* to a doubling of *Methanosarcina* and an increase of other hydrogenotrophic and methylotrophic species. Thus, cultivation by gas circulation (H_2 and CO_2) may increase the proportion of hydrogenotrophic methanogenic archaea, which would improve the subsequent efficiency of biofermentation.

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