Korean J. Chem. Eng., 32(10), 2067-2072 (2015)
DOI: 10.1007/s11814-015-0023-0

pISSN: 0256-1115
eISSN: 1975-7220

Factors affecting biological reduction of CO_2 into CH_4 using a hydrogenotrophic methanogen in a fixed bed reactor

Jae Hyung Kim*, Won Seok Chang, and Daewon Pak*,†**

*Graduate School of Energy and Environment, Seoul National University of Science and Technology, 172, Gongreung-dong, Nowon-gu, Seoul 139-743, Korea
**Korea District Heating Corp, Seongnam-si, Gyeonggy 463-908, Korea

(*Received 11 June 2014 • accepted 27 January 2015*)

Abstract−Biological conversion of CO_2 was examined in a fixed bed reactor inoculated with anaerobic mixed culture to investigate influencing factors, the type of packing material and the composition of the feeding gas mixture. During the operation of the fixed bed reactor by feeding the gas mixture (80% H_2 and 20% CO_2 based on volume basis), the volumetric CO_2 conversion rate was higher in the fixed bed reactor packed with sponge due to its large surface area and high mass transfer from gas to liquid phase compared with PS ball. Carbon dioxide loaded into the fixed bed reactor was not completely converted because some of H_2 was used for biomass growth. When a mole ratio of H_2 to CO_2 in the feeding gas mixture increased from 4 to 5, CO_2 was completely converted into CH_4. The packing material with large surface area is effective in treating gaseous substrate such as CO_2 and H_2. H_2, electron donor, should be providing more than required according to stoichiometry because some of it is used for biomass growth.

Keywords: CO_2 Conversion, Methane, Fixed Bed Reactor, Hydrogenotrophic Methanogens

INTRODUCTION

Biological carbon mitigation has utilized photosynthetic autotrophic organisms and plants for carbon sequestration. Photosynthetic organisms, including macroalgae, microalgae and cyanobacteria, utilize the process of carbon fixation as their food source by converting the CO_2 into organic carbon [1-4]. In culturing photosynthetic organisms using energy from light in the photosynthetically active radiation range, one of the main challenges is the capital cost due to the requirement of photobioreactor. The required photobioreactors are expensive. Pond production as an alternate process is less expensive to build and operate, but it is more difficult to keep culture axenic.

Instead of photosynthetic organisms, chemoautotrophs are also able to convert CO_2 into organic carbon. They use inorganic energy sources such as molecular hydrogen to reduce CO_2 into methane. Biologically produced methane is a clean energy source that is important for electrical generation by burning it as a fuel in a gas turbine or steam generator. Compared to other hydrocarbon fuels, burning methane produces less CO_2 for each unit of heat released. It also can be used as a vehicle fuel and is claimed to be more environmentally friendly than other fossil fuels such as gasoline and diesel.

As a biological carbon mitigation using chemoautotrophs, hydrogenotrophic methanogens are reported to have the capability to utilize CO_2 as an electron acceptor and be able to produce methane from hydrogen-carbon dioxide mixture without other organic carbon sources [5]. They are inhabitants of the anaerobic reactor, which has been regarded as an attractive process for the reduction of various types of compounds. Our previous study showed that CO_2 fed to the fixed bed reactor inoculated with anaerobic mixed culture from the anaerobic digestor of sewage treatment plant was reduced with H_2 without organic carbon source [6]. Methane formation commenced on the first day of operation of the fixed bed reactor. This indicates that the population of hydrogenotrophic methanogens grown under methanogenic environment is high enough to show their metabolic activity, and takes advantage of the attached growth system.

In many cases, higher metabolic activities were measured within the attached growth system compared to the suspended growth system [7]. Higher metabolic activity has been attributed to the higher amount of active biomass. Another explanation for higher activity is a physiological difference between attached and suspended microorganisms caused by the switching on of different genes [8]. The difference finds expression in a faster growth rate and increased metabolic activity. The attached growth system is beneficial to chemoautotrophic microorganisms such as hydrogenotrophic methanogens, which generally show slow growth rate compared with heterotrophic microorganisms.

When handling the gaseous substrate like H_2 and CO_2, the reactor configuration employing attached growth is important in determining their conversion efficiency. H_2 and CO_2 are hydrophobic and insoluble in water. The substrate, however, has to be solubilized or, otherwise, cannot participate in the biological conversion. From the engineering point of view, the packing material for fixed bed reactor is a very important factor in solubilizing gaseous substrate and designing the system. In the attached growth system like a fixed bed reactor, the selection of packing material depends on many factors, including the resistance to microbial degradation,

†To whom correspondence should be addressed.
E-mail: daewon@seoultech.ac.kr, dwpak@naver.com

mechanical strength, type of fluid and surface characteristics [7]. Especially, the type of packing material is an important factor in handling the gaseous substrate like CO_2 and H_2. Due to their low solubility, gaseous substrates have to stay in contact with aqueous medium as long as possible. Selection of packing material determines the path of gaseous substrates in the fixed bed reactor and the mass transfer from gas to aqueous phase. Depending on the mass transfer, the availability of gaseous substrates to the microorganisms will be changed and determine their conversion rates.

We tested two different types of packing materials to investigate how they affect the biological reduction of CO_2 with H_2 in the fixed bed reactor. The fixed bed reactors were designed, packed with different packing materials and continuously operated by feeding the gas mixture of H_2 and CO_2. The composition of the gas mixture fed to the reactor could be another factor affecting biological reduction of CO_2 with H_2. According to the stoichiometry, 4 mol of H_2 is needed to reduce 1 mol of CO_2 if the electron donor, H_2, is coupled only with the electron acceptor, CO_2. However some portion of electron donor can be used for energy to synthesize biomass. In addition, the availability of substrates to microorganisms can be determined by the mass transfer from gas to aqueous phase. Therefore, the composition of the feeding gas mixture can be an important factor that has to be figured out to maximize the conversion rate of CO_2. The mole ratio of H_2 to CO_2 in the feeding gas mixture was varied from 4 to 5. The volumetric loading and conversion rates of H_2 and CO_2 were estimated to determine the effect of those factors on biological conversion of CO_2 into CH_4.

MATERIAL AND METHODS

1. Continuous Operation of Anaerobic Fixed Bed Reactor

The experimental system, shown in Fig. 1, is composed of an up-flow anaerobic fixed bed reactor (7.8 L working volume) and gas cylinders (CO_2 and H_2). Internal diameter and height of the fixed bed reactor are 10 cm and 100 cm, respectively. The fixed bed reactor was packed with reticulated polyester urethane sponge (10 pore per inch), and the other with polystyrene ball (1-2 mm in diameter). The density of sponge and PS ball is 28-30 and 6.8-7.1 kg/m^3, respectively. Anaerobic bacteria consortium obtained from the anaerobic digester of Jungrang sewage treatment plant located in Seoul, Korea was used to inoculate the fixed bed reactors. 6.0 L of the anaerobic bacteria consortium (11,600 mg/*l* of volatile suspended solids) was transferred anaerobically to the fixed bed reactor packed with 1.8 L of sponge or PS ball. The sponge and PS ball acted as the support for biofilm growth and provided a different path of the gas bubbles in the reactor. The headspace of the reactor was filled with oxygen-free nitrogen before the operation and then spontaneously replaced with the gas products generated from the anaerobic mixed culture. Temperature of the reactor was maintained at 35 °C in a constant temperature chamber. Mixture of CO_2 and H_2 was provided to the bottom of the fixed bed reactor through the gas sparger. EBCT of gaseous substrates was changed by varying the flow rate of the gas mixture.

2. Analysis

250 μl of gas sample was taken from inlet and outlet of the fixed bed reactor to analyze CO_2, H_2 and CH_4 and injected into a gas chromatograph (GC 6000 series, Younglin, Korea) equipped with TCD (thermal conductivity detector). Argon was the carrier gas at a flow rate of 30 ml/min. Gas chromatography was optimized for analytes and the following parameters were used: oven temperature 35-210 °C (20 °C/min), injection temperature 220 °C, detector temperature 220 °C).

RESULTS AND DISCUSSION

1. Effect of Packing Material on the Conversion Rate of H_2 and CO_2

Two fixed bed reactors (one packed with sponge and the other

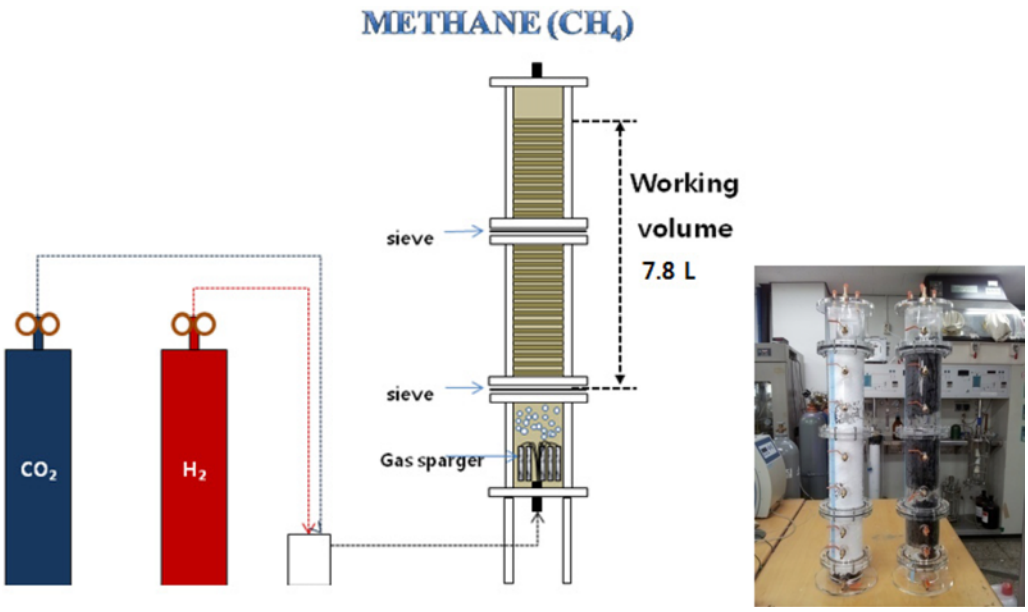

Fig. 1. Experimental set-up for biological conversion of CO_2 to CH_4.

packed with PS balls) were continuously operated by feeding the gas mixture of H_2 and CO_2. The mole ratio of H_2 to CO_2 in the feeding gas mixture was maintained at 4. The volumetric loading rates and conversion rates of H_2 and CO_2 in the fixed bed reactor packed with sponge are shown in Fig. 2. The volumetric loading rate of CO_2 was increased from 0.146 to 0.686 m^3/m^3/day, and the volumetric loading rate of H_2 was four times of CO_2 increasing from 0.584 to 2.743 m^3/m^3/day. EBCT of the gas mixture was decreased from 33 hours to 7 hours. When fed to the reactor, hydrogen was completely utilized by the microorganisms. The solubility of hydrogen in aqueous phase (the mole fraction solubility of 1.411×10^{-5} at 298.15 K) is much lower than CO_2 (the mole fraction solubility of 6.15×10^{-4} at 298.15 K) [9,10]. Due to its low solubility, the transfer of H_2 from the gaseous to the liquid phase can be a limiting step. However, H_2 was completely utilized by hydrogenotrophic methanogens as soon as available. Even though CO_2 has higher

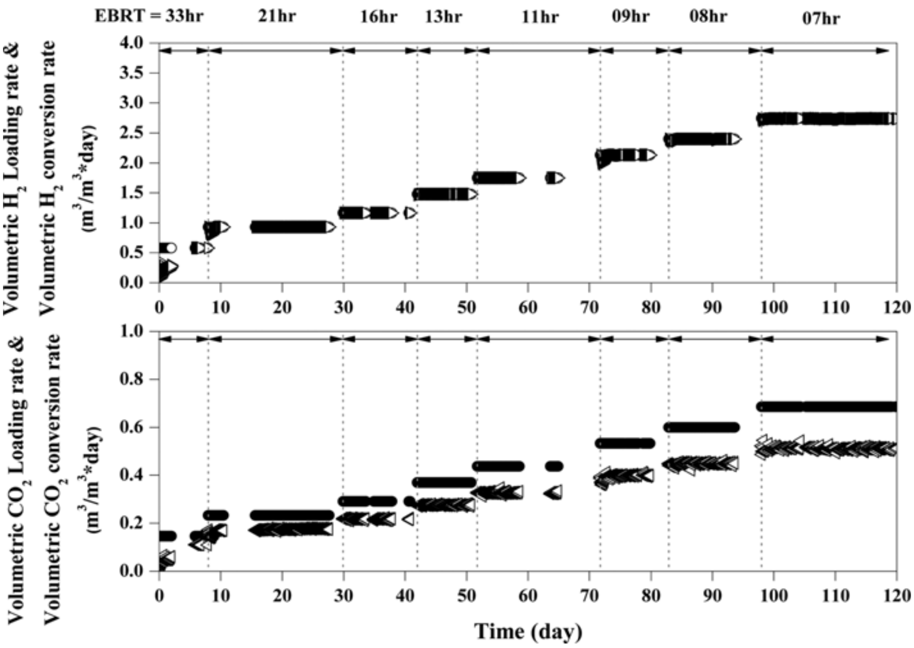

Fig. 2. Comparison between the volumetric loading rate and conversion rate of H_2 and CO_2 in the fixed bed reactor packed with sponge (○: volumetric H_2 loading rate, ▷: volumetric H_2 conversion rate, ●: volumetric CO_2 loading rate, ◁: volumetric CO_2 conversion rate).

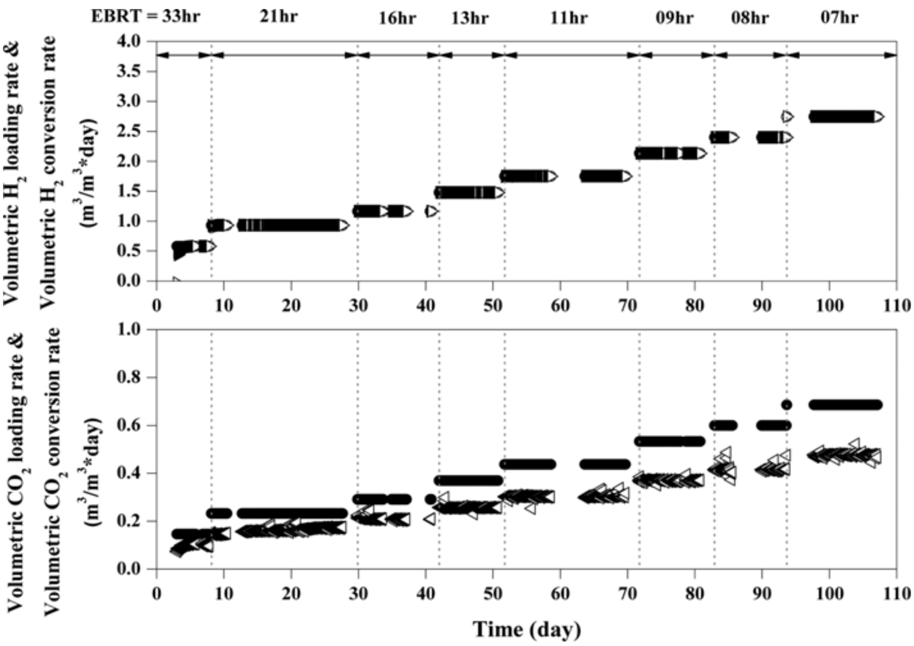

Fig. 3. Comparison between the volumetric loading rate and conversion rate of H_2 and CO_2 in the fixed bed reactor packed with PS ball (○: volumetric H_2 loading rate, ▷: volumetric H_2 conversion rate, ●: volumetric CO_2 loading rate, ◁: volumetric CO_2 conversion rate).

solubility in aqueous phase, it was not completely utilized by the microorganisms. This can be inferred from the difference between the volumetric loading rate and conversion rate of CO_2.

The volumetric loading rates and conversion rates of H_2 and CO_2 in the fixed bed reactor packed with PS balls are shown in Fig. 3. As seen in the fixed bed reactor packed with sponge, H_2 was completely utilized by hydrogenotrophic methanogens. The volumetric CO_2 conversion rate was low in the fixed bed reactor with PS balls compared with sponge. The comparison between sponge and PS ball in terms of the volumetric CO_2 conversion rate is shown in Table 1. At long EBRT (from 16 to 33 hours), there was no difference in the volumetric CO_2 conversion rate. However, when operated at short EBRT (from 13 to 7 hours), the fixed bed reactor with sponge has shown higher volumetric CO_2 conversion rate.

The ratios of volumetric CO_2 conversion rate to volumetric H_2 conversion rate in the fixed bed reactor operated at different EBRT are listed in Table 2 and Table 3. The average ratio is 0.19 and 0.17 for sponge and PS ball, respectively. This result indicates that under the same volumetric H_2 loading rate, the volumetric CO_2 conversion rate is higher in the fixed bed reactor packed with sponge. The higher volumetric CO_2 conversion rate could be attributed to higher CO_2 flux from gas to liquid phase. Considering a gas-liquid system, the flux of solute i (R_i) can be described as follows:

$$R_i = k_L\,(P_i/H_i - C_i) \qquad (1)$$

where P_i and C_i are partial pressure of the substrate i in gas phase

Table 1. Comparison between sponge and PS ball in term of volumetric CO_2 conversion rate

EBRT (hr)	Sponge (a) ($m^3/m^3/hr$)	PS ball (b) ($m^3/m^3/hr$)	a-b ($m^3/m^3/hr$)
33	0.004	0.004	-
21	0.007	0.007	-
16	0.009	0.009	-
13	0.011	0.010	0.001
11	0.014	0.013	0.001
9	0.016	0.015	0.001
8	0.019	0.017	0.002
7	0.021	0.020	0.001

Table 2. Comparison between volumetric conversion rate of H_2 and CO_2 in the fixed bed reactor packed with PS ball

EBRT (hr)	Volumetric H_2 conversion rate (a) (m^3/m^3*hr)	Volumetric CO_2 conversion rate (b) (m^3/m^3*hr)	b/a
33	0.023	0.004	0.17
21	0.039	0.007	0.17
16	0.049	0.009	0.18
13	0.062	0.011	0.17
11	0.073	0.013	0.17
9	0.089	0.015	0.16
8	0.100	0.017	0.17
7	0.114	0.020	0.17

Table 3. Comparison between volumetric conversion rate of H_2 and CO_2 in the fixed bed reactor packed with sponge

EBRT (hr)	Volumetric H_2 conversion rate (a) ($m^3/m^3/hr$)	Volumetric CO_2 conversion rate (b) ($m^3/m^3/hr$)	b/a
33	0.012	0.003	0.25
21	0.039	0.007	0.18
16	0.049	0.009	0.18
13	0.062	0.011	0.17
11	0.073	0.014	0.19
9	0.088	0.016	0.18
8	0.100	0.019	0.19
7	0.114	0.021	0.18

and concentration of substrate i in aqueous phase, respectively, while k_L and H_i are the mass transfer coefficient and Henry's constant of substrate i, respectively. From Eq. (1), the mass flux of CO_2 is proportional to its partial pressure. There was no difference in the partial pressure of CO_2 in the both reactor because of identical composition in the feeding gas mixture. The reason for higher volumetric CO_2 conversion rate could be that the sponge provides more surface area for mass transfer. This resulted into an increase in total amount of CO_2 transferred into the aqueous phase.

The average ratio of volumetric CO_2 conversion rate to volumetric H_2 conversion rate should be 0.25 according to the stoichiometry. However, the ratios obtained from continuous operation of both reactors were less than 0.25, likely because biomass growth and substrate utilization are coupled. If the stoichiometric equation for biomass growth can be written with the substrate as the basis, the generalized equation for microbial growth can be described as: [11]

$$\text{Carbon source+energy source+electron acceptor+nutrient} \rightarrow \text{Biomass+reduced acceptor+end product} \qquad (2)$$

All nonphotosynthetic microbial growth reactions consist of two components, one for synthesis and the other for energy. Using the concept of half-reactions, the overall stoichiometric equation (R) is the sum of three types of half-reactions: one for cell material (R_c), one for the electron donor (R_d) and one for electron acceptor (R_a):

$$R = R_d - f_e \cdot R_a - f_s \cdot R_c \qquad (3)$$

The term f_e represents the fraction of electron donor that is coupled with the electron acceptor, i.e., the portion used for energy, hence the subscript e, and f_s represents the fraction captured through synthesis. Furthermore, for Eq. (3) to balance:

$$f_e + f_s = 1.0 \qquad (4)$$

This equation is equivalent to stating that all electrons originally in the electron donor end up either in the biomass synthesized (f_s) or in the electron acceptor (f_e). For autotrophic growth like hydrogenotrophic methanogens in this study, the electron donor is an inorganic substance such as H_2. A portion of H_2 was used for biomass growth and the other for energy. This explains why the average ratio of volumetric CO_2 conversion rate to volumetric H_2 conversion rate was less than 0.25.

Table 4. Comparison of volumetric methane production rate between PS ball and sponge

EBRT (hr)	Volumetric methane production rate ($m^3/m^3/hr$)		Increasing rate (b-a)/a×100
	PS ball (a)	Sponge (b)	
21	163.19±9.67	169.41±10.35	3.81
16	209.63±12.90	217.25±2.00	3.63
13	250.69±5.51	275.62±1.99	7.37
11	303.60±5.39	326.64±3.35	7.59
9	369.94±4.12	393.84±10.25	6.46
8	417.25±24.85	447.95±5.04	7.36
7	475.79±6.95	510.86±6.04	7.37

In Table 4, the volumetric CH_4 production rates were compared between sponge and PS ball. Higher volumetric CO_2 conversion rate resulted in higher volumetric CH_4 production rate. During the operation of the fixed bed reactor packed with sponge at long EBRT (16 and 21 hours), volumetric CH_4 production rate was enhanced by 3.8% with sponge compared with PS ball. At short EBRT (from 7 to 13 hours), the volumetric CH_4 production rate was increased by 7.3%. A porous media like a sponge provides a very complicated path for gaseous substrates and large surface area. This could help the substrates transfer from gaseous to liquid phase, increase the total amount of substrate transferred and enhance the production rate of CH_4 in the reactor.

2. Effect of Mole Ratio of H_2 to CO_2 in the Feeding Gas Mixture

From the empirical formula for CH_4 production from H_2 and CO_2, the mole ratio of H_2 to CO_2 is 4. Five moles of the gas mixture is required to produce 1 mole of CH_4. The volume factions of H_2 and CO_2 in the feeding gas mixture were maintained at 0.8 and 0.2, respectively. Fig. 4 shows the comparison between the volumetric loading and conversion rate of H_2 and CO_2 in the fixed bed reactor packed with sponge operated at 6 hours of EBCT. During the operation, the volumetric H_2 loading rate was equal to its volumetric conversion rate. This indicates that H_2 provided to the fixed bed reactor was completely utilized by the microorganisms. However, volumetric CO_2 conversion rate was lower than its volumetric loading rate. From the results, H_2 appears to be the limiting substrate in the fixed bed reactor fed with the gas mixture consisting of 80% H_2 and 20% CO_2 based on volume base.

In Fig. 5 are compared the volumetric loading rate and conversion rate of H_2 and CO_2 in the fixed bed reactor packed with sponge operated at 6 hours of EBCT fed with the gas mixture (83.3% H_2 and 16.7% CO_2 based on volume basis). When the mole ratio of H_2 to CO_2 was increased from 4 to 5, CO_2 was almost completely reduced with H_2. This indicates that H_2 and CO_2 are not the limiting substrate any more. In Fig. 6, the volumetric CH_4 production rates were compared between the mole ratio of 4 and 5 in the feeding gas mixture. With the mole ratio of 5, the volumetric CH_4 production rate was higher than with mole ratio of 4 and was close to the volumetric CO_2 conversion rate.

From the results, H_2 as an electron donor for hydrogenotrophic methanogens has to be provided more than required according to the stoichiometry. About 80% of H_2 could be used for energy and reducing the electron acceptor, CO_2. Remaining 20% of H_2 could be used for biomass synthesis. For the complete conversion of CO_2, H_2 may be needed 20% more than that required according to the stoichiometry.

CONCLUSIONS

The factors affecting the biological reduction of CO_2 to CH_4, the

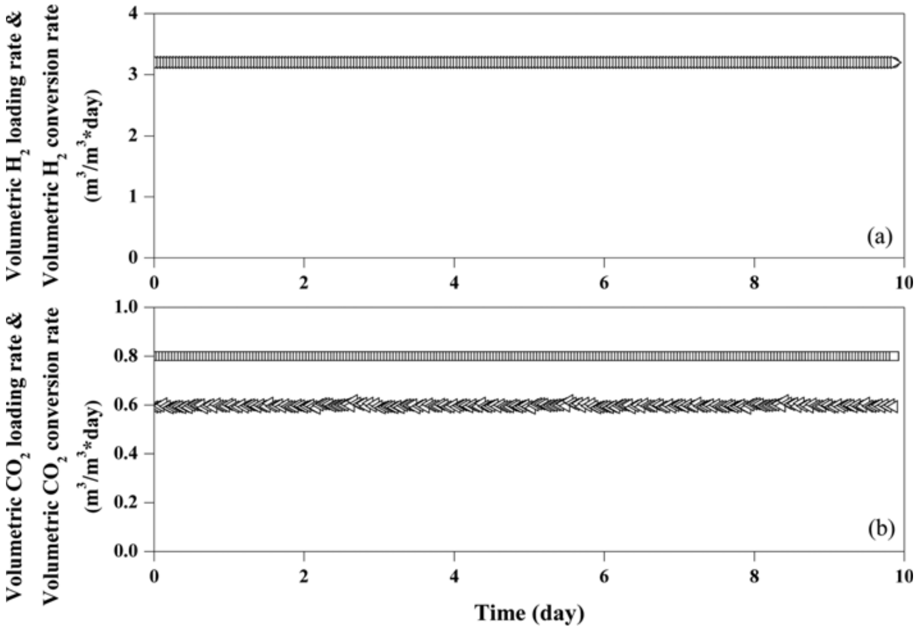

Fig. 4. Comparison between the volumetric loading rate and conversion rate of H_2 and CO_2 in the fixed bed reactor packed with sponge fed with gas mixture of 4 mole ratio of H_2 to CO_2 operated at 6 hours of empty bed contact time (○: volumetric H_2 loading rate, ▷: volumetric H_2 conversion rate, □: volumetric CO_2 loading rate, ◁: volumetric CO_2 conversion rate).

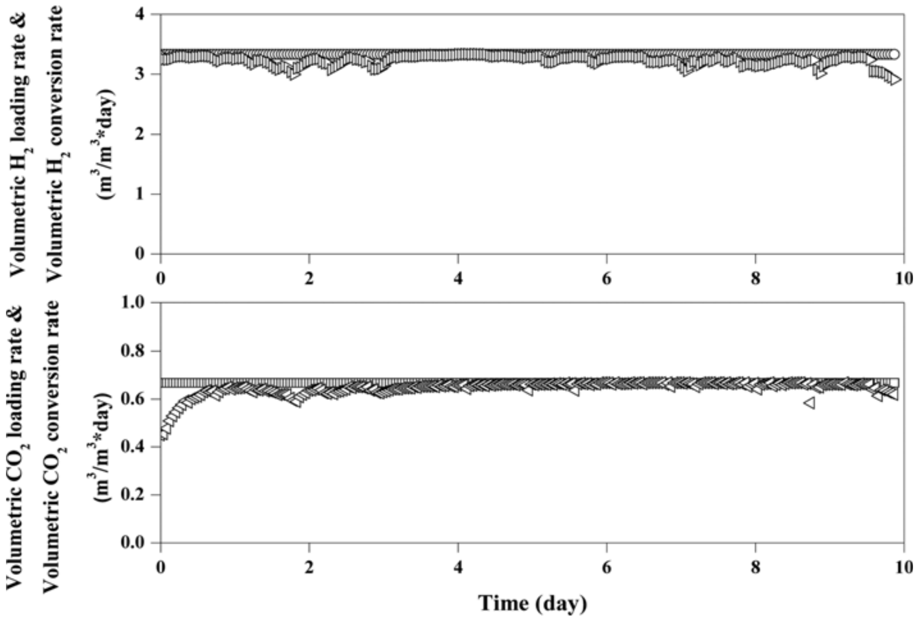

Fig. 5. Comparison between the volumetric loading rate and conversion rate of H_2 and CO_2 in the fixed bed reactor packed with sponge fed with gas mixture of 5 mole ratio of H_2 to CO_2 operated at 6 hours of empty bed contact time (○: volumetric H_2 loading rate, ▷: volumetric H_2 conversion rate, □: volumetric CO_2 loading rate, ◁: volumetric CO_2 conversion rate).

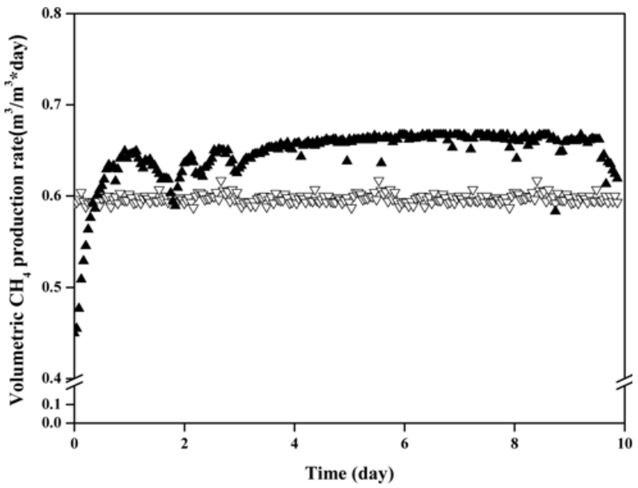

Fig. 6. Effect of mole ratio of H_2 to CO_2 in the feeding gas mixture on the volumetric CH_4 production rate from the fixed bed reactor packed with sponge operated at 6 hours of empty bed contact time (▲; 5 mol ratio of H_2/CO_2 (), ▽; 4 mole ratio of H_2/CO_2).

type of packing materials and mole ratio of H_2 to CO_2 in the feeding gas mixture, were investigated in the fixed bed reactor where hydrogenotrophic methanogens were enriched. Anaerobic mixed culture from the anaerobic digester of a sewage treatment plant was used to inoculate the fixed bed reactor, and hydrogenotrophic methanogens were enriched by feeding the gas mixture of CO_2 and H_2. During the operation of the fixed bed reactor, H_2 was completely utilized by hydrogenotrophic methanogens when fed at four times of CO_2 based on volume basis. The comparison between sponge and PS ball has shown that volumetric CO_2 conversion rate and CH_4 production rate were higher in the fixed bed reactor packed with sponge. This could be attributed to the larger surface area provided by sponge. The volumetric CO_2 conversion rate is lower than its loading rate in both reactors. The reason could be that H_2 was the limiting substrate because the portion of it was used for biomass growth. When the mole ratio of H_2 to CO_2 was increased to 5, CO_2 was completely utilized.

REFERENCES

1. D. J. Farrelly, C. D. Everard, C. C. Fagan and K. P. McDonnell, *Renewable and Sustainable Energy Reviews*, **21**, 712 (2013).
2. M. Anjos, B. D. Fernandes, A. A. Vicente, J. A. Teixeira and G. Dragone, *Bioresour. Technol.*, **139**, 149 (2013).
3. C.-L. Chiang, C.-M. Lee and P.-C. Chen, *Bioresour. Technol.*, **102**, 5400 (2011).
4. S.-H. Ho, C.-Y. Chen, D.-J. Lee and J.-S. Chang, *Biotechnol. Adv.*, **29**, 189 (2011).
5. A.-K. Kaster, J. Moll, K. Parey and R. K. Thauer, *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 2981 (2011).
6. J. C. Lee, J. H. Kim, W. S. Chang and D. Pak, *J. Chem. Technol. Biotechnol.*, **87**, 844 (2012).
7. Y. Cohen, *Bioresour. Technol.*, **77**, 257 (2001).
8. V. Lazarova and J. Manem, *Water Res.*, **29**, 2227 (1995).
9. R. Crovetto, *J. Phys. Chem. Ref. Data*, **20**, 575 (1991).
10. C. L. Young, IUPAC Solubility Data Series, Hydrogen and Deuterium, Pergamon Press, Oxford, England (1981).
11. C. P. Leslie Grady, G. T. Daigger and H. C. Lim, *Biological wastewater treatment*, 2nd Ed., Marcel Dekker, New York, 67 (1999).