

Full Length Research Paper

Effect of gas recirculation intensity and various temperatures on hydrogenotrophic methanogens activity in chemostat fermentation using H₂/CO₂ as substrate

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The influence of mixing H₂/CO₂ gas recirculation on the performance of hydrogenotrophic methanogens activity in continuous culture was studied at 37 and 20°C. Chemostat fermentation was used at laboratory scale to determine the bioconversion rate of H₂/CO₂ mixture gas to methane under different mixing rates. On comparison with continuous mixing, intermittent mixing at 45 min/h provided a better methane production, 1.94 ± 0.06 versus 1.83 ± 0.05 L for continuous mixing. When the temperature was progressively decreased to 20°C, the same configuration was observed. The methane production was lowered from 76.2, 80.8, 67.5, 61.2 to 27.9, 35.8, 29.6 and 26.7% for 60, 45, 30 and 15 min/h, respectively. The mixing at 45 min/h showed a stable methane production as compared to all proposed mixing duration especially at psychrophilic temperature. The results would facilitate an empiric model that could help to establish more economical biogas reactor model.

Key words: Hydrogenotrophic methanogens, mixing, H₂/CO₂ gas, bioconversion, temperature fluctuation.

INTRODUCTION

Bioenergy is a promising alternative to fossil fuel for a clean and reproducible nature (Angenent et al., 2004). Anaerobic digestion using various organic feedstocks has been investigated to well understand and develop the process converting biomass to energy. Inside the methanogenic anaerobic fermentation, mixing was shown as a performance enhancer in variable digesters type

(Kim et al., 2004; Vavilin and Angelidaki, 2005; Kaparaju et al., 2008).

Dahiya et al. (2015) have related the high rate biomethanation in a digester using mixing to a specific design features and the mode of mixing the digester contents. In addition, for energy efficiency in the design digester, some authors have attempted the biogas

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recirculation for mixing (Karim et al., 2005a) and compare the system with other mode of mixing (Karim et al., 2005b).

Although, the biogas recirculation technology is experienced, there is still uncertainty on the effect of mixing combined with factors such as limiting organic loading rate and temperature when gas H_2/CO_2 is recirculated as unique substrate on methanogens activity during methanogenesis. From economics view, anaerobic fermentation has demonstrated its capability to produce a useful byproduct, the vitamin B_{12} (Yang et al., 2004), but no research has yet been done on the effect of mixing on vitamin B_{12} production in methanogenesis.

Thus, the present study aimed to determine the methane production especially with acclimated mesophilic methanogenic culture cultivated at different mixing variations using recycling H_2/CO_2 as unique substrate and mixing source; then because the mesophilic temperature range represents the methanogens optimal activity temperature range (Bouallagui, 2004), the effect of temperature variation will be associated with the mixing variation.

MATERIALS AND METHODS

At laboratory scale, anaerobic sludge from a municipal anaerobic wastewater treatment plant was acclimated for 8 months under H_2/CO_2 (80:20, v/v) as unique substrate. Under epifluorescence microscopy analysis, the sludge presented long rod-shaped cells and cocci with a uniform blue-autofluorescence color (data not shown). The anaerobic growth culture was partitioned equitably (500 mL/reactor) in the four chemostat reactors for an experimental continuous cultivation.

Experimental procedures

The media composed of mineral nutrients and trace metals and also the protocol were identical with those used by Ako et al. (2008). The mineral nutrients and trace metals were separately boiled, cooled and sparged with mixture gas H_2/CO_2 (80:20, v/v) to remove O_2 traces. The pH values were 7.95 and 6.93 for mineral nutrients and trace metals, respectively. Nutrients and trace metals were applied to the growth of four reactors using a HRT set at 12 days for optimal hydrogenotrophic methanogens operation condition.

At mesophilic temperature (37°C)

The four chemostat reactors with acclimated hydrogenotrophic methanogens were connected to four aluminum tedlar gas bag containing H_2/CO_2 (80:20, v/v) gas supply at the rate of 12 L/reactor/d. The mixing inside each reactor was realized using four airtight pumps at 0.08 MPa and different mixing durations: 60, 45, 30 and 15 min/h. The daily gas production and composition were monitored at steady state cultivation; the pH and volatiles solids (VS) were measured for seven days.

At psychrophilic temperature (20°C)

The procedure is similar to the one use at mesophilic temperature

and the chemostat reactors temperature was progressively decreased ($-1^\circ\text{C}/\text{day}$) until it reached 20°C . Under 20°C , the chemostat steady state was realized then the biogas production and composition, pH and VS were monitored for 7 days.

Analysis methods

The pH was measured *in situ* with a pH-meter TPX-90 (Toko Chemical Laboratories Co. Ltd). The biogas composition was measured by GC-14B Shimadzu gas chromatograph, equipped with a thermal conductivity detector, connected to a C-R8A data analyzer. A high performance column packed with 50/80 mesh Porapak Q was used. The temperature of the injection column and detector was set at 100, 50 and 100°C , respectively. Argon was used as carrier gas at a flow rate of 50 mL/min and a pressure of 0.5 MPa.

The statistical analysis of the methane production during the 7 days sampling by means of 7 samples was done using the one way ANOVA data analysis. The least significant difference (LSD) between any four different mixing durations means at $p=0.05$ was applied to determine the differences.

The volatiles solids (VS) were analyzed using the standard methods protocol (APHA, 2005). The vitamin B_{12} was measured following the protocol developed by Yang et al. (2004).

RESULTS AND DISCUSSION

Time course of the process parameters

The pH and the bacteria concentration in the growth culture were monitored for all experiments duration. The results are presented in Figures 1 and 2, respectively. Figure 1 shows no significant variation of the pH in the four reactors for the variation of H_2/CO_2 recycling duration and also at decreasing temperature. That is probably due to the buffer effect of the nutrients and trace metals solution. The bacteria concentration results in Figure 2, presents a decrease of 94, 93, 94 and 92% for mixing duration of 60, 45, 30 and 15 min/h, respectively; that is, between the steady state under mesophilic condition and the steady state under psychrophilic condition. The results demonstrate that the utilization of organic wastes (Chae et al., 2008) or H_2/CO_2 (80:20, v/v) gas, in the present experiment, as substrate in anaerobic digestion obtains an activity reduction when the temperature is shifted from mesophilic range to psychrophilic range for hydrogenotrophic methanogens.

Effect of H_2/CO_2 mixing duration on methane production

At steady state mesophilic temperature

Results in Figure 3 show that intermittent mixing (45, 30 and 15 min/h) and continuous mixing (60 min/h) produce variable quantity of methane. The production of methane

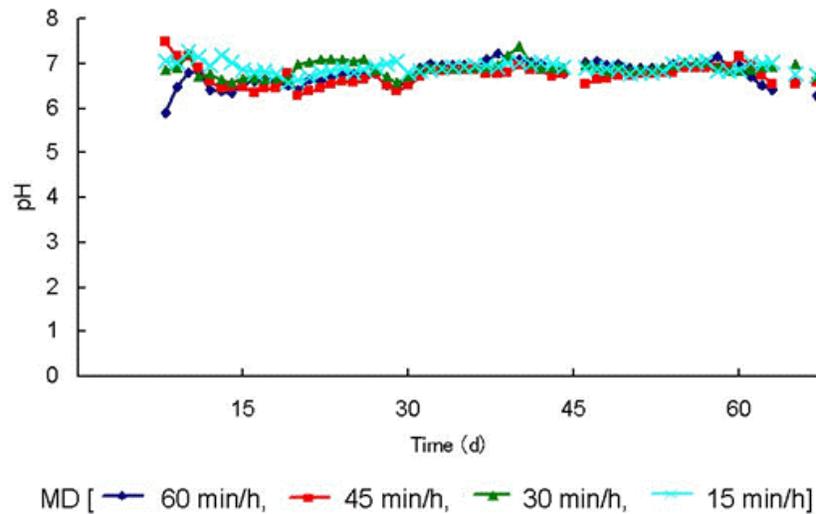


Figure 1. Time course of the pH evolution during the experiment at four different mixing durations (MD).

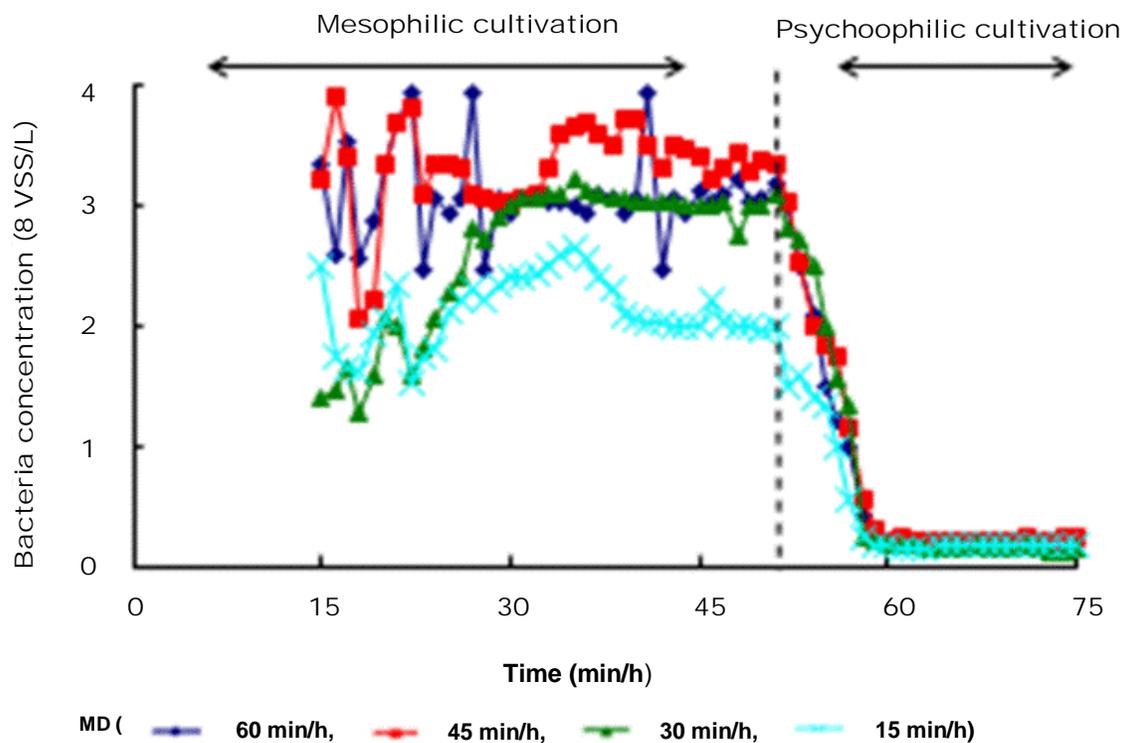


Figure 2. Time course of bacteria concentration during the experimental period under four different mixing durations (MD).

was found to be 1.83 ± 0.05 , 1.94 ± 0.06 , 1.62 ± 0.08 and 1.47 ± 0.04 L-CH₄/day for 60, 45, 30 and 15 min/h mixing duration, respectively. The highest methane production was found at 45 min/h mixing duration and the continuous mixing is presented as sensible inhibitor.

Recycling gas and limiting the effect of the organic loading rate by using inorganic nutrients in the present experiment shows similar methane production evolution as Karim et al. (2005a) where the organic loading rate was set to 50 g dry solids per liter of slurry.

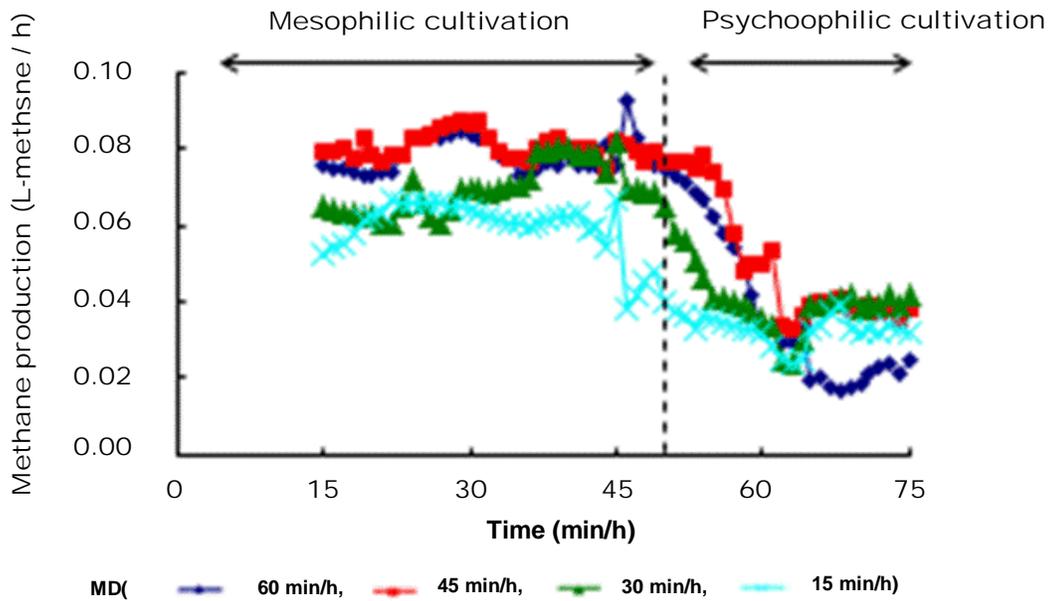


Figure 3. Time course of methane production for different mixing durations (MD).

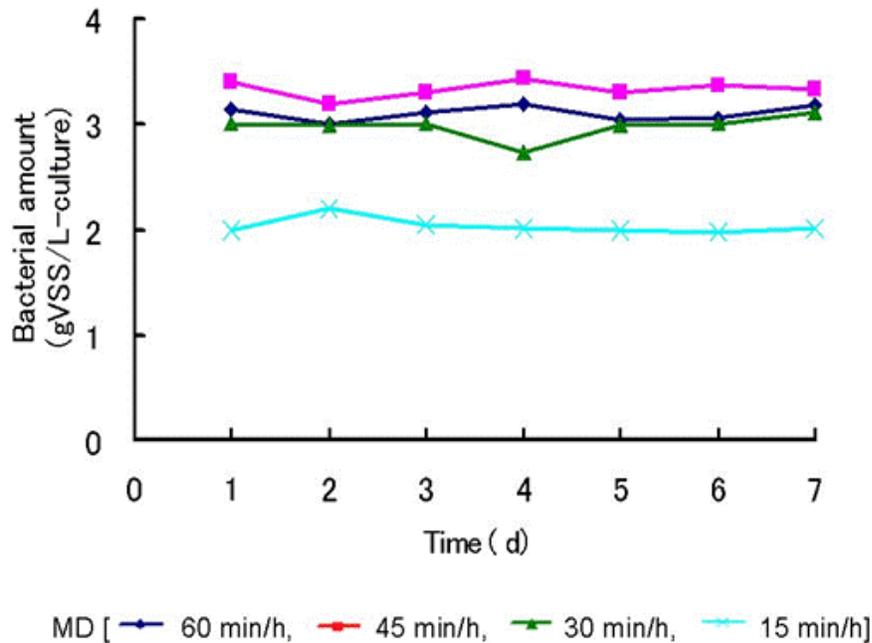


Figure 4. Hydrogenotrophic methanogens concentration during the steady state cultivation at 37°C for different mixing durations (MD).

Stroot et al. (2001) have related the change of the microbial population dynamics from an anaerobic co-digestion of municipal solid waste to the change of mixing level. The data in Figure 3 can be explained by the possible increase of methanogens cells damage during the

continuous mixing.

The bacteria concentration in the four mixing duration in Figures 2 and 4 conforms the assertion. Moreover, ultra microscopy used to observe the different mixing durations samples shows some difference in the cells

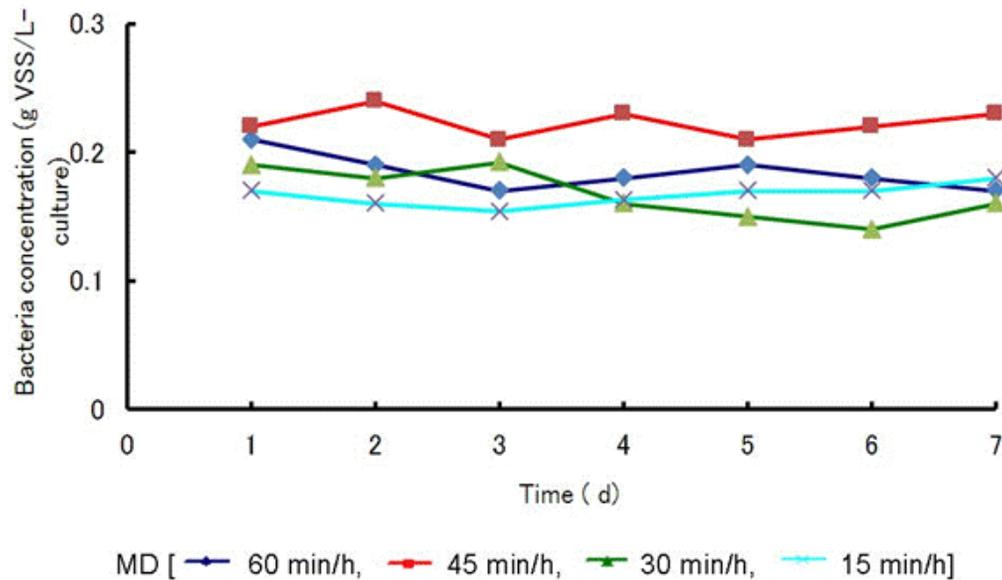


Figure 5. Time course of hydrogenotrophic methanogens concentration during the steady state cultivation at 20°C for different mixing durations (MD).

distribution inside samples (data not shown).

At steady state psychrophilic temperature

In Figure 5, due to the decline of bacteria concentration from mesophilic operation activity to the psychrophilic condition, the methane production was found to be the highest at 45 min/h mixing duration, 0.86 ± 0.01 L/day.

The temperature inhibits drastically the bacteria metabolic activity as compared to the mixing duration. Some authors have suggested a long period of acclimation to optimize the methanogens activity under psychrophilic condition (Torsten and Cavicchioli, 2000; King et al., 2011); it explains the large instability inside the same mixing duration. It is important to notice that at the beginning of the experiment, the methanogens were acclimated at 37°C for eight months not for the 20°C experiment.

Specific bioconversion capacity of carbon dioxide at various mixing duration

Due to carbon dioxide utilization as only carbon source during the experiment, the determination of the carbon dioxide depletion in the gas phase by means of the amount of carbon dioxide effectively utilized by the hydrogenotrophic and the fraction of dissolved carbon dioxide in the liquid shows that 76.2, 80.8, 67.5 and 61.2% of carbon dioxide were consumed during the mixing duration of 60, 45, 30 and 15 min/h, respectively. The present

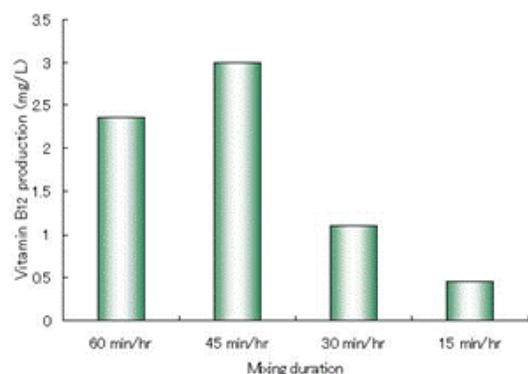
result may be crucial when hydrogenotrophic methanogens would be used for carbon dioxide absorption. At psychrophilic experiment, the capacity of carbon dioxide reduction was affected by the temperature change from the steady state mesophilic cultivation to the steady state psychrophilic cultivation from 76.2 to 27.9%, from 80.8 to 35.8%, from 67.5 to 29.6% and from 61.2 to 26.7% for 60, 45, 30 and 15 min/h, respectively; an activity reduction of about 45%.

Effect of mixing on vitamin B₁₂ production

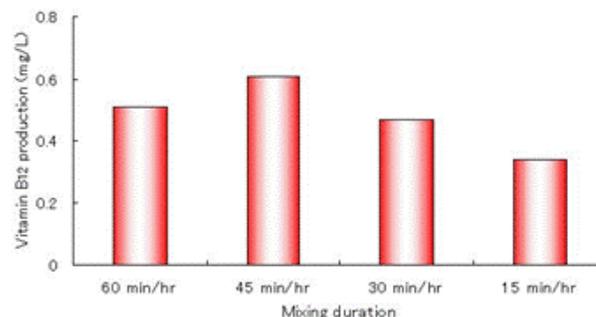
The present study attempts to observe the effect of the mixing duration variation on vitamin B₁₂ production. The result shows that the vitamin B₁₂ production follows the same evolution as the methane production. Figure 6 shows the maximum vitamin B₁₂ production was at 45 min/h mixing duration; the value is 3 mg/L effluent at mesophilic range and 0.61 mg/L at psychrophilic range. Values were effectively less than those obtained in fixed bed reactor (Zhang et al., 2004); despite the unknown effect and influence of CO₂ gas on vitamin B₁₂ production, the use of inorganic nutrients and substrate show the sensitivity of the methanogens to wash out in continuously stirred tank reactor digester type.

Conclusion

From the experimental operations, assertion can be made that correct media mixing is important in the



At mesophilic temperature (37°C)



At psychrophilic temperature (20°C)

Figure 6. Maximum vitamin B12 production at mesophilic and psychrophilic temperature.

success of gas H_2/CO_2 utilization as substrate. The investigation demonstrated that the continuous mixing achieved a high CO_2 gas dissolution rate but not the highest methane production. In fact, the suitable mixing duration was 45 min/h, which attain a methane production of 1.94 ± 0.05 L/d, about 80.8% of the carbon dioxide was converted to methane at mesophilic temperature. At 20°C, methane was the highest at 45 min/h, about 39.8% conversion of CO_2 to methane. The results should be useful for the CO_2 remediation from biogas; in addition, the possibility of vitamin B_{12} production under the standardize digester run was determined to initiate an economical cost.

Conflict of interest

The authors did not declare any conflict of interest.

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