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Rapid Bio-methanation of Syngas in a Reverse Membrane Bioreactor:

Membrane Encased Microorganisms

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Abstract

The performance of a novel reverse membrane bioreactor (RMBR) with encased microorganisms for syngas bio-methanation as well as a co-digestion process of syngas and organic substances was examined. The sachets were placed in the reactors and examined in repeated batch mode. Different temperatures and short retention time were studied. The digesting sludge encased in the PVDF membranes was able to convert syngas into methane at a retention time of one day and displayed a similar performance as the free cells in batch fermentation. The co-digestion of syngas and organic substances by the RMBR (the encased cells) showed a good performance without any observed negative effects. At thermophilic conditions, there was a higher conversion of pure syngas and co-digestion using the encased cells compared to at mesophilic conditions.

Keywords: Syngas fermentation; Co-digestion; Methane; Cell retention; Membrane bioreactor

1. Introduction

The global energy demand has constantly increased for several decades, and it has triggered the need for research and development on renewable energy sources. The potential to create renewable energy from waste material, including municipal solid waste (MSW), industrial waste, agricultural waste, and waste by-products has been developed (Fatih et al., 2011). Biogas or bio-methane is a renewable energy source with several applications in e.g., car fuel, heating, cooking, or electricity production. Biogas mainly consists of methane and carbon dioxide but may also contain minor impurities of other components (Deublein & Steinhauser, 2008).

There are different types of wastes used for methane production, which can be classified as easily degradable wastes, hard degradable wastes, and non-degradable wastes. In general, to obtain methane from easily degradable wastes such as food wastes, a biochemical approach called an anaerobic digestion process has been employed. On the other hand, the recalcitrance of the hardly degradable materials, such as crystalline cellulose and non-degradable materials such as lignin and plastic wastes cannot be decomposed by the microorganisms in an anaerobic digestion process (Nizami et al., 2009). Thermochemical processes however have the potential to convert this kind of wastes as well as non-degradable residues from the digestion process into methane. In this approach, feedstocks can be thermally gasified into intermediate gases, called syngas, through a partial oxidation process, at a relatively high temperature. Syngas or synthesis gas primarily contains carbon monoxide (CO), hydrogen (H_2), and carbon dioxide (CO_2). Raw syngas can be synthesized into methane by the use of metal catalysts, first introduced by Franz Fischer and Hans Tropsch. However, the high

manufacturing cost and challenges of the toxic impurities for the catalysts limit its economic feasibility (Fatih, 2009; Fatih et al., 2011).

Anaerobic microorganisms, primarily acetogencs, carboxytrops, and methanogencs are able to use CO and/or CO_2 sources as carbon sources and use H_2 as an energy source for their metabolism and produce different bio-products (Daniell et al., 2012). Thus, the combination of thermal and biological processes for the conversion of feedstocks into biofuel has been explored. This alternative method can generate a variety of products including methane from a wide variety of materials (Daniels et al., 1977; Kimmel et al., 1991; Klasson et al., 1992; Klasson et al., 1990). Using the fermentation processes and microorganisms offers several benefits over the catalytic process, such as being a more specific process, resulting in higher yields, having a lower energy consumption, being environmentally friendly, and having better robustness (Mohammadi et al., 2011; Munasinghe & Khanal, 2010).

The bio-methanation of CO, H_2, and CO_2 in the syngas by the microorganisms can be performed in two pathways (Kimmel et al., 1991). The first one utilizes an acetate pathway as a methane precursor. Thereafter, methanogenic microorganisms convert acetate into methane. The other pathway utilizes the H_2/CO_2 pathway. CO can be converted into CO_2 by the microorganisms (Henstra et al., 2007). The H_2 and CO_2 produced and initially present in the syngas are converted into methane by some methanogenic microorganisms (Kimmel et al., 1991). However, this microbial flora including methanogens requires a long retention time, since their growth rate is very low. Methanogenic microorganisms are also very sensitive to the process conditions;

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hence, the cells are easily washed out from the digester at high dilution rates. For these reasons, the population size of the microbial cells is easily reduced resulting in a decreased methane production. Moreover, fermentation processes with a low cell density need long start-up periods, and larger digesters are required for proper function, which means that the capital cost is high. To achieve a high process efficiency in converting syngas into methane using microorganisms in a fermentation process, retaining the microbial cells inside a compact reactor might be a solution to overcome these problems (Klasson et al., 1992). Immobilized cell technology, meaning the confinement of cells in a specific region or matrix, has been widely used in a variety of laboratory experiments and industrial applications (Raymond et al., 2004). These systems have been used to solve problems encountered in conventional bioreactors using suspended cell culture, including low biomass concentrations, low biomass productivity and product formation, inefficiency in continuous production, low stability to sudden fluctuation, and limited dilution rate in the case of continuous operation (Chinnayelka & McShane, 2004).

A membrane bioreactor (MBR) is the combination of a membrane process such as microfiltration with a suspended growth bioreactor (Judd & Judd, 2011). MBRs have been widely used for cell retention, especially in the municipal and industrial wastewater treatment. For example, MBR was studied by Badani et al. (2005) to treat textile wastewater, and it was found to perform perfectly compared to the conventional system. Kanai et al. (2010) employed a submerged anaerobic membrane bioreactor process in the anaerobic digestion of food wastes. They found that MBR enhanced the degradation of wastes, the rejection of toxics, and the reactor volumes could be scaled

down compared to the conventional system. This technology has several benefits over the conventional systems such as having low energy consumption, being environmentally friendly, and being easily implemented. Using MBR to retain the microbial cells for the enhancement of the bio-methanation of syngas could be an interesting system. However, currently available commercial MBR processes employed in biological process are designed to filter the liquid through the membranes, retaining the cells in the reactor to obtain a clarified and disinfected product. It is this type of MBR (the biomass rejection MBR), which is in primary focus of the most research in this field (Judd & Judd, 2011). In order to increase the conversion efficacy and bio-productivity, a new technique for retaining the cells inside the bioreactor is probably necessary for efficient bio-methanation systems of syngas.

The current work focuses on applying the reverse membrane bioreactor (RMBR) to retain the cells for the bio-conversion process of synthesis gas into methane. RMBR used in this work, the liquid permeate is not actively passes through the membrane, but the substrate diffuse through the membrane and the metabolic products diffuse back to the medium. It is a novel technique that uses membranes (PVDF) to enclose the microbial cells completely followed by immersion into a bioreactor in order to retain a high cell density in the bioreactor and increase the methane productivity, without the risk of a clogged outlet. In this work, the efficiency of the RMBR contained the encased cells and the resistance of using the membrane as a barrier for the syngas fermentation was tested compared to the free cells, in repeated batch mode with a shortened retention time and different temperatures. Moreover, the possibility of enhancing the methane

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productivity with addition of organic substances as a co-substrate with syngas using the

RMBR was also examined.

2. Materials and Methods

2.1. Anaerobic culture, medium, and syngas

An anaerobic culture was obtained from a 3,000 m^3 municipal solid waste digester,

operating under thermophilic (55ºC) conditions (Borås Energy and Environment AB,

Sweden). The inoculum was incubated at 55ºC for 3 days to keep the bacteria active

while consuming the carbon source provided by the inoculum. After incubation, the

inoculum was filtered through a sieve (1 mm pore size) to remove the large particles.

The digesting sludge was then centrifuged at 31,000×g for 15 minutes to separate the

solid inoculum (85% TS), the methane-producing microorganisms, which were loaded

into the membrane sachets. Two kinds of synthetic medium solutions for two different

experiments were prepared. One was for the co-digestion; the synthetic organic medium

contains acetate, propionate, butyrate, and vitamins solution (basal medium) with a ratio

of 3:1:1:1 (Osuna et al., 2003). The other was the vitamin solution, that is, the basal

medium for the pure syngas fermentation. Medium solutions were buffered to pH

7.0±0.2 with $NaHCO_3$ (Isci & Demirer, 2007). Synthetic syngas containing CO (55%

mole), H_2 (20% mole), and CO_2 (10% mole) (Kimmel et al., 1991) was purchased from

AGA gas (Borås, Sweden).

2.2. Membrane sachet preparations and cell containment procedure

The cell entrapment in the membranes was performed following a previously described

method (Youngsukkasem et al., 2013; Youngsukkasem et al., 2012). Flat plain PVDF

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(polyvinylidene fluoride, Durapore®) membranes (Thermo Fisher Scientific Inc., Sweden) were used as synthetic membranes. The PVDF membrane filters were hydrophile with the pore size, thickness and diameter of 0.1 µm, 125 µm and 90 mm, respectively. The membranes were cut into rectangular shapes of 6×6 cm and folded to create membrane pockets of 3×6 cm^2. They were then heat-sealed (HPL 450 AS, Hawo, Germany) on two sides with heating and cooling times of 4.5 and 4.5 s, leaving one side open for the insertion of the inoculum. Solid sludge inoculum (3 g per sachet) was then injected carefully into the synthetic membrane sachets, and the fourth side sealed. The sachets containing the inoculum were used immediately for the bio-methanation of the syngas and the co-digestion process.

2.3. Repeated batch fermentation process

The repeated batch fermentation processes (with cell reuse and medium replacement) were performed in order to preliminarily examine the performance of the RMBR (microorganisms encased in the synthetic membrane) for the syngas bio-methanation and the co-digestion of syngas and organic medium at different retention times. The schematic diagram of an experiment shows in Figure 1. In the experiment with the syngas bio-methanation, each reactor contained one sachet of inoculum and 30 mL of basal medium. For the experiment with the co-digestion process, each digester contained one sachet of inoculum and 30 mL of synthetic organic medium. The reactors used were serum glass bottles with 118 mL working volume, closed with butyl rubber seals and aluminum caps. The headspace of each bottle was flushed with syngas to obtain a sufficient amount of gas substrate at 1 atm. Reactors were inclined and carried out in a water bath with constant agitation of 100 rpm in order to provide good gas-

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liquid mass transfer. Anaerobic fermentations at temperatures of 35°C and 55°C were examined. The retention time was gradually shortened: 4, 4, 2, 2, and 1 day, respectively. The encased cells were reused; the syngas was exchanged with fresh syngas at 1 atm, and necessary nutrients were replaced prior to the start of every new batch. Anaerobic fermentations of the free cells with syngas were performed for nine days of retention time in parallel in order to examine the efficiency of the encased cells for the bio-methanation of syngas, especially, the membrane resistance, under identical conditions as the reference.

2.4. Analytical methods

Methane, hydrogen, carbon monoxide, and carbon dioxide were measured regularly, using a gas chromatograph (Perkin-Elmer, U.S.A.), equipped with a packed column (Carboxen^{TM} 1000, SUPELCO, 6'x1.8" OD, 60/80 Mesh, U.S.A) and a thermal conductivity detector (Perkin-Elmer, U.S.A.) with an injection temperature of 200°C. The carrier gas was nitrogen, with a flow rate of 30 mL/min at 75°C. A 250 µL gastight syringe (VICI, Precision Sampling Inc., U.S.A.) was used for the gas sampling. The obtained peak area was compared with a standard gas analyzed at the same condition (STP, 273.15°K and 101.325 kPa). The volatile fatty acids (VFA) were analyzed using a gas chromatograph (Auto System, Perkin-Elmer, U.S.A.) equipped with a capillary column (Zebron ZB-WAX plus, Polyethylen glycol (PEG), 30m x 0.25mm x 0.25µm, U.S.A.) and a flame ionized detector (Perkin-Elmer, U.S.A.) with an injection and detection temperature of 250°C and 300°C, respectively. The carrier gas was nitrogen, with a flow rate of 2 mL/min at a pressure of 20 psi. The experiment was performed in triplicate and the results were presented as mean ± standard deviation.

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3. Results and Discussion

3.1. The performance of the RMBR (digesting microorganisms encased in the PVDF

membranes) compared to the free cells for the bio-methanation of syngas

During the anaerobic bio-methanation process of syngas, it was observed that the

encased microorganisms performed smoothly. Sachets containing the cells stayed intact

and no leakage of cells occurred throughout the anaerobic conversion process.

Figure 2 shows the performance of the encased cells compared to the free cells in the

anaerobic bio-methanation of syngas. The encased microbial cells had a similar

accumulated methane level compared to the free cells in the thermophilic fermentation

in the 9-day batch. Methane was rapidly accumulated in the reactors with both the

encased and the free cells at thermophilic conditions (55°C), from the first day until the

3rd day of fermentation. Thereafter, the trend of methane production was stable until the

last day due to slow production (Figure 2a). The accumulated methane produced on the

9th day for the encased cells and the free cells at 55°C and 35°C were 0.72, 0.41, 0.51,

and zero mmol, respectively. The amount of H_2 and CO decreased dramatically during

the first three days. Under the thermophilic conditions, the free cells showed better

performance in rapid assimilation of the syngas compared to the encased cells. CO and

H_2 were completely consumed by the free cells already in the 2nd day of bio-

methanation. The anaerobic digesting sludge used as the inoculum contained different

species of microorganisms, and used for biogas production, as an active source of

methane-producing microorganisms. In addition, it has been found to be a potential

source of microorganisms for the syngas fermentation in different bioprocess systems

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(Sipma et al., 2003). The individual microbial cells (free cells) most likely have better contact to the medium with dissolved gases in the fermentation system compared to the compact cells encased in the membranes. However, cells encased in the PVDF membranes converted all H_2 into products in four days while CO required a longer time to be completely used up by the encased cells. The trend of CO_2 consumption was stable for all treatments (Figure 2d).

This experiment revealed that microorganisms encased in the PVDF membrane displayed a similar performance compared to the free cells in syngas bio-methanation, but the conversion rate of syngas was found to be a bit slower than for the free cells with the same process conditions. However, the results indicate that using the PVDF membrane to retain microbial cells for bio-methanation of syngas is feasible. Hence, it would be interesting to investigate this novel technique in a long-term process.

3.2. The efficiency of the RMBR (encased digesting microorganisms) in repeated batch process for bio-methanation of syngas

3.2.1 Effect of temperatures

Figure 3 shows the performance of the encased cells in the anaerobic bio-methanation of syngas at different retention times and temperatures compared to the control treatments with no syngas, under the same conditions. The result shows that at the first retention time of nine days, methane was produced continuously while the syngas concentration decreased. The remarkable result shows that methane was produced immediately from the first day of fermentation in all conditions. In addition, the interesting result from this experiment was that the encased cells, at both temperature conditions, produced more

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methane than the controls with no syngas at the same conditions. Furthermore, it was observed that under thermophilic conditions of 55°C, the encased cells had the highest methane production of 1.53 mmol on the 9th day compared to the mesophilic conditions at 35°C and the controls at both temperatures (0.78, 0.81, and 0.37 mmol, respectively). Kundiyana et al. (2011) studied the syngas fermentation using microbial cells at different temperatures and found that a mesophilic temperature (35°C) was the best condition for enhancing the gas solubility in the liquid medium and consequently enhancing the bio-productivity. However, in the current experiment, under mesophilic conditions (35°C), the encased cells produced less methane than those at thermophilic conditions. This means that the gas solubility may not be the main obstacle in increasing the process efficiency of syngas fermentation. The nature of the fermenting microorganisms, which might work better at thermophilic conditions, also plays an important role for this process. In this experiment, the active inoculum obtained from a thermophilic biogas plant is the likely reason for the improved bio-conversion of syngas. Thermophilic conditions generally stimulate the microbial metabolism compared to lower temperatures. Thus, faster bio-methanation of syngas occurred at thermophilic conditions with the community of microbial cells originating from the thermophilic biogas plant.

Both H_2 and CO have a lower solubility in the liquid phase compared to CO_2, but the amount of syngas, including H_2 and CO (Figures 3b and 3c), decreased continuously from the first day, at the same time as methane was produced (Figure 3a). The H_2 concentration of 0.7 mmol at 55°C anaerobic condition at the first day had decreased to 0.1 mmol at the 5th day and thereafter, was very low during the remainder of the

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fermentation period (Figure 3b). At 35°C, the encased cells had a lower conversion

efficiency of H_2 than at thermophilic condition. Here, the same amount of H_2 (0.7

mmol) decreased slowly to 0.06 mmol on the 9th day of fermentation. However, the

encased cells had no problems to take up H_2 for methane formation. H_2 is usually

contained in syngas at different concentrations depending on the composition of the

biomass used for the thermal gasification process and also on the process parameters

(Fatih, 2009). To produce methane, methanogenic microorganisms such as

Methanothermobacter thermoautotrophicus (Sipma et al., 2003) utilize H_2 together with

CO_2. However, precursors for methane-producing microorganisms, such as acetate, can

also be formed by microbial cells such as *Acetoanaerobium noterae* using H_2 (Liu et al.,

2012).

With carbon monoxide (Figure 3c), no problems regarding the inhibitory effects on the

cells were observed during the fermentation process. The encased cells performing at

thermoplilic conditions were able to also consume CO faster than the cells at mesophilic

conditions. The CO (1.8 mmol) was completely assimilated on the 7th day at 55°C. At

35°C, the same amount of CO was completely used up by the encased cells by the 9th

day. In order to mimic the raw syngas, CO_2 was added as one of the main gases for this

experiment. The result shows that the production trends of CO_2 by the encased cells

using syngas (35 and 55°C) were quite stable (Figure 3d). It may be because of CO_2,

even as a metabolic product (cf. controls on Figure 2d). The amount of CO_2 at the

conditions of 35°C and 55 °C were in the ranges of 0.25–0.50 and 0.33–0.59 mmol,

respectively. To form methane, the microbial cells probably used CO_2 from their

metabolisms, so they did not need the CO_2 supplied from the syngas. However, CO_2

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accumulation had no negative effect on the bio-methanation of syngas in this experiment.

3.2.2 Effect of short retention times

These results reveal that anaerobic microorganisms encased in the PVDF membrane were able to convert syngas into methane at the retention time of nine days. However, to investigate the efficiency of the encased cells in shorter periods of bio-methanation, the experiment was thereafter operated in repeated batch mode, in which the encased cells were reused and new syngas was replaced at the beginning of every new batch. The batch time was gradually shortened to 4, 4, 2, 1, and 1 day, respectively. The compositions of the syngas and methane were analyzed regularly.

The results showed that the encased cells still performed efficiently, and no negative effect was observed during the bio-methanation of the syngas. In addition, the encased microbial cells had better conversion efficiency of the syngas at anaerobic thermophilic conditions than at mesophilic conditions throughout all batches (Figure 3). Under thermophilic anaerobic conditions, CO was completely used up by the encased cells on the 3rd day of the batch. When the retention time was gradually shortened to two days, CO was still completely used up. With a retention time of one day, the encased cells used CO, but it was not enough time for the encased cells to completely use all CO (Figure 3c). For H_2, the amount decreased continuously from the beginning of the batches, especially, at the retention time of four days. Nevertheless, the retention times of 4, 2, and 1 day were not enough for the encased cell to entirely convert the H_2 into products. The trend for the CO_2 level was found to be the same throughout the process,

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even when the batch time was shortened. The accumulated methane production of the encased cells in thermophilic bio-methanation with batch times of 4, 4, 2, 1, and 1 day were 0.90, 0.88, 0.73, 0.35, and 0.35 mmol, respectively. The most interesting point from this experiment was that the reused encased cells performed well, and were still able to convert syngas into methane continuously when the retention time was shortened. The accumulated methane production was lower compared to the first period of nine days. However, a decrease was expected due to the used up methane potential of the inoculum.

All these results indicate that the digesting sludge retained in the hydrophilic PVDF membrane was able to convert syngas into methane. Furthermore, the encased cells were easy to handle when reused for the repeated batch fermentation process. Hydrophilic durapore® PVDF membrane allowed all gases, including syngas (substrate) and methane (product) together with the necessary liquid nutrients for the microbial cells to diffuse through the membrane layer. Consequently, the microbial cells encased in the PVDF membranes were able to take up syngas and produce methane. Thermophilic anaerobic conditions were found to enhance the conversion efficiency of syngas and facilitate faster methane production compared to at mesophilic conditions.

3.3. Enhancing the methane productivity using the encased microorganisms in
simultaneous bio-methanation of syngas and organic substances

In anaerobic digestion processing of biomass, co-digestion has been proven to be a powerful process due to its advantages in the improvement of productivity and process

efficiency. In this system, different kinds of biomass are put together in the same digester and digested simultaneously in an often synergistic manner (Abouelenien et al., 2014; Long et al., 2012). CO, H_2, and CO_2 in syngas, the intermediate gas, can be used for the metabolism of the microorganisms. However, syngas has a low energy, which may not be sufficient for the microbial cells to produce methane, thus, to increase the efficacy of the encased cells in bio-methanation of syngas, a simultaneous fermentation process for the syngas and organic substance to produce methane using the encased cells was studied. In this experiment, the digesting sludge was encased in the PVDF membranes and used as an inoculum. A synthetic organic medium containing: acetate, propionate, and butyrate, and syngas, containing CO, H_2 and CO_2, were used as a co-substrate. Mesophilic and thermophilic anaerobic conditions were examined and performed in repeated batch at different retention times. Syngas concentrations, total volatile fatty acids (VFA), and methane production were analyzed regularly. Syngas bio-methanation by the encased cells in the same condition was used for comparison.

The results show that the encased cells performed efficiently in the co-biomethanation of syngas and organic substances. The sachets got swollen during the anaerobic fermentation process, due to the high pressure of the methane produced. This means that the methanogenic microbes encased in the PVDF membranes had obtained sufficient substrates for product formation. Here, the thermophilic anaerobic conditions were found to be advantageous compared to the mesophilic conditions. Digesting sludge encased in the hydrophilic PVDF membranes was able to perform in the co-digestion process without any observed negative effects. In the first period of nine days, the trends of methane production by the encased cells in the co-digestion and in the pure syngas fermentation were similar. The highest accumulated methane production was obtained

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by the encased cells in the co-digestion and pure syngas fermentation (0.72 and 0.79 mmol) at thermophilic conditions (Figure 4a). Looking at the gas amounts in the same period, H_2 and CO were completely consumed in both processes by the encased cells on the 9th day. CO_2 was consumed a bit at the beginning and was thereafter stable.

Total volatile fatty acids (VFA) in the liquid nutrients were also measured on the last day of the different batches. After the first batch, with retention time of nine days, the VFA levels decreased from 0.97 g/L of pure syngas fermentation and 8.05 g/L of co-digestion under thermophilic conditions at the first day to 0.08 and 0.05 g/L (Table 1). Hence, the VFA utilizations were 92 and 99%, respectively. These results reveal that the encased cells can consume syngas simultaneously with the digestion of organic substrates. Nevertheless, the performance of the encased cells in the co-digestion process did not show that the co-digestion resulted in a higher methane production during the first batch period (Figure 4a). Thus, repeated anaerobic batch digestions with shorter retention times were performed.

When the encased cells were reused, the substrates (syngas and organic solution) were exchanged with new substrates, and the retention time was shortened to 4, 4, 2, and 1 days, interesting results were observed. It was found that the methane produced from the thermophilic co-digestion (55°C) showed an increasing trend (Figure 4a), even as the retention time was shortened. The microbial cells encased in the hydrophilic PVDF membranes produced the highest amount of methane from 4, 4, and 2 days of 0.42, 1.41, and 1.92 mmol in thermophilic co-digestion compared to the others. The encased microbial cells themselves may already have adapted from the first batch, so that they could perform better and produce methane faster in the following batch periods.

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However, the methane level was lower when the retention time was shortened to one day (1.14 and 1.59 mmol for the two batches, respectively) due to a too limited process time. In contrast, the methane production from the encased cells using only syngas as a substrate was lower than in the co-digestion process, and the produced amounts were similar in all batches except for the shortest batches, where the production rate was obviously too slow.

After nine days of fermentation, a new batch was started and the retention time was shortened, then H_2 was completely consumed by the encased cells in four days of two batches for thermophilic co-digestion process (Figure 4b). The encased cells were still able to use H_2 for their metabolism at the shorter retention time, but H_2 was not completely used up in the two and one day batches. CO was found to be consumed more rapidly by the encased cells in the thermophilic pure syngas fermentation compared to in the co-digestion (Figure 4c). It seems that the microorganisms encased in the PVDF membranes preferred to use the other sources of substrates compared to CO. The encased microorganisms managed to completely convert the CO that was present in two days. For the CO_2 values in this experiment (Figure 4d), it was shown to have an interesting trend. After the first period of nine days, the CO_2 was consumed rapidly by the encased cells under both mesophilic and thermophilic conditions of the co-digestion processes, but not in the systems with only syngas fermentation. It is presumed that the encased digesting sludge probably contains specific microorganisms such as *Methanothermobacter thermoautotrophicus,* which are able to utilize CO_2 together with gases such as H_2 to form precursors (acetate) or methane. Furthermore, the co-digestion process may provide suitable conditions for this microbial group, which was not the case with only syngas fermentation.

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According to the methane production and syngas consumption, the VFA concentration in the effluents from the co-digestion process was found to decrease (Table 1). The lower VFA concentrations in the system with the encased cells in thermophilic co-digestion compared to mesophilic conditions show that the encased cells had spent more organic substances for their metabolism, and it has been shown that the hydrophilic PVDF membrane allowed liquid organic substrates to diffuse through the membrane layer for the metabolism of the microorganism in the digestion process (Youngsukkasem et al., 2013). However, the cells in co-digestion were probably inhibited by the VFA in the first 2 and 3 batches and thereafter, they adapted and could quickly utilize the VFA as well as the syngas (Table 1).

The results presented here reveal that the microbial cells encased in the hydrophilic PVDF membranes were capable of simultaneous fermentation of the syngas and organic substrates for methane production, without any detected negative effects. These successful results show the feasibility of applying this novel technique in combined anaerobic organic digestion with syngas fermentation, in order to increase the methane productivity. In addition, the PVDF membranes turned out to be an interesting supporting material in this experiment. It was able to retain the microbial cells in the bioreactors, and at the same time it was able to allow both organic and gas substrates to pass through, which can provide the necessary nutrients for the metabolism of the microbial cells to produce methane.

4. Conclusion

Retaining microorganisms in the reverse membrane bioreactor (RMBR) using the PVDF membranes was a successful approach for bio-methanation of syngas, as well as simultaneous fermentation of syngas and organic substances. The PVDF membranes allowed the liquid and gas diffusion through the membrane surface. The encased cells in RMBR could convert CO, H_2, and CO_2 and produce methane in one day. Thermophilic conditions (55℃) enhanced the syngas fermentation, and the co-digestion using the encased cells improved the methane productivity. However, to develop RMBR system for the industrial scale, RMBR performs under continuous bio-methanation of syngas should be further developed.

Acknowledgements

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Table 1. The concentrations of total volatile fatty acids (VFAs) from the effluent of

each reactor at the start-up and at the end of the different batches.

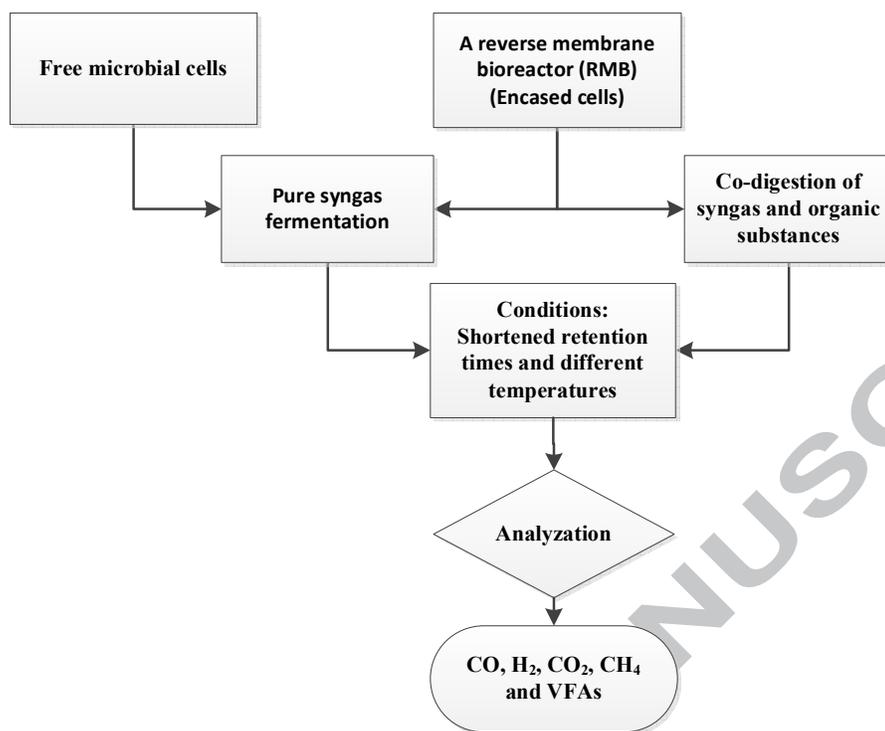| Retention time	Total volatile fatty acids (g/L)			
	Pure syngas fermentation		Co-digestion process	
(Days)	35°C	55°C	35°C	55°C
0	0.97±0.00	0.97±0.00	8.05±0.00	8.05±0.00
9	0.22±0.06	0.08±0.02	0.14±0.03	0.05±0.02
4	0.54±0.14	0.21±0.17	3.28±0.74	3.43±0.13
4	0.43±0.05	0.19±0.06	4.26±0.19	2.94±1.53
2	0.61±0.26	0.15±0.04	4.18±1.83	1.41±0.17
1	0.37±0.01	0.39±0.41	4.24±0.73	3.27±1.50
1	0.29±0.04	0.28±0.17	4.83±0.57	3.27±0.92

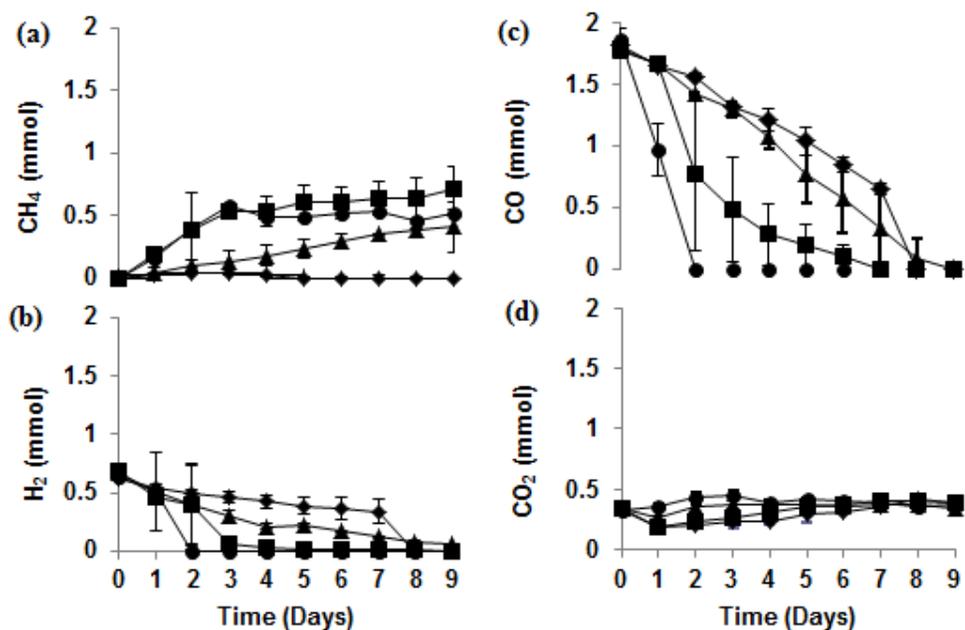**Fig. 1**. The schematic diagram of an experiment.

Fig. 2. Anaerobic bio-methanation of syngas using the RMBR (encased cells) compared to free cells. **(a)** Methane, **(b)** Hydrogen, **(c)** Carbon monoxide, and **(d)** Carbon dioxide. Symbols: Encased cells at 35°C (▲), Free cells at 35°C (♦), Encased cells at 55°C (■), and Free cells at 55°C (●).
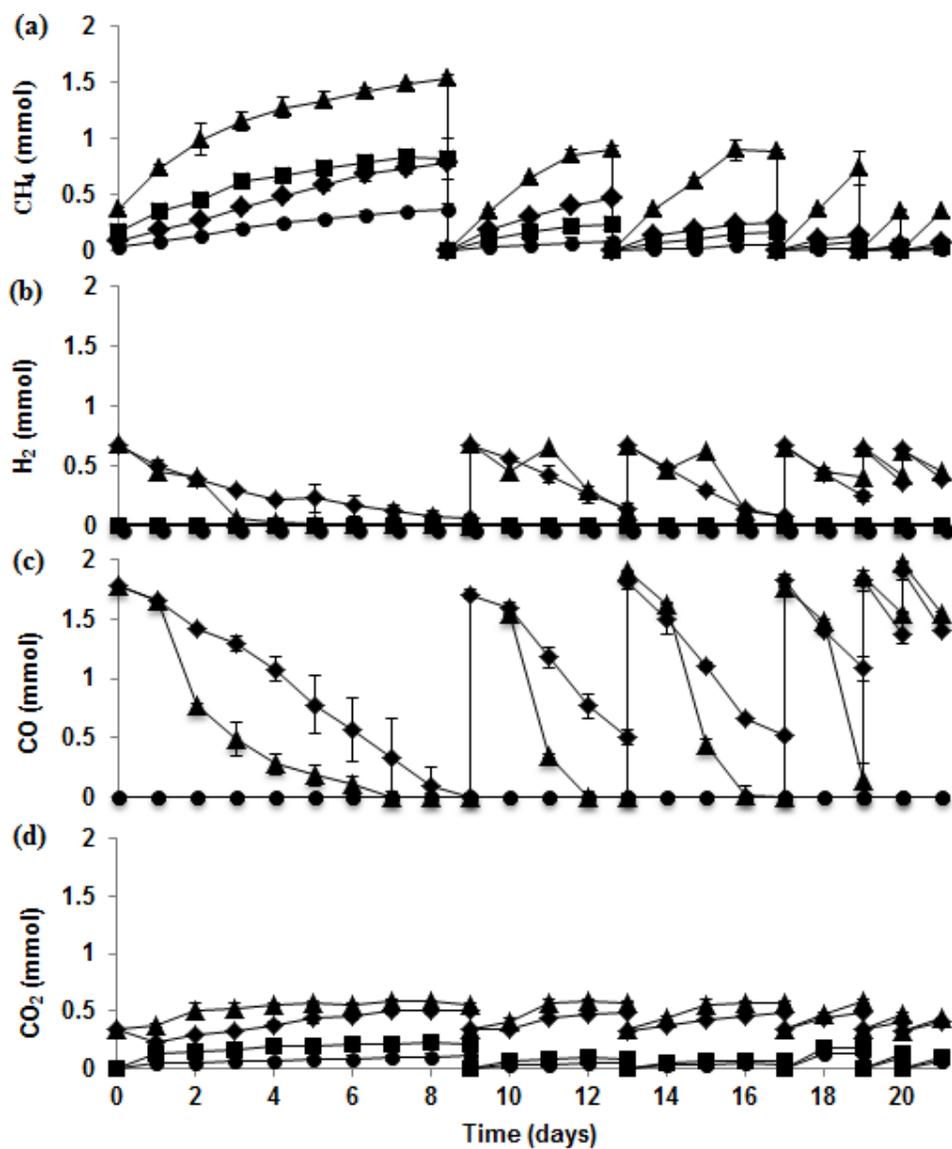
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Fig. 3. Performance of the RMBR in anaerobic repeated batch bio-methanation of syngas. **(a)** Methane production, **(b)** Hydrogen concentration, **(c)** Carbon monoxide concentration, and **(d)** Carbon dioxide concentration. Symbols: Syngas at 35°C (—■—), Blank at 35°C (—●—), Syngas at 55°C (—▲—), and Blank at 55°C (—◆—).
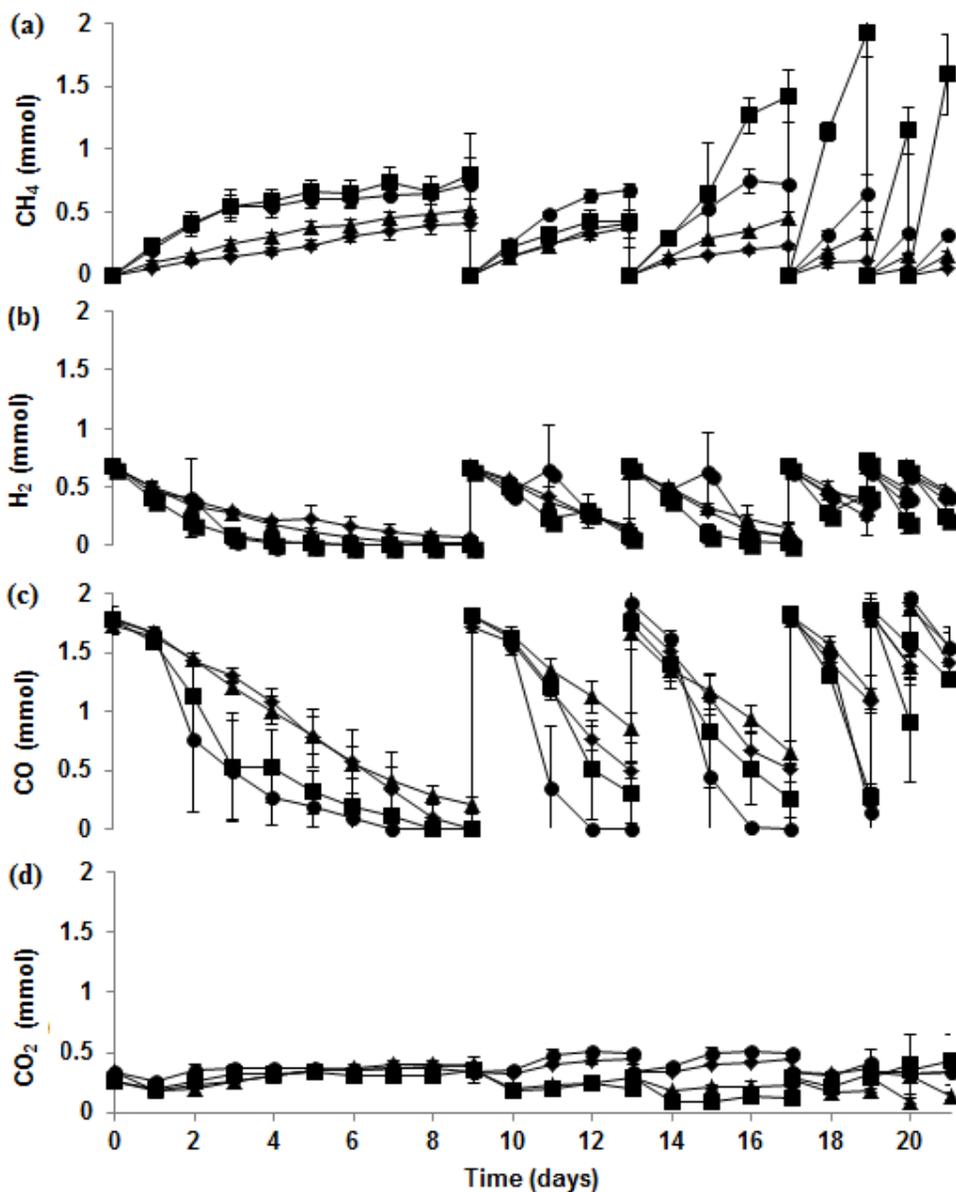
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Fig. 4. Comparison of syngas bio-methanation and co-digestion process of syngas and organic substances by the RMBR. **(a)** Methane, **(b)** Hydrogen, **(c)** Carbon monoxide, and **(d)** Carbon dioxide. Symbols: Pure syngas at 35°C (◆), Pure syngas at 55°C (●), Syngas+organic substances at 35°C (▲), and Syngas+organic substances at 55°C (■).

Highlights

- A reverse membrane bioreactor (RMBR) was applied for syngas bio-methanation.

- The cells encased in PVDF membrane could convert syngas and produce CH_4 in one day.

- Encased cells in the RMBR performed better at 55°C compared to 35°C.

- Addition of organic waste with syngas improved the methane productivity.

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