Global J. Environ. Sci. Manage. 9(SI): 35-46, Autumn 2023

Global Journal of Environmental Science and Management
(GJESM)

Homepage: https://www.gjesm.net/

Podcasts

SPECIAL ISSUE: Eco-friendly sustainable management
CASE STUDY

Effects of effluent recirculation on two-stage anaerobic digestion in treatment of biodegradable municipal solid waste

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ARTICLE INFO

Article History:
Received 19 April 2023
Revised 29 May 2023
Accepted 09 August 2023

Keywords:
Biodegradable solid waste
Fermentation
Recirculation
Two-stage anaerobic digestion

DOI: 10.22034/GJESM.2023.09.SI.03

ABSTRACT

BACKGROUND AND OBJECTIVES: Advantages such as high stability and high biogas production when recirculating the effluent stream in two-stage anaerobic digestion systems have been demonstrated on a variety of substrates, but there is limited information regarding the use of this practice on organic municipal waste. Therefore, this study aimed to investigate how effluent recirculation affects the two-stage anaerobic digestion of biodegradable municipal solid waste.

METHODS: Firstly, biodegradable municipal solid waste substrate was fermented under conditions of 12 percent initial total solids and a temperature of 36 degrees Celsius for 5 days. After that, the substrate continued to be diluted using tap water or the effluent stream with a rate of 2:1. In the case of using the effluent stream, the experiment was further performed with dilution rates of 3:1, 1:1, and 1:2. Then, the liquid part was collected and pumped into the methane reactor at an organic loading rate of 7.64 grams of total solids per liter per day at 36 degrees Celsius. The methane reactor was an up-flow reactor that contained both granular sludge and suspended sludge. The effectiveness of the experimental stages was evaluated through biogas production and chemical oxygen demand removal.

FINDINGS: In the fermentative reactor, using the effluent stream to dilute solid-state feedstock helped keep the reactor stable at pH 5.5 without alkali addition. In the case of using tap water for dilution, it required a dose of 115.8 grams and 75.3 grams of sodium hydroxide per kilogram of volatile solids to attain pH conditions at 6.5 and 5.5, respectively. Maintaining the reactor at pH 6.5 increased the concentration of fermentation products compared to pH 5.5, including 5.9 percent total chemical oxygen demand, 5.5 percent soluble chemical oxygen demand, and 10.6 percent total volatile fatty acids. In the case of recirculating the effluent stream in the methane reactor, increasing the dilution rate from 0.5 to 3.0 resulted in a methane yield of 227.5-278.9 milliliter per gram of volatile solids and 85-93 percent chemical oxygen demand removal. The methane reactor's best digestion performance was attained at recirculation rate 2. Methane formation mainly occurred in granular sludge via the hydrogenotrophic pathway. Methane formation in suspended sludge occurred in a secondary manner, mainly via both the hydrogenotrophic and acetotrophic pathways. Among methanogen families, Methanobacteriaceae was found to have the highest relative abundance (7.5 percent in granular sludge and 0.8 percent in suspended sludge).

CONCLUSION: Recirculating the effluent provided significant benefits, including the ability to stabilize the hydrolysis process and increase the methane yield. A recirculation rate of 2 to obtain a total chemical oxygen demand of 35.2 grams per liter was the best condition for methanogenesis. Acetotrophic methanogens were better adapted to difficult conditions than hydrogenotrophic methanogens. The formation of methane mainly occurred in granular sludge via a dominant hydrogenotrophic pathway. Methane formation in suspended sludge occurred in a secondary manner, mainly via both the hydrogenotrophic and acetotrophic pathways. Among methanogen families, Methanobacteriaceae was found to have the highest relative abundance.

NUMBER OF REFERENCES
52

NUMBER OF FIGURES
4

NUMBER OF TABLES
3

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Note: Discussion period for this manuscript open until April 1, 2024 on GJESM website at the "Show Article".

INTRODUCTION

Two-stage anaerobic digestion (TAD) is attracting the attention of researchers due to its stability, flexibility, and capacity to function effectively despite fluctuations in the waste stream (Dinh *et al.*, 2020). In the TAD process, the substrate is hydrolyzed in a fermentative reactor (FR), then the substrate stream is converted to methane (CH_4) in a methanogenic reactor (MR) (Dinh *et al.*, 2020; Srisowmeya *et al.*, 2020). In this way, the TAD system allows different groups of microorganisms to grow under their own optimal conditions (Dhayalan and Karuppasamy, 2021; Manjarrez Paba *et al.*, 2021; Samimi and Shahriari Moghadam, 2020; Nuryadin and Imai, 2021). This is the basis for a robust system that is able to resist fluctuations in characteristics such as potential hydrogen (pH), organic concentration, and organic loading rate (OLR) (Dinh *et al.*, 2020). In the FR, hydrolysis/acidogenesis converts the carbon of high-molecular-weight compounds into volatile fatty acids (VFAs). Although the microorganisms in charge of these processes could work well under acidic conditions, they require a longer retention time than usual to ferment substrates (Dinh *et al.*, 2020; Nabaterega *et al.*, 2021). In the MR, methane formation occurs due to the activities of strict anaerobes. They are extremely sensitive to pH conditions, being unable to operate at pH < 6.2 and potentially collapsing at pH <5.5 (Gerardi, 2003; Nabaterega *et al.*, 2021). Therefore, using alkali addition for pH control is necessary for anaerobic digestion (AD) systems. This effort would increase operational costs in full-scale plants (Notodarmojo *et al.*, 2022). To prevent the need for alkali addition in TAD, Romli *et al.* (1994) introduced the effluent from the MR to the FR in a wastewater treatment system. They reported that the use of the recirculation stream removed dissolved carbon dioxide (CO_2), (weak acids) from the gas phase, causing a decrease in caustic consumption. The effects of effluent recirculation on TAD systems have also been investigated for some organic wastes such as cattle feed (Kovalev *et al.*, 2021), starch and cotton (Aslanzadeh *et al.*, 2013), swine manure (Chen *et al.*, 2021), leafy waste materials (Zuo *et al.*, 2015), citrus waste (Wikandari *et al.*, 2018), food waste (Ding *et al.*, 2021), and vegetable waste (Zuo *et al.*, 2013). For starch and cotton, Aslanzadeh *et al.* (2013) demonstrated that using effluent recirculation provided considerable benefits in terms of improved CH_4 yield and process stability. For vegetable waste, Zuo *et al.* (2013) proved that effluent recirculation reduced volatile fatty acid (VFA) inhibition and increased biogas generation at a high organic loading rate (OLR) due to the effects of dilution and pH correction. For citrus waste, Wikandari *et al.* (2018) reported that the system using effluent recirculation produced a higher CH_4 yield compared to that without recirculation. For food waste, Ding *et al.* (2021) showed that the liquid effluent of the MR supplied base buffering and acid washing to the FR. Similar research on biodegradable municipal solid waste (BMSW) is still limited in the literature. The majority of the interest in TAD has been focused on MRs because methanogens have a much slower growth rate and are much more sensitive than other microbial groups. Many studies have shown that using granular sludge (GS) can help overcome those weaknesses due to advantages such as high microbial concentration, superior settling property, and high resistance to toxic compounds (Azizan *et al.*, 2022; Cruz-Salomón *et al.*, 2019). These dense particles consist of an intertwined mixture of symbiotic anaerobic microorganisms that work together. These microbial groups arrange themselves in an orderly fashion, forming a multilayered structure (McHugh *et al.*, 2003). The outside layer consists of an acidogenic bacterial group that acidifies complex organic matter into short-chain VFAs as food for the inner microbial layers. Moreover, the fact that free hydrogen-consuming microorganisms are found in the exterior layer helps to avoid hydrogen diffusion into the second layer (Pol *et al.*, 2004). Therefore, granular sludge might reduce the impacts of substrate fluctuations such as pH, organic concentration, and organic loading rate (Piri and Sepehr, 2022). The second layer contains acetogens and hydrogenotrophic methanogens. This layer surrounds the central core which contains acetotrophic methanogens. Therefore, sludge granules can be regarded as well-balanced microecosystems (McHugh *et al.*, 2003). However, studies of the application of anaerobic granular sludge to deal with BMSW are still limited in the literature. The objective of this study is to investigate the effects of effluent recirculation on the TAD of BMSW in an MR in the presence of GS. The assessment is based on the results of biogas quality, biogas quantity, and chemical oxygen demand (COD) removal. The experiment was conducted in Japan in 2019-2020 and data analysis was carried out in Vietnam in 2021-2022.

MATERIALS AND METHODS

Substrate, anaerobic microorganisms, and an anaerobic reactor are required to conduct any anaerobic digestion assay. In this study, the substrate was collected from BMSW sources. The microorganisms and the anaerobic reactor were in good working condition. The reactor was operated at different stages to suit the intended purpose. The evaluation results were based on the physicochemical analysis of the material flow.

Substrate

The substrate contained 90 percent (%) BMSW and 10% inoculum on a wet basis. It was sliced into small-sized particles and ground, then stored at 0-4 degrees Celsius (°C) until used. Characteristics of BMSW, inoculum, and feedstock are shown in Table 1.

Experimental setup

The experimental model simulating the TAD system is shown in Fig. 1. The system was operated at 37°C and consisted of one FR, one MR, and one buffer tank between these two reactors. Firstly, the feedstock was fermented in FR with a retention time (RT) of 5 days (d). According to the reviews of TAD systems by Dinh *et al*. (2020), the acidogenesis of BMSW in a mesophilic environment proved successful with RT ranging from 2 to 5 days. The acidogenesis in this study was conducted with a five-day RT for safety reasons. The hydrolysate was then diluted to lower the concentration before being filtered (1mm) to remove particles (nonhydrolyzed materials). Finally, the hydrolysate liquid was injected into MR at an organic loading rate (OLR) of 7.64 grams of volatile solids per liter per day (g-TS/(L.d)). The MR was an up-flow reactor that contained both GS and suspended sludge (SS). There were two effluent recirculation circles including R1 (dilution in FR) and R2 (dilution in MR), as shown in Fig. 1. While recirculation in FR (R1) was to adjust TSs of the fresh feedstock at a concentration of 120 grams per liter (g/L), recirculation in MR (R2) was to reduce the hydrolysate concentration. The recirculation rate (n) of R2 was set to 3:1 for stage EX3, then to 2:1, 1:1, and 0.5:1 for stages EX4, EX5, and EX6, respectively.

Nonrecirculation trials (EX1 and EX2) were carried out with tap water for dilution instead of using the effluent stream. During fermentation, the pH of trials EX1 and EX2 was adjusted to 6.5 and 5.5, respectively,

Table 1. Physicochemical properties of the materials

Characteristics	BMSW	Inoculum	Feedstock
Total solid - TS (%)	26.88	20.01	26.19
Volatile solid - VS (%TS)	66.24	76.82	67.05
Carbon - C (%TS)	44.81	45.22	44.84
Nitrogen - N (%TS)	2.54	1.24	2.44
Carbon to nitrogen (C/N)	17.64	36.37	18.37

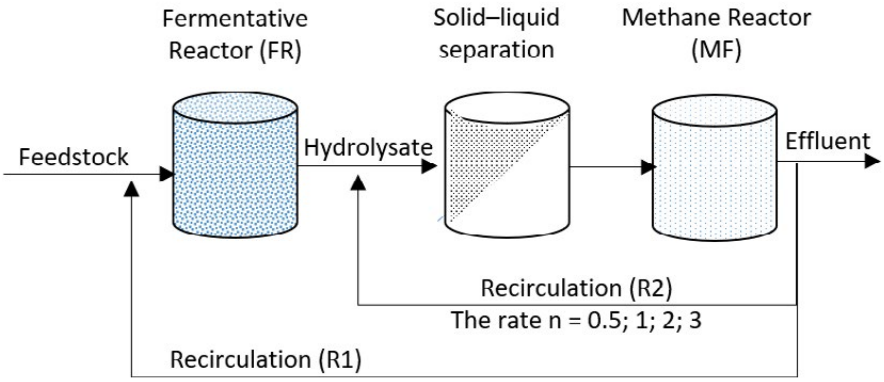

Fig. 1: Experimental model

using a 10 mol (M) solution of sodium hydroxide (NaOH).

Physicochemical analysis

For solid samples, the carbon, nitrogen, TS, and VS composition of the substrate were analyzed following standard methods, with the details presented by Dinh *et al.* (2018). For aqueous samples, pH was determined using a pH meter (Total Meter—Taiwan). Total COD (TCOD) and soluble COD (SCOD) were analyzed using a spectrophotometer (MD600, Lovibond, UK). VFA composition of the liquid hydrolysate was determined using a GC-14A gas chromatograph (Shimadzu, Japan). Biogas components were analyzed using a GC-2014 gas chromatograph (Shimadzu, Japan) equipped with a packed column and conductivity detector.

Microbiological analysis

Microbiological analysis was performed for both GS and SS. The details of deoxyribonucleic acid (DNA) extraction procedures are described in the literature. Cell lysis of 0.2 g wet-weight samples was achieved by beating them with sterile zirconium beads in the presence of sodium dodecyl sulfate (4% weight per volume), 0.5 mol sodium chloride, and 0.05 M ethylenediaminetetraacetic acid. Most of the impurities and the sodium dodecyl sulfate were then removed by precipitation with 10 M ammonium acetate. The nucleic acids were recovered by precipitation with isopropanol. After that, the isolation of genomic DNA was purified via sequential digestions with RNase and proteinase K, before using the QIAamp DNA Stool Mini Kit columns. The DNA amplification was performed following the method described by Nguyen *et al.* (2020). The method used a quantitative real-time polymerase chain reaction (PCR) targeting the V4 region of the 16S rRNA gene (forward: 5'-ACACTC TTTCCCTACACGACGCTCTTCCGATCTGTGCCAGCMGCCGCGGTAA-3'; reverse: 5'-TGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGACTACHVGGGTWTCTAAT-3'). Details of the protocol are attached in the report by Nguyen *et al.* (2020). The raw sequences from the archive were analyzed using the quantitative insights into microbial ecology (QIIME, version 1.9.1) program (Pagliano *et al.*, 2019). JMP software (version 11; SAS Institute) was used to perform statistical analysis on all data. A nonparametric Wilcoxon test was used to examine the statistical significance of the discrepancies.

RESULTS AND DISCUSSION

Due to its important role in defining the success and performance of methanogenesis, the microbial structure in the reactor is presented before the results are discussed according to the study objectives.

The microbial community in the methane reactor at the family level

The structure of the microbial community at the family level in GS and SS is presented in Fig. 2. The relative abundance (RA) of methanogens in GS accounted for 11.8% of the total microbial organisms, including *Methanobacteriaceae* (7.5%), *Methanosaetaceae* (2.9%), and *Methanomassiliicoccaceae* (1.4%). The RA of the methanogens in SS accounted for only 2.5%. The RA of methanogens in GS in this study was also significantly higher than that reported in the literature, for example, Guo *et al.* (2015) (5.6%), Qin *et al.* (2018) (3.18%), and Shin *et al.* (2019) (>1%). This suggests that GS favors the growth of methanogens due to its multilayer structure and symbiotic interaction mode. Within methanogens, *Methanobacteriaceae* is known as a hydrogenotrophic family, *Methanosaetaceae* is considered an acetotrophic one, and *Methanomassiliicoccaceae* represents a methylotrophic one (Söllinger and Urich, 2019; Ziganshin *et al.*, 2016). This was reflected in the fact that CH_4 formation mainly occurred in GS via a dominant hydrogenotrophic pathway using CO_2 and hydrogen (H_2) as substrates. Methane formation in suspended sludge occurred in a secondary manner, mainly via both the hydrogenotrophic and acetotrophic pathways. Methane synthesis by the methyl group in the reactor was not significant.

Excluding methanogens, the most abundant bacterial families found in GS in descending order were *Syntrophomonadaceae* (11.8%), *Porphyromonadaceae* (8.2%), *Ruminococcaceae* (6.5%), *Syntrophaceae* (5.8%), and *Anaerolineaceae* (4.9%). The family *Syntrophomonadaceae* plays the role of oxidizing fatty acids with 4-18 carbons into acetate and short-chain fatty acids (Schink and Muñoz, 2014). Members of this family are predominantly found in syntrophic associations with methanogens (Hashemi *et al.*, 2021; Schink and Muñoz, 2014). *Porphyromonadaceae* is known to be a family of acid-forming obligate anaerobic bacteria and has been previously identified in digesters dealing with municipal solid waste and animal waste (Chen *et*

Table 2: Operation of the TAD system

Test	Fermentation			Methanogenesis		
	TS	RT	pH	Dilution rate/ recirculation rate (n)	RT (days)	Total COD/soluble COD (g/L/g/L)
EX1	12 % (using tap water for dilution)	5 days	5.5	2.0	3.51	31.7/19.8
EX2			6.5	2.0	3.51	33.5/21.0
EX3	12 % (using effluent stream for dilution)	5 days	Uncontrolled	3.0	2.63	26.7/16.7
EX4				2.0	3.51	35.2/21.8
EX5				1.0	5.26	52.6/32.4
EX6				0.5	7.02	71.8/44.1

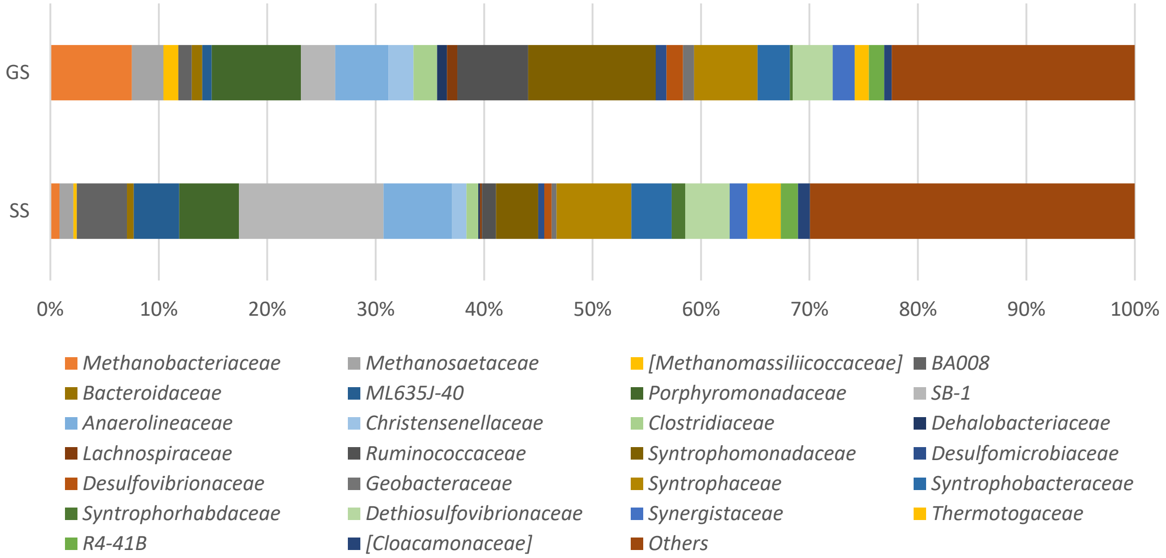

Fig. 2: Relative abundance of microorganisms in methane reactor at the family level

al., 2016). The family *Ruminococcaceae* has often been determined in the feces of animals (Pampillón-González *et al.*, 2017). They can hydrolyze a wide range of polysaccharides via several processes, including the production of cellulolytic enzymes (Morrison and Miron, 2000). They are also known to create VFAs and can ferment both hexoses and pentoses (Scott *et al.*, 2014). Blasco *et al.* (2020) found a positive significant relationship between VFA production and the RA of the microbial order *Clostridiales* containing the family *Ruminococcaceae*. They are also known to live in symbiosis with methanogens. The family *Syntrophaceae* contains four genera: *Syntrophus*, *Smithella*, *Desulfobacca,* and *Desulfomonile* (Kuever, 2014). *Syntrophus* and *Smithella* members are *chemoorganoheterotrophs* that may oxidize organic substrates partially to acetate or fully to CO_2. Members of the *Desulfobacca* and *Desulfomonile* genera are described as autotrophs that thrive on H_2 and CO_2 and can utilize sulfate, sulfite, and thiosulfate as electron acceptors that are reduced to sulfide. All members are mesophilic anaerobes and require anoxic media for growth (Nakasaki *et al.*, 2020). *Syntrophaceae* grows well in a long-chain fatty-acid-rich environment. The cultured *Anaerolineaceae* members ferment carbohydrates and/or peptides (Zhu *et al.*, 2017). Because they have a multicellular filamentous shape, *Anaerolineaceae* are significant bacteria for sludge granulation and granular structure maintenance. As a result, they have been discovered in both the outer layer and interior of sludge particles (Yamada and Sekiguchi, 2009). It has been

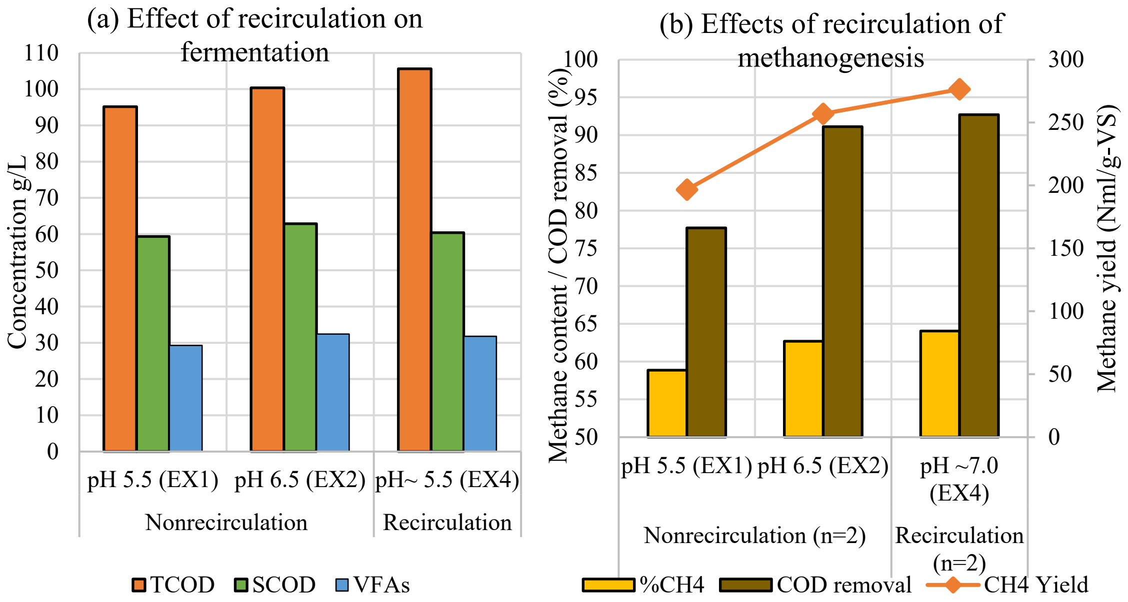

Fig. 3: Results at experimental stages

suggested that *Anaerolineaceae* has a symbiosis with *Methanosaeta* because *Methanosaeta* uses acetate produced by *Anaerolineaceae* (Owusu-Agyeman *et al*., 2019). The total RA of these acidogens/acetogens in SS (25%) was much lower than that obtained for those in GS (37%), reflecting that most VFAs were converted by microorganisms in GS.

Recirculation versus nonrecirculation

In the current study, maintaining the pH at 5.5 (EX1) required 74.4 grams of sodium hydroxide per kilogram of volatile solids (g/kg-VS). The alkali consumption increased to 114.4 g/kg.VS to attain a pH of 6.5 (EX2). For kitchen waste, Zhang *et al*. (2005) added a dosage of 272.1 mg-NaOH/g-TS to attain fermentation at pH 5. In another study, Sambusiti *et al*. (2013) reported that a dosage of 10 mg-NaOH/g-TS helped to maintain fermentation at pH 6.7 for wheat straw and at pH 6.3 for ensiled sorghum forage, respectively. Therefore, alkali consumption during fermentation depends not only on the pH level but also on the type of raw materials used. In experiment EX3, returning the effluent to the FR helped maintain a pH level comparable to that in EX1 without the need for alkali addition. This result can be explained by the following points. High-protein-content BMSW is converted into amino acids in the FR (Campuzano and González, 2016; Gerardi, 2003). In the MR, amino acids are further degraded to give ammonia (NH_3), which is a weak-base buffer (Chen *et al*., 2015; Gerardi, 2003). Furthermore, the majority of the VFAs fed into the MR are transformed into biogas. As a result of these factors, the effluent from the methane reactor has a high alkalinity. Thus, mixing the feedstock with the effluent stream can help to maintain a stable pH in the FR. Fig. 3(a) depicts the characteristics of the fermentative products from various experimental stages. The results from EX1 and EX4 did not significantly differ from one another. This reflects that the use of the effluent stream not only does not have any adverse effects on hydrolysis/acidogenesis but can also maintain pH conditions at a relevant level for fermentation. The influence of pH on fermentation was shown in the results between EX1 and EX2, whereby maintaining the reactor at pH 6.5 increased the fermentation products by 5.9% for TCOD, 5.5% for SCOD, and 10.6% for total VFAs when compared to the reactor maintained at pH 5.5. A similar trend was also reported by Veeken *et al*. (2000), who found that increasing the pH

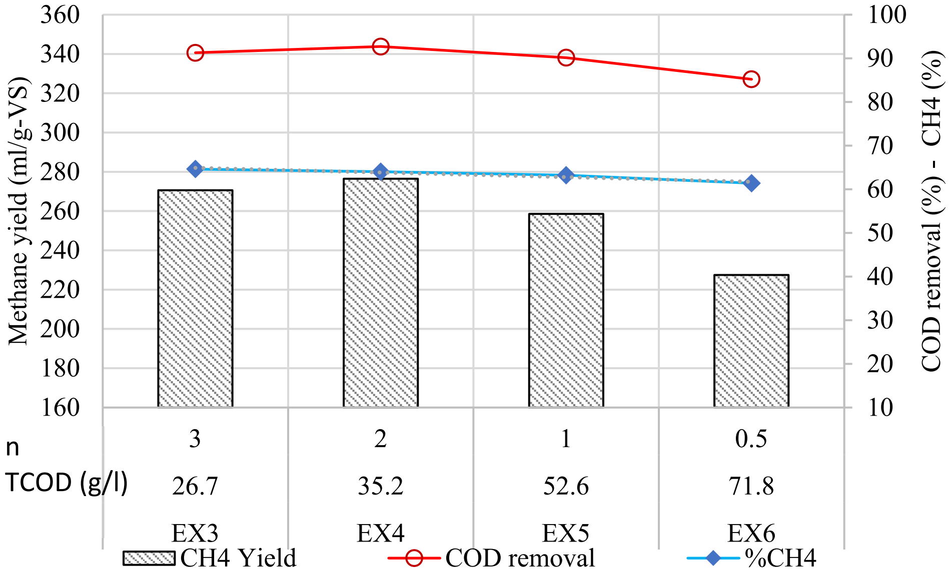

Fig. 4: Effects of different recirculation rates (n) on methanogenesis

from 5.0 to 7.0 greatly boosted the hydrolysis rate. These findings can be explained by the considerable positive association between enzymatic activity and pH in the range of 5.0-6.5 (Sanders, 2001). Following fermentation, the hydrolysate was diluted at a rate of n=2 before being introduced into the MR. While the pH in the nonrecirculation tests (EX1 and EX2) did not vary significantly, the pH condition in the recirculation examination (EX4) rose to 7.0. The various characteristics of the fermentation products produced differences in CH_4 yield, CH_4 concentration, and COD removal, as shown in Fig. 3(b). In general, the current investigation was consistent with the findings of Aslanzadeh *et al*. (2013) and Zuo *et al.* (2014), who found that recirculation helped obtain better performance than nonrecirculation.

The influent with a neutral pH (7.0) provided the best conditions in the MR with a CH_4 yield of 276.3 milliliters per gram of volatile solids (mL/g.VS). The influent with a slightly acidic pH (6.5) resulted in a 2% decrease in CH_4 concentration and a 7% decrease in CH_4 production. The influent with an acidic pH level (5.5) (EX1) exhibited a 28.8% and 8% reduction in CH_4 yield and CH_4 concentration, respectively. These findings indicate that methanogens thrive in pH-neutral environments. According to the literature, there are two main different reactions that produce CH_4: (1) acetotrophic methanogens digest acetic acid to produce CH_4 and CO_2, and (2) hydrogenotrophic methanogens create CH_4 from CO_2 (Dinh *et al*., 2020; Gerardi, 2003). These two microbial groups respond differently to environmental changes. The CH_4 composition of biogas changes as a result of the ratio of reaction (1) to reaction (2) altering. From the link between pH levels and CH_4%, it was observed that acetotrophic methanogens were better adapted to acidic circumstances than hydrogenotrophic methanogens.

Effects of different recirculation rates

Increasing the recirculation rate (n) from 0.5 to 3 led to changes in CH_4 yield (227.5-278.9 mL/g-VS) and COD removal (85.2-92.7%). Fig. 4 provides specific information regarding the impact of various recirculation rates on the performance of the MR.

The MR's best methanogenic performance was attained when the recirculation rate was n = 2 and the TCOD of the influent was 35.2 mg/L. Recirculation rate n = 3 offered benefits such as quicker substrate diffusion and interaction with lower substrate concentrations (TCOD = 26.7 g/L). However, operation at rate n=3 had a significantly lower retention time (RT) than operation at rate n=2. RT should be long enough for anaerobes to make contact with and break down substrates (Dinh *et al*., 2020; Mshandete *et al*., 2004). In this study, it was observed that operation at rate n=3 was slightly less effective than operation at n=2, probably due to the shorter contact period between the anaerobes and the substrate. Zuo *et al*. (2014) corroborated the same trend and observed that decreasing the recirculation rate to dilute the COD concentration from 21 grams of oxygen per liter (g-O_2/L) to 6.8 g-O_2/L resulted in a 6% drop in CH_4 yield and an 8% reduction in COD removal. Even during the highest recirculation rate, they discovered biomass washout. In a different study, Yu *et al*. (2000) confirmed that high n values would cause an excessive rise in the effective loading rate in the methanogenic reactor, which would then cause a gradual increase in the concentration of organic output and a reduction in efficiency. Dilution of the influent stream influences substrate diffusion or transmission and may result in a slower reaction rate, which lowers process efficiency. According to Mshandete *et al*. (2004), long retention times for anaerobic up-flow reactors produced a laminar flow that enhanced methanogenesis. However, a higher influent concentration is established at lower recirculation rates. Because of this, utilizing recirculation rates of n=1 and 0.5 resulted in a worse methanogenic performance than using n=2. At recirculation rate n=1, the influent with a TCOD of 52.6 g-O_2/L resulted in a 6.5% and 2.7% reduction in biogas yield and COD removal, respectively. At n=0.5, the TCOD concentration of the effluent was 71.8 g-O_2/L, leading to an 8.1% and 17.7% reduction

Table 3: The effects of effluent recirculation on TAD systems with different types of biodegradable wastes

Feedstock	TAD systems	Findings	Sources
Cattle feed	CSTR^{1st} – CSTR^{2nd} Thermophilic^{1st} – Thermophilic^{2nd}	A low recirculation rate can improve the performance of the TAD process. The best recirculation rate was 0.11 bringing a net energy of 7.7 kJ/g-VS.	Kovalev *et al*., 2021
Starch and cotton	CSTR^{1st} – UASB^{2nd} Thermophilic – Mesophilic^{2nd} OLR 4.0-10 g-VS/(L.d)	The use of effluent recirculation improved the overall performance and stability of the process.	Aslanzadeh *et al*., 2013
Swine manure	CSTR^{1st} – CSTR^{2nd} Thermophilic^{1st} – Mesophilic^{2nd} OLR 1.76 g-VS/(L.d)	Using the digestate recirculation increased CH_4, VS removal, and reaction rate by 9.92 ± 5.08, 5.22 ± 1.94, and 9.73 ± 12.60%, respectively.	Chen *et al*., 2021
Leafy waste materials	CSTR^{1st} – CSTR^{2nd} Mesophilic^{1st} – Mesophilic^{2nd} OLR 2.6 – 3.0 g-VS/(L.d)	The system without recirculation was susceptible to overloading and volatile fatty acid (VFA) utilization was inhibited in the methanogenic reactor.	Zuo *et al*., 2015
Citrus waste	CSTR^{1st} – UASB^{2nd} Mesophilic^{1st} – Mesophilic^{2nd} OLR 5.0 g-VS/(L.d)	The system using effluent recirculation produced a higher CH_4 yield and was more stable compared to that without recirculation.	Wikandari *et al*., 2018
Food waste	CSTR^{1st} – CSTR^{2nd} Mesophilic^{1st} – Mesophilic^{2nd} OLR ND	Compared to that without recirculation, the system with recirculation was better at buffering and more stable in operation.	Ding *et al*., 2021
Vegetable waste	CSTR^{1st} – Fixed-bed reactor^{2nd} Mesophilic^{1st} – Mesophilic^{2nd} OLR 1.7 g-VS/(L.d)	The use of recirculation helped to improve mass transfer capacity between two-stage reactors.	Zuo *et al*., 2014
Potato-waste leachate	Fixed-bed reactor^{1st} – Fixed-bed reactor^{2nd} Mesophilic^{1st} – Mesophilic^{2nd} OLR of 12 g-COD/(L.d).	The bioreactor with a low recirculation flow rate showed operational stability.	Mshandete *et al*., 2004
BMSW	CSTR^{1st} – UASB^{2nd} Mesophilic^{1st} – Mesophilic^{2nd} OLR 4.0 g-VS/(L.d)	Increasing the yield of biogas and CH_4 concentration while stabilizing the hydrolysis process without the use of alkaline chemicals. The optimal methanogenic conditions were achieved with a TCOD of 35.2 g-O_2/L using the recirculation rate.	The current study

Notes: ^{1st} first reactor (fermentative reactor – FR); ^{2nd} second reactor (methane reactor – MR) in COD removal and biogas production, respectively. More details about the impact of effluent recycling on TAD systems with various biodegradable waste types are provided in Table 3.

From reactions (1) and (2), the decrease in CH_4 concentration brought on by the reduction in recirculation rate demonstrated that acetogenotrophic methanogens were more adapted to high substrate concentrations than hydrogenotrophic ones. Romli *et al*. (1994) stated that CH_4 content declined with increasing n value, which is the opposite of this conclusion. However, in their report, the pH of the influent was decreased from 7.6 to 6.6, accompanied by an increase in the recirculation rate. As discussed in the previous section, lowering the pH also led to a reduction in CH_4. As a result, the change in CH_4 demonstrated that the impact of lowering the pH was greater than the effect of increasing the recirculation rate. In particular, the significant linear correlation between the CH_4% obtained and the COD concentration of the influent suggested the sensitivity of the anaerobes in the up-flow reactor. This could be explained by the direct contact between obligate anaerobes and the high concentration of the substrates. It differs from a CSTR in that high substrate concentrations are immediately diluted by low substrate concentrations inside (Dinh *et al*., 2020). As a result, Cavinato *et al*. (2011) reported no significant influence of COD input ranging from 16 to 49 g-O_2/L on CH_4% while employing a CSTR for methanogenesis.

CONCLUSION

Anaerobic granular sludge has been proven to have great potential in treating high-concentration substrates such as BMSW. Although growing in the same reactor, the structure of granular sludge helps the methanogens, acidogens, and acetogens thrive much more than in suspended sludge. The relative abundance of methanogens in granular sludge (11.8%) was fivefold that obtained in suspended sludge (2.5%). In addition, the relative abundance of the acidogens/acetogens in granular sludge (37%) was significantly higher than that in suspended sludge (25%). Among methanogen families, *Methanobacteriaceae* was found to have the highest relative abundance (7.5% in granular sludge and 0.8% in suspended sludge). The formation of CH_4 mainly occurred in granular sludge via a dominant hydrogenotrophic pathway. Methane formation in suspended sludge occurred in a secondary manner, mainly via both the hydrogenotrophic and acetotrophic pathways. Methane synthesis by the methylotrophic methanogens in the reactor was not significant. Recirculating the effluent provided significant benefits, including the ability to stabilize the hydrolysis process and increase the methane yield. In the fermentative reactor, using the effluent stream to maintain a state of 12% total solids helped keep the reactor stable at pH 5.5 without alkali addition. In the case of using tap water for dilution, it required a NaOH dose of 115.8 g/kg-VS and 75.3 g/kg-VS to maintain pH conditions at 6.5 and 5.5, respectively. The concentration of fermentation products in the reactor maintained at pH 6.5 increased by 5.9% TCOD, 5.5% SCOD, and 10.6% VFAs in comparison with the reactor maintained at pH 5.5. In the case of recirculating the effluent stream in the methane reactor, increasing the dilution rate from 0.5 to 3.0 resulted in a CH_4 yield in the range of 227.5-278.9 mL/g-VS and COD removal in the range of 85-93%. The methane reactor's best digestion performance was attained at a recirculation rate of 2 to obtain influent with a TCOD of 35.2 g-O_2/L. The decline in CH_4 concentration following a decrease in pH or an increase in substrate concentration reflected that acetotrophic methanogens are better adapted to difficult conditions than hydrogenotrophic methanogens.

AUTHOR CONTRIBUTIONS

D.V. Pham performed the experiments, analyzed the data, and wrote the original draft. T. Fujiwara supervised the experiments. P. Astrini Notodarmojo helped to perform the experiments. K.C. Tran revised the manuscript.

ACKNOWLEDGEMENT

This study is funded by the Vietnamese Ministry of Education and Training under [Grant number B2021-XDA-03]. The authors would like to thank the Hanoi University of Civil Engineering (HUCE) for helping us obtain funding. The authors also would like to thank Prof. Naoki Nishino and Dr. Nguyen Dang Qui from Okayama University for supporting us in the analysis of the microorganisms.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

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PUBLISHER'S NOTE

ABBREVIATIONS

%	Percentage
°C	Degrees Celsius
AD	Anaerobic digestion
BMSW	Biodegradable municipal solid waste
C	Carbon
C/N	Carbon to nitrogen
CH_4	Methane
CO_2	Carbon dioxide
COD	Chemical oxygen demand
CSTR	Continuous stirred tank reactor
d	Day
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EX	Examination
Fig.	Figure
FR	Fermentative reactor
g/kg.VS	Gram per kilogram of volatile solids
$g\text{-}O_2/L$	Oxygen gram per liter
GS	Granular sludge
g-VS/(L.d)	Gram of volatile solids per liter per day
H_2	Hydrogen
M	Mole
mL/g.VS	Milliliter per gram of volatile solids
MR	Methane reactor
n	Recirculation rate for methanogenesis
N	Nitrogen
NaOH	Sodium hydroxide
pH	Potential of hydrogen
RT	Retention time
SCOD	Soluble chemical oxygen demand
SS	Suspended sludge
TAD	Two-stage anaerobic digestion
TCOD	Total chemical oxygen demand
TS	Total solid
UASB	Up-flow anaerobic sludge blanket
VFA	Volatile fatty acid

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HOW TO CITE THIS ARTICLE

Dinh, P.V.; Fujiwara, T.; Peni, A.N.; Khanh, T.C., (2023). Effects of effluent recirculation on two-stage anaerobic digestion in treatment of biodegradable municipal solid waste. Global J. Environ. Sci. Manage., 9(SI): 35-46.

DOI: 10.22034/GJESM.2023.09.SI.03

URL: https://www.gjesm.net/article_706670.html