

Process Performance and Microbial Community Variation in High-Rate Anaerobic Continuous Stirred Tank Reactor Treating Palm Oil Mill Effluent at Temperatures Between 55 °C and 70 °C

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Research Article

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Abstract

Palm oil mill effluent (POME) is wastewater with a relatively high temperature (80 – 90 °C) that is generated from the extraction of oil from palm fruit and palm kernels. Owing to its high discharge temperatures, the thermophilic anaerobic digestion (AD) of POME could be advantageous as treatment at thermophilic temperatures can reduce loads for cooling the wastewater. In this study, the effects of stepwise temperature increments of 5 °C from 55 °C to 70 °C on the AD of POME were investigated in a continuous stirred tank reactor (CSTR) operated under high organic loading rates (OLRs). The process performance and microbial community structure at each temperature interval were evaluated. It was observed that the methane production rates of the CSTR increased with increasing OLRs up to values of 13.7 g/L d, 25.7 g/L d, and 26.5 g/L d at operating temperatures of 55 °C, 60 °C, and 65 °C, respectively. As a result of the increasing OLRs, the maximum rate of methane production increased from 3.8 L/L d at 55 °C to 4.4 L/L d at 60 °C and to 3.8 L/L d at 65 °C. The microbial community structure analysis showed that there were notable reductions in the gene copy number of the bacterial domain and the *Methanosarcinales* order with increasing temperatures from 55 °C to 60 °C and to 65 °C, whereas hydrogenotrophic methanogens, especially the genus *Methanobacterium*, in the order *Methanobacteriales* became dominant at 60 °C and 65 °C. Thus, the methanogenesis pathway was suggested to be a combination of acetoclastic and hydrogenotrophic methanogenesis at 55 °C and 60 °C with an increased contribution of hydrogenotrophic methanogenesis at 60 °C, whereas methane was mainly generated via hydrogenotrophic methanogenesis at 65 °C. The thermophilic AD of POME at 60 °C was found to be promising because the methane content in the biogas and the methane production rates were optimal, with an average methane content of approximately 73%.

Statement Of Novelty

This work illustrates a high-rate thermophilic anaerobic digestion of raw palm oil mill effluent (POME) with relatively high chemical oxygen demand and suspended solids in a stirred tank reactor at temperatures between 55 °C and 70 °C. The thermophilic anaerobic digestion of POME at 60 °C was found to be promising as the methane content in the biogas and the methane production rates were optimal, with an average methane production rate of 4.4 L/L d and methane content of approximately 73%, respectively.

1. Introduction

The palm oil industry is an important agricultural sector in Southeast Asia that produces palm oil to serve as an ingredient in food and personal care products worldwide. During the palm oil extraction process, a large amount of wastewater, known as palm oil mill effluent (POME), is generated. Anaerobic digestion (AD) is an effective method for treating POME at both the laboratory and industrial scales [1-11]. Currently, open anaerobic ponds are the most commonly used method for the industrial AD of POME [12]. The process is not environmentally friendly because a large amount of methane (CH₄) and carbon dioxide (CO₂) is released into the atmosphere [12]. The treatment of POME via the AD process in closed

reactors is an alternative method to convert organic compounds into CH₄ and then utilize the CH₄ to produce heat, electricity, biofuel, value-added chemicals, and hydrogen (H₂) [13].

Research regarding the AD of POME often aims to provide operational data for high-rate AD of POME in closed reactors [5,9], and evaluates the factors that affect the growth of anaerobic microbes [14]. It has been reported that temperature is a factor that influences both microbial communities [14] and the kinetics of reactions [15-17]. AD processes are typically carried out at two temperature levels: the mesophilic (35 – 40 °C) and thermophilic ranges (55 – 70 °C) [12]. A number of studies have achieved the successful thermophilic AD of POME at 55 °C [1-2, 5,7, 9-10, 18] and demonstrated that thermophilic AD of POME at 55 °C resulted in higher rates of biogas production and biogas yields than mesophilic AD [1, 4, 19]. Thermophilic AD at temperatures above 55 °C might be advantageous for treating POME because it could reduce loads for cooling the wastewater, which is typically discharged at 80 – 90 °C [10]. However, the bioreactor performance of POME thermophilic AD at 60 – 70 °C has never been reported.

It has been shown that small temperature fluctuations (approximately 5 °C) in thermophilic AD could alter the dominant methanogens in thermophilic AD or even inhibit some methanogens [20-24]. For example, it has been reported that the growth of acetoclastic *Methanosarcinara* was optimal at temperatures ranging from 55 °C to 60 °C but was completely inhibited at 65 °C [25]. Conversely, hydrogenotrophic methanogenesis, which involves the conversion of H₂ and CO₂ into CH₄, was optimal at 65 °C [20-21, 24].

Therefore, in this study, the effect of stepwise temperature increments of 5 °C from 55 °C to 70 °C on the microbial community structure and process performance of a continuous stirred tank reactor (CSTR) treating POME was investigated. Both the process performance and microbial community profiles with their population dynamics at each temperature interval were evaluated. It was illustrated that a temperature of 60 °C provided the highest methane content in the biogas and the highest rate of methane production from POME, and that hydrogenotrophic methanogenesis played an important role in the increased methane production at 60 °C, as evidenced by the increased ratio of the gene copy number of the order *Methanobacteriales* (MBT) to that of the order *Methanosarcinales* (MSL).

2. Material And Methods

2.1 Materials

The CSTR used in this study was previously described by Khemkhao et al. [4]. Mesophilic anaerobic digested sludge obtained from a water quality control plant in Bangkok (Thailand) was used as the inoculum for the CSTR. The sludge had a total solids (TS) volume of 54.45 ± 0.14 g/L, volatile solids (VS) volume of 29.33 ± 0.07 g/L, suspended solids (SS) volume of 47.83 ± 0.67 g/L, volatile suspended solids (VSS) volume of 27.35 ± 0.28 g/L, a sludge volume index (SVI) of 19.10 mL/g as SS, and a VSS/SS ratio of 0.57. The POME sample was collected from a palm oil factory for the experiments before being transferred to a sedimentation tank. The raw POME had a pH of 4.62 ± 0.07, a total chemical oxygen demand (tCOD) of 89.35 ± 5.78 g/L, soluble chemical oxygen demand (sCOD) of 66.55 ± 4.35 g/L, TS of

68.23 ±1.73 g/L, VS of 58.63 ±1.59 g/L, SS of 55.05 ±0.85 g/L, and VSS of 49.48 ± 0.67 g/L. The POME was diluted with water to obtain COD values in the range of 6.6 – 89.4 g/L.

2.2 Start-up and experimental run

A 5.5-L working volume CSTR was initially inoculated with 10 g/L of VSS in the sludge. Throughout the experiment, the feed flow rate into the reactor was 1.67 L/d to achieve a hydraulic retention time of 3.3 d. The start-up and operation of the reactor followed the procedure described by Khemkhao et al. [4]. The CSTR was initially operated at 55 °C under an organic loading rate (OLR) of 2 g/L d. The OLR was increased stepwise by increasing the COD of the feed. At each OLR, the reactor was operated until both the biogas production and COD removal rates were steady for at least seven days. The OLR was increased repeatedly until the biogas production rate did not further increase. When the highest OLR was achieved at 55 °C, a stepwise increase in temperature from 55 °C to 60 °C was performed. Subsequently, stepwise increases in the OLR were performed. This process was repeated for a stepwise increase in the temperature from 60 °C to 65 °C and from 65 °C to 70 °C.

2.3 Analytical methods for physical and chemical properties

The gas counter method was used to measure the gas produced from the reactor. The biogas composition was examined using a gas chromatograph coupled with a thermal conductivity detector (SHIMADZU, GC-2014, Japan) equipped with a stainless-steel packed column, and an argon carrier gas with a flow rate of 50 mL/min. The volumes of biogas and methane reported were corrected to the volumes under standard conditions of 273 K and 1 atm pressure. For the liquid effluent sample, the total alkalinity (TA) as CaCO₃, total volatile acidity (TVA) as CH₃COOH, TS, SS, VS, VSS, tCOD, and sCOD were analyzed according to the procedures described in [26]. The compositions of the volatile fatty acids (VFAs) in the liquid effluent were examined using a gas chromatograph coupled with a flame ionization detector (SHIMADZU, GC-2010, Japan) equipped with a capillary column (Restek, Stabilwax, USA) and a nitrogen carrier gas with an average linear velocity of 35 cm/s.

2.4 DNA extraction and denaturing gradient gel electrophoresis analysis

Representative samples of the sludge were taken from the reactor operating under the highest OLR of each operating temperature. Genomic DNA was extracted using a Presto™ Soil DNA Extraction Kit (Geneaid, Taiwan). The bacterial and archaeal community structures of the sludge samples were examined using nested polymerase chain reaction (PCR) and PCR- denaturing gradient gel electrophoresis (DGGE), as described in Khemkhao et al. [3]. The 16S rRNA gene sequences of the representative samples were compared with those in the National Center for Biotechnology Information database using the Basic Local Alignment Search Tool [27].

2.5 Quantitative real-time PCR

The primer set of domain bacteria (BAC) and three primer sets of methanogens in the MSL, MBT, and *Methanomicrobiales* (MMB) orders were used to quantify the copy numbers of genomic DNA of the representative sludge samples. A real-time PCR assay was conducted as reported by Khemkhao et al. [28]. The details of the primer sets were previously reported by Yu et al. [29] and the equipment used is described in Khemkhao et al. [28]. The copy number of the 16S rRNA gene/volume was used to indicate the 16S rRNA gene copy number in the sludge sample [30].

2.6 Tests of statistical significance

All measurements were performed in triplicate, including the real-time PCR assay. Tests of statistically significant differences between the groups were performed using a one-way variance analysis with a p value of < 0.05 for statistical significance.

3. Results

3.1 Process performances of the CSTR

Fig. 1 shows the methane content in the biogas and the biogas and methane production rates from POME in the CSTR operated for 335 d under thermophilic temperatures between 55 °C and 70 °C. For each operating temperature, the reactor was operated at a series of OLRs as follows: 1.6 g/L d to 19.0 g/L d at 55 °C, 14.0 g/L d to 25.7 g/L d at 60 °C, 15.8 g/L d to 26.5 g/L d at 65 °C, and 13.7 g/L d to 26.8 g/L d at 70 °C. The OLR was increased to increase the methane production rates. As shown in Fig. 2a, at an operating temperature of 55 °C, the methane production rate markedly increased from 1.3 L/L d to 3.8 L/L d when the OLR increased from 11.4 g/L d to 13.7 g/L d. However, the methane production rate did not further increase with increasing OLR in the range of 13.7 – 19.0 g/L d. At 60 °C, the methane production rate increased almost linearly as the OLR was increased from 14.0 g/L d to 25.7 g/L d, and a maximum methane production rate of 4.4 L/L d was obtained at an OLR of 25.7 g/L d (Fig. 2b). Meanwhile, at 65 °C, the methane production rate increased from 2.7 L/L d to 3.8 L/L d when the OLR increased from 15.8 g/L d to 26.5 g/L d (Fig. 2c). Conversely, at 70 °C, the OLR increase from 20.4 g/L d to 26.8 g/L d caused a significant decrease in the methane production rate (Fig. 2d), which indicates system failure.

The methane contents in the biogas and the methane yields at different operating temperatures were compared for an OLR of 20 ± 1 g/L day. It was found that the methane content increased with a temperature increase from 55 °C to 60 °C. However, when the temperature further increased from 60 °C to 65 °C and from 65 °C to 70 °C, the methane content in the biogas gradually declined. The average methane contents at this OLR range were $69.8\% \pm 1.7\%$, $72.9\% \pm 2.9\%$, $71.1\% \pm 2.4\%$, and $68.9\% \pm 2.4\%$ at temperatures of 55 °C, 60 °C, 65 °C, and 70 °C, respectively. The average methane yields at an OLR of 20 ± 1 g/L day were not significantly different, i.e., 0.272 L/g COD removed, 0.264 L/g COD removed, 0.273 L/g COD removed, and 0.284 L/g COD removed at temperatures of 55 °C, 60 °C, 65 °C, and 70 °C, respectively, whereas the average methane yields at the highest OLRs were 0.272 L/g COD removed at an

OLR of 19.0 g/L d at 55 °C, 0.343 L/g COD removed at an OLR of 25.7 g/L d at 60 °C, and 0.264 L/g COD removed at an OLR of 26.5 g/L d at 65 °C.

Fig. 3 shows the concentration of each VFA in the effluent. Regardless of the operating conditions, acetate was the dominant VFA. When the reactor was operated at 55 °C and the OLR ranged from 1.6 g/L d to 19.0 g/L d, the acetate concentrations were in a range of 0.74 – 3.93 g/L, while those of propionate and butyrate were relatively small. When the reactor was operated at 60 °C at an OLR range of 14.0 g/L d to 25.7 g/L d, the concentrations of acetate and other VFAs were relatively unchanged. However, there was a slight increase in the levels of propionate and butyrate when the OLR increased from 19.4 g/L d to 25.7 g/L d. When the temperature was increased stepwise to 65 °C, there was a notable increase in the concentrations of propionate and butyrate. Similarly, at 70 °C, the concentrations of all the VFAs increased, regardless of the OLR. In particular, at 70 °C under an OLR of 26.8 g/L d, the VFA accumulations increased significantly with rapid decreases in both biogas production and the methane content in the biogas (Fig. 1).

Fig. 4 shows the pH profile and the ratio of VFA to TA (VFA/TA) during reactor operation. The VFA/TA ratios were relatively high during the first 30 d of the operation and then decreased from > 1.0 to values in the range of 0.2 – 0.7. The VFA/TA ratios increased above 1.0 again when the CSTR was operated at 65 °C under an OLR of 26.5 g/L day and at 70 °C under an OLR of 26.8 g/L day. The average pH of the effluent during reactor operation at 55 °C, 60 °C, and 65 °C ranged from 7.5 to 7.7. Conversely, there were relatively large fluctuations in the pH of the effluent when the reactor was operated at 70 °C. Specifically, the pH dropped below 6.5 on days 332 and 334 when the reactor was operated at 70 °C under an OLR of 26.8 g/L d. This large pH fluctuation was an early sign of process instability and subsequent system failure when the reactor was operated at 70 °C.

The efficiency of SS reduction in the reactor could be an indicator of the efficiency of the anaerobic hydrolysis reactions [31]. Increased temperatures can stimulate hydrolysis reactions as thermal degradation can alter the structure of biopolymers, leading to enhanced enzymatic digestibility [32]. However, in this study, it was found that an increase in temperature above 55 °C had a negative effect on the SS reduction efficiency, as there were notable decreasing trends for both the SS and tCOD reductions with increasing temperature (Figs. 5a and 5b). There was also a decrease in the sCOD reduction efficiency with increasing temperature, especially when the operating temperature increased to 65 °C and 70 °C (Fig. 5b). A possible explanation for the decreased efficiency of SS and tCOD reductions in the reactor could be a negative effect of the increased temperatures on the community of hydrolytic bacteria in the reactor, which will be discussed in Section 3.2.

3.2 Microbial community profiles and population dynamics of bacteria and methanogens

DGGE and real-time PCR were conducted to investigate the changes in microbial community profiles and population dynamics of bacteria and methanogens as a result of stepwise temperature increases of 5 °C from 55 °C to 70 °C. Fig. 6 shows the DGGE profiles of the bacteria and methanogens in the inoculums collected at 55 °C, 60 °C, 65 °C, and 70 °C. Throughout the experiment, the members of *Firmicutes* were

the most dominant bacterial phyla (Fig. 6a), while the MBT order was the dominant *archaea* taxa (Fig. 6b). Meanwhile, the microbial diversity at the taxonomic genus level changed greatly with increasing operational temperatures.

Fig. 6a shows the DGGE profiles of the bacterial communities. In lane 1 (55 °C), the dominant DGGE bands were identified as bacteria in the genera *Acinetobacter* (phylum *Proteobacteria*), *Clostridium* (phylum *Firmicutes*), *Exiguobacterium* (phylum *Firmicutes*), and *Prevotella* (phylum *Bacteroidetes*). In lane 2 (60 °C), the sequences of the dominant DGGE bands belonged to the bacterial genera *Clostridium* and *Acetomicrobium* (phylum *Synergistetes*). In lane 3 (65 °C), the sequences of the dominant DGGE bands belonged to the bacterial genera *Acetomicrobium* and *Prevotella*. In lane 4 (70 °C), the sequences of the dominant DGGE bands belonged to the bacterial genera *Clostridium*, *Prevotella*, *Dialister*, and *Megasphaera* (phylum *Firmicutes*). Thus, the diversity of the bacteria decreased with an increase in temperature from 55 °C to 60 °C and from 60 °C to 65 °C, but the bacterial community was more abundant at 70 °C than at 60 °C and 65 °C. In addition, the dominant bacteria at 70 °C were associated with the conversion of fatty acids into propionate. For example, *Dialister succinatiphilus* converts decarboxylate succinate to propionate [33], while *Megasphaera elsdenii* degrades lactate to butyrate, acetate, and propionate [34]. However, the hydrolytic activity of the major bacteria identified at 70 °C has not yet been reported.

Fig. 6b shows the DGGE profiles of the archaeal communities. The results show that there were variations in the effects of the operation temperature on microbial diversity at both the taxonomic and genus levels. Regarding the genera in the MSL order, the DGGE bands were the sharpest in lane 1 (55 °C), but the bands faded with increasing temperatures. The disappearance of the DGGE band of *Methanosaeta thermophila* in lane 4 (70 °C) also indicates that the genera in the MSL order could only survive in an environment in which the temperature was below 70 °C. Further, both the disappearance of the DGGE band of *M. thermophila* and the large build-up of acetate in the reactor occurred when the operating temperature increased from 65 °C to 70 °C (Fig. 3). Regarding the genera in the MBT order, there were variations in the appearance of the DGGE bands. That is, the DGGE bands of the genus *Methanobacterium* were sharp and intense at all operating temperatures, while the sharpness of the bands belonging to *Methanothermobacter marburgensis* increased with increasing operation temperature. Conversely, the DGGE band of *Methanothermobacter thermautotrophicus* disappeared at 70 °C (lane 4). These results suggest that the genera in the MBT order might have different responses to increasing temperatures. Finally, the DGGE bands of the genera in the MMB order appeared indistinctly in all lanes, indicating that the genera in this were the minority population among the three orders of methanogens.

Fig. 7 illustrates changes in the 16S rRNA gene copy number due to shifts in the operating temperatures. At 55 °C, the 16S rRNA gene copy number of the BAC, and the MBT, MSL, and MMB orders were $6.24 \pm 0.99 \times 10^{12}$, $1.21 \pm 0.31 \times 10^{11}$, $2.73 \pm 0.17 \times 10^9$, and $9.63 \pm 0.16 \times 10^7$ copies/mL, respectively. The increase in the operation temperature from 55 °C to 60 °C reduced the gene copy number of the BAC, MBT, MMB, and MSL sets by 7.61-, 4.92-, 2.61-, and 18.44 times, respectively. In addition, a further increase in the operation temperature to 65 °C increased the gene copy number of the MBT and MMB by

7.93 and 3.87 fold, respectively. Conversely, the gene copy number of the BAC and MSL sets decreased by 2.02 and 6.46 fold, respectively, as compared with those at 60 °C. Meanwhile, at 70 °C, only the gene copy number of the BAC set increased markedly up to $1.96 \pm 0.38 \times 10^{13}$ copies/mL, while the gene copy number of the other three methanogen orders declined from those at 65 °C.

4. Discussion

The microbial community profiles derived from the DGGE and the population dynamics obtained from real-time PCR data were in good agreement, indicating that the bacterial diversity and the 16S rRNA gene copy number of the BAC were higher at the operation temperature of 55 °C than at 60 °C and 65 °C. Meanwhile, the decrease in the gene copy number of the MSL order with increasing temperature was in good agreement with the fading of the DGGE band of *M. thermophila* at temperatures higher than 55 °C.

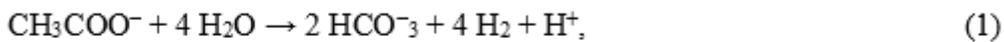
There was also a good agreement between the gene copy number of the BAC and the SS reduction efficiency of the reactor, revealing that the 16S rRNA gene copy number of the BAC and the SS reduction efficiency of the reactor were the highest at an operational temperature of 55 °C and decreased with increasing operation temperatures. Thus, a temperature of 55 °C may be optimal for bacteria with hydrolytic activities. Further, the increase in the gene copy number of the BAC (some bacterial species in the genera *Clostridium* and *Prevotella* and the species *D. succinatiphilus* and *M. elsdenii*) at 70 °C did not to provide any benefits to the SS reduction efficiency of the reactor.

It has been reported in the literature that the VFA accumulation level is related to the absence/presence of acetoclastic methanogens [35-36]. Lins et al. [35] observed that a sharp decrease in the amount of accumulated VFAs with increases in the total biogas and CH₄ production, which were found to be related to the presence of *Methanosarcina* sp., a genus of acetoclastic methanogens that convert acetate into CH₄. Similar results were reported by Ho et al. [36], who found that VFA accumulation in a stirred tank reactor was related to the absence of acetoclastic *Methanosarcina*. In this study, the decrease in gene copy number of the MSL order with increasing operation temperature was also in good agreement with VFA accumulation in the reactor. That is, the average acetate concentration was 3.6 mg/L at 55 °C when the 16S rRNA gene copy number of the MSL order was the highest. Conversely, the average acetate concentration increased to above 4.4 mg/L when the reactor was operated at 65 °C and 70 °C, at which the gene copy number of the MSL order substantially decreased (Fig. 8).

At 55 °C, there was a relatively high gene copy number for the MSL and MBT orders and the presence of dominant bands was associated with both acetoclastic (*Methanosaeta*) and hydrogenotrophic methanogens (*Methanobacterium* and *Methanothermobacter*). Regarding process performance, the rates of methane production at these operating temperatures were relatively high, and the VFA levels were low. Based on both the microbial community profile and process performance, the methanogenesis pathway at 55 °C was suggested to be a combination of acetoclastic and hydrogenotrophic methanogenesis. Both methanogenesis pathways have been reported to occur at temperatures between 55 °C and 60 °C. However, the methane production rate did not further increase with increasing OLR (in the range of 13.7 –

19.0 g/L d) despite the relatively high gene copy number of the MSL and MBT orders at this temperature. A possible reason is that the acetoclastic *Methanosaeta*, which are the dominant methanogens at this temperature, are vulnerable to the increased concentrations of SSs [37], oil and grease [38], and sulfides [39]. These substances have been reported to be potential microbial inhibitors during POME bioconversion [14].

At 60 °C, there was a decrease in both the gene copy number of the MSL and MBT orders, whereas the acetate concentration remained at a low level. The ratio of the gene copy number of the MBT order to that of the MSL order increased from 44 at 55 °C to 166 at 60 °C. The relatively low acetate concentration at 60 °C was possibly because of the synergy between syntrophic acetate oxidation and hydrogenotrophic methanogenesis [40], as shown in Equations (1) and (2):



A few studies have reported that when the temperature increased to > 50 °C, syntrophic acetate oxidation ($\Delta G^0 = +104.6$ kJ/mol) became energetically favorable, and the association of syntrophic acetate oxidation and hydrogenotrophic methanogenesis was the main methanogenesis pathway in several high-temperature anaerobic digestion systems [41-43].

At 65 °C, methane production decreased with increasing VFA levels, during which a substantial decrease in the gene copy number of the MSL set with low intensity *Methanosaeta* also occurred. Conversely, the gene copy number of the MBT set increased, and *Methanothermobacter* became dominant. This implies that methane was mainly generated via the hydrogenotrophic pathway. It has been reported in the literature that a temperature of 65 °C is suitable for hydrogenotrophic methanogenesis, but not for acetoclastic methanogenesis. For example, Ho et al. [36] found that the absence of *Methanosaeta* in a stirred tank reactor was attributed to both an increase in temperature from 55 °C to 65 °C and a short solid retention time of three days. In contrast, hydrogenotrophic *Methanothermobacter* became dominant at 60 – 65 °C, and the synergy between syntrophic acetate oxidation and hydrogenotrophic methanogenesis became dominant at > 60 °C. Furthermore, the exclusion of acetoclastic methanogenesis led to VFA accumulation. Thang et al. [44] found that methane formation via the acetoclastic pathway was suppressed at > 65 °C, at which methane generation via the hydrogenotrophic methanogenesis pathway became dominant. In addition, the populations of *Methanothermobacter* and *Methanobacterium* were stable at temperatures between 55 °C and 65 °C [45].

At 70 °C, the DGGE band of *Methanosaeta* faded, and that of *Methanothermobacter* became dominant. These results were consistent with data reported in [21, 46-47], indicating that hydrogenotrophic methanogens outcompete acetoclastic methanogens and homoacetogens under extreme thermophilic conditions.

Conclusions

During the CSTR treatment of POME, the methane content in the biogas and the methane production rates were the highest at 60 °C at, on average, approximately 73% and 4.4 L/L d at an OLR of 25.7 g/L d, respectively. The results from both the DGGE and real-time PCR analyses suggested that hydrogenotrophic methanogens, especially the genus *Methanobacterium* in the MBT order, were responsible for the increased methane production at the operating temperatures of 60 °C and 65 °C. The thermophilic AD of POME at 60 °C was found to be promising because the SS reduction efficiencies of the CSTR operated at 60 °C and OLRs below 19 g/L d were not significantly different from those at 55 °C, while the methane content in the biogas and the methane production rates were optimal, with an average methane content of approximately 73%. The high methane production rates at 65 °C were mainly due to hydrogenotrophic methanogenesis. In particular, the gene copy number of the MBT set was the highest at 65 °C. However, the efficiencies of the hydrolysis reactions at 65 °C were greatly reduced, as evidenced by the decreased bacterial diversity and gene copy number of the BAC-set and low SS removal efficiency. In future research, the thermophilic AD of POME at 60 °C should be investigated in long-term experiments, and certain process performance parameters, such as the removal efficiencies of COD, biological oxygen demand, oil and grease removal efficiencies, and any potential microbial inhibition should be investigated. In addition, thermophilic AD at 65 °C should be examined for treating decanted POME, in which most free fat, oil, grease, and SSs have been removed.

Declarations

We wish to confirm that there are no known conflicts of interest associated with this manuscript and there has no significant financial support for this work that could have influenced its outcome.

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Figures

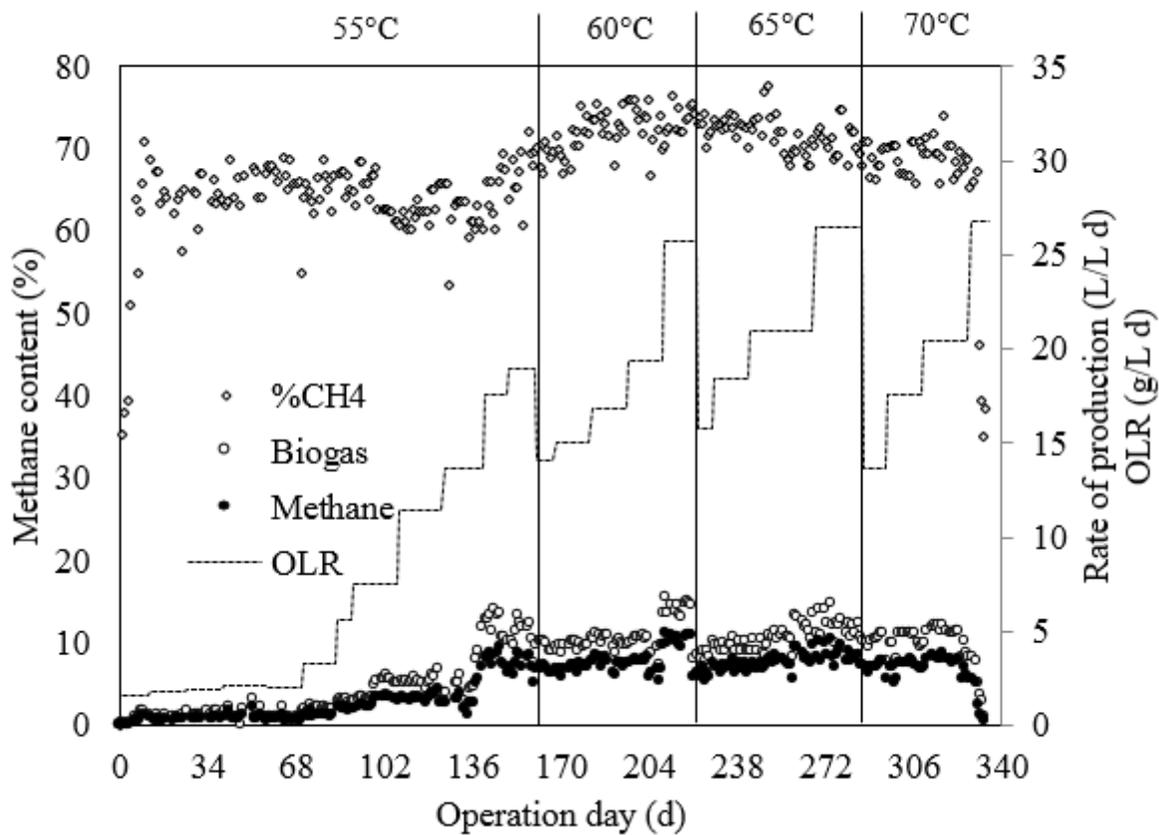


Figure 1

OLR and rates of biogas and methane productions and methane content in biogas from the CSTR operated under thermophilic temperatures between 55 to 70 °C.

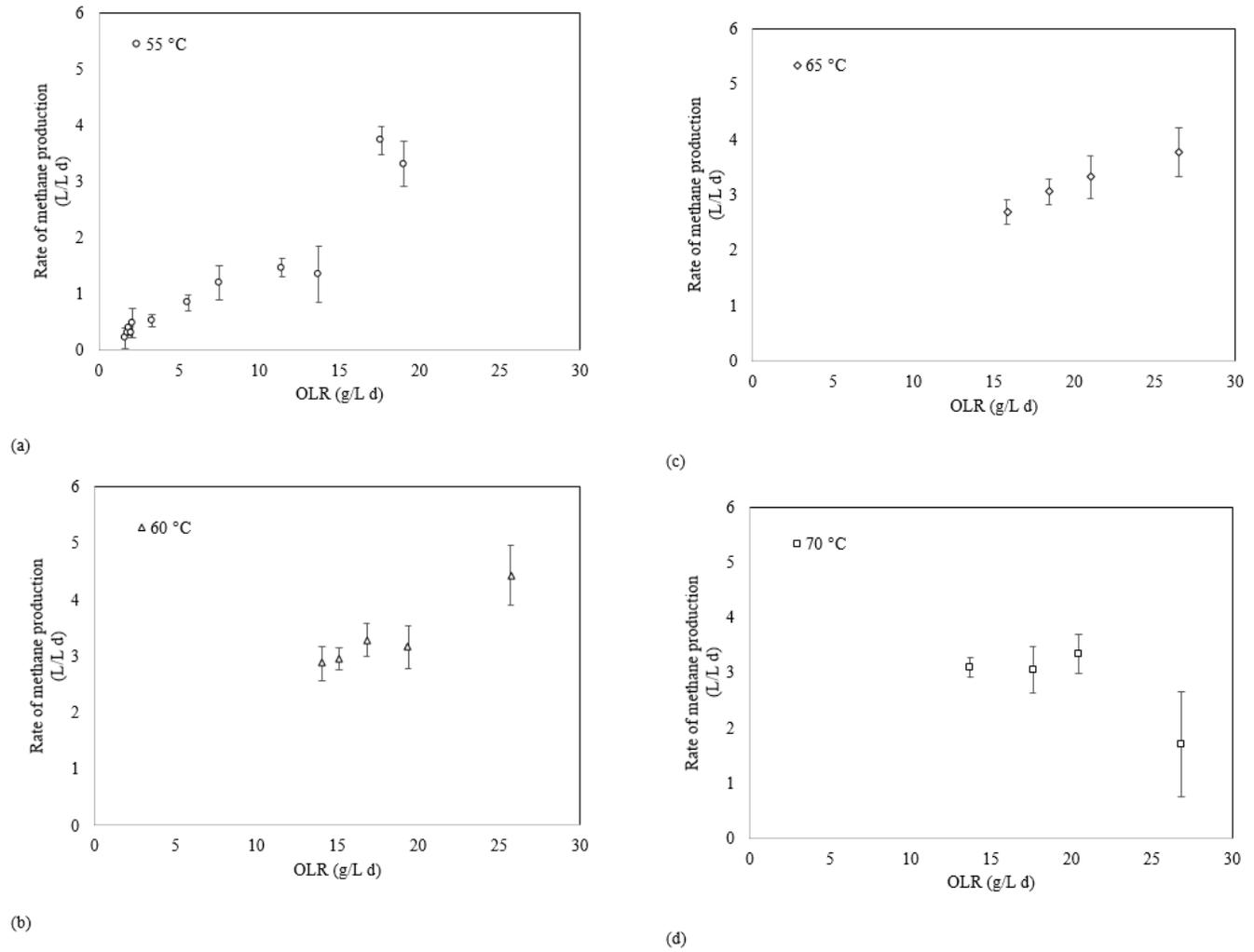


Figure 2

Rates of methane production as a function of OLRs: (a) at 55 °C; (b) 60 °C; (c) 65 °C and (d) 70 °C.

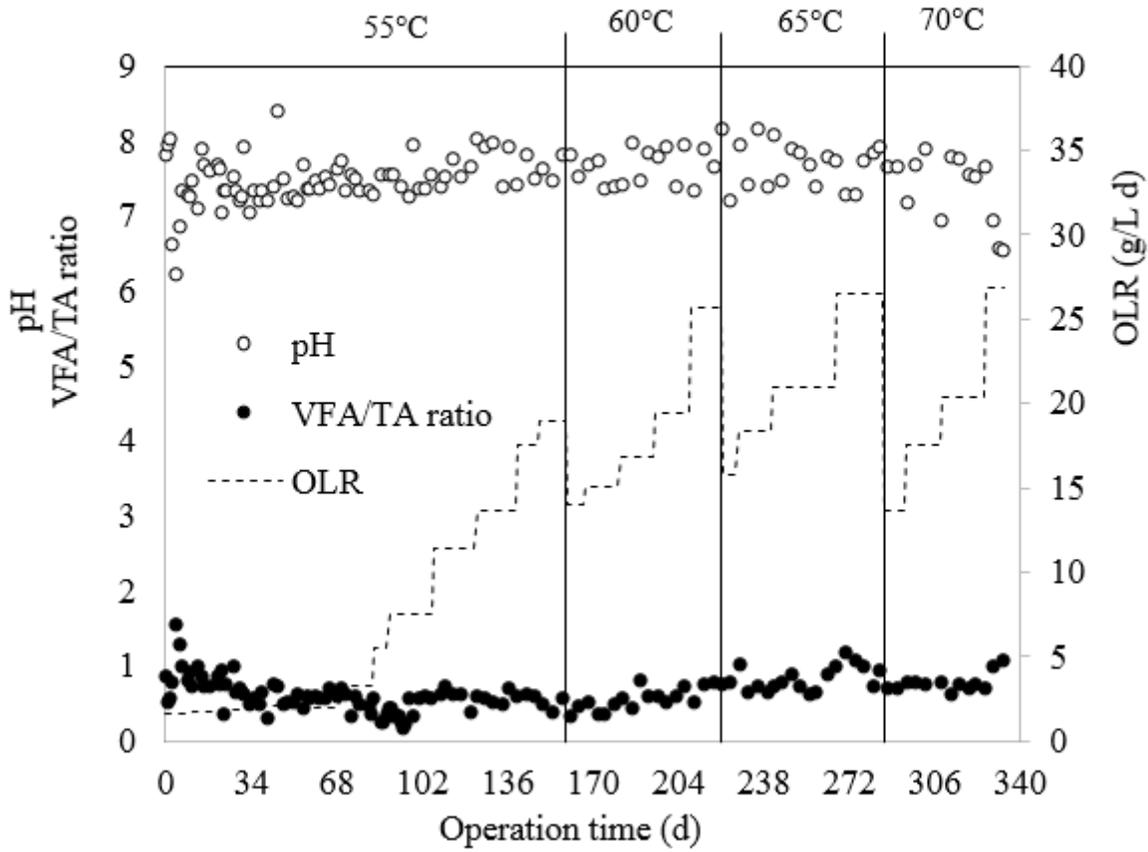


Figure 3

pH and VFA/TA ratio in the effluent from the CSTR operated under thermophilic temperatures between 55 to 70 °C.

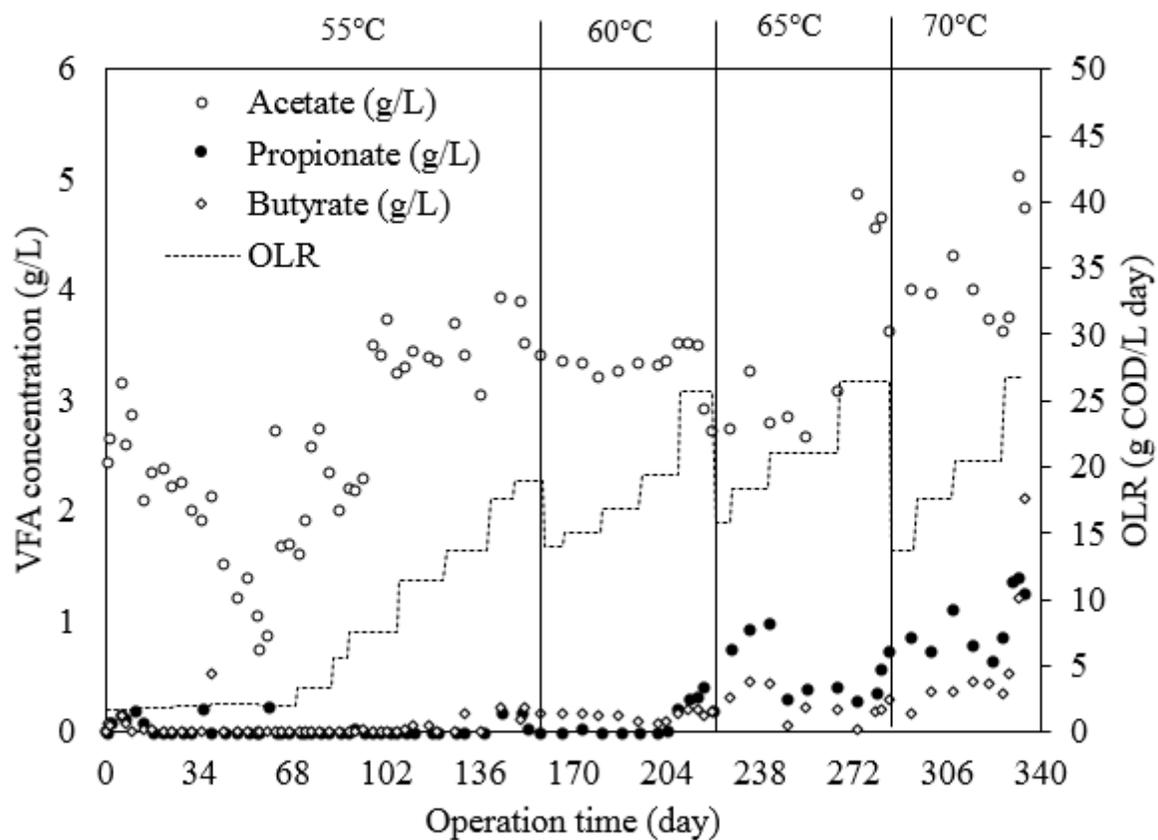
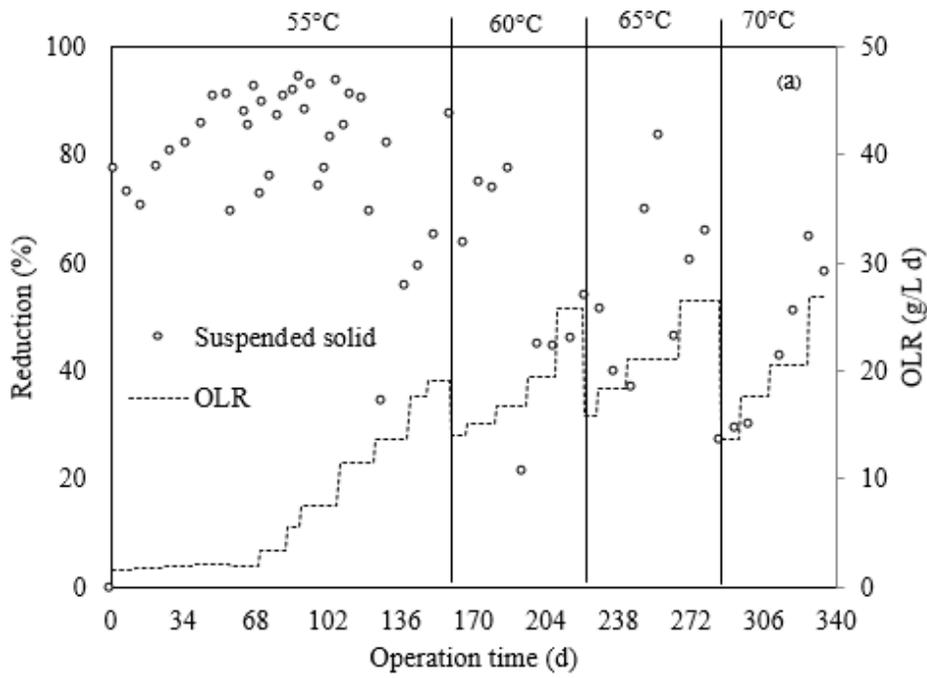
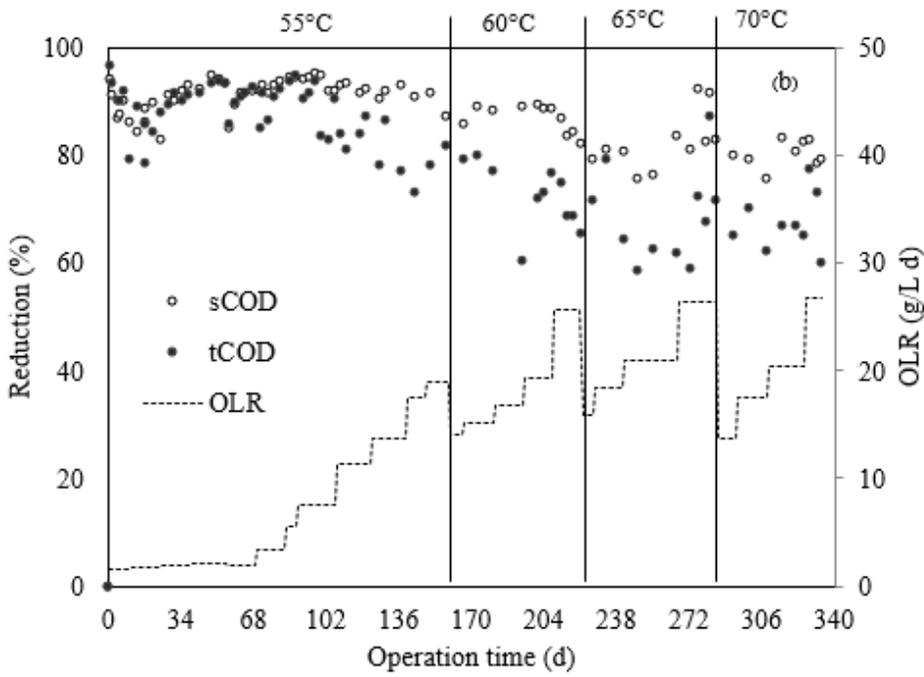


Figure 4

VFA concentrations in the effluent from the CSTR operated under thermophilic temperatures between 55 to 70 °C.



(a)



(b)

Figure 5

Removal efficiency of the CSTR (a) suspended solids and (b) tCOD and sCOD.

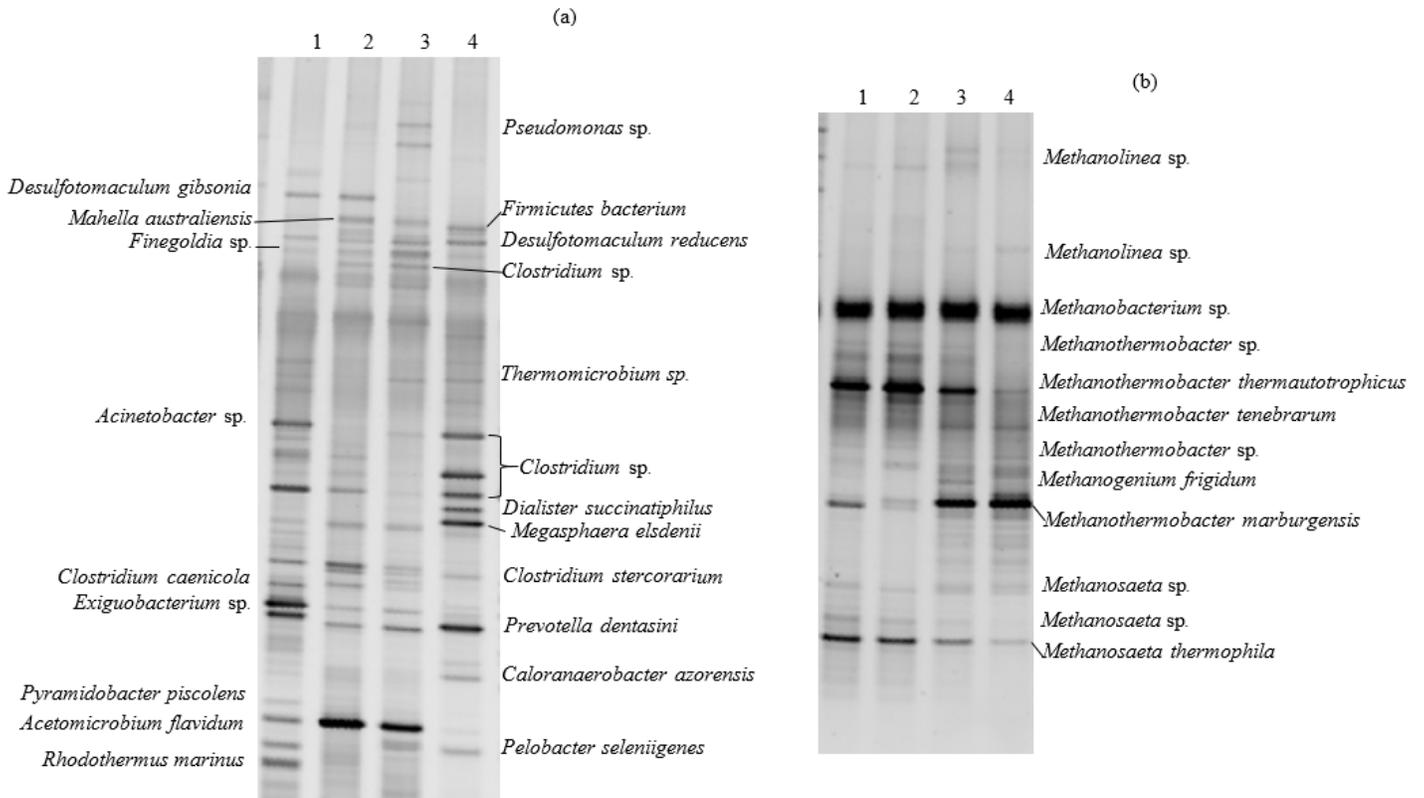


Figure 6

DGGE profiles of microbial community: (a) bacteria domain; (b) archaea domain (1st lane 55 °C; 2nd lane 60 °C; 3rd lane 65 °C; 4th lane 70 °C).

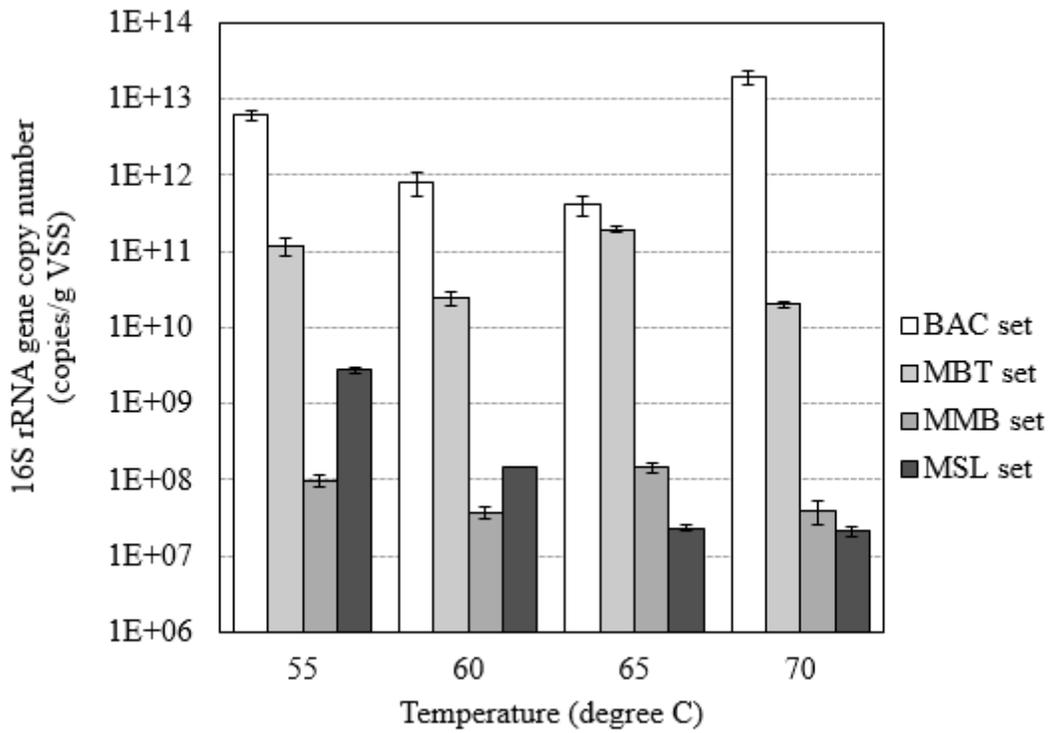


Figure 7

Number of gene copies of the representative bacteria domain and methanogen orders in the acclimated sludge collected from the CSTR at different operation temperatures.

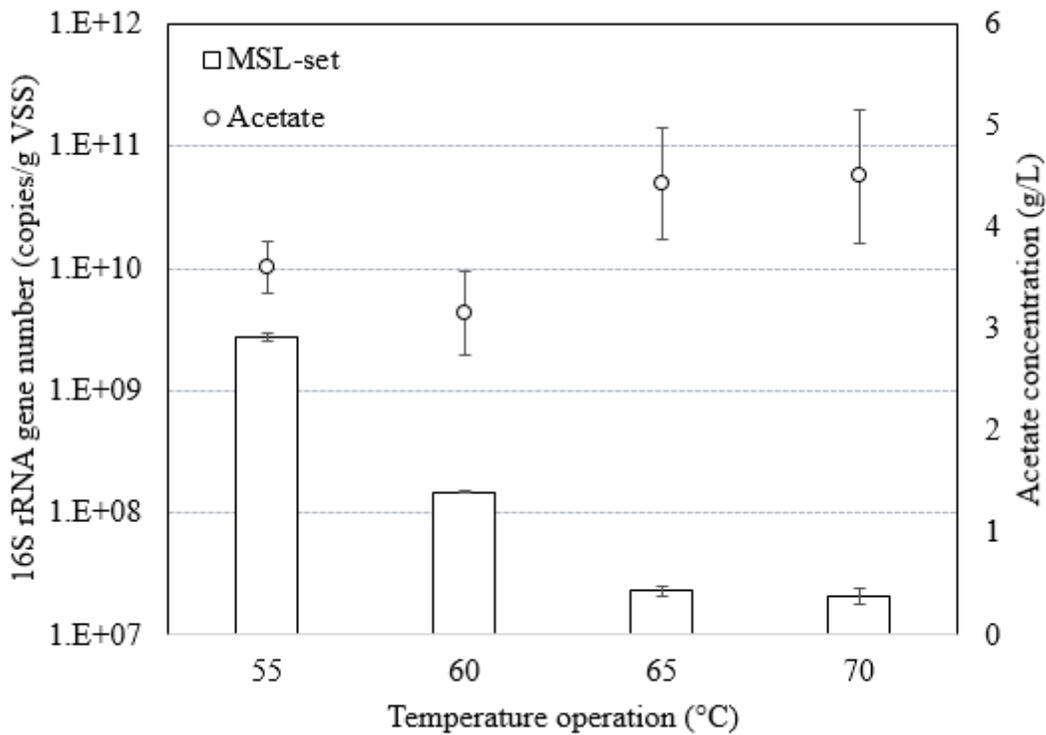


Figure 8

Gene concentrations associated with the MSL-set in the sludges and the average concentration of VFA in the CSTR during the operation at 55 °C, 60 °C, 65 °C and 70 °C.

Supplementary Files

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