Inhibitory effect of long-chain fatty acids on biogas production and the protective effect of reverse membrane bioreactors (RMBR)

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Abstract

Anaerobic digestion of lipid-containing wastes for biogas production is often hampered by the inhibitory effect of long chain fatty acids (LCFAs). In this study, the inhibitory effects of LCFAs (palmitic, stearic, and oleic acid) on biogas production as well as the protective effect of a reverse membrane bioreactor (RMBR) against LCFAs was examined in thermophilic batch digesters. The results showed that palmitic and oleic acid with concentrations of 3.0 and 4.5 g/L resulted in >50% inhibition on the biogas production, while stearic acid had an even stronger inhibitory effect. The encased cells in the RMBR system were able to perform better in the presence of LCFAs. This system exhibited a significantly lower percentage of inhibition than the free cell system, not reaching over 50% at any LCFA concentration tested.

Keywords: Anaerobic digestion, biogas, membrane bioreactor, LCFAs, inhibitor

1. Introduction

Prolog biogas dan waste-sedikit saja

Biogas or bio-methane is a renewable energy source that can be directly used as a car fuel, for heating, or indirectly used to generate electricity [1]. Biogas production through anaerobic digestion is a complex process, which can be divided into four phases: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Each step is carried out by different consortia of microorganisms, partly standing in syntrophic interrelation with each other [2]. Biogas can be produced from various kinds of waste materials, including municipal solid waste (MSW), industrial waste, agricultural waste, and waste by-products. Among these wastes, lipid-rich wastes, which are released from e.g., dairy products industry, slaughterhouses, edible oil processing industry, olive oil mills, and wool scouring facilities are produced in high amounts each year [3,4,5,6]. If the wastes are left untreated, they can have severe negative impacts on the environment.

Commented [B1]: Comprising mainly methane, biogas is a ...

Tentantang lipida

Among the nutritional compounds present in the lipid-rich wastes, lipids are the main constituents and play the most significant role in anaerobic digestion for biogas production due to their high energy content [7]. Lipids are long-chain fatty acids (LCFA) bonded to glycerol, alcohols, or other groups by an ester or ether linkage. During the first stage of anaerobic digestion, lipids are rapidly hydrolyzed into monomers such as glycerol and LCFAs [7]. LCFAs are, in turn, degraded into short organic acids via β-oxidation. The short organic acids are subsequently converted into acetate and hydrogen. The acetate and hydrogen are finally converted into methane and

carbon dioxide [1]. Theoretically, lipid degradation in anaerobic digestion would yield high biogas quality, meaning that the biogas should have a high methane content, compared to production from carbohydrates and proteins [7]. However, utilization of lipid-rich waste as a main substrate for biogas production is challenging.

Challenge caused by lipid

The anaerobic digestion of fat rich wastes may be subjected to inhibition caused by excessive organic loading or LCFAs [8,9]. It has been reported that LCFAs inhibit several reactions during the anaerobic degradation process [10,11]. LCFAs also have severe inhibitory effects on the microorganisms in anaerobic digestion. In particular, it has been shown that both methanogens and acetogens, the two main groups involved in LCFA degradation for methane production, can also become severely inhibited by the LCFAs [12]. The adsorption of LCFAs onto the microbial cell wall or the membrane causes damage in the microorganism's transport and protective functions. The inhibitory effects of LCFA are already visible at concentrations as low as 50 mg/L [12].

Another challenge in the anaerobic digestion process of lipid-containing wastes is that the cells are easily washed out from the digester at high organic loading rates. This can happen since the methanogens in the biogas production process require a long retention time in the digesters, but their growth rate is very low and the microorganisms are also very sensitive to the harsh process conditions [13]. Sousa et al. [14] reported that methanogens in the anaerobic digestion of lipid containing wastes are often considered to be highly sensitive to the LCFAs derived from the lipid hydrolysis.

Tentang MBR-sedikit lebih ringkas (1 paragraph)

For these reasons, the population size of the microorganisms is easily reduced, resulting in a decreased methane production [13,15). Moreover, anaerobic digestion processes with a low cell density need long start-up periods, and larger digesters are required for appropriate function, meaning that the capital cost is high. To achieve high process efficiency in converting lipid-rich wastes into biogas using microorganisms in an anaerobic digestion process, protecting and retaining the microbial cells inside a compact reactor might be a solution to overcome these problems.

As a solution to the above-mentioned issues, a ~~reverse~~ membrane bioreactor (RMBR) has been developed. This system employs a technique that uses membranes to enclose the microbial cells, followed by immersion into a bioreactor without the risk of a clogged outlet. It has been reported that using the microorganisms encased in a semi-permeable polyvinylidene fluoride (PVDF) membrane, the biogas production could be improved [16,17,18]. The PVDF membrane was able to retain the cells in the reactor and allowed the liquid substrate as well as the gaseous product to pass through the membrane layer. This method provided a better system for preventing the wash out of the cells in semi-continuous digestion processes at high organic loading rate [17,19]. Also, it has been tested with substrates containing inhibitors such as fruit flavor compounds, showing a protective effect on the encased microorganisms. However, applying this technique to reduce the inhibitory effect of LCFAs on microbial cells for biogas production has not yet been investigated.

The aim of this work was to investigate the inhibitory effect of LCFAs on free microbial cells for biogas production under thermophilic anaerobic batch digestion. Saturated (palmitic acid - $C_{16:0}$ and stearic acid - $C_{18:0}$) and unsaturated (oleic acid - $C_{18:1}$) LCFAs were used as model LCFA inhibitors. The protective performance of the RMBR system,

cells encased in membranes, against the toxicity of the LCFAs, under identical condition as used for the free cells, was also examined.

2. Materials and Methods

2.1 Anaerobic culture preparation

An anaerobic culture was obtained from a 3,000 m^3 municipal solid waste digester operating at thermophilic (55°C) conditions (Borås Energy and Environment AB, Sweden). The culture was acclimated in an incubator at 55°C for 3 days prior to use. The acclimated sludge was homogenized and filtered through a sieve with a pore size of 1.0 mm in order to remove any remaining large particles. The sludge was thereafter centrifuged (Carl Padberg 77933 LE, Huber and Moser, Germany) at 30,000 rpm for 15 minutes to separate the supernatant and suspended sludge. The suspended sludge was later used as an inoculum for cell containment in membrane sachets, or as free cells.

2.2. Synthetic medium, membrane and inhibitors

The synthetic medium used for this experiment was prepared as previously described [20]. It contained D-glucose, yeast extract, and nutrient broth with a concentration of 20 g/L in distilled water. The solution was homogenized and filtered using 0.2 µm membrane filters. Flat plain PVDF (polyvinylidene fluoride, Durapore®) membranes were obtained from Thermo Fisher Scientific Inc. (Sweden) and used for cell encasement. PVDF membranes have a hydrophilic surface, with pore size and thickness of 0.1 µm and 125 µm, respectively.

Palmitic, stearic, and oleic acid were used as model LCFA inhibitors and were purchased from Sigma Aldrich (Sweden). These inhibitors were first dissolved in 2.5

mL methanol (reagent grade) in order to obtain a homogeneous solution in the reactors; thereafter, it was added to the reactors for investigation in four concentrations of 0, 1.5, 3.0, and 4.5 g/L.

2.3. Membrane sachet preparation and cell containment procedure

A cell containment technique was conducted following the method described in a previous study [17]. The PVDF membranes were cut into rectangular shapes 6 x 6 cm and folded to create membrane pockets of 3 x 6 cm. The pockets were heat-sealed (HPL 450 AS, Hawo, Germany) on two sides with heating and cooling times of 4.0 and 3.5 s, leaving one side open for insertion of the inoculums. Solid inoculums (3 g), prepared as described above, were carefully injected into the synthetic membrane pockets. The remaining open side of the sachet was sealed and the inoculum inside was carefully spread out. The inoculum containing sachets were immediately used for biogas production.

2.4. Anaerobic batch digestion process set up

In order to examine the performance of the encased cells in the presence of LCFAs, batch digestion processes were set up. The use of free inoculum and inoculum encased in membrane, as well as different concentrations of the LCFAs, were used as independent variables.

The experiment was conducted using 100 mL serum glass bottles with total working volume of 43.5 mL. Each reactor contained 1 mL of synthetic medium, 1 membrane sachet containing 3 g of inoculum, 2.5 mL methanol, inhibitor solution with the concentrations 0 (control), 1.5, 3.0, and 4.5 g/L and 40 mL distilled water. The reactors were closed with 20 mm aluminum crimp caps with inserted gray PTFE/butyl rubber

septa. The headspace of each bottle was flushed with 80% N_2 and 20% CO_2 gas mix to obtain anaerobic conditions and help to keep the pH neutral at the beginning of the process. The anaerobic digestion process was carried out under thermophilic conditions at 55°C ± 1°C in an incubator until the gas production was constant at a low level, indicating that most of the substrate had been converted. During the biogas production process, the digesters were manually shaken daily. The reactors of the free cells were performed in parallel, with otherwise identical conditions.

2.5 Analytical methods

Methane was quantified regularly using a Varian 450 gas chromatograph with a capillary column equipped with a thermal conductivity detector (TCD). N_2 was used as a carrier gas, and the instrument was operated with injector and oven temperature at 75°C and 50°C, nitrogen column flow 2.0 mL/min and detector temperature at 250°C. A 0.25 mL pressure lock syringe (VICI, U.S.A.) was used for the gas sampling.

The percentage of inhibition from each treatment was used as an indicator of the inhibitory effects caused by the LCFA in this work. It was calculated according to equation 1.

$$\text{Inhibition (\%)} = (x - y) / x \times 100 \quad (1)$$

Where, x : Methane production rate from control reactor

y : Methane production rate from samples

Volatile fatty acids (VFAs) were analyzed using a Waters® High Performance Liquid Chromatography (HPLC) system with a BIORAD Aminex® HPX-87H, 300mm x 7.8 mm column and 5 mM of sulphuric acid as mobile phase. It was operated at 0.6 mL/min

isocratic mobile phase flow; the column temperature was set at 50°C and the VFAs were detected using a UV detector at a wavelength of 210 nm. The experiment was performed in triplicate, and the results were presented as mean ± standard deviation.

3. Results and Discussion

LCFAs have an inhibitory effect on the anaerobic digestion processes. This has been attributed to permanent toxicity resulting from cell damage and is known to affect both syntrophic acetogens and methanogens [21]. Moreover, wash out of the microbial population commonly occurs during the continuous processes at a high organic loading rate. The resulting low density of the microorganisms during anaerobic biodegradation of lipid rich wastes leads to an unstable process and low biogas productivity. Thus, the experiments in this work were designed in order to investigate the inhibitory effects of the LCFAs on biogas production and utilization of the RMBR systems, cells encased in membranes, as means of cell protection and cell retention.

3.1. Inhibitory effects of LCFAs on biogas production

It has been demonstrated that the LCFAs are inhibitory to microbes in the anaerobic digestion systems, leading to low biogas productivity. Shin et al. [22] reported that in anaerobic biodegradation, the inhibitory effects of major long-chain fatty acids (LCFA), which have 16 or 18 carbons, did not only have an effect on the acetate degradation, but also on the propionate degradation and β-oxidation. However, the LCFA concentration that leads to inhibition depends on many factors, e.g., the type and concentration of the LCFAs present. Palmitic and stearic acids are the principal saturated LCFAs to be accumulated. They are known to be degraded five times slower than the unsaturated acids [23]. However, among the various kinds of LCFAs, oleic acid has the highest

toxicity level, with a minimum inhibitory concentration (MIC) of 50-75 mg/L under mesophilic conditions [22,24,25,26]. In this experiment, the effects of the different types of LCFAs at different concentrations on thermophilic anaerobic digestion for biogas production were investigated.

The inhibitory effects of palmitic acid at different concentrations on the cumulative methane production of free cells are shown in Fig. 1a. The results show that the methane production increased sharply in all the reactors during the first 6 days of incubation. After six days, the methane production continued at a lower rate, indicating that the easy to digest material was consumed. The methane yield at the beginning of the digestion in the medium containing 1.5 g/L of palmitic acid was not different from the control without an inhibitor (0.75 and 0.75 m^3/kgVS, respectively). However, from the 6th day of incubation until the last day, a lower methane yield than in the control was measured. At the end of the digestion, the accumulated methane yield of the control and the reactor containing 1.5 g/L of palmitic acid were 1.4 and 1.1 m^3/kgVS, respectively.

In medium containing 3.0 g/L of palmitic acid, the free microbial cells exhibited a methane yield of 0.6 m^3/kgVS, which was lower than the control and 1.5 g/L of palmitic acid, but still higher than the blank. This shows that the cells were still active, albeit at a lower level due to the inhibition by the LCFA. An even higher palmitic acid concentration of 4.5 g/L was also tested. However, it seems that the maximum inhibitory effect of palmitic acid on biogas production was reached at 3.0 g/L of palmitic acid. At the end of the experiment, the methane yield with 4.5 g/L palmitic acid was 0.6 m^3/kgVS, similar to that at 3.0 g/L.

It has been reported that the addition of palmitic acid at a concentration of >1.1 g/L inhibited the performance of anaerobic digestion by about 50% under mesophilic conditions [27]. This experiment confirms that palmitic acid also has an inhibitory effect on the thermophilic anaerobic digestion process, and that palmitic acid at a concentration of ≥1.5 g/L negatively influenced the performance of the microorganisms in the digestion system. Increasing the palmitic acid concentration added to the medium led to a further decreased methane yield. However, the highest concentration of palmitic acid tested, 4.5 g/L, did not increase the negative effect on the microorganisms more than the second highest concentration.

Stearic acid ($C_{18:0}$) plays an important role as an inhibitor of biogas production from lipid-rich wastes. To investigate the concentration dependence on its inhibition of the biogas production, stearic acid was added at different concentrations to the reactors with the free cells.

The cumulative methane yield produced from the conventional system of free microbial cells containing stearic acid is shown in Fig. 1b. Initially, methane was produced in all of the batches, showing that the free microbial cells were able to perform at all concentrations of stearic acid. The results also showed that with increasing concentration of stearic acid, the methane yield was reduced. On the last day of the digestion, the methane yield of the control and the media containing stearic acid at 1.5, 3.0, and 4.5 g/L were 3.4, 1.5, 1.2, and 0.9 m^3/kgVS, respectively.

In the current experiment, the stearic acid at a concentration of 1.5, 3.0, and 4.5 g/L inhibited more than 50 percent of the anaerobic digestion performance under thermophilic conditions (Fig. 2b). It has previously been reported that stearic acid at a

concentration of 1.5 g/L could inhibit 50 percent of the anaerobic performance under mesophilic conditions [27]. The results show clearly that free microbial cells under thermophilic anaerobic digestion conditions were at least as inhibited by the addition of stearic acid, as were cells under mesophilic conditions.

Oleic acid ($C_{18:1}$) is one of the most common LCFAs [28]. Oleic acid has a large impact on biogas production from fat-containing wastes due to its strong inhibitory effect on acetate degradation in the anaerobic digestion process. Thus, this experiment was designed to investigate the inhibitory effects of oleic acid on thermophilic anaerobic digestion for biogas production.

Fig. 1c presents the cumulative methane yield produced from the conventional system of free cells containing oleic acid. The results show that methane was produced in all of the reactors. However, the addition of oleic acid to the anaerobic digesters resulted in a lower methane yield compared to the control. The free cells were already strongly inhibited by the addition of oleic acid at the lowest concentration of 1.5 g/L. Increasing the concentration of oleic acid to 3.0 and 4.5 g/L resulted in an even lower methane yield. The methane yields produced in the reactor with the free cells containing oleic acid with the concentrations of 0, 1.5, 3.0, and 4.5 g/L were 6.7, 3.5, 1.8, and 2.0 m^3/kgVS, respectively. It has been reported that oleic acid at a concentration of 0.05 – 0.07 g/L could inhibit the digestion performance by about 50 percent under mesophilic conditions [25]. The results from the current experiment also showed that oleic acid at a higher concentration had a strong inhibitory effect on the thermophilic anaerobic microorganisms for biogas production.

3.2 Performance of the reverse membrane bioreactor (RMBR) with encased cells in the presence of inhibitory LCFAs for biogas production

The RMBR has been intensively studied in both batch and continuous digestion processes in order to improve the process efficiency and biogas productivity under harsh anaerobic process conditions ([16,17; 18,19]. The system has showed the ability to retain a high cell density, prevent wash out of microbial cells, as well as protect the anaerobic microbes from toxic media, resulting in high biogas productivity in stable anaerobic processes.

The previous section showed the strong inhibition of LCFAs on methane production under thermophilic digestion. In this work, the encased cells in the RMBR system were studied in batch digestion processes, in order to preliminarily investigate the application of this system as an aid against the inhibitory effects of LCFAs (palmitic, stearic, and oleic acid). A conventional reactor with the free cells was used as a control system, run under the same conditions. The factors used for comparisons between the RMBR system and that with the free cells were the percentage of inhibition, the accumulation of VFAs and pH.

Palmitic, stearic, and oleic acid had a strong inhibitory effect on the free cells under thermophilic anaerobic conditions as shown in section 3.1, resulting in low methane yields when the concentration of the acid was increased. We hypothesized that the RMBR system could be applied as a solution to decrease the inhibition effect of these LCFAs on the anaerobic biodegradable process for biogas production. Under identical conditions with the free cell reactors in this work, the protective effect of the RMBR,

with microbial cells encased in the PVDF membranes for biogas production were tested in synthetic media containing LCFAs at different concentrations.

Palmitic acid was the main LCFA that inhibited the activity of the microbial cells by accumulation in the anaerobic sludge during the anaerobic digestion [27]. The accumulated methane production in the RMBR with the encased cells is shown in Fig. 2a. The results showed that methane production from the RMBR containing 1.5 and 3.0 g/L were not significantly different from the control without an inhibitor. It means that encased microorganisms were not affected by the palmitic acid at concentrations of 1.5 and 3.0 g/L. However, adding a higher palmitic acid concentration of 4.5 g/L resulted in a lower methane yield.

Fig. 3a presents the percentage of inhibition calculated from the reactors with the encased and the free cells containing palmitic acid. The results show clearly that increasing the concentration of the palmitic acid in the RMBR resulted in a lower methane production (higher inhibition). However, the percentage of inhibition was found to be lower in the RMBR compared to in the reactor with the free cells at all concentrations of palmitic acid. The percentage of inhibition in the RMBR was 1.4, 5.0, and 42.3 % at concentrations of 1.5, 3.0, and 4.5 g/L, respectively, whereas the percentage of inhibition in the reactors with the free cells containing palmitic acid at concentrations of 1.5, 3.0, and 4.5 g/L were 26.1%, 70.2%, and 73.9%, respectively (Fig. 3a). This shows that the cells in the RMBR system were significantly less affected by the presence of palmitic acid in the medium as compared to the conventional system with the free cells.

As shown in section 3.1, stearic acid, a saturated LCFA, had a strong inhibitory effect on the free cells under thermophilic anaerobic conditions, resulting in low methane yields when the concentration of the acid was increased. Fig. 2(b) presents the methane production from the medium containing stearic acid by the encased cells. The methane yield in the RMBR containing stearic acid of 1.5 g/L was not significantly different from the control. It was 1.70 and 2.1 m^3/kgVS, respectively. This showed that encased cells could tolerate stearic acid with a concentration of 1.5 g/L, while the free cells failed under the same condition (Fig. 1b). Increasing the stearic acid concentration to 3.0 and 4.5 g/L exhibited a lower methane yield than the control.

In addition, the protective effect of the RMBR against the stearic acid compared to the reactor with the free cells can also be observed in Fig. 3(b). The percentage of inhibition in the bioreactor with the free cells at concentrations of 1.5, 3.0, and 4.5 g/L stearic acid were 54.8, 63.6, and 69.0%, respectively. The cells in the RMBR system, with otherwise the same conditions as the free cells, exhibited a significantly lower percentage of inhibition than the free cell system (9.1, 30.0, and 38.2 %, respectively). This showed clearly that the encased cells in the RMBR experienced less inhibition from the stearic acid compared to the free cells during the thermophilic anaerobic digestion processes.

In the systems containing the oleic acid, the results showed that increasing the concentration added to the reactors with the free cells did indeed lead to a stronger inhibition (Fig. 1c). In this section, the results presented that methane produced from the medium containing 1.5 g/L of oleic acid in the RMBR was not significantly different from the control. However, the higher oleic acid concentrations of 3.0 and 4.5 g/L exhibited a lower methane yield. The methane yield produced from the RMBR

containing the oleic acid with the concentrations of 0, 1.5, 3.0, and 4.5 g/L were 2.7, 2.2, 1.8, and 1.8 m3/kgVS, respectively.

When the performance of the RMBR was presented in the percentage of inhibition compared to the conventional system, the results showed that the percentage of inhibition in the RMBRs containing the oleic acid was less than 50% at all concentrations of the LCFA. The free microbial cells in the conventional system were more severely affected by the oleic acid already at a concentration of 1.5 g/L, and the inhibition was more than 50% when the oleic acid concentrations were increased to 3.0 and 4.5 g/L. In addition, it was observed that there was no significant difference in the inhibition by the oleic acid present at 3.0 or 4.5 g/L (Fig. 3c). Sousa et al. [14] reported that oleic acid had more severe effects on the methanogens than the saturated LCFAs. Furthermore, Shin et al. (2003) reported that unsaturated oleic acid was more inhibitory than the saturated stearate and palmitate on the acetate degradation.

In an anaerobic treatment, lipids are first hydrolyzed to glycerol and free LCFAs by the acidogenic bacteria. Glycerol is further converted into acetate by acidogenesis, while the LCFAs are converted into hydrogen, acetate, and/or propionate through the β-oxidation pathway (syntrophic acetogenesis) [29]. During the last stage of methanogenesis, the products of the previous stage are further degraded to principally carbon dioxide and methane. Under ideal operating conditions, the acid production and gas production are in balance, with the volatile acids being broken down as quickly as they are produced [30]. Thus, VFAs and pH have been widely used as fast indicators of unstable anaerobic digestion processes. In the experiments in the current study, the VFAs and pH were analyzed and used as indicators of a balanced system in order to

investigate the performance of the encased cells in the RMBR system in comparison to the free cells, for the degradation of the LCFA containing wastes.

Tables 1 and 2 show the total VFA concentrations and pH on the last day of digestion in both the systems with the palmitic acid. The results show that the total VFA concentration and the pH in both systems were not different from the control. The VFA concentrations in the RMBR systems with the encased cells were in the range of 2.4 – 2.8 g/L, while the reactors with the free cells were in the range of 2.1 – 3.2 g/L. However, for the highest concentration of the palmitic acid, a lower VFAs concentration was observed in the RMBR with the encased cells compared to the reactor with the free cells.

In the reactors with the stearic acid, the results show that increasing the stearic acid concentration added to both the systems led to an accumulation of the total VFAs, especially at concentrations of 3.0 and 4.5 g/L in both the systems, where the VFA concentrations were significantly different from the control. The results also revealed that the digestion of the media containing the stearic acid by the encased cells in the RMBR led to a lower VFA accumulation than in the reactor with the free cells. Thus, the VFAs could be converted into biogas by the encased methanogens.

It has been reported that the accumulation of VFAs above 4 g/L in the digester leads to an imbalance of the anaerobic digestion process [10,13]. In this experiment, with the addition of stearic acid at concentrations of 3.0 and 4.5 g/L the reactor with the free cells resulted in an accumulation of VFA compounds to 4.4 and 5.4 g/L, respectively. In the RMBR reactor, on the other hand, the VFA concentrations of 2.6 and 2.4 g/L were measured at the same concentrations of stearic acid. The pH (Table 2) in both the

systems with the stearic acid decreased during the incubation period, following the concentration of VFAs (Table 1). Higher pH values were found in the RMBR system compared to the free cell system. The addition of stearic acid at different concentrations to the bioreactor with the free cell led to decreases in the pH from 5.97 in the control to 4.81 in the reactors with 4.5 g/L stearic acid. The pH in the reactors with the encased cells, however, decreased only from 6.23 in the control to 5.79 in the reactor with the highest concentration of stearic acid. Based on the above results, it is apparent that the RMBR was able to balance the inhibitory system, with a lower risk of an unstable digestion process as compared to the conventional system with the free cells.

The VFAs concentration and pH at the end of the digestion process in the reactors containing oleic acid are presented in Tables 1 and 2. Increasing the concentration of the oleic acid added to both the systems led to an increase in the total VFA concentration. As for the other LCFAs investigated, and probably for the same reasons, the VFA accumulation was higher in the conventional system with the free cells compared to the RMBR system with the encased cells. VFA concentrations of > 4 g/L in the reactors with the free cells were found in the reactor with a concentration of 1.5 g/L and with increasing concentrations until the highest oleic acid concentration of 4.5 g/L. In contrast, VFA concentrations of > 4 g/L in the RMBR reactors were observed only in the reactors containing oleic acid at concentrations of 3.0 and 4.5 g/L. However, the pH values measured at the end of the incubations in both the systems were not different. These results indicated that the RMBR system with the encased cells was superior to the conventional system with the free cells when it came to performing under the inhibitory conditions created by the presence of the oleic acid under thermophilic anaerobic biodegradation.

In this study, the microbial cells encased in the PVDF membranes displayed less inhibition with palmitic, stearic, and oleic acid compared to the free cells. In the conventional system, the free microbial cells probably had a more direct contact with the inhibitors leading to an adsorption of the inhibitors onto the cell membrane. Gerardi [13] reported that the cell walls of methanogens lacking protective envelopes resulted in inhibitor sensitive cells. This can cause damage to the cells and lead to an unstable digestion process with a low biogas production [7]. At the end of the digestion, the higher VFA concentration in the reactor with the free cells can also be due to the fast degradation by the free cells, being readily exposed to the substrates, including the LCFAs. This resulted in high VFA concentrations in the end, since the more sensitive methanogens could not convert the VFAs as fast as they were produced by the less sensitive acid-forming bacteria. High VFA concentrations in the reactors can inhibit the activity of the methane-forming microorganisms leading to unstable digestion processes [5,10,22].

The encased cells in the RMBRs, on the other hand, were protected by the polymeric membranes enclosing the microorganisms. The membrane could likely limit the diffusion of the inhibitors to the cells. Thereby, the microorganisms had a longer time to detoxify the medium by utilizing the LCFAs and VFAs for biogas production and maintaining them at a low concentration close to the cells. At the end of the digestion, the lower VFAs concentration allowed the encased cells to perform efficiently without any negative effect from the high concentrations of VFAs; thus, a stable digestion process could be maintained. In addition, the method of retaining the microbial cells in the membranes provides a high cell density, meaning that the cell to LCFA ratio is high, thus, enabling a better acclimatization and detoxification. In reports by [24,31], an

anaerobic fixed-bed reactor was used to prevent cell washout. It was shown that the retention of the cells improved the tolerance of the system in the presence of high concentrations of LCFAs in the wastewater.

It is also possible that the inhibitors could not pass through the cell pellet inside the pouches of the RMBR easily, meaning that only a portion of the cells were affected by the adsorption of the inhibitors onto the cell membrane. Protection by the outer layer of the cells in a dense cell pellet has previously been reported as a reason for the higher tolerance of encapsulated and flocculating yeast cells to convertible inhibitors during a second generation bioethanol production [32,33]. Similar phenomena are likely to be present also for the encased anaerobic sludge, tightly packed in between the membrane layers.

At the same time, using membranes as cell supporting material in the RMBR may lead to mass transfer limitations during the biodegradation process, especially so in static reactors only shaken once per day. This was evident from the results, with lower methane yields in the RMBRs compared to the reactors with the free cells. RMBRs in continuous processes, or in batch reactors with continuous flow of the medium, would however likely display a better performance, enhancing the biogas productivity in the presence of the LCFAs compared to the free cells, as previously observed for other inhibitory substances [16,17,18,19] .

From the above results, it can concluded that using the PVDF membrane to enclose the microbial cells in the RMBRs reduced the inhibitory effect of the palmitic, stearic, and oleic acid on the performance of the microbial cells in thermophilic anaerobic degradation systems for biogas production. Thus, the degradation of lipid containing

wastes for biogas production can be run in a better balanced system as compared to the conventional system with the free cells.

4. Conclusion

Increasing the concentration of LCFAs (palmitic, stearic, and oleic acid) to thermophilic anaerobic batch digesters led to stronger inhibitory effects on the microorganisms. Retaining the cells in a reverse membrane bioreactor (RMBR) was a successful approach to decrease the inhibitory effect of LCFAs, since a lower percentage of inhibition and more stable VFA concentration and pH value were found in the RMBR compared to the conventional system with the free cells. However, to develop the RMBR on an industrial scale, further investigations of the RMBR in continuous digestion process should be done.

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References

[1] S.G. Pavlostathis, G. Misra, M. Prytula, D. Yeh,. Anaerobic processes, Water Environ. Res. 68 (4) (1996) 479-497.

[2] I. Angelidaki, B.K. Ahring, Thermophilic anaerobic digestion of livestock waste: the effect of ammonia, Appl. Microbiol. Biotechnol. 38 (4) 1993 560-564.

[3] P. Becker, D. Köster, M.N. Popov, S. Markossian, G. Antranikian, H. Märkl, The biodegradation of olive oil and the treatment of lipid-rich wool scouring wastewater under aerobic thermophilic conditions, Water Res. 33 (3) (1999) 653-660.

[4] S.H. Kim, S.K. Han, H.S. Shin, Two-phase anaerobic treatment system for fat-containing wastewater, J. Chem. Technol. Biotechnol. 79 (1) (2004) 63-71.

[5] M. Quéméneur, Y. Marty, Fatty acids and sterols in domestic wastewaters, Water Res. 28 (5) (1994) 1217-1226.

[6] S. Sayed, J. van der Zanden, R. Wijffels, G. Lettinga, Anaerobic degradation of the various fractions of slaughterhouse wastewater, Biol. Wastes, 23 (2) (1988) 117-142.

[7] K. Hanaki, M. O'Nagase, T. Matsuo, Mechanism of inhibition caused by long-chain fatty acids in anaerobic digestion process, Biotechnol. Bioeng. 23(7) (1981) 1591-1610.

[8] O. Stabnikova, S.-S. Ang, X.-Y. Liu, V. Ivanov, J.-H. Tay, J.-Y. Wang, The use of hybrid anaerobic solid–liquid (HASL) system for the treatment of lipid-containing food waste, J. Chem. Technol. Biotechnol. 80 (4))2005) 455-461.

[9] L.-J. Wu, T. Kobayashi, Y.-Y. Li, K.-Q. Xu, Comparison of single-stage and temperature-phased two-stage anaerobic digestion of oily food waste, Energy Convers. Manage. 106 (2015) 1174-1182.

[10] I.W. Koster, A. Cramer, Inhibition of methanogenesis from acetate in granular sludge by long-chain fatty acids, Appl. Environ. Microbiol. 53 (2) (1987a) 403-409.

[11] I.W. Koster, A. Cramer, Inhibition of methanogenesis from acetate in granular sludge by long-chain Fatty acids, Appl. Environ. Microbiol. 53 (2) (1987b) 403-9.

[12] A. Rinzema, M. Boone, K. van Knippenberg, G. Lettinga, Bactericidal effect of long chain fatty acids in anaerobic digestion, Water Environ. Res. 66 (1) (1994) 40-49.

[13] M.H. Gerardi, The microbiology of Anaerobic Digesters, John Wiley & Sons,Inc., Hoboken, New Jersey, 2003.

[14] D.Z. Sousa, A.F. Salvador, J. Ramos, A.P. Guedes, S. Barbosa, A.M.S. Stams, M.M. Alves, M.A. Pereira, Activity and viability of methanogens in anaerobic digestion of unsaturated and saturated long-chain fatty acids, Appl. Environ. Microbiol. 79 (14) (2013) 4239-4245.

[15] D. Deublein, A. Steinhauser, Biogas from Waste and Renewable Resources, Wiley-VCH Verlag GmbH & Co. KGaA, Germany, 2008.

[16] R., Wikandari, S., Youngsukkasem, R., Millati, M.J. Taherzadeh, Performance of semi-continuous membrane bioreactor in biogas production from toxic feedstock containing d-Limonene, Bioresour. Technol. 170 (2014) 350-355.

[17] S. Youngsukkasem, J. Akinbomi, S.K. Rakshit, M.J. Taherzadeh, Biogas production by encased bacteria in synthetic membranes: protective effects in toxic media and high loading rates, Environ. Technol. 34 (13-14) (2013a) 2077-2084.

[18] S. Youngsukkasem, K. Chandolias, M.J. Taherzadeh, Rapid bio-methanation of syngas in a reverse membrane bioreactor: Membrane encased microorganisms, Bioresour. Technol. 178 (2015) 334-340.

[19] S. Youngsukkasem, H. Barghi, S. Rakshit, M.J. Taherzadeh, Rapid biogas production by compact multi-layer membrane bioreactor: efficiency of synthetic polymeric membranes, Energies 6 (12) (2013b) 6211.

[20] R. Wikandari, S. Gudipudi, I. Pandiyan, R. Millati, M.J. Taherzadeh, Inhibitory effects of fruit flavors on methane production during anaerobic digestion, Bioresour. Technol. 145 (2013) 188-192.

[21] H.N. Chanakya, H.K. Khuntia, N. Mukherjee, R. Aniruddha, J.R. Mudakavi, P. Thimmaraju, The physicochemical characteristics and anaerobic degradability of desiccated coconut industry waste water, Environ. Monit. Assess. 187 (12) (2015) 772.

[22] H.S. Shin, S.H. Kim, C.Y. Lee, S.Y. Nam, Inhibitory effects of long-chain fatty acids on VFA degradation and β-oxidation, Water Sci. Technol. 47 (2003) 139-146.

[23] G. Silvestre, J. Illa, B. Fernández, A. Bonmatí, Thermophilic anaerobic co-digestion of sewage sludge with grease waste: effect of long chain fatty acids in the methane yield and its dewatering properties, Appl. Energy 117 (2014) 87-94.

[24] M.M., Alves, J.A., Mota Vieira, R.M., Alvares Pereira, M.A., Pereira, M. Mota, Effects of lipids and oleic acid on biomass development in anaerobic fixed-bed reactors. part II: oleic acid toxicity and biodegradability, Water Res. 35 (1) (2001b) 264-270.

[25] C.S. Hwu, B. Donlon, G. Lettinga, Comparative toxicity of long-chain fatty acid to anaerobic sludges from various origins, Water Sci. Technol. 34 (1996) 351-358.

[26] A. Pereira, M. Mota, M. Alves, Degradation of oleic acid in anaerobic filters: The effect of inoculum acclimatization and biomass recirculation, Water Environ. Res. 73(5) (2001) 612-621.

[27] M.A. Pereira, O.C. Pires, M. Mota, M.M. Alves, Anaerobic biodegradation of oleic and palmitic acids: Evidence of mass transfer limitations caused by long chain fatty acid accumulation onto the anaerobic sludge. Biotechnol. Bioeng. 92 (1) (2005) 15-23.

[28] J.A., Lalman, D.M. Bagley, Anaerobic degradation and inhibitory effects of linoleic acid, Water Res. 34 (17) (2000) 4220-4228.

[29] D.G. Cirne, X. Paloumet, L. Björnsson, M.M. Alves, B. Mattiasson, Anaerobic digestion of lipid-rich waste—effects of lipid concentration, Renewable Energy 32 (6) (2007) 965-975.

[30] C.-N., Weng, J.S. Jeris, Biochemical mechanisms in the methane fermentation of glutamic and oleic acids, Water Res. 10 (1) (1976) 9-18.

[31] M.M. Alves, J.A. Mota Vieira, R.M. Alvares Pereira, M.A. Pereira, M. Mota, Effect of lipids and oleic acid on biomass development in anaerobic fixed-bed reactors. Part I: Biofilm growth and activity, Water Res. 35 (1) (2001a) 255-263.

[32] J.O. Westman, N. Bonander, M.J. Taherzadeh, C.J. Franzén, Improved sugar co-utilisation by encapsulation of a recombinant *Saccharomyces cerevisiae* strain in alginate-chitosan capsules, Biotechnol. Biofuels 7 (1) (2014a) 102.

[33] J.O. Westman, V. Mapelli, M.J. Taherzadeh, C.J. Franzén, Flocculation causes inhibitor tolerance in *Saccharomyces cerevisiae* for second-generation bioethanol production, Appl. Environ. Microbiol. 80 (22) (2014b) 6908-6918.

FIGURE CAPTIONS

Fig. 1. Cumulative methane yield from reactors of free cells containing the LCFAs. (a) Palmitic acid, (b) Stearic acid, and (c) Oleic acid.

Fig. 2. Cumulative methane yield from the RMBR with the encased cells containing the LCFAs. (a) Palmitic acid, (b) Stearic acid, and (c) Oleic acid.

Fig. 3. Percentage of inhibition in the reactors with the free cells and RMBRs with the encased cells in the presence of LCFAs. (a) Palmitic acid, (b) Stearic acid, and (c) Oleic acid.

TABLE LEGENDS

Table 1 Total VFA concentration on the last day of the experiment in the reactors with the free cells and the RMBRs with the encased cells containing different LCFAs at different concentrations.

Table 2 pH value on the last day of the experiment in the reactors with the free cells and the RMBRs with the encased cells containing different LCFAs at different concentrations.

Tables and Figures

Figure 1.

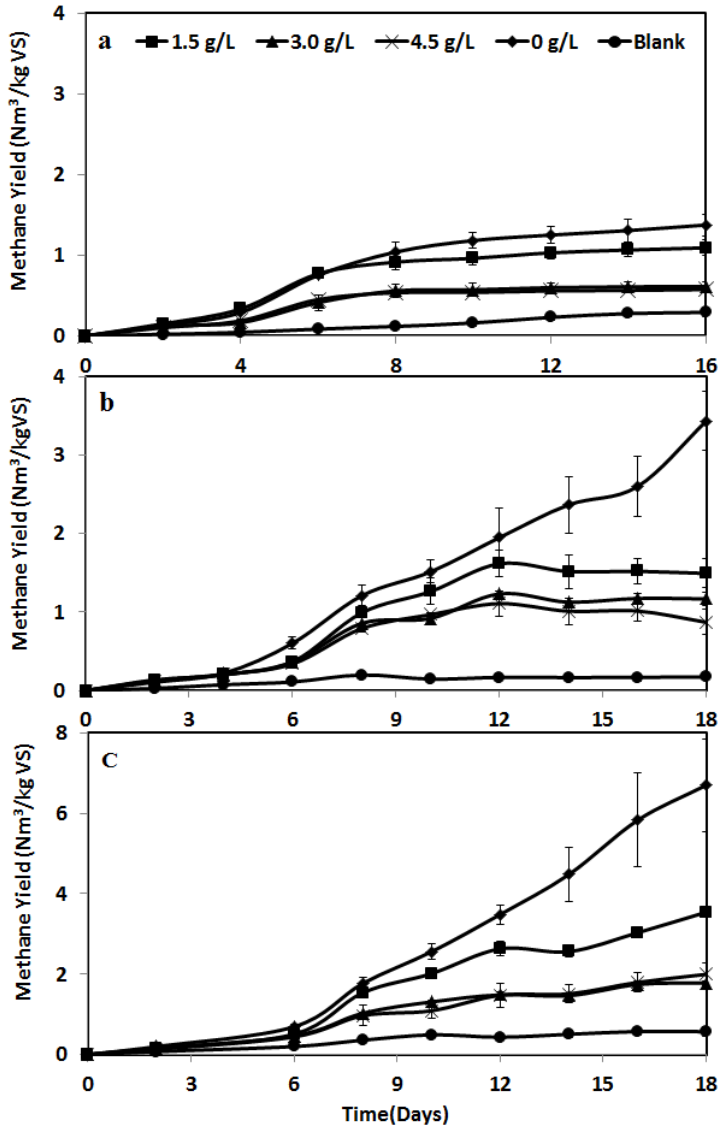

Figure 2

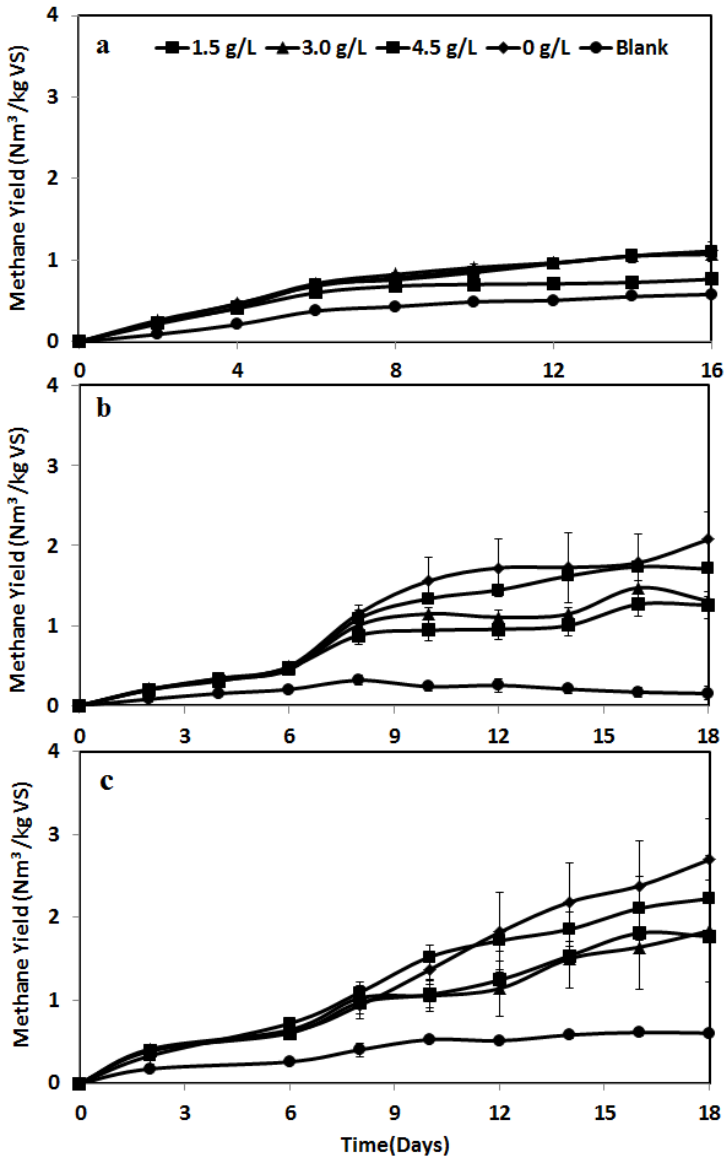
a
1.5 g/L
3.0 g/L
4.5 g/L
0 g/L
Blank
Methane Yield (Nm³ /kg VS)
b
c
Time(Days)

Figure 3

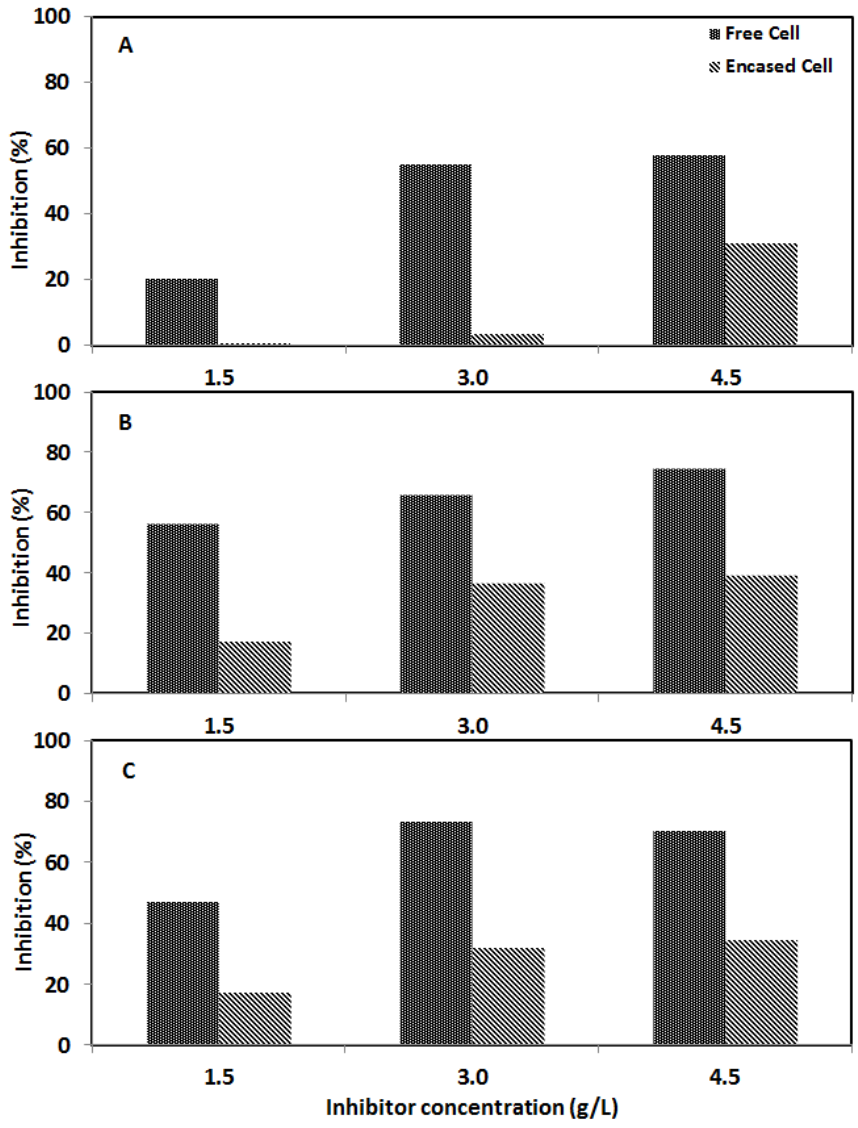
A
Free Cell
Encased Cell
Inhibition (%)
100
80
60
40
20
0
1.5
3.0
4.5
B
Inhibition (%)
C
Inhibition (%)
Inhibitor concentration (g/L)

Table 1

LCFAs		Total VFA Concentration (g/L)	
		Free cells	Encased cells
Palmitic acid Conc. (g/L)	Control	2.1 ± 0.6[a]	2.4± 0.1[a]
	1.5	2.5 ±0.0[a]	2.6 ± 0.0[a]
	3.0	2.6 ±0.0[a]	2.8 ± 0.2[a]
	4.5	3.2 ± 0.0[a]	2.8 ± 0.1[a]
Stearic acid Conc. (g/L)	Control	2.6 ± 0.5[a]	1.8 ± 0.1[a]
	1.5	2.7 ± 0.1[a]	1.9 ± 0.0[a]
	3.0	4.4 ±0.1[b]	2.6 ± 0.0[b]
	4.5	5.4 ±0.2[b]	2.4 ± 0.0[b]
Oleic acid Conc. (g/L)	Control	4.0 ± 0.5[a]	3.4 ± 1.1[a]
	1.5	4.6 ± 0.5[a]	3.8 ± 0.1[a]
	3.0	4.5 ± 0.1[a]	4.0 ± 0.4[a]
	4.5	5.1 ± 0.2[b]	4.2 ± 1.2[b]

[a] Not significantly different from control.
[b] Significantly different from the control, $p<0.05$, $n = 3$.

Table 2

LCFAs		pH	
		Free cells	Encased cells
Palmitic acid Conc. (g/L)	Control	5.65	5.38
	1.5	5.29	5.11
	3.0	5.14	5.10
	4.5	5.18	4.90
Stearic acid Conc. (g/L)	Control	5.97	6.23
	1.5	5.75	6.01
	3.0	5.48	5.72
	4.5	4.81	5.79
Oleic acid Conc. (g/L)	Control	5.12	5.84
	1.5	5.61	5.76
	3.0	5.27	5.16
	4.5	5.25	5.20