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MANAGEMENT OF HYDROGEN SULFIDE IN ANAEROBIC DIGESTION OF ENZYME PRETREATED MARINE MACRO-ALGAE

Vätesulfidhantering vid rötning av enzymförbehandlade marina makroalger

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Abstract

Enzymatic pretreatment of algae by means of cellulose degrading enzyme was evaluated through lab-scale and pilot-scale experiments. The degradation efficiency of the enzyme depended on the initial physical quality of the algae. Lab-scale batch anaerobic digestion experiments showed comparatively low methane potential for the pretreated algae at both mesophilic and thermophilic temperatures. However, the raw algae (cut into small pieces) were found to be hardly hydrolysable. The methane potential of raw algae in thermophilic and mesophilic digestion was about 17 NmL/g VS and –36 NmL/g VS respectively. Presence of inhibitory agent(s) was obvious at both temperatures. Very fast growth of sulfate-reducing bacteria was noticed in the continuous digestion, so that in less than 20 days, hydrogen sulfide concentrations over 10000 ppm were observed in both meso- and thermophilic reactors. Inhibition of methanogenesis in the thermophilic reactor occurred at unionized dissolved sulfide concentration of about 22 mg/L (10000 ppm in the biogas) while it was mainly non-SRB acetogens that were inhibited in the mesophilic reactor at unionized sulfide concentrations as high as 50 mg/L (17000 ppm in the biogas). This shows that probably thermophilic digestion is more prone to be inhibited at high sulfide concentrations. Micro-aeration was found to be more efficient in the thermophilic reactor while its effect on the mesophilic process was negligible.

Key words – Anaerobic digestion, Sulfate, Hydrogen sulfide, Enzymatic pretreatment, Marine macro-algae, Micro-aeration

Sammanfattning

Enzymatisk förbehandling av alger (med cellulosanedbrytande enzym) utvärderades genom satsvisa och kontiuerliga experiment. Nedbrytningens effektivitet av enzymet berodde på algernas initiala fysiska kvalitet. Satsvisa rötningsförsök i labskala visade jämförelsevis låg metanpotential för de förbehandlade algerna både vid mesofil och termofil temperatur. De råa (obehandlade) algerna (skurna i små bitar) visade sig dock vara knappt hydrolyserbara. Metanpotential av råa alger i termofil och mesofil rötning var ca 17 NmL/g VS respektive –36 NmL/g VS. Närvaro av hämmande ämnen var uppenbar vid båda temperaturerna. Mycket snabb tillväxt av sulfat-reducerande bakterier noterades i det kontinuerliga försöket, så att på mindre än 20 dagar, observerades svavelvätekoncentrationer över 10000 ppm i både de meso-och termofila reaktorerna. Hämning av metanogener i den termofila reaktorn skedde vid upplöst sulfidjonkoncentration av ca 22 mg/L (10000 ppm i biogasen) medan det var huvudsakligen icke-SRB acetogener som inhiberades i den mesofila reaktorn vid sulfidjon-koncentrationer så höga som 50 mg/L (17000 ppm i biogasen). Detta visar förmodligen att metanogener i termofil rötning är mer benägna att inhiberas vid höga koncentrationer av sulfid. Mikro-luftning visade sig vara mer effektiv i den termofila reaktorn medan dess effekt på den mesofila processen var försumbar.

Introduction

Recent elevated demand for renewable energy as a substitute for fossil fuels has conducted attentions towards anaerobic digestion. It is estimated that about 20000 anaerobic digestion plants will be installed in Europe by 2015 (Korz, 2009). However, obstacles such as hydrogen sulfide, high carbon dioxide content, presence of water vapor, slow hydrolysis rates under anaerobic conditions, and high sensitivity of anaerobic microorganisms to changes in substrate composition; restrict the full industrial application of biogas (Noyala et al., 2006; Leitão et al., 2006).

Hydrogen sulfide production in the biogas from sulfur (sulfate)-rich substrates, such as the effluents from the industries that use sulfuric acid (Lens et al., 1998) or marine substrates (Jasińska et al., 2012), may cause serious problems in application of biogas. Hydrogen sulfide, besides its unpleasant smell and corrosive nature which reduces the lifespan of pipework and other different installations in biogas industry, is also highly toxic to living beings (Guidotti, 2010). Reportedly, hydrogen sulfide toxicity inhibits the methanogens in anaerobic digestion process (Chen et al., 2008). So it is crucial to look for applicable methods and techniques in order to solve the hydrogen sulfide issue to be able to introduce new sulfur-rich substrates into anaerobic digestion.

Accumulation of large piles of seaweed on beaches (Figure 1) may cause unpleasant odor (Charlier et al., 2007) due to predomination of anaerobic conditions hence formation of gaseous sulfur-containing compounds such as hydrogen sulfide. The problem with accumulation of algae is not only restricted to bad odor but also some health problems may be caused by such emissions due to continuous inhalation of sulfide (Peu et al., 2011). In order to meet the problem authorities collect the piled up macro-algae on the beaches and store it temporarily during spring and summer and, later on, in autumn and winter release them back into the sea. However, algae can be used as fertilizer via spread on agricultural lands but its salinity, sometimes high cadmium (Cd) content and high amount of trapped sand limit such an application. Algae are counted as toxic wastes in Sweden because of their occasional high Cd content (Nkemka and Murto, 2010).

Aim

In this study, technical feasibility of digestion of marine macro-algae through continuous anaerobic digestion was looked into. One of the crucial issues to tackle was to propose a way in order to convert the algal matter into a free-flowing substrate, appropriate for continuous wet digestion. Additionally, evaluation of the problems caused by hydrogen sulfide was considered as a major aim in this study.

Since sulfide production is a biological process done by sulfate-reducing bacteria (SRB) which consume mutual substrates with methanogens; changing process parameters such as hydraulic retention time (HRT), temperature, pH, and organic loading rate (OLR) for driving the competition towards the interest of methanogens, seemed to be interesting. Also precipitation of sulfide via oxygen, i.e. micro-aeration, (Cirne et al., 2008) was tested.

Figure 1. *Piled seaweed on the Baltic coast, southern Sweden (Skåre).*

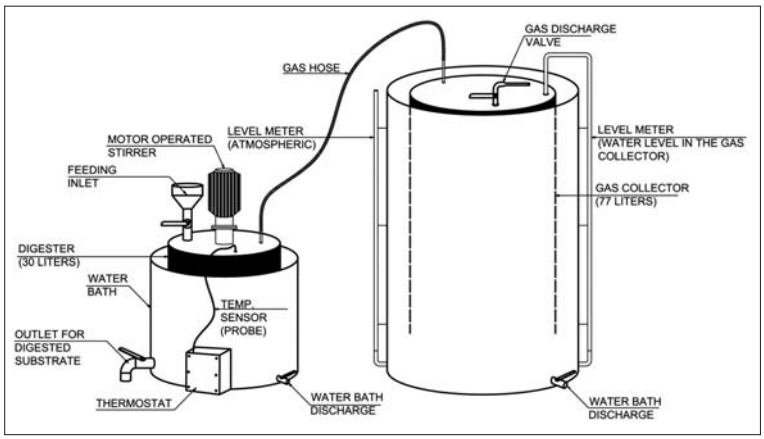

Figure 2. *Schematic of the pilot-scale reactors used in the experiment.*

Materials and methods

Two pilot-scale reactors located at Sjölunda Wastewater Treatment Plant (WWTP) in Malmö, Sweden, were used in the study operated at mesophilic (35°C) and thermophilic (55°C) temperatures. The total volume of the reactors was about 30 L from which 20 L was occupied by the substrate and 10 L were left as head space. The reactors were equipped with stirrers operated continuously at a constant speed of 50 rpm. Feeding substrate and withdrawal of digested matter were done manually once a day at a certain time so that the HRT was adjusted to about 15 days. The temperature in the reactors was regulated by a thermostat connected to a heater. The digestion chamber was surrounded by a water bath which heated up the reactors. Produced biogas was collected in a bell-shape gas collector filled with water. Change in the water level inside the gas bells represented the volume of the produced gas as well as its pressure which was then used to convert the volumes to Standard Temperature and Pressure (STP) condition. Schematic drawing of the reactors is shown in Figure 2.

Some parameters of the digestion process as well as operational characteristics were measured in-situ at the location of pilot-scale reactors. pH of the digested matter from the reactors was measured using a digital pH-meter (pH 3110 SET 2 incl. SenTiz® 41) calibrated based on a two point-calibration at pH levels of 4 and 7. Gas fractions including methane, carbon dioxide, oxygen and hydrogen sulfide (up to 2000 ppm) were measured using a portable gas-meter (SEWERIN SR2-DO). For higher hydrogen sulfide contents (above 2000 ppm), Dräger tube *Hydrogen Sulfide 0.2 %/A* with order code of CH28101 was employed (range from 0.2 vol.-% to 7 vol.-%.)

Samples of digested matter as well as substrate were taken to the laboratories at the Department of Chemical Engineering, Lund University, for further analyses. HACH LANGE test tubes were used for measuring COD (LCK 114), ammonium (LCK 303), sulfate (LCK 153), iron (LCK 320), and phosphate (LCK 049). Prepared tubes were analyzed with HACH LANGE spectrophotometer (model DR 2800).

All the samples stated above were centrifuged for 15 minutes at the speed of 10000 rpm and filtrated through general purpose filter papers with 6-10 µm pore size before further analysis.

Furthermore, total solids (TS) content of digested matter and the substrate were measured after samples were dried for 24 h at 105°C. Volatile solids (VS) content was estimated after burning the already dried samples at 550°C for 2 h (SIS, 2000).

Biological Methane Potential (BMP) tests were done in 2-liter lab-scale batch reactors (as triplicates) and evaluated according to the method suggested by Hansen et al. (2004). The reactors were inoculated and fed with substrate with the proportions of 60:40 inoculum to substrate, on VS basis. Methane content of the biogas produced in the batch reactors were measured with gas-chromatograph, Varian 3800 Gas Chromatograph, equipped with TCD (thermal conductivity detector) and a column with the dimensions: 2.0 m x 0.3 mm x 2.0 mm. Volatile fatty acids (VFA) content of samples were analyzed with gas-chromatography using Agilent 6850 Series GC System equipped with FID (Flame Ionization Detector) and a column with the dimensions: 25 m x 0.32 µm x 0.5 µm.

The inoculum for the mesophilic reactor was taken from the digesters at the WWTP in Helsingborg, Sweden, (operated at 35°C) and the thermophilic inoculum was collected from the digesters at Kävlinge WWTP, Sweden, (operated at 55°C).

Needed algae for the experiment were collected at Skåre harbor, approximately 7 kilometers west of Trelle-

borg in southern Scania, Sweden. Collected algal mass, shown in Figure 3, consisted of various species such as *Fucus vesiculosus*, *Fucus serratus*, *Furcellaria lumbricalis*, *Polysiphonia sp.*, *Ceramium sp.* and *Zostera marina* (a species of seagrass). Mixture of algae had a TS of 15–25 % from which between 75–80 % was measured as VS fraction.

Enzymatic pretreatment of marine algae was carried out using *Cellic® CTec2* in order to hydrolyze higher amounts of algal mass to get higher organic content of degradable COD. *Cellic CTec2* enzyme converts cellulose and hemicellulose, containing polymeric forms of sugar, into hydrolyzed fermentable monomers (Novozyme, 2010). Accordingly, peak performance of the enzyme is obtained at 45–50°C and pH 5–5.5. Frozen algae were used for evaluation of enzymatic pretreatment in a lab-scale experiment. The enzymatic pretreatment was done at 50°C for five days with three different doses of enzyme 15, 30, and 45 FPU/g TS (*Filter Paper Unit* (FPU) is the unit used for expressing enzymes' activity) as well as a reactor with no enzyme as the reference. Distilled water was added into the reactors after pretreatment ended in order to compensate the effect of evaporation; afterwards the content was sieved through 4 mm pore size sieve in order to obtain a homogenous matter.

Results and discussions

Pretreatment and BMP estimation

In order to evaluate the efficacy of enzymatic pretreatment, dissolved COD (SCOD), total COD (TCOD), ammonium, and sulfate contents were measured in the end-product of the pretreatment reactors after passed through 4 mm sieve. According to the results from the lab-scale enzymatic pretreatment, higher dosages of en-

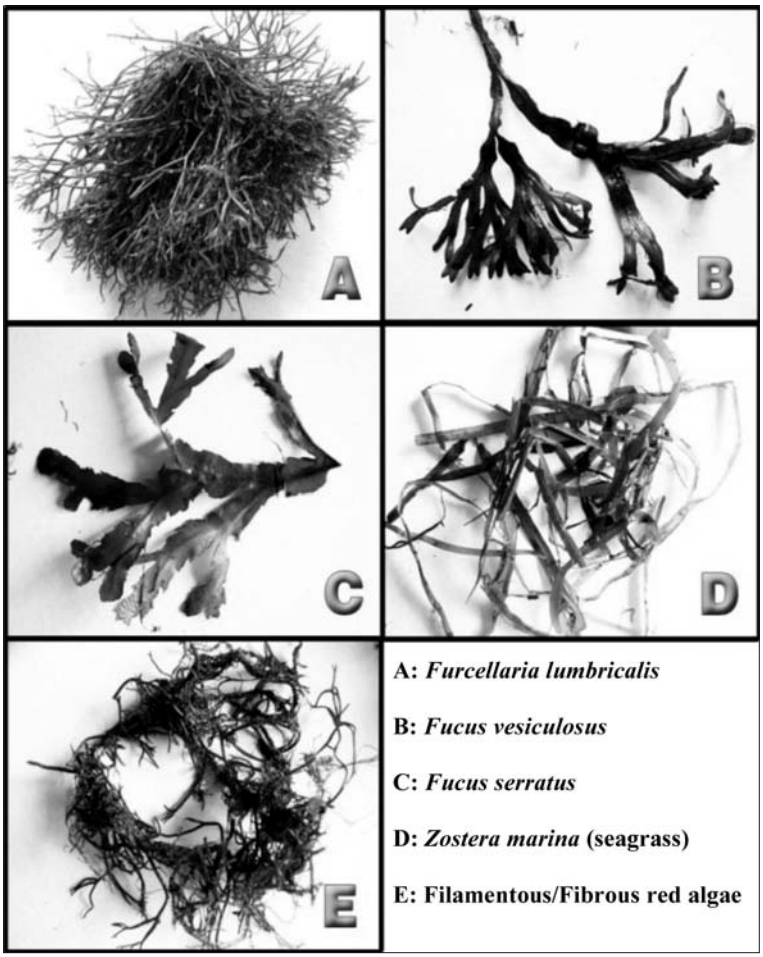

Figure 3. *Different species of algae included in the experiment.*

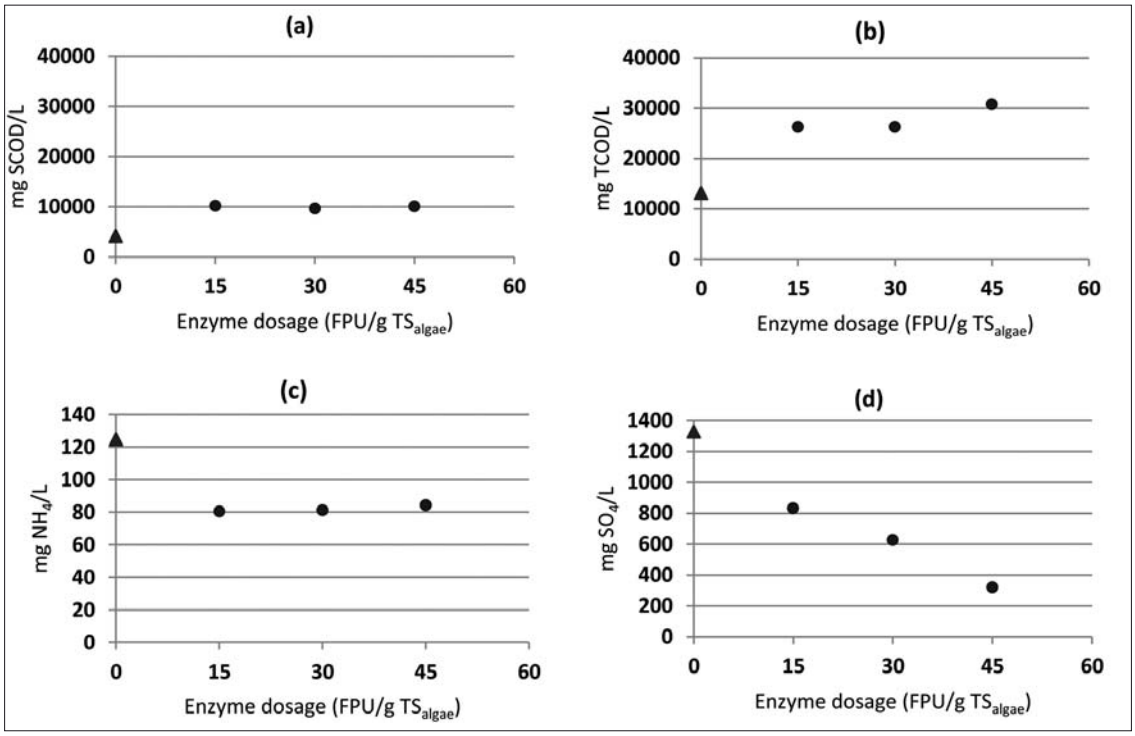

Figure 4. *Results from the lab-scale enzymatic pretreatment experiment for three different doses of enzyme as 15, 30, and 45 FPU/g TS_{algae}. The triangular marker on the vertical axis shows the value from the reference reactor with no enzyme addition. (a): Dissolved COD, (b): Total COD, (c): Ammonium, (d): Sulfate.*

zyme do not lead to further disintegration of solid matter so that the same amount of COD and ammonium is obtained in all cases (Figure 4a, b, c). Surprisingly, it was observed that less sulfate is present in the reactors with higher dosages of enzyme (Figure 4d). The same trend was seen for acetate as well (data not shown). Similar decreasing trends for sulfate and acetate can probably be a sign for presence of acetotrophic SRB utilizing both substrates to produce hydrogen sulfide. This has to be investigated furthermore because no anaerobic conditions were guaranteed during the experiment.

The end-product from enzymatic pretreatment was then digested in lab-scale batch experiment at mesophilic (37°C) and thermophilic (55°) temperatures according to the method for estimation of BMP. Digestion was operated for approximately 50 days and the resulting methane yields are presented in Figures 5 and 6. The results of the experiment suggest very low net methane potential for both mesophilic digestion (12 NmL/g VS_{in}) and thermophilic (100 NmL/g VS_{in}) in comparison with municipal sewage sludge, the conventional digestion substrate which normally gives yields around 400 NmL/g VS_{in} (Davidsson, 2007). It should be noted that the methane potential values mentioned above are net values exclusive of the yield contributed by the added enzyme and of the inoculum.

Observations as presented in Figures 5 and 6, demonstrate presence of strong inhibitory agent in the process. As it can be noticed in Figure 5, no considerable methane yield is found at mesophilic digestion of enzyme pretreated algae and the process was inhibited after 8 days of digestion when the yield reaches 82 NmL/g VS_{in}.

At thermophilic batch digestion of enzyme pretreated algae, maximum yield is observed after 5 days of digestion about 135 NmL/g VS_{in} (Figure 6). Digestion of untreated algae at thermophilic temperature was also inhibited in the start of the process but later on showed a tendency for recovery as positive yields, although very small, were obtained.

Digestion of untreated algae at mesophilic temperature was inhibited constantly leading to the methane potential of –36 NmL/g VS_{in}. This illustrates stronger inhibitory effect of the inhibition source in the untreated substrate. Note that the accumulative methane yield in the BMP tests can be negative or may have a decreasing

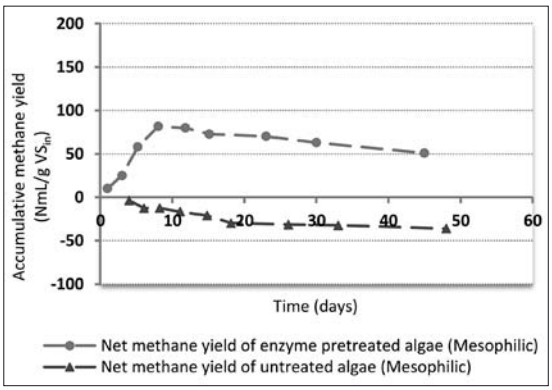

Figure 5. *Accumulative methane production from mesophilic batch experiment). About 40 NmL/g VS_{in} of the found methane potential is contributed by the added enzyme. (30 FPU of enzyme/g TS_{algae} were used for pretreatment).*

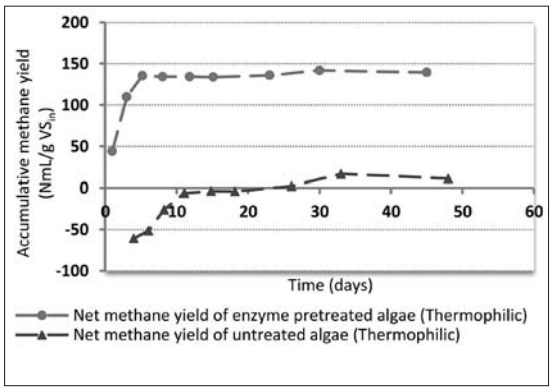

Figure 6. *Accumulative methane production from thermophilic batch experiment. About 40 NmL/g VS_{in} of the found methane potential is contributed by the added enzyme (45 FPU of enzyme/g TS_{algae} were used for pretreatment).*

trend when the yield from the substrate-inoculum reactor is lower than from only inoculum reactor.

Reportedly, different derivatives of lignin, specifically aldehyde groups and those with apolar side chain, can be highly toxic to methanogens (Benjamin et al., 1984; Sierra-Alvarez and Lettinga, 1991; Chen et al., 2008). Presence of true lignin cells in a specific species of red algae, *Calliarthron cheilosporioides,* has also been reported (Martone et al., 2009). Although there is a chance that lignin causes the inhibition; lack of data regarding cellular structure of the digested algae as well as degradability of lignin by means of the employed enzyme, makes it impossible to draw reliable conclusions about that.

Nevertheless analyses of reactors' content after digestion period of about 60 days indicate no accumulation of VFA in the batches with untreated algae. In order to justify the phenomenon, two different speculations presented below may be considered:

a) SRB outcompeted methanogens in competition over acetate due to higher amounts of sulfate released from un-pretreated algae, i.e. lower COD/SO_4^{2-} ratio. On the other hand due to the fact that the methane yield in mesophilic reactor is negative could be another argument for the hypothesis. This could be in agreement with Shin et al. (1996) demonstrating that at lower temperatures the amount of COD degraded by SRB increases. However it is not possible to verify the hypothesis since hydrogen sulfide production was not measured in the batches.
b) Hydrolysis is reported to be the limiting step in anaerobic digestion. It seems very probable that the un-pretreated algae have not been hydrolyzed sufficiently. Ocular examination of digestates from the batches also proved that not much had happened to the structure of the fed algae. Therefore, it could be speculated that insufficient hydrolysis of algal mass (perhaps due to protective membranes) besides presence of inhibitory agent(s) may have led to very low methane yield in thermophilic and negative yield for mesophilic batches.

Continuous digestion of enzyme pretreated algae

Two digesters set at mesophilic (35°C) and thermophilic (55°C) temperatures where inoculated and fed with 2 L of enzyme pretreated algae on daily basis. Disregarding the data from first 10 days, temperatures of the reactors were maintained throughout the experiment at 36.01±0.49 (°C) and 55.05±0.71 (°C) for mesophilic

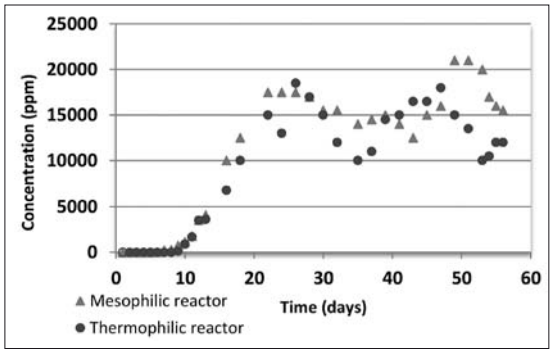

Figure 7. *Hydrogen sulfide content of produced biogas measured as parts per million (ppm). For concentrations below 2000 ppm portable gas meter was used while for higher concentrations Dräger tubes were employed.*

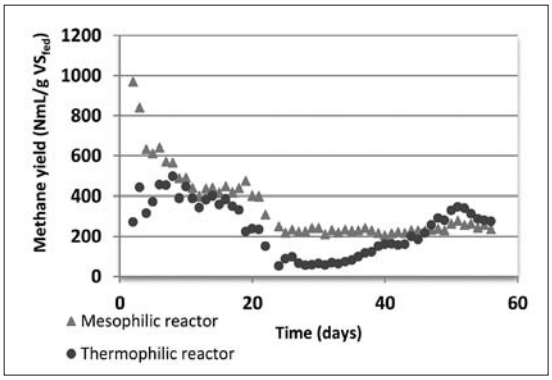

Figure 8. *Methane yield as NmL/g VS_{fed} for thermophilic and mesophilic reactors.*

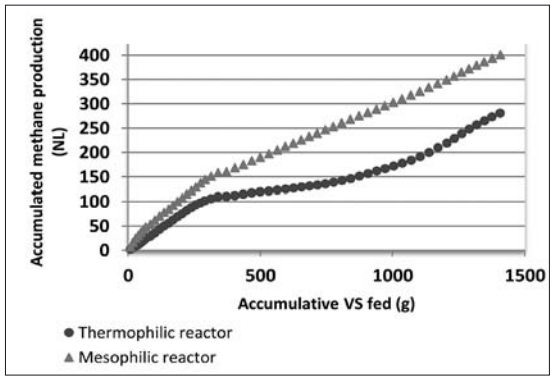

Figure 9. *Accumulated methane production against accumulative VS fed is illustrated in this figure.*

and thermophilic reactors, respectively. Methane production reached about 7 NL/day (about 400 NmL/g VS_{fed}) after 10 days of inoculation in both reactors. Meanwhile the concentration of hydrogen sulfide in the produced biogas was increasing rapidly so that over 10000 ppm was reached in less than 20 days (Figure 7).

Considering the data on methane yield (Figure 8) and accumulated methane production (Figure 9) it can be noticed that the major inhibition in the thermophilic reactor has occurred on day 18 while the significant drop in methane yield of the mesophilic reactor occurred a few days later on day 21 corresponding to about 22 mg/L (10000 ppm in gas phase) and 50 mg/L (17000 ppm in gas phase) of unionized dissolved sulfide, respectively. Earlier inhibition of methanogens in the thermophilic reactor, in spite of its lower sulfide content, demonstrates that methanogens are more prone to sulfide inhibition at thermophilic temperatures. However, stronger resistance of mesophilic reactor against higher sulfide concentrations can be a sign of acclimatization of the methanogens to high sulfide levels. This is very likely to be the reason in this case, since the inoculum for mesophilic reactor was taken from the digesters at the WWTP in Helsingborg which have had relatively high hydrogen sulfide content in the produced biogas for a long period of time.

Considerable consumption of acetate (Figure 10a) in the mesophilic reactor reveals that either both or at least one of the acetotrophic groups, methanogens or SRB, are not affected significantly by sulfide toxicity throughout the experiment. Studying the propionate variation during the first 30 days of the experiment (Figure 10b) suggests that propionate oxidation is also partly inhibited, perhaps because of high sulfide content. Since the amount of propionate in the thermophilic reactor is not considerable (700 mg COD/L), it can be mentioned that SRB are not considerably affected by sulfide toxicity. On the other hand, propionate accumulation in the mesophilic reactor is observed on day 27 and tends to increase up to about 1500 mg COD/L in 3 days (day 30). Also, a considerable drop in methane yield of the mesophilic reactor is noticed on day 22. The drop in methane yield (Figure 8) can therefore be linked to lack of acetate meaning that not sulfide toxicity, but insufficient oxidation of propionate causes the starvation of

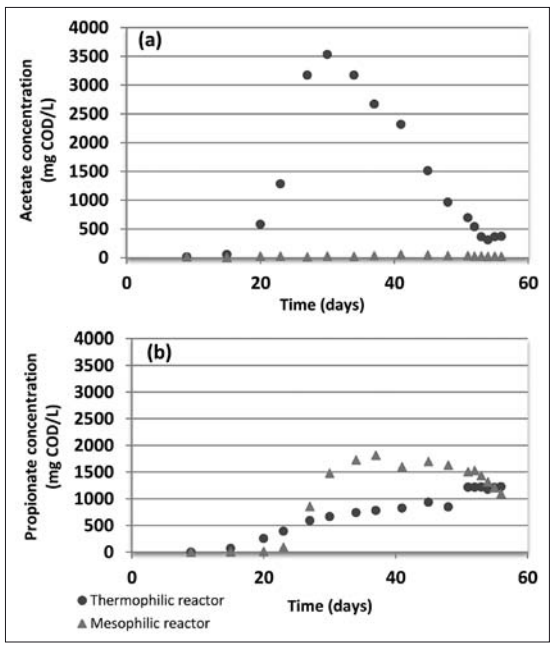

Figure 10. *Volatile fatty-acids concentration during the pilot-scale experiments.*

methanogens. In other words, if inhibition of SRB is the reason for accumulation of propionate then a significant decrease in sulfide yield should have occurred as well. Nevertheless, normalizing the amount of hydrogen sulfide against the amount of sulfate fed per day shows that no significant reduction in sulfide yield has happened. This shows that no inhibition of SRB has taken place in the reactor.

Consequently, accepting that lack of acetate, due to incomplete oxidation of propionate, is the cause of methane yield drop, the effect of sulfide toxicity on non-SRB acetogens may be the reason. It is also reported that under sulfate rich conditions (low propionate/sulfate ratios) acetate is most favored by SRB (Uberoi and Bhattacharya, 1995). This, somehow, explains high degradation level of acetate in the mesophilic reactor.

As a sulfide treatment method, micro-aeration started to be applied at the rate of 2 L air/day, injected into the reactors' headspaces starting on day 23. As it can be seen from the data presented in Figure 7, aeration has resulted in better sulfide reduction in the thermophilic reactor. Hydrogen sulfide fraction decreased considerably in about 12 days from about 18000 ppm down to 10000 ppm. It should be noted that the inhibition in thermophilic reactor due to sulfide toxicity occurred at around 10000 ppm.

During the same period (from day 23 to 35), the methane yield was leveled out at about 65 NmL CH_4/g VS_{fed} more or less unaffected (see Figure 8). After this period, methane yield tended to increase gradually until day 50, having recovered the yield to about 330 NmL CH_4/g VS_{fed}. Simultaneously hydrogen sulfide production in the thermophilic reactor increased as well.

Unlike the thermophilic reactor, the effect of micro-aeration in the mesophilic reactor is negligible. The decrease observed in hydrogen sulfide content of biogas can be linked to the dilution of gas due to injection of air (about 13 %). However the amount of oxygen consumed per day was calculated about 300 NmL/day (approximately 75 % of the injected oxygen was consumed while the value for the thermophilic reactor was found to be about 62 %). The methane yield for the mesophilic reactor remained unchanged during micro-aeration period (see Figure 8).

Conclusion

Comparative batch experiments with enzyme pretreated algae and untreated frozen cut algae, revealed that hydrolysis of untreated algae takes place at minor rates, especially at mesophilic temperatures. Additionally presence of an inhibitory agent in digestion of untreated algae was suggested.

Digestion of enzyme pretreated algae in continuous digestion showed relatively acceptable methane yields (about 400 NmL CH_4/g VS_{fed}) for both thermophilic and mesophilic reactors before inhibition occurred due to high hydrogen sulfide levels. Inhibition of methanogens by sulfide toxicity was only observed in the thermophilic reactor despite the fact that the level of dissolved sulfide was lower according to Henry's law at thermophilic temperatures. Methanogenesis inhibition in the thermophilic reactor – linked to considerable acetate accumulation – was initiated at dissolved sulfide concentration of 22 mg/L (10000 ppm H_2S in gas phase) while the SRB were found to be unaffected. No sulfide toxicity on SRB was observed in the mesophilic and thermophilic reactors. Relatively more resistant methanogens in the mesophilic reactor may have been a result of acclimatization.

Micro-aeration of the thermophilic reactor at the rate of 2 L air/day led to improvement of methane yield up to about 330 NmL CH_4/g VS_{fed}. Oppositely, the mesophilic reactor remained more or less unaffected regarding the methane yield during the micro-aeration period.

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