Optimization of Anaerobic Digestion of Sewage Sludge Using Thermophilic Anaerobic Pre-Treatment

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Thermophilic anaerobic pre-treatment for hydrolysis and hygienization of sewage sludge

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ABSTRACT—Thermophilic anaerobic pre-treatment at temperatures of 55, 60, 65, 70, 75 and 80°C, respectively, and for retention times of 0.5, 1.0, 1.5, 2.0 2.5 and 3.0 days, respectively, was studied. Experimental results show that, to achieve the simultaneous sufficient hydrolysis enhancement and satisfactory pathogen reduction effect, temperature should be in the range of 70-75°C, and retention time in the range of 1.75-2.5 day when feeding/withdrawing frequency was set at 24 times per day. Conducting the pre-treatment within these temperature and RT ranges, about 29.0% to 33.8% of the organic solids in the sludge can be hydrolyzed in terms of COD, and 47.1% to 70.2% of the hydrolysis products are in the form of VFA. At the same time, the pathogen indicator microorganism, Faecal streptococci, could be totally eliminated.

KEY WORDS—temperature, retention time, feeding/withdrawing frequency, thermophilic, anaerobic, pre-treatment, sludge

INTRODUCTION
As an established technology, anaerobic digestion (AD) is a biological engineering process that has long been used for stabilization of sewage sludge in wastewater treatment plant. Currently, AD of sewage sludge is normally carried out in one reactor either at mesophilic temperatures (30-40°C) for a retention time (RT) of 30 days or at thermophilic temperatures (50-60°C) for a RT of 15 days (Gujer and Zehnder, 1983; Hamer et al., 1985; Ahring, 1994). Because organic material in the sludge is in the form
of particulates that make hydrolysis the rate-limiting step of the whole AD process (Eastman and Fergusson, 1981; Li and Noike, 1992; Ghosh et al., 1995), only about 50% of the organic material is degraded in either of the above-mentioned operation conditions. And therefore, to enhance hydrolysis and thus increase the degradation efficiency is of great importance for both biogas production and sludge stabilization (Ahring, 2003).

Meanwhile, the final disposal of the digested sludge is still a worldwide problem. Other than landfill and incineration, a sustainable outlet for the disposal of AD effluent can be to use it as fertilizer and hence to recycle the plant nutrients such as P and N back to the farmland (Ahring, 2003). However, because sewage sludge usually contains chemical pollutants such as heavy metals and xenobiotics and pathogenic microorganisms, there is a great danger of spreading epidemic diseases. Therefore, land application at the moment is restricted by the national or regional legislations such as the US Federal regulation (US EPA, 2003) and EEC directive (EEC, 1986), and sometimes is opposed by the public (Gibbs et al., 1995; Verstraete et al., 2005). Heavy metals and xenobiotics can be eliminated or reduced to a level that meets the legislation requirements by implementing stricter discharging limits or by prohibiting their exist in the market (Jensen and Jepsen, 2005), while complete elimination of the pathogens such as *Salmonella typhi*, *enteroviruses*, parasite ova and etc. can only be attained during the sludge treatment process since they are originated in the excretion of human beings and animals.

Previous studies show that pre-treating the sludge before AD using mechanical, thermal, chemical and biological methods all can to a great extend increase the degradability of
the organic matters in the sludge, even though each method has, in one way or the other, different advantages and disadvantages (Müller, 2001; Kim et al., 2003). Thermal pre-treatment at temperatures from 60°C to 80°C for a short period can enhance the hydrolysis of organic material in the sludge and increase biogas yield by more than 30% according to Hiraoka et al. (1984). Since the operating temperature is lower than water boiling point, it is convenient for process control (Wang et al., 1999). Meanwhile, there widely exist in nature thermophilic anaerobic bacteria, the optimal temperature of which are in the range of 57°C to 80°C, possessing high activities in hydrolyzing and acidifying the organic polymers such as cellulose, hemicellulose, protein, pectin, and etc. (Kristjansson and Stetter, 1992; Wiegel, 1992). So, if these thermophiles are inoculated into the pre-treatment reactor, they will contribute to the hydrolysis of organic solids as well. Since thermal and biological effects belong to different mechanisms, conducting a pre-treatment anaerobically and at thermophilic temperatures will obtain these two effects simultaneously.

Most of the pre-treatment methods that have been reported are mainly focused on hydrolysis enhancement, and pathogen reduction effect is normally not taken into consideration. Pathogen reduction effect of anaerobic digestion for organic waste treatment has long been recognized, but total elimination of pathogens can not be obtained by the conventional single stage AD process (Lee et al., 1989; Burtscher et al., 1998, Bendixen, 1999). There are several studies on thermal pre-treatment or two-phase AD in which PRE are considered (Dichtl, 1997; Huyard et al., 2000; Skiadas et al.,
In this study, six anaerobic completely stirred tank reactors (CSTR) were run at temperatures of 55, 60, 65, 70, 75 and 80°C, respectively, and for RTs of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 days, respectively. The purposes are to examine the effect of thermophilic anaerobic pre-treatment at different temperature and RT conditions on the enhancement organic solid hydrolysis and on the pathogen reduction effect, and to determine the most suitable temperature and RT to conduct this pre-treatment.

**MATERIAL AND METHODS**

**Sewage sludge**

Sewage sludge used in this study was the mixture of primary and waste activated sludge (0.8:0.2, DW) from Lundtofte WWTP, Denmark. To keep the identity of the sludge used for the whole study period so that experimental results can be used for comparison (Andreasen, et al., 1997; Bouzas et al. 2002; Ferreiro and Soto, 2003; Chyi and Dague, 2005), the sludge sample was taken in one collection and stored at -20°C in 1-litter plastic bags. Once a day, a certain amount of the frozen sludge was thawed at ambient temperature and fed into the influent bottles of the reactors.

**Inoculum cultivation**

The original inoculum used in this study were taken from a single-phase thermophilic anaerobic CSTR reactor, which had been using mixed sludge as feed substrate and
running stably at 55°C and a RT of 15 days in our lab for more than one year. For enrichment of the thermophilic anaerobic bacteria that could selectively grow at different thermophilic temperatures and for their acclimation to the sampled mixed sludge to be used in the experiment, the inoculum was distributed together with the mixed sludge at a ratio of 0.5:0.5 (v:v) into 6 simplified 1000-ml CSTR reactors with a working volume of 667 ml for each and incubated at 55, 60, 65, 70, 75 and 80°C, respectively. It was assumed that the anaerobic thermophiles that could benefit the hydrolysis of the organic polymers had already existed in the original inoculum or could be continuously introduced from the raw sludge, and therefore could be selectively enriched at the above-mentioned temperatures. All the reactors were run at RT of 3 days and manually fed with the mixed sludge once a day for a period of two months.

**The pathogen indicator organisms**

In order to examine the pathogen reduction effect under different operational conditions, *Fecal Streptococci* (FS) was used as indicator organism in this study. This was based on the fact that most pathogenic bacteria such as *Salmonella*, *Shigella*, *Listeria*, and most viruses and helminth ova are inactivated or looses their viability long time before FS has been eliminated (Jepsen et al., 1997; Bendixen, 1999). So, if this more resistant indicator organism has been destroyed, it can be regarded that the less resistant pathogenic organisms will have already been destroyed as well (Carrington, 2001). Because the concentration of viable cells in the influent sludge was found decreasing along with the storage time, the pure culture of *S. faecalis* (Strain 25v-2), a specie of FS genus (Dalgaard et al, 2003) provided by Danish Research Institute for Fisheries, was continuously spiked
into the influent sludge to keep the number of the indicator organism at the same level so that the values of pathogen reduction effect obtained at different operational conditions could be used for comparison.

**Start-up and operation of the pretreatment reactors**

Six 1000-ml CSTR reactors with a working volume of 667 ml for each, marked as R₁, R₂, R₃, R₄, R₅ and R₆, respectively, were run in two series: in Series I, R₁, R₂ and R₃ were run at 55, 60 and 65°C, respectively, and in Series II, R₄, R₅ and R₆ were run at 70, 75 and 80°C, respectively. Figure 1 shows the reactor setups. The reactors were inoculated with the cultures that had been prepared at corresponding temperatures, respectively. For each series of the reactors, RT was set at 3.0 days in the beginning and then gradually shifted to 2.5, 2.0, 1.5, 1.0 and 0.5 day in the stepwise order. To avoid the shortcut of fresh influent that might contaminate the effluent (Farrell *et al.*, 1988), the mode of influent feeding immediately after effluent withdrawing was adopted. The reactors were mixed intermittently for 30 second in each 30 minutes by 8mm-magnetic stirrers set at 300 rpm. Both the stirrers for the reactors and the stirrers for the influent bottles were switched on during the period when effluent withdrawing and influent feeding were proceeding so as to keep the homogeneity of the effluent and the influent. Since withdrawing/feeding frequency can greatly affect the pathogen reduction effect (Lee *et al.*, 1989; Huyard *et al.*, 2000), the several frequencies including once per hour, as it was in the wastewater treatment plant where the experimental sludge was sampled, was tested. A computer installed with Lab-view Software was used for the automatic operation of each series of
the reactors. At steady stage, successive sets of gaseous and liquid samples were taken from each of the reactors at reasonable and regular intervals.

**Analytical methods**

pH was measured by pH meter (Metrohm 744, Switzerland). TS, VS, TSS and VSS were determined directly according to standard methods (APHA, 1992).

For the determination of total COD, the standard method for COD measurement (APHA, 1992) was modified before use as previously described (Miron et al., 2000). For the determination of soluble COD (s-COD), the sludge sample was centrifuged at 13,000 RPM for 20 minutes and then the liquid fraction filtered by 0.45 μm membrane filter was used for analysis according to standard method (APHA, 1992).

For the determination of volatile fatty acids (VFA), 1ml of the sludge sample was mixed with 30 μl of 17% phosphoric acid (w/v) for decreasing the pH lower than 2 in Eppendorf tube and then centrifuged at 10,000 RPM for 10 minutes. The liquid fraction after filtration by glass fiber filter (Millipore, Ireland) was used for analysis by gas chromatograph (Hewlett Packard 5890-II, USA) according to the method described by Sørensen et al. (1991).

For the numeration of FS number, 0.1 ml the sludge sample or its decimal dilutions were sowed on the selective agar prepared according to Slanetz and Bartley (1957). The
characteristic red colonies were counted after incubation at 44°C for 48 hours. A suitable number of the colonies were verified by microscopy (Jepsen et al., 1997).

Biogas flow was quantified by liquid displacement technique using paraffin oil as liquid in a U-tube with a working volume of 10 ml. Concentrations of CH₄ and H₂ in the biogas were monitored according to the methods used by Sørensen et al. (1991) and Mladenovska and Ahring (1997), respectively.

**Calculations**

The following conversion factors were used during calculation:
- 1 mole CH₄ is equivalent to 64g COD;
- 1g of volatile fatty acids, such as acetic, propionic, butyric and valeric acids is equivalent to 1.07, 1.51, 1.86 and 1.04g COD, respectively.

CH₄ production:
For convenient comparison of how much s-COD had been lost to the gas phase (via VFA), CH₄ production was accounted as mg-COD/l-influent sludge:

$$CH_4_{COD} = (V_{CH_4} \times C_{CH_4}) / (22.436 \times 64)$$  
(Eq.1)

Where $V_{CH_4}$ is the volume of biogas produced by 1litter of influent sludge under standard conditions, ml/l-inf.;
$C_{CH_4}$ is the CH₄ concentration in biogas, %;

Increase of non-VFA part of soluble COD:
- $\Delta S_{COD,non-VFA} = (S_{COD,inf.} - S_{VFA,inf.}) - (S_{COD,eff.} - S_{VFA,eff.})$

Where $S_{COD,inf.}$ and $S_{COD,eff.}$ are the soluble COD concentrations in the influent and effluent sludge, respectively, mg-COD/l;
$S_{VFA,inf.}$ and $S_{VFA,eff.}$ are the VFA concentrations in the influent and effluent sludge, respectively, mg-COD/l.

Value of PRE:
- $PRE = \log_{10}(C_{FS,inf.}) - \log_{10}(C_{FS,eff.})$

Where $C_{FS,inf.}$ and $C_{FS,eff.}$ are *Fecal Streptococci* concentrations in the influent and effluent sludge samples, respectively, CFU/ml.
RESULTS AND DISCUSSION

Characterization of the sewage sludge

In this study, using of one collection of the sludge sample and saving it at -20°C did have kept the identity of the sludge characteristics during the whole experiment period except for the content of viable FS. By spiking the prepared pure culture of *S. faecalis* (Strain 25v-2), the number of FS in the influent sludge had been kept at a relatively constant level of $8.5 \times 10^3$ CFU/ml. The characteristic of the influent sludge is shown in Table 1.

Reactor stability during shift of RT

After the six CSTR reactors had been inoculated with the corresponding cultivated inoculum and started to run with a RT of 3.0 days at temperatures of 55, 60, 65, 70, 75 and 80°C, respectively, it took 2-3 weeks for all of the reactors to reach their steady-states characterized by stable pH value, concentration of s-COD, concentration and composition of VFA in the effluent, and gas production and composition. During the stepwise shifts of RT, variations in biogas production, VFA and dissolved COD concentrations were noticed during transition period. However, the variation was decreased less than 5% of the corresponding mean value measured after a period of about 3-8 of the corresponding RT.

Hydrolysis enhancement

*Production of s-COD.* Hydrolysis products were measured as s-COD. The concentrations of s-COD in the sludge after pre-treatment at different temperatures and RTs are shown in Table 2. It can be seen that the production of s-COD was affected by both temperature
and RT. For the same RT, the higher the temperature was, the higher s-COD concentration was obtained. For the temperature of 55°C, s-COD concentration increased along with the increase of RT from 0.5 to 1.0 day and then decreased from 1.0 to 3.0 days due to increased methanogenic activity. For the rest of the temperatures, the longer the RT was, the higher the s-COD was obtained. The lower s-COD concentration was obtained at 55°C and RT of 3 days, which was 5.37g/l; the highest s-COD concentration was obtained at temperature of 80°C and RT of 3 days, which was 14.09g/l. In comparison to the raw sludge, the highest increase in s-COD concentration was 320.6%, accounting for 33.7% of the influent solid COD.

It was though that pathogens in both primary sludge and waste activated sludge should be eliminated and in practice this should be conducted in one process to save equipment installation and operation, so mixed sludge was used in this study. In the other studies on pre-treatment only for sludge hydrolysis enhancement, primary sludge or waste activated sludge was studied individually. According to Miron et al.(2000) hydrolysis products could be increase by 13% if primary sludge was anaerobically pre-treated at 25°C for a RT of 3 days. At a higher temperature, 37°C, for the same RT, hydrolysis products could be increased by 17% (Eastman and Ferguson, 1981). Ghosh et al. (1995) reported that for waste activated sludge a 3.1-day pre-treatment at 49.8°C gave an increase of hydrolysis product about 20%. In comparison to these studies, thermophilic anaerobic was much more efficient.
Production of VFA and CH$_4$. Like in the other anaerobic digestion process, in our study it was found that hydrolysis was accompanied by acidogenesis and methanogenesis in the reactors for at all of the temperatures and RTs tested. Table 3 shows the VFA concentrations at different temperatures and RTs. It can be seen that for the same RT, with the only two exceptional cases that might be caused by error of analysis, VFA concentration increased along with the temperature elevation from 55°C to 70°C and then decreased from 70°C to 80°C. For the temperature of 55°C, VFA concentration increased along with the increase of RT from 0.5 to 1.5 day, and then decreased along with RT from 1.5 to 3.0 days. For the temperatures of 60, 65 70, 75 and 80°C, VFA concentrations increased along with the increase of RT. The production of VFA might come from the fermentation of the hydrolysis products or from the lysis of the microbial cells, especially from aerobe cells in the waste activated sludge, where VFA such as acetic acid was saved as metabolic intermediates. At temperatures higher than 70°C, VFA might come from the lysis of the microbial cells since biological activity on acidification was very low (Lu et al., 2006).

Since the existence of methanogenesis, hydrolysis products were lost (via acetic acid) to the gas phase. Table 4 shows the percentage of s-COD that had been lost. Since the growth of acetate-utilizing methanogens was naturally slow in comparison to thermophilic bacteria and it increases along with temperature and decreases rapidly at temperature higher 60°C (Madigan et al., 2003), for the same RT, the production of CH$_4$ decreased along with the elevation of temperature, and for the same temperature, it increased along with the increase of RT. When RT was set at 3.0 days, the highest CH$_4$
production was obtained at 55°C, which was 18.49 g-COD/l-influent sludge, accounting for 78.2% of the total s-COD generated by the pre-treatment. When temperature was set at 75°C and 80°C, the production of CH₄ was negligible. Besides CH₄ in the biogas, trace amount of H₂ (0.5 to 1.5%) was detected except at the temperatures of 55°C for RTs of 2.0, 2.5 and 3.0 days. The low H₂ concentration could be attributed to the high activity of hydrogen-utilizing methanogens at thermophilic temperatures, by which the hydrogen produced had been simultaneously converted into CH₄ (Madigan et al., 2003).

295 The mechanism of thermophilic anaerobic hydrolysis. It had been assumed that the enhancement of hydrolysis during thermophilic anaerobic pre-treatment could be the contributions of both thermal and biological effects. However, from the data obtained in this study, it was not possible to differentiate the biological effect and the thermal effect. The composition of organic solids in sewage sludge is very complicated and the biological and thermal effects on different compounds such as lipids, proteins and carbohydrates are different at different temperatures. A specific study on differentiating the these two effects was carried out in our lab showing that the hydrolysis of lipids and proteins depended more on the biological effect than on thermal effect, while the hydrolysis of carbohydrates depended more on thermal effect. Batch experiment demonstrated that the involvement of biological effect within 3 days could increase 33.6% and 31.5% of the hydrolysis efficiency at 60 and 70°C, respectively, and for 80°C, the biological effect was negligible (Lu et al., 2007).
As it is discussed in the beginning, during AD of sewage sludge, hydrolysis, instead of acidogenesis or methanogenesis, is normally the rate-limiting step of the whole AD process. As shown in Table 1, the concentration of VFA and d-COD in the influent sludge was 1.87g/l and 3.07g/l, respectively. The non-VFA part of d-COD was 1.29g/l. After pre-treatment, there was accumulation of non-VFA part of d-COD for all of the temperatures and RT tested, as shown in Table 5. This indicates that acidogenesis, instead of hydrolysis, was the rate-limiting step. This result was contrary to that obtained by Eastman and Ferguson (1981). In their study, they set the experimental temperature at 35°C and RTs at 9, 18, 36 and 72 hours, respectively. They found that the non-VFA part of d-COD for each RT were uniformly consumed for all of the reactor runs, so they concluded that hydrolysis was the rate-limiting step of the whole process in the acid-phase reactor. The difference between their finding and ours could be attributed to the fact that in our study both thermal effect and/or higher microbial activities at thermophilic temperatures could have helped the rate of hydrolysis process to overtake that of the acidogenesis process. Similarly, for all temperatures and RTs tested in this study, VFA concentration in the pre-treated sludge was increased in comparison to that in the influent sludge, indicating that methanogenesis was the rate-limiting step in stead of acidogenesis.

**Pathogen reduction effect**

The mechanism of temperature effect on pathogen reduction is that at a certain temperature for a long enough acting period critical protein of the pathogenic microorganism is inactivated by heat (Madigan, et al., 2001). When the reactor is semi-
continuously fed at constant feeding frequency, it is the feeding interval that determines
the real acting time. When RT is fixed, pathogen reduction effect decreases along with
the feeding frequency, exhibiting a first order kinetics (Lee, et al., 1989). But, when
feeding frequency is set constant, RT determines the amount of raw sludge fed into the
reactor each time, which will further affect the amplitude of temporary drop of
temperature in the reactor just after feeding (Huyard, et al., 2000) and the dilution rate of
the pathogenic microorganisms if they have not been inactivated during the interval of
feeding. Besides temperature, RT and frequency, the concentration of VFA in the reactor
is also reported to have contribution to inactivation of pathogens. According to the
studies carried out by Kunte, et al. (1998, 2000a, 2000b), higher log reduction of
pathogens such as *Salmonella typhi*, *Shigella dysenteria* and *Vibrio cholerae* was
obtained in the reactors with higher VFA concentration (5000-8000mg/l) than in the
reactors with lower VFA concentration (100-500mg/l). As mentioned above, the VFA
concentration was higher than 5000mg/l for most of the temperatures and RTs in our
study, so the contribution of high VFA concentration could take effect as well.

In this study, feeding frequencies such as 4, 6, 8, 12 and 24 times per day were tested for
different RTs. Our results confirmed that PRE value decreased along the feeding
frequency. Table 6 shows the PRE values for each of temperature and RT combinations,
i.e., when the frequency of withdrawing/feeding was set at 24 times per day. It can be
seen that, for the same temperature, longer RTs achieved better pathogen reduction
effects; and for the same RT, higher temperatures achieved better pathogen reduction
effects. The temperature and RT combination of 70°C and 2 days seems to be a key point,
at which FS was totally eliminated. So, to thoroughly eliminate FS, RT should be set at least 2.0 days at the temperature of 70°C, and for the temperatures of 75°C and 80°C the RT of 0.5 day was enough.

Determination of the most suitable temperature and RT for pre-treatment

As we have discussed in the beginning, the purpose of conducting pre-treatment under thermophilic anaerobic conditions was to obtain both enhanced hydrolysis and satisfactory pathogen reduction so that sewage sludge can be better stabilized, more biogas produced and the digested sludge recycled to the farmland without fear of epidemic diseases. So, the choice of temperature and RT should guarantee the elimination of pathogens and high concentration of s-COD. In addition, since the present of VFA in the influent can increase the microbial activity and the thus improve the performance of the next step AD reactor (Zhang and Noike, 1991; Skiadas et al., 2005; Lu and Ahring, 2006), higher VFA concentration in the pre-treated sludge is preferred. Besides, methanogenesis consumes VFA, so it should be avoided. And therefore, the hierarchy adopted here in determining the suitable temperature and RT for thermophilic anaerobic pre-treatment follows the order of a thorough elimination of pathogens, a higher s-COD concentration, a higher VFA concentration and finally a low s-COD loss to the gas phase.

Since the highest values for d-COD concentration, VFA concentration and pathogen reduction effect and lowest value for s-COD loss were not obtained at the same temperature and RT combination, only a range of the most suitable temperature and RT for thermophilic anaerobic pre-treatment was actually able to be determined from this
study. It can be seen that, for pathogen reduction, only temperature is higher than 70°C and RT longer than 2 day, or temperature higher than 75°C and RT longer than 0.5 days, can the indicator organism be totally eliminated. Relatively higher s-COD concentration (not lower than 85% of the highest value) can be obtained at temperatures of 65°C, 70°C, 75°C and 80°C when RT in the range of 2.5, 1.5, 1.0 and 0.5 to 3.0 days, respectively. Relatively high VFA concentration (not lower than 85% of the highest value) can be obtained in the temperature range of 60°C, 65°C and 70°C when RT was set in the range of 2.5, 2.5 and 2.0 to 3.0 days, respectively. To limit biogas production (lower than 10% of the total s-COD produced), at 55°C, 60°C and 65°C, RT should be shorter than 0.5, 2.0 and 2.5 day, respectively. Figure 2 shows the compromised boundary for choosing temperature and RT to conduct a satisfactory pre-treatment. It can be estimated from the data in Table 2 and 3, if temperature and RT is chosen within the boundary, s-COD concentration will be in the range of 12.1 to 14.1g/l, corresponding that 29.0% to 33.8% of the solids can be hydrolyzed in terms of COD, and 47.1% to 70.2% of the hydrolysis products are in the form of VFA.

In practice, the choice should keep temperature and RT values as low as possible so as to reduce the operation cost and to reduce the reactor volume, for example, setting temperature at 73°C and RT for 2.0 days, so that operation energy (heat) and reactor volume can be kept as low and small as possible.
CONCLUSIONS

In this study, thermophilic anaerobic pre-treatment at temperatures of 55, 60, 65, 70, 75 and 80°C, respectively, and for retention times of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 days, respectively, was examined. It was confirmed that thermophilic anaerobic pre-treatment is virtually a very promising pre-treatment method for hydrolysis and hygienization of sewage sludge. By properly manipulating the operational parameters, such as temperature, RT and feeding/withdrawing frequency, desired hydrolysis enhancement and pathogen reduction effect could be obtained simultaneously. Experimental results show that, when temperature was in the range of 70-75°C, and retention time in the range of 1.75-2.5 day with a feeding/withdrawing frequency of 24 times per day, about 29.0% to 33.8% of the organic solids in the sludge can be hydrolyzed in terms of COD, and 47.1% to 70.2% of the hydrolysis products are in the form of VFA. At the same time, the pathogen indicator microorganism, Faecal streptococci, could be totally eliminated.

ACKNOWLEDGEMENT

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REFERENCES


### TABLES

#### Table 1 Characteristics of the raw sludge

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Mean (SD, N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS</td>
<td>g/l</td>
<td>43.95 (1.09, 3)</td>
</tr>
<tr>
<td>TSS</td>
<td>g/l</td>
<td>40.05 (1.17, 3)</td>
</tr>
<tr>
<td>VS</td>
<td>g/l</td>
<td>30.21 (1.62, 3)</td>
</tr>
<tr>
<td>VSS</td>
<td>g/l</td>
<td>28.94 (1.96, 3)</td>
</tr>
<tr>
<td>Total COD</td>
<td>g/l</td>
<td>44.84 (1.85, 3)</td>
</tr>
<tr>
<td>Soluble COD</td>
<td>g/l</td>
<td>3.07 (0.29, 9)</td>
</tr>
<tr>
<td>VFA</td>
<td>g-COD/l</td>
<td>1.87 (0.19, 9)</td>
</tr>
<tr>
<td>pH</td>
<td>----</td>
<td>6.90 (0.07, 3)</td>
</tr>
<tr>
<td>FS</td>
<td>CFU × 10⁶/ml</td>
<td>8.5 (0.3, 34)</td>
</tr>
</tbody>
</table>

a. Standard deviation;  
b. Number of measurements.

#### Table 2 s-COD concentrations in the pre-treated sludge

<table>
<thead>
<tr>
<th>Retention time (day)</th>
<th>55</th>
<th>60</th>
<th>65</th>
<th>70</th>
<th>75</th>
<th>80</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>8627 ± 137 a</td>
<td>8758 ± 98</td>
<td>9512 ± 285</td>
<td>10512 ± 243</td>
<td>11925 ± 222</td>
<td>12425 ± 158</td>
</tr>
<tr>
<td>1.0</td>
<td>9059 ± 88</td>
<td>9686 ± 148</td>
<td>10651 ± 131</td>
<td>11619 ± 77</td>
<td>12726 ± 351</td>
<td>13033 ± 165</td>
</tr>
<tr>
<td>1.5</td>
<td>8109 ± 68</td>
<td>10407 ± 231</td>
<td>11240 ± 327</td>
<td>12051 ± 62</td>
<td>12920 ± 340</td>
<td>13503 ± 354</td>
</tr>
<tr>
<td>2.0</td>
<td>6931 ± 291</td>
<td>10976 ± 333</td>
<td>11699 ± 105</td>
<td>12395 ± 271</td>
<td>13146 ± 202</td>
<td>13678 ± 111</td>
</tr>
<tr>
<td>2.5</td>
<td>5415 ± 301</td>
<td>11441 ± 59</td>
<td>12083 ± 76</td>
<td>12920 ± 212</td>
<td>13416 ± 211</td>
<td>13954 ± 207</td>
</tr>
<tr>
<td>3.0</td>
<td>5367 ± 441</td>
<td>11911 ± 120</td>
<td>12334 ± 87</td>
<td>13333 ± 243</td>
<td>13572 ± 376</td>
<td>14085 ± 431</td>
</tr>
</tbody>
</table>

a. Values are the average of triplicates with standard deviation.
Table 3 VFA concentrations in the effluent sludge after pre-treatment at different temperatures for different RTs. Unit: mg-COD/l

<table>
<thead>
<tr>
<th>Retention time (day)</th>
<th>Temperature (°C)</th>
<th>Unit: mg-COD/l</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>55</td>
<td>60</td>
</tr>
<tr>
<td>0.5</td>
<td>5556 ± 5°</td>
<td>5446 ± 27</td>
</tr>
<tr>
<td>1.0</td>
<td>5572 ± 3</td>
<td>6257 ± 26</td>
</tr>
<tr>
<td>1.5</td>
<td>5716 ± 27</td>
<td>7260 ± 54</td>
</tr>
<tr>
<td>2.0</td>
<td>5276 ± 42</td>
<td>8407 ± 58</td>
</tr>
<tr>
<td>2.5</td>
<td>3707 ± 64</td>
<td>9144 ± 56</td>
</tr>
<tr>
<td>3.0</td>
<td>3218 ± 71</td>
<td>9716 ± 32</td>
</tr>
</tbody>
</table>

a. Values are the average of triplicate with standard deviation.

Table 4 Percentage of s-COD loss to gas phase (%)

<table>
<thead>
<tr>
<th>RT (day)</th>
<th>55°C</th>
<th>60°C</th>
<th>65°C</th>
<th>70°C</th>
<th>75°C</th>
<th>80°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>4.90</td>
<td>2.03</td>
<td>1.45</td>
<td>0.41</td>
<td>0.08</td>
<td>0.03</td>
</tr>
<tr>
<td>1.0</td>
<td>12.41</td>
<td>4.18</td>
<td>2.76</td>
<td>1.01</td>
<td>0.27</td>
<td>0.11</td>
</tr>
<tr>
<td>1.5</td>
<td>27.02</td>
<td>6.41</td>
<td>4.71</td>
<td>3.04</td>
<td>0.58</td>
<td>0.15</td>
</tr>
<tr>
<td>2.0</td>
<td>47.60</td>
<td>8.61</td>
<td>5.92</td>
<td>5.09</td>
<td>1.15</td>
<td>0.31</td>
</tr>
<tr>
<td>2.5</td>
<td>69.00</td>
<td>10.08</td>
<td>7.38</td>
<td>7.06</td>
<td>2.64</td>
<td>0.55</td>
</tr>
<tr>
<td>3.0</td>
<td>78.38</td>
<td>13.74</td>
<td>9.90</td>
<td>8.12</td>
<td>4.39</td>
<td>0.85</td>
</tr>
</tbody>
</table>
Table 5 Increase in Non-VFA d-COD concentration after pre-treatment

<table>
<thead>
<tr>
<th>Retention time (day)</th>
<th>Temperature (°C)</th>
<th>Unit: mg-COD/l</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>55</td>
<td>60</td>
</tr>
<tr>
<td>0.5</td>
<td>1780</td>
<td>2021</td>
</tr>
<tr>
<td>1.0</td>
<td>2196</td>
<td>2138</td>
</tr>
<tr>
<td>1.5</td>
<td>1102</td>
<td>1856</td>
</tr>
<tr>
<td>2.0</td>
<td>364</td>
<td>1278</td>
</tr>
<tr>
<td>2.5</td>
<td>417</td>
<td>1006</td>
</tr>
<tr>
<td>3.0</td>
<td>858</td>
<td>904</td>
</tr>
</tbody>
</table>

Table 6. Hygienic effects under different temperature and retention time conditions

<table>
<thead>
<tr>
<th>RT (day)</th>
<th>Temperature (°C)</th>
<th>Unit of FS concentration: CFU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>55</td>
<td>60</td>
</tr>
<tr>
<td>0.5</td>
<td>14300 ± 1100 (^a) (0.8) (^b)</td>
<td>750 ± 50 (2.1)</td>
</tr>
<tr>
<td>1.0</td>
<td>9500 ± 300 (1.0)</td>
<td>430 ± 20 (2.3)</td>
</tr>
<tr>
<td>1.5</td>
<td>4700 ± 200 (1.3)</td>
<td>110 ± 10 (2.9)</td>
</tr>
<tr>
<td>2.0</td>
<td>3200 ± 300 (1.4)</td>
<td>70 ± 12 (3.1)</td>
</tr>
<tr>
<td>2.5</td>
<td>2500 ± 300 (1.5)</td>
<td>70 ± 12 (3.1)</td>
</tr>
<tr>
<td>3.0</td>
<td>1800 ± 300 (1.7)</td>
<td>5 ± 1 (4.2)</td>
</tr>
</tbody>
</table>

\(^a\) Values are triplicates with standard deviation;  
\(^b\) Value in bracket is the decades reduction of FS;  
\(^c\) Decades reduction of FS equals to \(\infty\), meaning FS were totally eliminated.
**FIGURE LEGENDS**

Figure 1. Reactor set-ups for the continuous experiment.

Figure 2. The optimal temperature and RT boundary for thermophilic anaerobic treatment.

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**Figure 1**

1-Reactor; 2-Reactor stirrer; 3-Gas meter; 4-Water bath; 5-Influent pump; 6-Effluent pump
7-Liquid sampling port; 8-Gas sampling port; 9-Influent bottle; 10-Effluent bottle
11-Influent stirrer; 12-Gas buffering bag; 13-Computer control; 14-N2/CO2 gas bag

**Figure 2**

![Graph showing temperature and RT boundary boundaries for pathogen reduction, hydrolysis, VFA, biogas prevention, and compromisation.](image)
Re: Thermophilic anaerobic pre-treatment for hydrolysis and hygienization of sewage sludge

To Whom It May Concern:

Attached is our paper for possible publication in Journal of Environmental Engineering. Thank you for your attention, and I am looking forward to receiving your positive reply.

Sincerely yours,

Jingquan Lu

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