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

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Biological and thermal effects of thermophilic anaerobic pre-treatment on the hydrolysis of organic solids in sewage sludge

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ABSTRACT

Biological and thermal effects involved in thermophilic anaerobic pre-treatment of primary sludge and waste activated sludge were investigated at temperatures of 60°C, 70°C and 80°C, respectively. The study revealed that solubilization of lipids, proteins and carbohydrates were improved by both biological and thermal effects. However, the solubilization of lipids and proteins was more dependent on microbial impact while the solubilization of carbohydrates more dependent on thermal impact. At 60°C, 70°C when biological activity was high, significantly larger fractions of lipids and proteins were solubilized than at 80°C. Batch experiment for a period of 72 hours shown that, due to biological effect, for primary sludge, the production of d-COD could be increased by 49.5% and 48.3% at 60°C and 70°C, respectively; and for waste activated sludge, it could be increased by 50.7% and 46.0% at 60°C and 70°C, respectively. At 80°C, biological effect was negligible at 80°C, and the thermal hydrolysis of carbohydrates was the dominant mechanism of the primary sludge degradation, and the hydrolysis of both proteins and carbohydrates was the dominant mechanism for waste activated sludge, due to the release of the inner cellular material by thermal lysis of microbial cells.
Key words—biological, thermal, effect, thermophilic, anaerobic, pre-treatment, primary sludge, waste activated sludge, lipids, proteins, carbohydrates
Introduction

Organic material in sewage sludge, for both primary and waste activated sludge, is in the form of particulates, and this makes hydrolysis of these particulates the obstacle when anaerobic digestion (AD) process is employed for treatment (Ghosh et al., 1975; Eastman and Fergusson, 1981; Li and Noike, 1992). By using various kinds of pre-treatment methods, such as mechanical, thermal, chemical, biological and the combination of these methods, hydrolysis of the organic particulates can be enhanced and thus make the AD process more efficient both in organic material degradation and in biogas production (Müller, 2001).

In our previous study (Lu and Ahring, 2005), it has been found that thermophilic anaerobic pre-treatment, which is anaerobically conducted at the temperatures of 55-80°C for a short retention time not longer than 3.0 days, has a very promising feature in treating sewage sludge in a sustainable way. This is because this pre-treatment method, at the same time of enhancing hydrolysis as the other pre-treatment methods do, also carries out a significant pathogen reduction effect (PRE) that makes it possible to re-use the anaerobic digestate of sewage sludge as fertilizer and soil conditioner on the farmland without in fear of spreading diseases.

Although effects of high volatile fatty acids (VFA) concentration on the reduction pathogenic microbes such as Salmonella typhi, Shigella dysenteriae and Vibrio cholerae, have been reported (Kunte et al., 1998; 2000; 2004), PRE is normally regarded as the contribution mainly from thermal effect (Huyard et al., 2000; Carrington, 2001). Besides for the purpose of PRE, another reason for conducting the pre-treatment under thermophilic anaerobic conditions was to take full advantage of both thermal effect and biological effect on hydrolysis enhancement simultaneously (Lu and Ahring, 2005). Hiraoka et al. (1984) demonstrated that thermal pre-treatment at temperatures of 60-100 °C for a holding time within 2 hours was efficient for the hydrolysis of waste activated sludge and at higher temperatures from 120°C to 175°C thermal effect can be carried out at RTs as short as 30 minutes according to Li and Noike (1992). Biological effect for sludge treatment is by way of enzymes secreted by the microorganisms (Wiegel, 1991; Stetter, 1998). Since different microbes may have different optimal temperature for growth, the biological effect at different
temperatures can be different as well. In addition, methanogens are active at thermophilic temperatures (Kristjansson and Stetter, 1991; Ahring, 2003). Running the pre-treatment reactor with the presence of methanogenesis to remove H₂ may be in favor of more efficient hydrolysis of lipids and proteins (Novak and Carlson, 1970; Palenzuela-Rollon, 1999). However, to maintain the reactor to be a biological system rather than a simple thermal process, more sophisticated control system is needed to keep proper anaerobic environment, pH level, organic loading rate, feeding, and agitation and so on. The contributions of biological effect and thermal effect to the hydrolysis should be clarified in order to evaluate the advantages of thermophilic anaerobic pre-treatment over a simple thermal pre-treatment.

In this study, we used anaerobic cultures that had been enriched in and acclimatized to primary sludge and waste activated sludge, respectively, at thermophilic temperatures of 60°C, 70°C and 80°C, respectively, to determine how biological activity and thermal impact contribute to the solubilization of organic solids in primary sludge and waste activated sludge, and to investigate how hydrolysis of organic compounds such as lipids, proteins and carbohydrates are affected.

**MATERIAL AND METHODS**

**The sludge used in the study**

Both of the primary sludge and the waste activated sludge used in this study were obtained from Lundtofte Wastewater Treatment Plant, Lyngby, Denmark. Immediately after sampling, the sludges were dispensed into 500 ml plastic bags, and then stored at -20°C. Each day, a required portion of the sludge was thawed at room temperature and used in the experiments.

**Continuous reactor experiment**

Continuous reactor experiments were carried out in two series by employing six 1-litter completely stirred tank reactors (CSTR), the working volume of which was 800 ml for each. In Series I, three of the reactors running at 60°C, 70°C and 80°C, respectively, were fed with primary sludge as feedstock. In Series II, the other three reactors running also at 60°C, 70°C and 80°C, respectively, were fed with waste activated sludge as feedstock. The hydraulic retention time (HRT) of the reactors was set to be the same, i.e. 2.0 days, and the feeding frequency four times per day. The
reactors were intermittently mixed by magnetic stick and stirrer combinations at a frequency of 1 min per hour.

The inoculum used to start up the reactors was taken from an anaerobic reactor running at 73°C and with HRT of 2.0 days. Before the inoculum was taken, this reactor had been adapted to the mixture of the primary and secondary sludges as feed and reached the steady-state for more than one year.

Biogas flow was quantified by liquid displacement technique using paraffin oil as liquid in a 10 ml U-tube. The concentrations of CH₄ and H₂ in the biogas, and pH, VFA, COD, carbohydrates, lipids and proteins in the effluents were monitored since the start-up of the reactors. When the reactors reached their steady-states, three successive sets of sampling were carried out for the determination of the above-mentioned parameters.

**Batch experiment**

To compare efficiency of the thermophilic anaerobic pre-treatment (with both biological and thermal impacts) with that of the thermal pre-treatment (with only thermal impact) at identical temperatures in solubilizing the organic particulates in the sludge, batch experiments were carried out. The inoculum used in the batch setups were taken from the continuous reactors, respectively, under their steady-states. Beside the test vials, in which both inoculum and raw primary or waste activated sludge were contained, three kinds of controls were employed. To quantify the contributions of the biological impact coming from the inoculum and the raw sludge, Control 1 and Control 2 were involved, in which only inoculum and raw sludge was contained in the vials, respectively. To quantify the thermal impact when biological impact was inhibited, NaN₃ was dosed to the vials in Control 3 with a final concentration of 0.1% to prevent the biological activity of the anaerobes that had existed in the raw sludge (Slanetz and Bartley, 1957). Since the ratio of biomass to inoculum and the medium composition could affect the biodegradation test, the same amount of inoculum was used and the final volume in all of the control vials was adjusted to the same as the test vial by adding BA medium (Moreno et al., 1999; German, 2002). After pH had been adjusted to the respective inoculum pH and the headspace was degassed with N₂/CO₂, (20%/80%), the vials were closed with butyl
rubber stoppers and aluminum crimps, and finally put in the water bath that had been set at 60°C, 70°C and 80°C, respectively. The composition of batch setups is shown in Table 1.

To avoid these drawbacks such as adherence of the material to the vial wall, insufficiency of mixing during sampling and blockage of sampling needles, ‘multiple flasks’ method (Sanders, 2002) was followed. At each sampling time, three vials from each kind of the vial setups were taken out of the water bath and immediately quenched in a –20°C freezer. At the end of the experiment, all of the vials were taken out of the freezer and thawed at room temperature for immediate analysis.

**Analytical methods**

The content of total organic solids in the sludges was measured as volatile suspended solids (VSS) and COD, and lipids, proteins and carbohydrates were determined as the main organic compounds, as done in many of the other similar studies (Li & Noike, 1992; Miron et al., 2000; Mahmoud et al., 2004). Soluble COD and VFA were measured for the soluble components. Because there existed simultaneous acidogenesis and methanogenesis during the pre-treatment, biogas volume and the concentrations of CH₄ and H₂ were also measured.

To separate the solid and the soluble components in the sludge samples, the previously described method (Eastman and Ferguson, 1981) was improved. A 40-ml sludge sample was first centrifuged at 4,000 rev/min for 20 minutes to separate the bulk of the suspended solids. The supernatant was centrifuged again at 13,000 for 10 minutes. The second supernatant was then filtered by 0.45 μm membrane filter, and the filtrate was used for soluble COD and VFA analysis. All of the centrifugation- and filtration-captured solids were washed by distilled water for two times so that there was no soluble organic matter remained. This was done by re-suspending with distilled water and repeating the above-mentioned centrifugations and filtration (Hasegawa et al., 2000). The contents of VSS, COD, lipids, proteins and carbohydrates were determined for the finally obtained solid sample.
VSS, COD, lipids, Kjeldahl-N and ammonia-N were analyzed according to the standard methods (APHA, 1995). The content of proteins was obtained the difference of Kjeldahl-N and ammonia-N according to Mahmoud et al. (2004).

Since lipids, proteins and carbohydrates account for more than 95% of the total volatile suspended solids for both primary sludge and secondary sludge (Eastman and Ferguson, 1981; Elefsiniotis and Oldham, 1994; Hiraoka et al., 1984), the content of carbohydrates was estimated by subtracting the lipids and proteins from VSS.

For the measurements of VFA in the filtrate and CH\(_4\) and H\(_2\) concentrations in the biogas, methods as previously described (Sørensen et al., 1991; Mladenovska and Ahring, 1997) were followed.

**Conversion factors**

For simplification, methanogenic and acidogenic products were converted to COD basis according to the following factors:

- 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;
- 1 l CH\(_4\) (Standard T & P) is equivalent to 2.86g COD;
- 1 l H\(_2\) (Standard T & P) is equivalent to 0.71g COD.

The main organic components were converted to COD basis according to the following factors:

- 1g lipids is equivalent to 2.91g COD (Mahmoud et al., 2004)
- 1 g proteins (assumed as \((C_4H_6O_{12})_x\) is equivalent to 1.5g COD (Eastman and Ferguson, 1981)
- 1g carbohydrates (assumed as C\(_6\)H\(_{12}\)O\(_6\)) is equivalent to 1.07g COD (Miron et al., 2000)

**Calculations**

Solubilization rates of carbohydrates, lipids, proteins and VSS and the total soluble COD production for the continuous experiments were calculated by the following equations:
In the batch experiment, the production of soluble COD in the testing vials came from six parts: A. the biological hydrolysis of the biomass in the inoculum by the microbes in the inoculum; B. the biological hydrolysis of the biomass in the raw sludge by the microbes in the raw sludge; C. the biological hydrolysis of the biomass in the raw sludge by the microbes in the inoculum; D. the thermal hydrolysis of the biomass in the raw sludge; E. the biological hydrolysis of biomass in the inoculum by the microbes in the raw sludge, and F. the thermal hydrolysis of the biomass in the inoculum. Since the amount of thermally and biologically hydrolysable biomass in inoculum is much smaller than that in the raw sludge, and the microbial activity in raw sludge was much smaller than that in the inoculum, so Part E and Part F could be ignored. Part A could be obtained by Control 1. Part B could be obtained by subtracting Control 2 from Control 3. Finally, soluble COD produced from the biomass in the raw sludge by both biological and thermal effects (i.e. Part C and Part D, respectively) could be obtained by subtracting Part A and Part B from the soluble COD measured in the testing vial.

The total soluble COD yields with both biological and thermal impacts and with thermal effect only were calculated by the following equations:

\[ Y_{sCOD,bio+ther.} = (S_{COD,test,t} - S_{COD,cont-1,t})/M_{VSS,0} \]  
\[ Y_{sCOD,ther.} = (S_{COD,cont-2,t} - S_{COD,cont-1,0})/M_{VSS,0} \]  

The index “0” and “t” refers the influent and the effluent, respectively.
RESULTS AND DISCUSSION

Characteristics of the primary sludge and waste activated sludge

Table 2 shows the characteristics of the primary sludge and waste activated sludge used in this study. In primary sludge, the order of the component contents is carbohydrates>lipids>proteins. Carbohydrates were the dominant organic compound, accounting for 59.7% of the total VSS, while lipids and proteins had comparable contents, accounting for 22.1% and 18.1% of the total VSS, respectively. In waste activated sludge, the order is proteins>carbohydrates>lipids. Proteins are the dominant organic compound, accounting for 54.8% of the total VSS, and carbohydrates for 40.6%, while lipids are negligible, only accounting for less than 4.6% of the total VSS.

Continuous reactor experiment

*Hydrolysis, acidogenesis, methanogenesis and VSS solubilization*

After start-up, it took about two months for all of the continuous reactors to reach steady stage, indicated the stable biogas production and composition and stable concentrations of soluble COD and VFA. Properties of the pre-treatment of primary sludge waste active sludge during steady stage are summarized in Table 3 and 4, respectively.

Figure 1 depicts the production of CH$_4$, VFA and soluble COD and the solubilization rate of VSS as a function of temperature for primary sludge and secondary sludge, respectively. It can be seen that, even though the operation condition was not favorable for CH$_4$ production, 9.2% and 11.4% of the total solid COD was produced when the reactors were run at 60°C for primary sludge and waste activated sludge, respectively. At 70°C, the production was 6.2% and 8.5%, respectively. The production of CH$_4$ at 80°C was rather low, only account for 0.3 % and 0.4% for primary sludge and waste activated sludge, respectively. The decrease of CH$_4$ production indicates that methanogenesis activities decrease along with the increase of temperature in thermophilic temperature range.
Besides CH₄, trace amount of H₂ was also detectable in the 70°C reactor fed with primary sludge and in the 80°C reactor fed with waste activated sludge from time to time. Since the production only accounted for 0.2% of the total solid COD, it was ignored in calculating the solubilization of organic solids.

Similar to the production of CH₄, the production of VFA was also decreasing along with the increase of temperature for both primary sludge and waste activated sludge. At 80°C, the production was significantly lower than at 60°C and 70°C. Since VFA production in relation to the biological activities of fermentation and/or β oxidation (Novak and Carlson, 1970; McInernery, 1988), it can be concluded that at 80°C the activities of thermophilic bacteria for hydrolysis and acidogenesis were very low even though it had been expected.

For both primary sludge and waste activated sludge, the decrease of total soluble COD was not as sharp as the decrease of CH₄ and VFA. It seems that the decrease of biological activity did not seriously affect the production of soluble. The measured soluble COD in the pre-treated was actually increased along with the increase of temperature.

For primary sludge, the decrease of VSS solubilization was not parallel with the production of soluble COD. Especially at 80°C, the decrease of soluble COD production was much faster than the decrease of VSS even though soluble COD was from the VSS. This phenomenon was not shown for the waste activated sludge.

**Degradation of lipids, proteins and carbohydrates**

Organic compounds such as lipids, proteins and carbohydrates can be biologically degraded. Under anaerobic conditions, carbohydrates are hydrolyzed by cellulase or xylanase to simple sugars and subsequently fermented to volatile acids (Cohen, 1982). Proteins are hydrolyzed by protease to amino acids and further degraded to VFA either via anaerobic oxidation linked to hydrogen production or via fermentation according to Stickland reaction (McInerney, 1988). The former is dependent on the presence of hydrogen-utilizing methanogens while the latter is independent on the methanogenic activities in the anaerobic ecosystem (Nagase and Matsuo, 1982). The polymeric lipids, such as glycerol esters, are first hydrolyzed with the release of free...
fatty acids. Glycerol, galactose, choline and other non-fatty acid components released by the hydrolysis are fermented mainly to volatile fatty acids by the fermentative bacteria. Free fatty acids including oils and greases are further oxidized via oxidation to acetate or propionate by H$_2$-producing syntrophic bacteria (Bryant, 1979). The presence of H$_2$ indicated that the activity of H$_2$-utilizing methanogens was not high enough to convert the H$_2$ produced during fermentation of the hydrolysis products to CH$_4$.

As it can be seen in Figure 2, for both primary sludge and waste activated sludge, the degradation rate of carbohydrates, the theoretical COD potential of which is 1.07 g-COD/g-dw, increased along with the elevation of temperature, while the degradation of lipids and proteins, the COD potential of which is 2.88 g-COD/g-dw and 1.50 g-COD/g-dw, respectively, decreased along with the elevation of temperature. So, even though relatively less VSS reduction was obtained at lower temperatures, higher d-COD yields could still be obtained due to the degradation of compounds with high COD potential. It can be seen that the degradation of carbohydrates is more dependent on thermal impact than the degradation of proteins and lipids, while the degradation of proteins and lipids is more dependent on the microbial activities than that of carbohydrates. By using the individual measurements on the abovementioned organic compounds and their corresponding COD potential, the calculated VSS degradation rates and the d-COD yields for both primary sludge and waste activated sludge were obtained, which dovetail very well with the direct measurements as plotted in Figure 2.

It should be also noticed the decrease in the degradation rate of the proteins in primary sludge is much faster than in waste activated sludge, and the degradation rate at all temperatures are much lower than that in waste activated sludge. The degradation rate of the proteins in waste activated sludge at 80°C is even higher than that in primary sludge at 60°C. The VSS reduction rates at all the temperatures in waste activated sludge are much higher than those in primary sludge. All of these phenomena can be explained by the fact that the proteins in waste activated sludge were different from those in primary sludge. The microbial cells in waste activated sludge contained a large amount of easily degradable proteins. When the cells were lysed by heat and/or enzyme attack, large amount of easily degradable substance
including proteins was released, resulting higher degradation rate of proteins and higher VSS reduction rate.

**Differentiation of biological effect and thermal effect**

After calculation to omit the interferences from the biomass in inoculum and from the microbial activity in the raw sludge, the soluble COD yield from biological effect and thermal effect on the biomass of raw sludge in the test vial, indicated as inoculated sludge and the soluble COD yield from thermal effect in Control 3, indicated as inhibited sludge, were plotted in Figure 3. For primary sludge, it can be seen that due to the introduction of biological activities, the yield of soluble COD can be significantly increased by 49.5% and 48.3% at 60°C and 70°C, respectively, while at 80°C, due to negligible biological activity involved, the increase in d-COD yield is not so significant between the inoculated sludge and the inhibited sludge. To word this quantitively, during thermophilic anaerobic pre-treatment of primary sludge in batch at 60°C for a period of 72 hours, 33.1% of the hydrolysate was caused by biological activity and the rest, 69.9%, is caused by thermal effect. At 70°C for the same period, 32.6% is caused by biological activity, and 67.4% is caused by thermal effect. At 80°C, almost the entire soluble COD yield was from thermal effect.

For waste activated sludge, the increase of soluble COD yield due to biological activity was 50.7% and 46.0% for 60°C and 70°C, respectively. Also, it can be said that during thermophilic anaerobic pre-treatment of waste activated sludge in batch at 60°C for a period of 72 hours, 33.6% of the hydrolysate is caused by biological activity and the rest, 66.4%, was caused by thermal effect. At 70°C for the same period, 31.5% was caused by biological activity, and 68.5% is caused by thermal effect. At 80°C, almost the entire soluble COD yield was from thermal effect.

From Figure 3, it can be noticed that the yield of d-COD from waste activated sludge is higher than that from primary sludge at each of the corresponding temperatures, and at 80°C, the decrease in d-COD yield from waste activated sludge was not so significant compared with the counter part from primary sludge. These findings also prove that the additional mechanism, thermal lysis of microbial cell, did exist during thermophilic anaerobic pre-treatment of waste activated sludge, by which soluble or easily hydrolyzed substances were released from the microbial cells.
CONCLUSION

Thermophilic anaerobic pre-treatment is an efficient pre-treatment method. By running completely stirred tank reactors for a retention time of 2 day at 60 °C, 70 °C and 80 °C, respectively, and using primary sludge and waste activated sludge, respectively, it was confirmed that both biological and thermal effects contribute to the hydrolysis of organic particulates. For waste activated sludge, the additional mechanism, thermal lysis of microbial cell, was proved.

For the hydrolysis of different types of organic material, i.e. lipids, proteins and carbohydrates, their dependency on thermal effect and biological effect were different. The hydrolysis of lipids and proteins was more dependent on biological activity than the hydrolysis of carbohydrates, while the hydrolysis of carbohydrates was more dependent on thermal effect than lipids and proteins. For both of primary sludge and waste activated sludge, at 60 °C and 70 °C, i.e., at the temperatures of which microbial activity was high, larger percent of lipids and proteins were hydrolyzed than at 80 °C. For primary sludge, at 80 °C, the biological effect is negligible, and high percent of carbohydrates are hydrolyzed by thermal effect. For waste activated sludge, at 80 °C, thermal lysis of microbial cell was the dominant mechanism resulting in the high degradation rate of proteins released from the microbial cells.

In differentiating the contributions to hydrolysis from biological effect and thermal effect, batch experiment for a period of 72 hours shown that, for the pre-treatment of primary sludge, an increase of 49.5% and 48.3% of hydrolysis product could be obtained due to biological activity at 60 °C and 70 °C, respectively. At 60 °C, 33.1% of the hydrolysate was caused by biological activity and the rest, 69.9%, is caused by thermal effect. At 70 °C, 32.6% is caused by biological activity, and 67.4% is caused by thermal effect.

For the pre-treatment of waste activated sludge, an increase of 50.7% and 46.0% of hydrolysis product could be obtained due to biological activity at 60 °C and 70 °C, respectively. At 60 °C, 33.6% of the hydrolysate was caused by biological activity and
the rest, 66.4%, is caused by thermal effect. At 70°C, 31.5% is caused by biological activity, and 68.5% is caused by thermal effect.

At 80°C, for the pre-treatment of both primary sludge and waste activated sludge, almost all of the hydrolysis is caused by thermal effect. Biological effect is negligible. The weak biological effect might be due to the failure in enriching the microbes that can grow at this high temperature or might be the microbes were enriched, but with low activity at this high temperature. This temperature can be recommended as the upper limit for thermophilic anaerobic pre-treatment for sewage sludge.

ACKNOWLEDGEMENTS
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REFERENCE


### Table 1 Composition in the experimental batch set-ups

<table>
<thead>
<tr>
<th>Vials</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test vials</td>
<td>60 ml sludge + 20 ml Inocula</td>
</tr>
<tr>
<td>Control vials</td>
<td></td>
</tr>
<tr>
<td>Control 1</td>
<td>20 ml inocula + 60 ml BA medium</td>
</tr>
<tr>
<td>Control 2</td>
<td>60 ml sludge + 20 ml BA medium</td>
</tr>
<tr>
<td>Control 3</td>
<td>60 ml sludge + 20 ml BA medium + 0.1% NaN</td>
</tr>
</tbody>
</table>

### Table 2 Properties of the primary sludge and waste activated sludge

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Primary sludge</th>
<th>Waste activated sludge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Components in solid form</td>
<td></td>
<td>Primary sludge</td>
<td>Waste activated sludge</td>
</tr>
<tr>
<td>TSS</td>
<td>g/l</td>
<td>24.54 ± 0.22</td>
<td>17.06 ± 0.70</td>
</tr>
<tr>
<td>VSS</td>
<td>g/l</td>
<td>17.39 ± 0.17</td>
<td>9.61 ± 0.35</td>
</tr>
<tr>
<td>Lipids</td>
<td>g/l</td>
<td>3.85 ± 0.27</td>
<td>0.44 ± 0.10</td>
</tr>
<tr>
<td>Proteins</td>
<td>g/l</td>
<td>3.15 ± 0.06</td>
<td>5.27 ± 0.12</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>g/l</td>
<td>10.39 ± 0.21</td>
<td>1.46 ± 0.14</td>
</tr>
<tr>
<td>COD</td>
<td>g/l</td>
<td>27.91 ± 1.25</td>
<td>14.09 ± 0.86</td>
</tr>
<tr>
<td>Components in soluble form</td>
<td></td>
<td>Primary sludge</td>
<td>Waste activated sludge</td>
</tr>
<tr>
<td>COD</td>
<td>g/l</td>
<td>1.02 ± 0.03</td>
<td>0.64 ± 0.04</td>
</tr>
<tr>
<td>VFA</td>
<td>g/l</td>
<td>0.55 ± 0.01</td>
<td>0.23 ± 0.01</td>
</tr>
</tbody>
</table>

a. Values are average of three measurements with standard deviation
### Table 3 Properties of the primary sludge pre-treated at different temperatures

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Temperature of reactor (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>60</td>
</tr>
<tr>
<td>pH</td>
<td>----</td>
<td>6.53 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Biogas production</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume</td>
<td>ml/l-influent</td>
<td>2756 ± 120</td>
</tr>
<tr>
<td>H&lt;sub&gt;2&lt;/sub&gt; concentration</td>
<td>%</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CH&lt;sub&gt;4&lt;/sub&gt; concentration</td>
<td>%</td>
<td>32.51 ± 1.21</td>
</tr>
<tr>
<td>Components in soluble form</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soluble COD</td>
<td>g/l</td>
<td>7.72 ± 0.32</td>
</tr>
<tr>
<td>VFA</td>
<td>g/l</td>
<td>2.55 ± 0.05</td>
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<tr>
<td>Components in solid form</td>
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<td></td>
</tr>
<tr>
<td>VSS</td>
<td>g/l</td>
<td>12.15 ± 0.01</td>
</tr>
<tr>
<td>Lipids</td>
<td>g/l</td>
<td>2.96 ± 0.12</td>
</tr>
<tr>
<td>Proteins</td>
<td>g/l</td>
<td>2.15 ± 0.04</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>g/l</td>
<td>7.04 ± 0.12</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values are average of three measurements with standard deviation; <sup>b</sup> Not detectable.

### Table 4 Properties of the waste activated sludge pre-treated at different temperatures

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Temperature of reactor (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>60</td>
</tr>
<tr>
<td>pH</td>
<td>----</td>
<td>6.60 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Biogas production</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume</td>
<td>ml/l-influent</td>
<td>1538 ± 404</td>
</tr>
<tr>
<td>H&lt;sub&gt;2&lt;/sub&gt; concentration</td>
<td>%</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>CH&lt;sub&gt;4&lt;/sub&gt; concentration</td>
<td>%</td>
<td>36.66 ± 1.23</td>
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<tr>
<td>Components in soluble form</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soluble COD</td>
<td>g/l</td>
<td>4.12 ± 0.23</td>
</tr>
<tr>
<td>VFA</td>
<td>g/l</td>
<td>2.05 ± 0.00</td>
</tr>
<tr>
<td>Components in solid form</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VSS</td>
<td>g/l</td>
<td>5.96 ± 0.21</td>
</tr>
<tr>
<td>Lipids</td>
<td>g/l</td>
<td>0.33 ± 0.07</td>
</tr>
<tr>
<td>Proteins</td>
<td>g/l</td>
<td>2.51 ± 0.15</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>g/l</td>
<td>3.12 ± 0.07</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values are average of three measurements with standard deviation; <sup>b</sup> Not detectable.
FIGURE LEGENDS

Figure 1. Percentage of hydrolysis, acidogenesis and methanogenesis products (i.e. s-COD, VFA and CH₄, respectively) on account of total solid form COD in the influent sludge, and the solubilization rate of VSS after pre-treatment at different temperatures for primary sludge (a) and waste activated sludge (b). Methanogenesis products are included in acidogenesis products, and the acidogenesis products are included in hydrolysis products.

Figure 2. Solubilization rates of the main organic compounds in comparison with the production of total soluble COD for primary sludge (a) and waste activated sludge (b).

Figure 3. Comparison of pre-treatment with and without biological effects on soluble COD yield. Soluble COD converted to CH₄ was included. (a₁, a₂ and a₃ are for primary sludge at 60°C, 70°C and 80°C, respectively; b₁, b₂ and b₃ are for waste activated sludge at 60°C, 70°C and 80°C, respectively).
Figure 1

![Graph showing CH4, VFA, d-COD, and VSS against temperature.](image)

Figure 2

![Graph showing solubilization rate and COD concentration against temperature.](image)

Lipids

Proteins

Carbohydrates

Calculated soluble COD

Measured soluble COD
Figure 3

- □ - Inoculated sludge
- ○ - Inhibited sludge
June 5, 2007

Re: Biological and thermal effects of thermophilic anaerobic pre-treatment on the hydrolysis of organic solids in sewage sludge

To Whom It May Concern:

Attached is our paper for possible publication in Water Research. Thank you for your attention, and I am looking forward to receiving your positive reply.

Sincerely yours,

Jingquan Lu

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