Anaerobic digestion of sludge differing in inorganic solids content: performance comparison and the effect of inorganic suspended solids content on degradation
Nina Duan, Xiaohu Dai, Bin Dong and Lingling Dai

ABSTRACT
High inorganic suspended solids (ISS) content of sludge in many areas (especially with combined sewage systems) results in low VS/TS (volatile solids, VS; total solids, TS) levels and raises concerns about its effect on anaerobic digestion. The performances of sludge anaerobic digestion with different feeding VS/TS levels as well as the effect of ISS content on the anaerobic degradation process were investigated in completely stirred tank reactors by semi-continuous and batch experiments. In semi-continuous experiment with sludge at VS/TS of 61.4%, 45.0, 30.0% and 15.0%, biogas yield, VS reduction and methane content decreased logarithmically with the feeding VS/TS decreasing; slightly higher volatile fatty acid concentration was observed at VS/TS 15%. Results of the batch experiments suggested that acetogenesis and methanogenesis are obviously affected by high ISS addition, while hydrolysis is less affected. The retardment of substrate conversion rate is probably attributed to decreased mass transfer efficiency at high ISS content.

INTRODUCTION
In developed countries, anaerobic digestion has been widely used in the treatment of sewage sludge, especially in Europe. However, this technology has not been successfully implemented in China. For example, up to 2015, among the 3,000 wastewater treatment plants (WWTPs) in China, 60 were designed with anaerobic digestion systems, but only 15 of them were still in operation. The main problems coming up with the existing sludge anaerobic digesters are low efficiency of energy recovery and large amount of sands and grits accumulated in anaerobic digesters. Those two problems come from the characteristics of sewage sludge in China, which is featured with low VS/TS (volatile solids, VS; total solids, TS) levels and high inorganic suspended solids (ISS) content. In many areas, especially those in undeveloped or developing countries like China, VS content of sludge was much lower (usually 20–55% of TS) than that in developed countries (usually higher than 60%) due to high ISS influent to WWTPs, which may be attributed to the wide spread of existing combined sewage systems, development of infrastructure construction, inadequate function of grit chambers in WWTPs and so on.

In fact, it has already been found in anaerobic project cases of sewage sludge that VS reduction decreased with VS/TS value of the feeding sludge (Roediger et al. 1990). Also, from the data on VS reduction and biogas yield in the references listed in Table 1, a similar trend is obtained. There is hardly any research to explain the reasons for the decreased VS reduction and biogas yield with low organic (high ISS) sludge. Generally, several explanations are preferred. One holds that part of the organic matters may degrade during long-distance pipeline distribution, which would result in lower VS degradation in the following anaerobic digestion unit. Another explanation could be the difference in organic composition of the sludge. Another is that high concentrations of ISS enter the anaerobic digestion process with low-VS feeding sludge and interfere with the degradation process, which finally leads to lower VS reduction and biogas production. The actual reasons may be more than one of them, yet each one needs to be confirmed by scientific research.

Recently, high-solids anaerobic digestion of dewatered sludge has been proved feasible (Duan et al. 2012) and could be one viable option to improve organic loading rates and reduce sand accumulation (for increased TS and viscosity to prevent inorganic solid particles settling down)
in countries with low VS/TS sludge. If the low VS/TS dewatered sludge was collected and centrally processed in a high-solids anaerobic digester, ISS should be one possible factor influencing the performance of the system.

So far, research related to the influence of inorganic suspended particles on anaerobic digestion have mostly focused on inert packing materials (clay, activated carbon, and zeolite) and their positive effect on anaerobic digestion by immobilizing microorganisms (Hansen et al. 1998; Sawayama et al. 2004; Milán et al. 2005; Sasaki et al. 2011; Rajagopal et al. 2013; Jiménez et al. 2015). Neither the performance of anaerobic digestion of sludge with different VS/TS levels caused by ISS content nor the effect of non-adsorptive ISS has been investigated.

In this study, the performances of anaerobic digestion of sludge at different VS/TS levels were investigated by semi-continuous experiment. The effect of ISS on the anaerobic degradation process of sludge in batch experiment was analyzed by a modified Gompertz model (Zwietering et al. 1990; Lay 2000). The influence of ISS on each step (hydrolysis, acidification and methanation) during the conversion of substrate to methane was also explored.

**METHODS**

**Substrate and inocula**

Dewatered sewage sludge from Anting WWTP (Shanghai, China) was used as substrate for the present study. The dewatered sludge was stored at 2°C after sufficient mixing. Two batches of dewatered sludge were collected during the experiments. Sludge of Batch 1 was used as daily feeding substrate for continuous runs, while Batch 2 for substrate of batch runs, which will be described in detail later. The mesophilic seed sludge was collected from laboratory-scale anaerobic digesters previously operated. Characteristics of substrate and inocula are listed in Table 2.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>TS (%)</th>
<th>VS/TS (%)</th>
<th>TSS/TS (%)</th>
<th>VSS/VS (%)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dewatered sludge (Batch 1)</td>
<td>18.60 ± 0.03</td>
<td>61.44 ± 0.16</td>
<td>97.10 ± 0.21</td>
<td>95.92 ± 0.19</td>
<td>7.3 ± 0.1</td>
</tr>
<tr>
<td>Dewatered sludge (Batch 2)</td>
<td>25.41 ± 0.05</td>
<td>54.93 ± 0.12</td>
<td>98.24 ± 0.18</td>
<td>96.55 ± 0.27</td>
<td>7.1 ± 0.1</td>
</tr>
<tr>
<td>Inocula</td>
<td>14.73 ± 0.12</td>
<td>37.66 ± 0.23</td>
<td>–</td>
<td>–</td>
<td>7.8 ± 0.1</td>
</tr>
</tbody>
</table>

TSS, total suspended solids; VSS, volatile suspended solids; –, not determined.
Reactors and operation

Reactors with liquid working volume of 6.0 L and equipped with helix-type stirrers were used to provide sufficient mixing for substrates. The rotation speed was set at 0.5 s⁻¹ with 1 minute stirring and 19 minutes break continuously. A simplified drawing and photograph of the reactor can be found in our previous study (Dai et al. 2015).

For semi-continuous operation, 6.0 L seed sludge was added to each reactor on the first day of the experiment. Daily feeding was carried out by pushing substrate materials through the feeding piston, and daily draw-off by opening the discharge valve at the bottom. Biogas volumes were measured by wet gas meters every day. For batch operation, seed sludge and substrate with a total volume of 6.0 L were added to the reactor. Cumulative biogas production was recorded during the experiment.

Effect of feeding VS/TS on digestion performance of semi-continuous systems

Powdered SiO₂ (medium particle size: 2.03 ± 1.03 μm) and de-ionized water were used to adjust ISS content of the dewatered sludge collected in Batch 1. SiO₂ addition dosage was determined to simulate dewatered sludge with VS/TS levels of 15–61% which covered typical VS/TS levels in large-scale applications. Data on characteristics of the feeding substrate with different SiO₂ dosage and operation conditions for four reactors (R1–R4) are shown in Table 3. Performance data of this experiment were collected after each reactor had been operating in semi-continuous mode at 15-day SRT for 50 days (longer than 3 SRTs), when pseudo-steady state was obtained.

Effect of ISS addition on sludge degradation

Batch experiments were conducted to evaluate the effect of ISS content on sludge degradation. Dewatered sludge (1,500 g) collected in Batch 2, 3,000 g seed sludge and 1,500 g water were added to each of five reactors (numbered r1–r5). SiO₂ was added to r1–r5 with a dosage of 0, 300, 600, 900 and 1,500 g, respectively. Detailed information is shown in Table 4. Substrate concentration and ratio of substrate to inocula were the same in r1–r5, to eliminate their influence on degradation rate. It is important to note that, as the feeding substrate in this batch experiment, sludge collected in Batch 2 has lower VS content than that of Batch 1, which made it impossible to reproduce the same scale of VS/TS levels (15.0–61.4%) as in the semi-continuous experiment. However, in this batch experiment, ISS content in the reactors was 7–26%, which could cover the scale of that in the semi-continuous experiment (7–15%).

A modified Gompertz equation (Equation (1)) was used to estimate experimental parameters (Zwietering et al. 1990; Lay 2000).

\[
P_{CH₄}(t) = P_{max} \times \exp \left\{ -\exp \left[ \frac{R_{max}}{P_{max}} \times (\lambda - t) + 1 \right] \right\}
\]

where \(P_{CH₄}(t)\) is the cumulative methane production at time \(t\) (L), \(P_{max}\) is methane production potential (L), \(R_{max}\) is the maximum methane production rate (L/d) and \(\lambda\) is lag-phase time (d).

It is well known that sludge anaerobic digestion usually undergoes hydrolysis, acidification and methanation. To investigate effect of ISS content on the three steps, the activities of their representative enzymes including protease, acetate kinase (AK) and coenzyme F₄₂₀ were analyzed.

Effect of ISS on acetoclastic methanogenesis

As acetic acid was the main volatile fatty acid (VFA) and preferred substrate for methane production, a test for

<table>
<thead>
<tr>
<th>Parameters R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS of feeding substrate (%, w/w)</td>
<td>18.6</td>
<td>18.6</td>
<td>18.6</td>
</tr>
<tr>
<td>ISS of feeding substrate (%, w/w)</td>
<td>7</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>VS/TS of feeding substrate (%, w/w)</td>
<td>61.4</td>
<td>45.0</td>
<td>30.0</td>
</tr>
<tr>
<td>Organic loading rate (kg VS/(m³·d))</td>
<td>7.6</td>
<td>5.7</td>
<td>3.9</td>
</tr>
<tr>
<td>Solids retention time (d)</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
</tbody>
</table>
conversion rate was carried out by acetate addition to the digestate of reactors r1–r5 after batch experiment of sludge degradation. Sodium acetate corresponding to 1.8 g/L acetic acid in the digestate was added. After addition of sodium acetate, cumulative biogas production with degradation time was recorded.

**Effect of ISS content on mass transfer property**

Electrical conductivity of the digestate in r1–r5 was measured after the experiments described above. An extra experiment was conducted to qualitatively verify the effect of powdered SiO2 on the mass transfer property of electrolyte solutions. Powdered SiO2 was added to four beakers each with 500 mL NaCl solution (with the same NaCl concentration of 2.0 mol/L), respectively. The mass-based ratios of SiO2 to water were 0%, 7%, 16% and 26%, in accordance with the general ISS content scale in r1–r5. Then electrical conductivity was measured under the same mixing intensity.

**Analytical methods**

Parameters indicating digestion performance including methane content, VFA and VS/TS were measured according to our previous study (Duan et al. 2012).

Protease activity was determined as described in Ledoux & Lamy (1986). For determining the AK activity, 5 mL of fermentation mixture was taken out of the anaerobic fermentation reactor and then washed and resuspended in 10 mL of 100 mmol/L sodium phosphate buffer (pH 7.4). The suspension was sonicated at 20 kHz for 10 min to break down the cells of acidogenic bacteria and then centrifuged at 10,000 rpm and 4 °C for 20 min to remove the waste debris. The extracts were kept cold on ice before they were used for enzyme activity assays (Allen et al. 1964). Coenzyme F420 was assayed by spectrophotometric method (Delafontaine et al. 1986).

**Statistical analysis**

All assays were conducted in triplicate. The data were expressed as mean ± standard deviation of triple assays. An analysis of variance by SPSS (Statistical Package for the Social Science, version 19.0) was used to test the significance of the results and P < 0.05 was considered statistically significant.

**RESULTS AND DISCUSSION**

**Effect of feeding VS/TS on digestion performance of semi-continuous systems**

In this study, powdered SiO2 and de-ionized water were used to adjust ISS concentration of the dewatered sludge collected, which aimed to simulate dewatered sludge with different VS/TS levels but the same organic composition and property. For semi-continuous systems fed with sewage sludge of different VS/TS levels (15.0%, 30.0%, 45.0% and 61.2%), the variations in biogas yield, VS reduction, methane content, specific biogas production (SBP) rate (i.e., biogas production per VS removed) and VFA concentrations are shown in Figure 1.

As shown in Figure 1, biogas yield, VS reduction and methane content of the biogas all showed increasing trend with VS/TS of the substrate, while biogas production based on VS removed (SBP) did not exhibit significant variations with different VS/TS. As VS/TS decreased from 61% to 15%, biogas yield, VS reduction and methane content decreased 29%, 29% and 4%, respectively. The decrease of biogas yield and VS reduction indicated that ISS content of the feeding sludge may affect the degradation rate of organic matters, and interfere with biomass reproduction rate or substrate utilization rate. Great fluctuations of biogas yield were observed with VS/TS below 50% (indicated by error bars), which may be due to the influence of inorganic particles on mass transfer of intermediate products or biogas stripping from liquid to gas phase. Relatively higher concentrations of acetic acid at VS/TS of 15.0% and propionic acid at VS/TS of 30.0% and 15.0% are also observed in Figure 1. Slight accumulation of acetic acid and propionic acid at low VS/TS indicated that the methanogenesis process may be influenced by high ISS content. Relevance analysis by SPSS showed that biogas yield, VS reduction and methane content were strongly correlated with VS/TS level of the substrate (P0.05 = 0.000, 0.000 and 0.000, respectively). Regression analysis (Equation (2) and Equation (3)) showed that logarithmic relationships were fitted between performance parameters (biogas yield (Y) and VS reduction (VSr)) and VS/TS levels. The correlation of Y with VS/TS is relatively lower than that of VSr with VS/TS, which was probably due to the higher fluctuations of Y than VSr at low VS/TS values as shown by error bars in Figure 1 and scatter data in Figure 2. From the fitted curves shown in Figure 2, when VS/TS is 80%, biogas yield and VS reduction are
calculated to be 0.45 L/(L·d) and 45%, respectively. When VS/TS goes to 0%, biogas yield and VS reduction tend to be infinitesimal, which matches with the fact.

\[
Y = 0.107 + 0.078\ln(\frac{\text{VS}}{\text{TS}}) \quad R^2 = 0.854 \quad (2)
\]

\[
\text{VSr} = 5.649 + 9.035\ln(\frac{\text{VS}}{\text{TS}}) \quad R^2 = 0.941 \quad (3)
\]
In order to investigate the effect of ISS on the degradation process of sludge, batch experiments and enzyme activity tests were conducted and are discussed in the following sections.

**Effect of ISS addition on sludge degradation**

Characteristics of biogas production, VFA accumulation and enzyme activities during sludge degradation were investigated in the batch experiment. The cumulative methane yields based on added VS, acetic acid concentration, propionic acid concentration, and instantaneous biogas production speed in r1–r5 are shown in Figure 3. In accordance with the results in semi-continuous experiments, the accumulative methane yield decreases with ISS increasing, especially with ISS higher than 21% (VS/TS lower than 15%).

Table 5 presents the parameters estimated (based on added VS) by Gompertz equation. In this experiment, maximum methane production rate $R_{max}$ was negatively influenced by increasing ISS content. ISS content ranging from 7% to 26% showed no effect on lag-phase time $\lambda$ and slight negative effect on methane yield potential $P_{max}$. It indicated that, in the beginning of the degradation, when biomass in the seed sludge was sufficient for the existing easily biodegradable organic matters, the effect of ISS content was neglectable, showing no differences in lag-phase time $\lambda$. As degradation progressed and biomass increased, variations in $R_{max}$ showed negative correlation with ISS content, reflecting the influence of ISS on the interaction between organics and biomass, which finally led to slight drop in $P_{max}$. The result proved that the conversion speed of organic substrate was affected by ISS addition.

Acetic and propionic acid concentrations, as well as instantaneous biogas production speed, are also shown in Figure 3. Basically, acetic and propionic acids showed increasing trends with ISS content, while instantaneous biogas production rate showed decreasing trend with ISS content. The result is consistent with that in semi-continuous experiment, where biogas yield was lower and acetic and propionic acids were higher in high ISS (low VS/TS) systems. It seems from Figure 3 that the speed of VFA conversion was affected by ISS addition.

During the first couple of days, acids accumulated rapidly and the concentration increased with ISS content, from which it can be speculated that during the first few days methanogenesis was the rate-limiting step, and the process of organic matters converting to VFAs was much faster and thus hardly influenced by ISS addition. Generally, among the three typical steps of anaerobic digestion, the methanogenesis step requires a more rigid micro-environment and is more susceptible to influences of external factors. In contrast, hydrolysis and acidogenesis steps are done by fermentation bacteria, which are less vulnerable than methanogens.

During Day 2 to Day 8, acetic acid concentrations decreased with biogas production rates increasing, indicating that after the lag time (first couple of days), methanogen reproduction rate increased. Still, in reactors with higher ISS addition, acetic acid concentration was higher, while biogas production rate was lower. It should also be noticed that the peak value of biogas production rate decreased with ISS addition, and the time that peak values appeared lagged with higher ISS concentration as well, indicating the influence of ISS on acetic conversion. During this period,
While acetic acid rapidly converted to biogas, propionic acid concentration continued increasing. After Day 8, biogas was gradually produced from the remaining VFAs, especially propionic acid. Higher biogas production rate after Day 12 in r5 was attributed to its higher previous VFA accumulation.

It is well known that sludge anaerobic digestion usually undergoes hydrolysis, acidification and methanation. The activities of their representative enzymes, including protease, AK and coenzyme F420, were analyzed. Samples were collected during Day 5 to Day 8, when both VFA degradation and biogas production rates were kept at high levels. Results are shown as enzyme activity relative to that in r1 (with no extra ISS addition), in the form of the percentage that certain enzyme activity in r2–r5 accounted for that in r1, respectively (Figure 4).

From the results in Figure 4 it is obvious that enzyme activity representing each of the three steps is influenced by ISS addition. However, protease activity was obviously influenced only at high ISS addition (ISS > 21%, VS/TS < 15%), while the activities of AK and F420 decreased more obviously with ISS concentration, which were consistent with the analysis about the batch degradation process of sewage sludge. The result suggested that, during the degradation of sewage sludge, acetogenesis and methanogenesis are obviously affected by high ISS addition, while hydrolysis is less affected.

From the view of process kinetics, the acidogenesis is the quickest step of the anaerobic conversion of complex organic matter in liquid phase digestions. Thus, changes in acidogenic rate could hardly influence the biogas production rate. The slowest steps in anaerobic digestion with suspended or dissolved wastes are normally considered to be the hydrolysis of solids or the methanogenesis (Vavilin et al. 1996). Hydrolysis is normally rate-limiting if the
substrate is in particulate form (Vavilin et al. 1996). The first step in anaerobic biodegradation is the conversion of the complex waste (particulate and soluble polymers) into soluble products by extracellular enzymes. As extracellular enzymes are believed to be in excess (Hobson 1987; Batstone et al. 2002; Vavilin et al. 2008), activities of enzymes in the hydrolysis step are not vulnerable to external non-chemical influences. In the reactor with high ISS addition (ISS > 21%), the decrease of protease activity may indicate that inorganic solid particles occupied parts of the binding sites of extracellular enzymes and led to insufficient contact between organic particles and extracellular enzymes. Acetogenesis and methanogenesis are intracellular bio-reactions, and the decrease in enzyme activities of AK and F420 with ISS addition indicated the reduction in biomass. One possible reason is that high ISS concentration hinders the conversion rate of substrate and intermediate matter, which leads to lower microbial reproduction rate.

**Effect of ISS on acetoclastic methanogenesis**

Since acetoclastic methanogenesis generates most of the CH4 in anaerobic digestion, acetic acid is considered as the most important precursor of biogas. The effect of ISS content on the conversion rate of acetic acid to biogas was investigated, for further confirmation of the finding in batch degradation of sludge that methanogenesis step was hindered. Figure 5 shows the conversion of acetic acid in each reactor. In this experiment, ISS content < 21% (VS/TS > 5%) exhibited no significant differences on the total conversion of acetic acid, which was in accordance with the results in batch experiment of sludge degradation, whereas ISS increasing from 21% to 26% (VS/TS from 15% to 11%) led to significant decrease in conversion rate, represented by the slope in the data trend. Although the conversion of substrate during the methanation step was affected at high ISS (low VS/TS) levels, final biogas yields of acetic acid were not influenced obviously, which was in accordance with the semi-continuous performance shown in Figure 1 that no serious VFA accumulation was observed in the four reactors.

**Effect of ISS on mass transfer property**

As discussed above, during sludge degradation, the hydrolysis step was slightly influenced whereas acetogenesis and methanogenesis steps were obviously influenced by ISS addition. One possible reason could be the influence of ISS particles on the mass transfer property of the bulk liquid phase. To qualitatively identify the mass transfer property for digestion systems with different VS/TS, electrical conductivity of the digestate, representing the ion transfer property, was measured after the batch experiments of sludge degradation. At the same time, an extra experiment was conducted to qualitatively verify the effect of powdered SiO2 on the ion transfer property of electrolyte solutions. Figure 6 shows the electrical conductivity of the digestate and NaCl solutions.

The electrical conductivity of both the digestate and NaCl solutions showed decreasing trend with increasing content of ISS. Although the electrical conductivity data of the digestate could not represent the real mass transfer property, the data of NaCl solutions proved that, with the same
ion concentration and at the same mixing intensity, ISS particle did affect the iron transfer efficiency. Therefore, it is safe to assume that, apart from ions, mass transfer of other substances such as intermediate products may also be slightly retarded, which would further reduce the rates of substrate conversion and microbial reproduction and finally result in loss of biogas production and VS reduction performances.

It is worth noting that although similar trends of performance data with ISS were obtained in both semi-continuous and batch experiments, data of the former were more distinct, which is probably attributed to the difference in reproduction time (duration of experiments) for biomass in either mode of the experiment. In batch experiment, the initial biomass came from the inocula and is considered sufficient for the batch-fed substrate. It is also known that methanogens hold relatively longer generation time. In addition, substrate in the batch experiment only retains one degradation period. In this short period, the variation of biomass at different ISS content was not demonstrated obviously. As a consequence, the conversion performance of substrate by the biomass did not show great variations as well. However, in semi-continuous experiment (draw-off and feeding every day), after three sludge retention periods when the seed sludge was almost completely replaced by daily feeding substrate, both biomass and substrate reduction in R1–R4 obtained ISS-influenced steady state respectively, which was then obviously shown in performance parameters. It may also be understood that the relatively more distinct difference between different VS/TS groups in semi-continuous experiment reflected the slowed biomass production rate or substrate utilization rate at high ISS content.

CONCLUSIONS

The effect of ISS on anaerobic digestion in both batch and semi-continuous modes was investigated. In semi-continuous anaerobic digestion of sludge at VS/TS 61.4%, 45.0%, 30.0% and 15.0%, it was shown that biogas yield, VS reduction and methane content decreased logarithmically with VS/TS of the feeding sludge decreasing; slightly higher VFA concentration was observed at VS/TS 15%. Results of the batch experiments suggested that acetogenesis and methanogenesis are obviously affected by high ISS addition, whereas hydrolysis is less affected. The retardment of substrate conversion rate is probably attributed to decreased mass transfer efficiency at high ISS content.

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