Biogas production from thermophilic codigestion of air-dried rice straw and animal manure

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SUMMARY

In order to evaluate the effects of organic loading rate (OLR) on thermophilic codigestion of air-dried rice straw (RS) with pig manure (PM), cow manure (CM), and chicken manure (CHM), continuous bench experiments (40 L) were carried out at OLRs of 3.0, 3.6, 4.2, 4.8, 6.0, 8.0, and 12.0 kg VS/(m³ · d). Stable biogas production without inhibition by volatile fatty acids (VFA) or ammonia and foaming was achieved at OLRs of 3–12, 3–6, and 3–4.8 for the codigestions of RS + PM, RS + CM, and RS + CHM, respectively. Maximum average volumetric biogas production rates of 4.98, 2.64, and 2.03 m³/(m³ · d) were obtained at OLRs of 12, 6, and 4.8 kg VS/(m³ · d) for the codigestions of RS + PM, RS + CM, and RS + CHM. Foaming occurred at OLRs of 8 kg VS/(m³ · day) for the codigestion of RS + CM. The codigestion of RS + CHM was inhibited by the accumulation of ammonia instead of VFA when the OLR was ≥6 kg VS/(m³ · d). This study provided references for the engineering application of codigestion of RS and animal manure. Copyright © 2016 John Wiley & Sons, Ltd.

KEY WORDS

chicken manure; codigestion; cow manure; foaming; inhibition; pig manure; rice straw

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1. INTRODUCTION

Agricultural residues and animal manure are the two most important types of organic waste. China, which ranks first in the world in crop residue production, is estimated to produce over 800 million tons of crop residues per year [1]. In terms of the distribution of crop residues throughout China, rice straw (RS) is more popular in southern China than in other locations. As additional waste materials, the total production of animal manure from large-scale centralized farms is about 837 million tons/year of which pig manure (PM), cow manure (CM), and chicken manure (CHM) account for 208 mill., 382 mill., and 126.5 mill. tons, respectively [2].

Anaerobic digestion (AD) has been widely used to produce biogas from animal manure and corn silage. There are few biogas plants using air-dried RS as material, owing to the intrinsic characteristics of RS, including unbalanced nutritional properties (i.e. high carbon/nitrogen [C/N] ratios) and hard degradable lignocellulosic structure. For nutrition regulation, codigestion with animal manure is considered a more cost-effective method compared to the addition of nitrogen-containing chemical reagents, such as urea or ammonium bicarbonate. For hard hydrolysis properties, thermophilic condition can strengthen the hydrolysis of straw and increase biogas production. Thermophilic straw digestion has been shown to have 36% higher methane yield, with a 106% increase in the hydrolysis rate constant [3].

Thermophilic codigestion has been used to treat mixed materials. Zhang et al. investigated the influence of material ratio and initial pH on thermophilic anaerobic co-digestion of swine manure and maize stalk, and found that the maximum total biogas production was achieved at TS ration of 7:3 and initial pH 6.8 [4]. Sarker et al. conducted thermophilic co-digestion of Laminaria digitata and cattle manure and obtained maximum methane yield at condition of 41% L. digitata based on TS [5]. Astals et al. studied the process performance and digestate
stability of thermophilic co-digestion of PM and crude glycerol. The results showed that the specific biogas production of co-digestion of PM supplemented with 3% of glycerol, on weight basis, was 180% higher than that of monodigestion of PM [6]. Sharma et al. evaluated the effects of material ratio on thermophilic co-digestion of poultry litter and thin stillage. The results showed that 40% and 60% thin stillage increased methane production, while 80% thin stillage decreased stability of co-digestion [7]. However, there was no thermophilic codigestion of air-dried RS with animal manure. These codigestion studies have been carried out primarily using batch and bottle experiments. Continuous bench experiments may be able to simulate biogas production in engineering scale. Additionally, for engineering applications, the organic loading rate (OLR) is a key running parameter affecting the volumetric biogas production rate (VBPR).

In the present study, bench continuous thermophilic codigestions of RS plus PM, CM, or CHM were carried out at different OLRs. The digestion performance in terms of specific biogas production (SBP) and VBPR, and the process stability using pH, volatile fatty acids (VFA), ammonia, total alkalinity, and foaming as stability indicators were evaluated.

2. MATERIALS AND METHODS

2.1. Substrates and inoculum

RS was obtained from rural Guangzhou, China. The collected RS was chopped and ground into small particles that were less than 1 mm in size. Fresh PM, CM, and CHM were separately collected from their respective farms. Collection of animal manure was conducted in two sets for continuous digestion with OLRs of 3.0–4.8 and 6–12 kg VS/(m³·d). After removing visible bristles, the manure was stored at 4 °C.

The original inoculum was obtained from a mesophilic anaerobic digester fed with PM. The thermophilic inoculum was obtained by gradually increasing the temperature of the original inoculum from 37 °C to 55 °C and adding PM for 30 days. This inoculum was screened by 1-mm sieve before using in order to remove large particles.

2.2. Experimental setup and design

Continuous AD was conducted in a bench-scale reactor with a total volume of 40 L (Figure 1). The reactor contained five ports for various purposes such as online pH monitoring, mechanical agitation, temperature control, and gas and liquid sampling. To avoid biogas outlet tube blockage from AD foaming, the working volume was restricted to 30 L. AD was carried out at 55 ± 2 °C, and the contents of the reactor were mixed six times per day at 80 rpm for 30 min.

According to the previous study on optimization of raw material ratio [8,9], the mixed substrates with a fixed volatile solid (VS) ratio of 1:1 (RS + PM, RS + CM, and RS + CHM) were added to the reactor every day in the continuous bench experiment. Anaerobic codigestion was carried out at OLRs of 3.0, 3.6, 4.2, 4.8, 6.0, 8.0, and 12.0 kg VS/(m³·d). The daily substrate feed amounts for different OLRs are listed in Table II.

2.3. Analytical methods

TS, VS, organic carbon, total Kjeldahl nitrogen (TKN), and total ammonium nitrogen (TAN) analysis for raw material were determined using standard techniques [10]. The pH was determined using a pHS-3C pH meter (Shanghai Precision & Scientific Instrument Co., Ltd., China). Biogas production was estimated using an LML-1 wet gas meter (Changchun Automobile Filter Co., Ltd, China). Biogas composition analysis was performed using an Agilent 6890 gas chromatography (GC) system (Agilent Technologies, Santa Clara, CA, USA) with a thermal conductivity detector and a 2-m stainless steel column packed with Porapak Q (50/80 mesh). The operating temperatures at the injection port, column oven, and detector were 100 °C, 70 °C, and 150 °C, respectively. Argon at a flow rate of 30 mL/min was used as the carrier gas.

Liquid samples were centrifuged at 12 000 rpm for 10 min at room temperature and filtered with a 0.45-μm membrane filter for analyzing the ammonia, total alkalinity, VFA, and soluble chemical oxygen demand (SCOD). The total ammonium nitrogen (TAN) was determined

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Table I. Main characteristics of the substrates and inoculum.

| Characteristic | Inoculum | Rice straw | Pig manure | Cow manure | Chicken manure |
|---------------|----------|------------|------------|------------|----------------|
|                | 3–12     | 3–4.8      | 6–12       | 3–4.8      | 6–12           |
| OLR (kg VS/m³·d) | 42.8     | 937.2 ± 21.1 | 334.3 ± 12.4 | 254.8 ± 8.5 | 125.4 ± 4.6 |
|                | VS (g/kg TS) | 690.0     | 952.6 ± 23.6 | 703.6 ± 21.6 | 710.8 ± 23.9 |
| Organic carbon (g/kg VS) | NA       | 400.9 ± 11.2 | 558.4 ± 18.9 | 482.7 ± 15.5 | 498.8 ± 16.9 |
| TKN (g/kg VS) | NA       | 8.4 ± 0.3  | 47.9 ± 1.7  | 41.2 ± 1.2  | 36.6 ± 1.3  |
| C/N           | NA       | 47.7 ± 1.6 | 11.7 ± 0.5  | 11.7 ± 0.4  | 13.6 ± 0.5  |
| TAN (g/kg VS) | NA       | 7.5 ± 0.3  | 7.1 ± 0.2   | 8.1 ± 0.3   | 8.6 ± 0.4   |

Note: NA, no analysis.
using an FC-100 ammonia analyzer (Shanghai Super Info. Tech. Co. Ltd., China) with an electrochemical sensor. Total alkalinity was determined using the titration method with Bromocresol green-methyl red as the indicator [11]. The concentration of SCOD was determined using a DRB200 digestion device and DR2700 spectrophotometer (Hach Company, Loveland, CO, USA).

Concentrations of VFAs (including acetate, propionate, butyrate, iso-butyrate, valerate, and iso-valerate) in the supernatant were measured after acidification with 6 mol/L of HCl using the Agilent 6820 GC equipped with a flame ionization detector and an Agilent DB-FFAP 30 m × 0.25 mm × 0.25 μm capillary column. The temperatures at the injection port and detector were 250 °C and 300 °C, respectively. The initial temperature of the column oven was maintained at 50 °C for 5 min, after which the temperature was increased at a rate of 10 °C/min to a final temperature of 250 °C, which was maintained for

| OLR (kg VS/ (m³ d)) | Daily feed amount of RS + PM (g) | Daily feed amount of RS + CM (g) | Daily feed amount of RS + CHM (g) |
|--------------------|-------------------------------|-------------------------------|-------------------------------|
|                   | RS   | PM    | Water | RS   | CM    | Water | RS   | CHM    | Water |
| 3                  | 54   | 192   | 1354  | 54   | 446   | 1100  | 54   | 245    | 1301  |
| 3.6                | 65   | 230   | 1305  | 65   | 535   | 1000  | 65   | 294    | 1241  |
| 4.2                | 76   | 268   | 1256  | 76   | 624   | 900   | 76   | 350    | 1174  |
| 4.8                | 87   | 306   | 1207  | 87   | 713   | 800   | 87   | 400    | 1113  |
| 6                  | 108  | 480   | 1012  | 108  | 732   | 760   | 108  | 442    | 1050  |
| 8                  | 146  | 642   | 812   | 146  | 909   | 545   | 146  | 588    | 866   |
| 12                 | 216  | 994   | 390   | 216  | 1364  | 20    | 216  | 882    | 502   |
5 min. Nitrogen at a flow rate of 1 mL/min was used as the carrier gas.

2.4. Calculation methods

Free ammonia (FAN) and free VFA (FVFA) were calculated using Eqs. (1) and (2) [12]:

\[
[H_A] = \frac{C_T[H^+]}{K_A} \frac{[H^+]}{[C_{138}]} \quad (1)
\]

\[
[NH_3] = \frac{K_B C_{Total}}{K_B} \frac{[H^+]}{[C_{138}]} \quad (2)
\]

where \([H_A]\) and \([NH_3]\) are the concentrations (mg/L) of FVFA and FAN, respectively; \(C_T\) and \(C_{Total}\) are the concentrations (mg/L) of total VFA (TVFA) and total ammonia (TAN), respectively; \([H^+]\) is the hydrogen ion concentration (mol/L); and \(K_A\) and \(K_B\) are the dissociation equilibrium constants for VFA and ammonia, respectively.

The proportion of FVFA depended on pH rather than the temperature because \(K_A\) was negligible over the range from 0 to 60 °C. The proportion of FAN depended on both pH and temperature because temperature had a significant effect on \(K_B\), with a higher temperature resulting in a higher proportion of FAN. The \(pK_A\) and \(pK_B\) were 4.8 and 8.42, respectively, at 55 °C [12].

The experimental and fitted data were analyzed and plotted using Origin Lab software version 7.5 (OriginLab, Northampton, MA, USA).

3. RESULTS AND DISCUSSION

3.1. Effects of OLR on biogas production by codigestion

SBP and VBPR are two important indices used to evaluate the performance of AD systems. SBP is an indicator of raw material utilization efficiency, whereas VBPR is an indicator of the reactor efficiency. Figure 2 shows the values of SBP and VBPR at various OLRs for the codigestion of RS plus PM, CM, or CHM.

For the codigestion of RS and PM, when the OLR ranged from 3 to 12 kg VS/(m³·d), stable biogas production was achieved, with an SBP of 350–500 L/kg VS and methane content of 52%–57%. There was no relation between OLR and SBP with average SBP of 434.2 L/kg.
VS. This result was different from the previous. Xie et al. have observed a decreasing of SBP along with increasing OLR in their study, where the mesophilic continuous codigestion of separated solid fraction of PM and dried grass silage with VS ratio of 6:4 was conducted with OLR of 1, 1.5, 2, and 3 kg VS/(m³·d) [13]. The VBPR increased with increasing OLR, and a maximum average VBPR of 4.98 m³/(m³·d) was obtained at an OLR of 12 kg VS/(m³·d).

For the codigestion of RS and CM, when the OLR ranged from 3 to 6 kg VS/(m³·d), stable biogas production was achieved, with an SBP of 400–550 L/kg VS. When the OLR was 8 kg VS/(m³·d), serious foaming occurred, resulting in decreases in the SBP and VBPR. The whole reactor was occupied by inflated sludge, and the biogas outlet tube was clogged. On day 63, feeding was stopped, and the reactor was cleaned. Feeding was restarted on day 64, with an OLR of 8 kg VS/(m³·d). After restarting the feeding, biogas production gradually recovered, and the maximum average VBPR of 5.26 m³/(m³·d) was obtained at an OLR of 12 kg VS/(m³·d). The average SBP and methane content were 455.4 L/kg VS and 50%–53%, respectively, for the entire process. Comino et al. have conducted a similar study on mesophilic continuous co-digestion of CM and crop silage with OLR of 4.45, 5.15 and 7.78 kg VS/(m³·d) [14]. The stable biogas production was observed in OLR of 4.45 and 5.15 kg VS/(m³·d), while the failure of biogas production with methane content of 39.9% was found in OLR of 7.78 kg VS/(m³·d). The SBP, VBPR, and methane content were 464.9 L/kg VS, 2.06 m³/(m³·d), and 51.1% for OLR of 4.45 kg VS/(m³·d), and they were 477.2 L/kg VS, 2.46 m³/(m³·d), and 52.2% for OLR of 5.15 kg VS/(m³·d).

For the codigestion of RS and CHM, when the OLR ranged from 3 to 4.8 kg VS/(m³·d), stable biogas production was achieved with a fluctuant SBP of about 450 L/kg VS. An VBPR of 1.37 and 1.54 m³/(m³·d) was obtained in OLR of 3.0 and 3.6 kg VS/(m³·d), which was higher than the other result. Yaldiz et al. have carried out a study on mesophilic continuous co-digestion of CHM and plant waste with VS ratio of 1:1. An VBPR of 1.05 m³/(m³·d) was reached at OLR of 3.4 kg VS/(m³·d). The raise of VBPR could be because of the thermophilic condition.

When the OLR increased to 6 kg VS/(m³·d), SBP continued to decline and TAN concentration sharply increased. When the OLR increased from 3 to 6 kg VS/(m³·d), the average methane content was decreased from 53% to 47%. However, there was no AD foaming and increasing of VFA concentration. These results suggested that the anaerobic codigestion process was slightly overloaded. In order to mitigate the inhibition of methanogens, feeding was stopped on day 55, and 5 L of material in the reactor was replaced with 5 L of water. Four days later, feeding was restarted at an OLR of 6 kg VS/(m³·d).

When the OLR ranged from 6 to 8 kg VS/(m³·d), the SBP increased from the lowest value (i.e., 381 L/kg VS) to the highest value (621 L/kg VS), and the average methane content increased to 50.7%. When the OLR increased to 12 kg VS/(m³·d), SBP decreased to the lowest value of 274 L/kg VS after a brief fluctuation. Besides SBP decreasing, further accumulation of VFA and TAN (Figure 3) and the obvious AD foaming were observed. These results suggested that the anaerobic codigestion process was severely overloaded.

3.2. Effects of OLR on the production of intermediate metabolites during codigestion

VFA and ammonia are key intermediate metabolites produced during AD. VFA, including acetate, propionate, butyrate, iso-butyrate, valerate, and iso-valerate, are products of hydrolysis and acidogenesis and substrates for methanogenesis. A stable VFA concentration indicates a correct balance between hydrolysis/acidogenesis and methanogenesis. For stable AD, the VFA concentration stabilizes at a lower value because the VFA produced from hydrolysis and acidogenesis can be consumed by methanogens over time. Excessive accumulation of VFA inhibits methane production.

Figure 3a shows the results of VFA concentration analysis during the codigestion. For the codigestion of RS and PM, acetate was the primary intermediate metabolite, with a total VFA concentration below 150 mg/L for an OLR of 3–12 kg VS/(m³·d). Similar results were obtained for the codigestion of RS and CM. For the codigestion of RS and CHM, acetate and propionate were the primary intermediate metabolites, with a total VFA concentration between 916 and 1110 mg/L for an OLR of 3–6 kg VS/(m³·d). When the OLRS were increased to 8 and 12 kg VS/(m³·d), the total VFA concentrations were increased to 3458 and 4478 mg/L, with propionate concentrations of 447 and 1632 mg/L, respectively.

Dogan et al. [15] reported that roughly 50% and 100% inhibition occur at acetate concentrations of 13 000 and 25 000 mg/L, butyrate concentrations of 15 000 and 25 000 mg/L, and propionate concentrations of 3500 and 5000 mg/L, respectively. However, other studies have shown much lower concentrations of propionic acid (900–1500 mg/L) that may cause inhibition of methane production [16,17]. This contradiction was because of the experiments were carried out under different process conditions, especially under different pH. Because the real inhibitor is free VFA, the inhibition of VFA on methane production would be discussed in section 3.3.

Ammonia originates from the degradation of proteins, peptides, and amino acids. Ammonia is an important nitrogen source for the growth of biogas-producing microorganisms and is a key pH-stabilizing agent for the neutralization of VFA. However, high concentrations of ammonia can inhibit methanogens. Analysis of the ammonia concentration is shown in Figure 3b.

For the codigestion of RS and PM, a quadratic polynomial equation could be used to simulate the change in TAN concentration. During the low OLR of 3 and 3.6 kg VS/(m³·d), the fed ammonia was less than discharged ammonia, resulting in a decrease of ammonia concentration.
in reactor. While during the high OLR of 4.2–12 kg VS/(m³ · d), the feeded ammonia was more than discharged ammonia, resulting in an increase of ammonia concentration in reactor. The maximum ammonia concentration was 1622 mg/L, which was much lower than the inhibition level of 3860 mg/L [18]. Similar results were obtained for the codigestion of RS and CM.

For the codigestion of RS and CHM, during the first stage of 0–54 days, the TAN concentration increased with increasing OLR. A positive linear equation could be used to predict the change in TAN concentration. There was no decrease of ammonia concentration in reactor during the low OLR of 3 and 3.6 kg VS/(m³ · d), which was different from the case of codigestion of RS and PM. This was because that the TAN of CHM was much higher than that of PM. The feeded ammonia was more than discharged ammonia, resulting in a increase of ammonia concentration in reactor. 

Similar results were obtained during the second stage of 59–82 days. The maximum ammonia concentrations were 5171 and 4804 mg/L for the first and second stages, respectively. These concentrations were much greater than the inhibition level of 3860 mg/L [18]. Therefore, we concluded that the AD system of RS and CHM with OLRs of 6 and 12 kg VS/(m³ · d) was inhibited by the accumulation of ammonia instead of VFA.

Figure 3c shows the results of the effluent SCOD concentration analysis. The effluent SCOD concentrations increased with increasing OLRs for codigestion of three types of mixed substrates. The maximum effluent SCOD concentrations of 10 320 and 12 180 mg/L were obtained at an OLR of 12 kg VS/(m³ · d) for codigestion of RS + PM and RS + CM, respectively. Because of the inhibition by accumulated ammonia, the maximum effluent SCODs of 22 580 and 33 000 mg/L were obtained during the first and second stages, respectively, for codigestion of RS + CHM.

3.3. Effects of OLR on inhibition during codigestion

The pH is typically used as an indicator of the health of an AD system. When the pH ranges from 6.8 to 8.0, the system is considered normal. In this study, the pH stabilized at 7.2–
7.7 for OLRs of 3–12 kg VS/(m³·d) for the codigestion of RS + PM and RS + CM (Figure 4a), while it stabilized at 7.6–8.0 for the codigestion of RS + CHM. According Figure 3(a), Figure 3(b), and Figure 4(a), it could be concluded that, for codigestion of RS + PM and RS + CM, pH was controlled by ammonia concentration instead of low VFA concentrations, while for codigestion of RS + CHM, pH is synergetic controlled by ammonia and VFA concentrations.

The pH of the AD system was controlled using a buffer system, and total alkalinity was used to quantify the buffering capacity. The variations in total alkalinity were similar to variation in TAN concentrations because ammonia was the primary alkaline substance (Figure 4b). The ratio of VFA to total alkalinity can be used to determine the stability of AD systems [19]. When the value is less than 0.4, the system is considered stable; in contrast, values between 0.4 and 0.8 indicate that the system is unstable, and values greater than 0.8 indicate that the system is severely unstable. In this study, the ratio of VFA to total alkalinity was less than 0.05 during the entire digestion process for the codigestions of RS + PM and RS + CM. For the codigestion of RS + CHM, the ratios of VFA to total alkalinity at OLRs of 8–12 kg VS/(m³·d) were greater than 0.4. These results indicated that the AD system was prone to failure at OLRs of 8–12 kg VS/(m³·d).

Compared with ionized ammonia and VFA, FAN and FVFA have been suggested to be the main causes of inhibition because they are freely membrane permeant [20]. The hydrophobic FAN and FVFA molecules may diffuse passively into cells, causing proton imbalances and/or potassium deficiency [21]. Inhibition of methanogens by FVFA is stronger than that of acidogens; the concentration at which inhibition of acidogens occurs is 2400–3000 mg/L, while that for methanogens is 30–60 mg/L [20,22]. FAN mainly inhibits methanogens, particularly acetoclastic methanogens, but does not have a significant effect on hydrogenotrophic methanogens or acidogens [23]. Such inhibition is reversible and adaptive; it can be relieved by diluting the digested liquid or by acclimatizing the methanogens. Moreover, temperature also has a great influence on FAN inhibition. Table III lists the reported half-maximal inhibitory concentrations (IC₅₀) of TAN and FAN under different digestion conditions.

Based on Eqs. (1) and (2) and the information presented above, a diagram of the inhibition of aerobic methane production by VFA and ammonia at 55 °C is shown in Figure 5. For an acclimated thermophilic AD system, the inhibition thresholds (IC₅₀ values) of FVFA (60 mg/L) and FAN (700 mg/L) were adopted in this diagram. In this study, the maximum FVFA concentrations were all below the inhibition thresholds for codigestions of RS + PM, RS + CM, and RS + CHM. With regard to FAN, the maximum FAN concentrations were below the inhibition thresholds for codigestions of RS + PM and RS + CM. However, these concentrations were higher than the inhibition thresholds for two

![Figure 4](image-url)
AD foaming is another important factor that affects the stability of an anaerobic digester [29]. AD foaming dramatically reduces the effective volume of the digester and can clog the biogas outlet tube, further reducing biogas production. In this study, AD foaming was observed through Plexiglas. There was no AD foaming during the entire digestion process for the codigestion of RS + PM. AD foaming was observed at OLRs of 8 kg VS/(m³ · d) for the codigestion of RS + CM, while it was observed at OLRs of 12 kg VS/(m³ · d) for codigestion of RS + CHM.

The causes of anaerobic foaming include (i) overloading, which is the main factor affecting foam formation [30]; (ii) feedstock composition, including the concentrations of proteins, amino acids, and lipids [31]; (iii) the presence of filamentous bacteria, including Nocardia sp., Gordonia sp., and Microthrix sp. [32]; (iv) inappropriate mixing patterns [33]; and (v) inappropriate reactor configurations [34]. Addition of an antifoaming agent, improved mixing, and optimal design of the digester are usually used to resolve AD foaming when the system is operated at high OLRs. Therefore, improved mixing and optimal design of the digester should be considered to prevent AD foaming when performing thermophilic codigestion of RS and animal manure at high OLRs. For example, egg-shaped digesters have been designed to solve scum and foaming problems [34].

### 3.4. Effects of OLR on foaming during codigestion

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NOMENCLATURE

AD = anaerobic digestion  
CHM = chicken manure  
CM = cow manure  
FAN = free ammonia  
FVFA = free volatile fatty acids  
OLR = organic loading rate  
PM = pig manure  
RS = air-dried rice straw  
SBP = specific biogas production  
SCOD = soluble chemical oxygen demand  
TVFA = total volatile fatty acids  
TAN = total ammonia  
TS = total solid  
VBPR = volumetric biogas production rate  
VFA = volatile fatty acids  
VS = volatile solid

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