Comparison of digestate from solid anaerobic digesters and dewatered effluent from liquid anaerobic digesters as inocula for solid state anaerobic digestion of yard trimmings

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Abstract

To select a proper inoculum for the solid state anaerobic digestion (SS-AD) of yard trimmings, digestate from solid anaerobic digesters and dewatered effluent from liquid anaerobic digesters were compared at substrate-to-inoculum (S/I) ratios from 0.2 to 2, and total solids (TS) contents from 20% to 35%. The highest methane yield of around 244 L/kgVS_{feed} was obtained at an S/I of 0.2 and TS content of 20% for both types of inoculum. The highest volumetric methane productivity was obtained with dewatered effluent at an S/I ratio of 0.6 and TS content of 24%. The two types of inoculum were found comparable regarding methane yields and volumetric methane productivities at each S/I ratio, while using dewatered effluent as inoculum reduced the startup time. An S/I ratio of 1 was determined to be a critical level and should be set as the upper limit for mesophilic SS-AD of yard trimmings.

Key words: Biogas, methane, inoculation ratio, total solids content, wood chips, grass

1. Introduction

Anaerobic digestion (AD) is a biological process that converts organic matter into biogas (about 70% CH₄ and 30% CO₂) under oxygen-free conditions. AD is believed to be a cost-effective technology that both produces renewable energy and reduces the volume of organic waste (Forster-Carneiro et al., 2008). Depending on the operating total solids (TS) content of the digester substrate, AD systems can be categorized into liquid AD (L-AD) and solid-state AD (SS-AD). L-AD systems typically operate with a TS content of less than 15%, while SS-AD operates with a TS content higher than 15%. In the past decades, L-AD systems have been successfully used to convert liquid organic wastes such as animal manure and food processing waste into bioenergy. However, a barrier for the wide application of L-AD technology is the high cost of handling its large quantities of effluent, which can account for more than 30% of the operating costs for L-AD systems (Mata-Alvarez et al., 2000). In contrast, SS-AD systems are suitable to treat solid organic wastes and lignocellulosic biomass without the floating and stratification problems associated with fibrous materials in L-AD (Li et al., 2011a). SS-AD also has advantages of a relatively small digester volume and higher volumetric loading capacity and methane productivity (Brown et al., 2012), which can potentially reduce the cost for construction and heating (Guendouz et al., 2010). Due to the low water content, SS-AD generates a compost-like end product which can be used as a soil amendment (Li et al., 2011b).

SS-AD currently represents 70% of the total installed AD capacity in Europe for treating organic fraction of municipal solid waste (OFMSW) and other solid organic materials, such as energy crops (De Baere and Mattheeuws, 2012; European Bioplastic, 2015). Compared to L-AD, SS-AD tends to be more difficult to start-up and control, thus inoculation is essential for this process (Le

Hyaric et al., 2012). It was reported that in commercial SS-AD digesters, about 50%-70% (wet based) of the finished solid materials (digestate) need to be recycled as inoculum, significantly reducing digester volume for feedstocks (Karthikeyan and Visvanathan, 2013). A recent lab scale study on continuous SS-AD used a digestate recycling ratio (by fresh weight) of around 55%, and found that a TS content of about 26% and above would cause volatile fatty acid (VFA) accumulation and a pH drop (Benbelkacem et al., 2015). However, currently very limited information can be found for the optimal substrate to inoculum (S/I) ratio and the operating TS content using recycled digestate as inoculum.

Several researches have reported that the best inoculum for SS-AD is the finished material (effluent) from L-AD digesters (Forster-Carneiro et al., 2007; Karthikeyan and Visvanathan, 2013). L-AD effluent has been widely used in scientific research as inoculum for SS-AD, as well as to supplement nitrogen, water, trace elements, and alkalinity to the system (Shi et al., 2014; Yang et al., 2015). However, L-AD effluent has not been widely used as the inoculum in commercial scale SS-AD, due to difficulty of transporting L-AD effluent to the SS-AD site. A pilot scale integrated anaerobic digestion system (*iADs*) has been built in Zanesville, OH (Li et al., 2011a). In the *iADs*, L-AD and SS-AD systems are constructed side-by-side and L-AD effluent is used to inoculate solid organic waste (e.g., crop residues, yard waste, and municipal solid waste) for biogas production in the SS-AD reactors.

Both digestate and dewatered effluent have advantages and disadvantages as inoculum for SS-AD. Inoculating SS-AD with L-AD effluent, as adopted in the *iADs*, has the potential to reduce effluent treatment costs of L-AD, and rapidly initiate the SS-AD process (Li et al., 2011a). On the other hand, using SS-AD digestate as inoculum makes the system more self-sustainable, allows for a higher TS content, and provides a microbial community acclimated to the feedstock

and operating conditions. Currently, there has been no side-by-side comparison of these two inoculation options, and the optimal TS range and substrate to inoculation ratio (S/I) for using SS-AD digestate as inoculum have not been studied scientifically. Thus the objective of this research was to determine the optimal range of the operating TS content and S/I ratio using recycled digestate as an inoculum for SS-AD of yard trimmings, and to compare the effects of inoculating SS-AD with L-AD effluent or SS-AD digestate on methane yield, volumetric productivity, and startup time, in order to facilitate the design and operation of large scale continuous SS-AD systems.

2. Materials and methods

2.1 Substrate and inoculum

The yard trimmings used as substrate was a mixture of three common components of urban yard waste: wood chips, lawn grass, and maple leaves (Table 1). The percentages of these three components have seasonal variations, such as more grass in summer and more leaves in fall. For this study, the yard trimmings components were mixed at a ratio of 1:1:1 (wet basis), which had been found to achieve a good nutrient balance and high methane yield in thermophilic SS-AD (Lin et al., 2015). The mixed substrate did not require nitrogen supplementation because its C/N ratio of 24.6 was within the optimal range for microbial growth (Li et al., 2011b). The three yard trimming components were collected in November, 2013 from the Ohio Agricultural Research and Development Center campus in Wooster, OH, USA. Upon receipt, all components were dried at 40 °C for 48 h in a convection oven (Precision Thelco Model 18, Waltham, MA, USA) to reduce the moisture content to less than 10%, ground with a hammer mill to pass through a 9 mm screen sieve (Mighty Mac, MacKissic Inc., Parker Ford, PA, USA) and then stored in air-tight containers.

Effluent from a mesophilic liquid anaerobic digester (KB BioEnergy, Inc., Akron, OH, USA) fed with municipal sewage sludge was used to prepare both types of inoculum. The digestate was prepared by mixing the effluent with yard trimmings at a substrate/inoculum (S/I) ratio of 2 (based on dry weight) and a TS content of around 20%, and anaerobically digesting it at 37°C for 44 days in a 20 gallon digester. The dewatered effluent was prepared by centrifuging (10000 rpm for 15 min) the effluent to increase its TS content to be similar with the digestate. Both types of inoculum were stored in air-tight buckets at 4 °C and activated at 37°C for 1 week prior to use. Characteristics of the substrate and inocula are shown in Table 1.

2.2 Solid-state batch anaerobic digestion

Each SS-AD reactor (1 L glass bottle with working volume 700 ml) was loaded with a mixture of yard trimmings and inoculum mixed at S/I ratios of 0.2 to 2.0 (based on dry weight) and TS content ranged from 20% to 35% (Table 2). The water added (if any) was counted toward the wet weight of feedstock. The high TS content of the inoculum allowed the SS-AD to achieve a high operating TS content of more than 40%; however, the data for SS-AD with 40% TS is not shown in this paper because acidification occurred at a TS of 30% and above. To exclude the effect of TS content, another set of SS-AD were conducted with S/I ratios from 0.2 to 2.0, but the TS content of all reactors was adjusted to 20% by adding deionized water. Reactors containing only inoculum (TS about 18%) were used as controls. Each reactor was sealed with a rubber stopper and placed in a 37±1 °C walk-in incubator for 40 days. Biogas was collected in a 5-L gas bag (CEL Scientific Tedlar gas bag, Santa Fe Springs, CA, USA) attached to the outlet of the reactor. The Tedlar bags were emptied every 2-4 days to measure the biogas composition and volume. All tests were conducted in duplicate.

2.3 Analytical methods

Biogas was sampled by a syringe for composition (CO_2 , CH_4 , N_2 , and O_2) analysis by gas chromatography (GC) (Agilent Technologies, HP 6890, Wilmington, DE, USA) equipped with a $30\text{ m} \times 0.53\text{ mm} \times 10\text{ }\mu\text{m}$ alumina/KCl deactivation column and a thermal conductivity detector (TCD). Helium gas was used as a carrier gas at a flow rate of 5.2 ml/min. The temperature of the detector was set at 200 °C. The volume of biogas collected in the Tedlar bags was measured by a drum-type gas meter (Ritter, TG 5, Bochum, Germany).

Substrate, inoculum, and reactor contents at the beginning and the end of the SS-AD process were sampled and characterized. TS and volatile solids (VS) contents were analyzed according to the Standard Methods for the Examination of Water and Wastewater (American Public Health Association., 2005). Total carbon and total nitrogen contents were determined by an elemental analyzer (Vario Max CNS, Elementar Americas, Mt. Laurel, NJ, USA) to calculate the carbon-to-nitrogen (C/N) ratio. Total ammonia nitrogen (TAN) content was determined by a modified distillation and titration method (ISO 5564, 1984) using 4% boric acid. Five grams of solid sample were mixed with 50 ml deionized water and extracted at 4 °C for 20 min to determine pH values, and then titrate with 0.1 N hydrochloride acid to determine the concentration of VFAs and alkalinity (McGhee, 1968) using an auto-titrator (Mettler Toledo, DL22 Food & Beverage Analyzer, Columbus, OH, USA). Extractives were determined based on the NREL Laboratory Analytical Procedure using a soxhlet extraction method (Sluiter et al., 2005). Extractive-free samples were used to determine cellulose and hemicellulose contents using a two-step acid hydrolysis process according to the NREL Laboratory Analytical Procedure (Sluiter et al., 2010). Monomeric sugar (glucose, xylose, galactose, arabinose, and mannose) and cellobiose contents were measured by high-performance liquid chromatography (HPLC)

(Shimadzu LC-20AB, Columbia, MD, USA) equipped with a BioradAminex HPX-87P column and a refractive index detector (RID). Deionized water was used as the mobile phase at a flow rate of 0.6 ml/min. The temperatures of the column and detector were maintained at 80 °C and 55 °C, respectively. Statistical analysis was conducted using a two-sample t-test with a threshold p-value of 0.05 using R-project software 3.0.2.

3. Results and discussion

3.1. Characteristicsof raw materials

Each component of the combined yard trimmings had distinctive characteristics (Table 1). Among them, the lawn grass hadthe highest methane potential, probably due to the high extractives and cellulose contents, as well as the lowest lignin content(Lin et al., 2015). In contrast, wood chips and maple leaves wererelatively hard to degrade due to the high lignin content (Liew et al., 2012). It is noteworthy that all feedstocks had acidic pH, which can consume the digester alkalinity and negatively impact the SS-AD process. Acidic pH is commonly found in woody biomass (Choon and Roffael, 1990), in which acetyl groups, uronic acid residues, and some organic acids and estersin the extractives were reported to be responsible for the acidic pH(Pulido et al., 1998; Uçar and Uçar, 2012). The effects of the acidic substrate on reactor initial pH and alkalinity are shown in Table 3. With the S/I ratio increased from 0.2 to 2, the initial pH was decreased from 8.8 to 7.6 (with digestate as inoculum) and 8.2 to 7.5 (with dewatered effluent as inoculum), and the initial alkalinity also decreased accordingly from 8.0-8.2 gCaCO₃/kg to 1.6-2.3 gCaCO₃/kg.

As for the inoculum, the dewatered effluent had a lower dry basis VS content (54% of TS) than the digestate (70% of TS), which wasprobably caused by the high inorganic contents such as

sand and salts in the L-AD effluent (Table 1). Although the digestate had relatively high dry basis VS content, a large portion of its VS content was from the remaining lignocellulosic biomass residues from the previous SS-AD process, including most of the lignin and part of the cellulose and hemicellulose that are not easily degradable (Motte et al., 2015). As a result, the VS of the digestate could not be used as an indicator of the abundance of microorganisms, and it is difficult to compare these two inocula based on VS. Moreover, in this study, inocula with the same TS had comparable physical and chemical characteristics, such as density, alkalinity, and pH (Table 1). The S/I ratios calculated based on TS, total weight, and VS are shown in Table 2.

3.2 Comparison of two inocula at different substrate-to-inoculum ratios

The cumulative and daily methane yields of yard trimmings inoculated with different inoculum at seven different S/I ratios are shown in Fig. 1. The highest methane yield of around 244 L/kg VS_{feed} was achieved at an S/I ratio of 0.2. When the S/I ratio was increased gradually from 0.2 to 2, cumulative methane yields of yard trimmings decreased to less than 5 L/kg VS_{feed} due to acidification. However, there were no significant differences ($p < 0.05$) between cumulative methane yields obtained by these two types of inoculum at each S/I ratio (Fig. 1 a and b). One exception was at an S/I ratio of 1.0, where reactors inoculated by dewatered effluent stopped producing methane after the second day due to the accumulation of VFAs and acidic pH (Table 3). In comparison, at an S/I ratio of 1.0, reactors using solid digestate as inoculum experienced an initial inhibition stage and a prolonged lag phase (Fig. 1 c), but still produced a significant amount of methane with an average cumulative methane yield of 140 L/kg VS_{feed} . One possible reason might be that the solid digestate had a slightly higher initial alkalinity than the dewatered effluent, which may have prevented the pH from dropping below 7 (Tables 1 and 3). The upper limit of the TS-based S/I ratio was about 1.0 to 1.5 in this study, which was equivalent to a VS

182 based inoculation ratio of around 2 (Table 2), which was lower than that in previous studies
183 where the digester usually failed at S/I ratios (VS based) higher than 4 when using L-AD effluent
184 (not dewatered) as inoculum (Xu et al., 2014a). One possible reason might be that the acidic
185 feedstock consumed the buffering capacity of the reactor. According to this study, alkalinity
186 above 3.4 gCaCO₃/kg was needed for successful AD processes (Table 3).

187 The reduced cumulative methane yield with increased S/I ratio in this study (Fig. 1 a) has
188 been commonly observed in SS-AD. One reason for this yield reduction was proposed to be the
189 lower populations of methanogens and the low alkalinity at high S/I ratios reduced the reactor
190 capacity to consume VFAs produced in the early stage of SS-AD, resulting in a pH drop and
191 reactor failure (Shi et al., 2014). In this study, the increased S/I ratio also increased the TS content.
192 Higher TS content has been shown by several researchers to significantly decrease methane yield
193 due to the compromised mass transfer of solutes in the solid matrix (Bollon et al., 2013; Xu et al.,
194 2014b). As a result, the decreased methane yield with increased S/I ratios in this study was
195 attributed to the combined effects of less inoculum, lower alkalinity, and higher TS content.

196 Although similar cumulative methane yields were obtained by the two types of inoculum (except
197 for that at the critical S/I ratio of 1.0), the daily methane yield was distinctively different,
198 indicating that the type of the inoculum affected the methane production kinetics. According to
199 Fig. 1 c, when using digestate as inoculum, reducing the inoculation size resulted in retarded
200 reactor startup, indicated by the delayed peak occurrence from the 10th day to the 16th day when
201 the S/I ratio increased from 0.2 to 1.0. The peak values of the daily methane yields also decreased
202 as the S/I ratio increased. When the S/I ratio was higher than 0.8, daily methane yields of reactors
203 started to decrease on the 4th day, indicating an initial inhibition to methanogens due to the

accumulation of VFAs. This phenomenon usually appears when the methanogenesis rate is lower than the hydrolysis rate (Ma et al., 2013). It is also possible that the solid digestate had higher hydrolysis abilities due to the acclimation of the microbial community to lignocellulosic biomass (Gu et al., 2014).

In contrast, when using dewatered effluent as inoculum, no initial inhibition was observed and the lag phase for methane production was much shorter than that of using solid digestate as inoculum (Figs. 1 c and d). All reactors reached the daily methane production peak within 6 days, although the peak values decreased as the S/I ratio increased. The faster startup may indicate that the dewatered effluent had higher concentrations of methanogens that reduced the risk of severe VFA accumulation at the initial stage of SS-AD.

3.3 Cumulative methane yield and methane production kinetics with controlled TS content

As discussed in the previous section, reducing inoculation size not only decreased the microbial population and alkalinity of the reactor, but also increased the TS content of the entire reactor. To exclude the effect of changing TS content, and also to investigate the robustness of the dry basis S/I ratio at a wider TS range, another batch of experiments were done in which the TS content was adjusted to 20% (Table 2). When the TS was controlled, reactors with different S/I ratios were shown to be separated into three groups (Fig. 2 a and b): The first group, which had an S/I ratio of 0.2, had a significantly higher cumulative methane yield than other reactors ($p < 0.05$); the second group, which had S/I ratios of 0.4 to 0.8 (1.0 for dewatered effluent), had final cumulative methane yields ranging from 174 to 195 L/kgVS_{feed}; and the third group, which was composed of those reactors with an S/I ratio of more than 1.0 (1.5 for dewatered effluent), failed from the second day due to VFA accumulation and pH drop.

As seen by comparing Fig. 1 and 2, reducing TS content generally had positive effects on the 40-day cumulative methane yield of yard trimmings. For example, reducing the TS content from 26% to 20% resulted in an average of 7% and 12% increase in cumulative methane yields of yard trimmings using digestate or dewatered effluent, respectively, as inoculum. However, this improvement was not very prominent for reactors that originally had a TS content of less than 24%. Interestingly, at the critical S/I ratio ($S/I = 1.0$), reducing TS content from 28% to 20% caused reactor failure when using digestate as inoculum (Fig. 2a), while it significantly increased methane yield when using dewatered effluent as inoculum (Fig. 2b). At the critical S/I ratio of 1.0, reactors using solid digestate as inoculum achieved a methane yield of 140 L/kgVS_{feed} at a TS content of 28%, while the reactors failed to produce methane when the TS was reduced to 20%, probably because at a TS content of 20%, the enhanced production and diffusion of VFAs inhibited methanogens. In contrast, reactors using dewatered effluent as inoculum achieved a cumulative methane yield of 176 L/kgVS_{feed} at 20% TS, possibly because the additional water slightly diluted the VFAs produced during startup and enhanced the consumption of VFAs by anaerobic microbes (Bollon et al., 2011; Liotta et al., 2014).

Reducing TS content was found to also increase the peak values of the daily methane yield, especially for reactors that previously had TS contents of 26% and 28% (Fig. 2 c and d). The increase of the daily methane yields might be mainly caused by the enhanced hydrolysis and VFA production due to the addition of water, which was also evidenced by the high intensity of daily CO₂ production at a lower TS content (Fig. 3). It is generally accepted that the rate of CO₂ production can indicate the intensity of the fermentation process. In this research, it was observed that reducing the TS content from above 26% to 20% can increase the CO₂ production rate during the first five days, which may have also boosted the methane production rate (Fig. 1 c

and d, Fig 2 c and d) due to the high concentrations of acetate produced. It has been reported that reducing TS content increases the methane production rate in SS-AD (Pommier et al., 2007; Xu et al., 2014b). Le Hyaric et al. also found the specific methanogenic activity declined when the TS content increased from 18% to 35% (Le Hyaric et al., 2011; Le Hyaric et al., 2012). Different reasons have been provided for the decreased methane production rate with increased TS content, including decreased hydrolysis rate (Abbassi-Guendouz et al., 2012), diffusion rate (Bollon et al., 2013; Xu et al., 2014b), and methanogenesis rate (Le Hyaric et al., 2011; Le Hyaric et al., 2012; Pommier et al., 2007). In this study, the reduced TS content appeared to influence the hydrolysis/fermentation rate, which may in turn, have increased the methanogenesis rate.

Adjusting the TS content to 20% also reduced the VFA concentration by up to 50% in failed reactors (Table 3). This might be mainly attributed to the dilution effect from the additional water. In addition, the diffusion limitations of VFAs may also be reduced at a TS content of 20%, thus VFAs might be more accessible to anaerobic microbes as substrates. At both high and low TS contents, acetic acid was the major VFA that accumulated during SS-AD, and butyric acid was the second largest contributor to VFA accumulation in acidified reactors (Data not shown).

3.4 Volumetric methane productivity

Volumetric methane productivity is a parameter that measures how much methane can be produced per unit reactor volume. The volumetric methane productivity at different S/I ratios is shown in Fig. 4. Without TS adjustment, the highest volumetric productivity was obtained at an S/I of 0.6 - 0.8 for both inocula. Adjusting the TS content to 20%, although slightly increased the methane yield, reduced the volumetric productivity by 7-10% for reactors with S/I ratios of less than 0.8, because the reactor volume was partially occupied by the additional water. Different

from methane yield, the volumetric productivity had an increasing trend with S/I ratio after TS adjustment, until reaching the S/I ratio that caused reactor failure.

Volumetric methane productivity is an important parameter from an engineering point of view, as it indicates the utilization efficiency of the digester volume. Although it is preferred to minimize the inoculum size and feed the digester as much substrate as possible, there should be an optimal inoculation size that can obtain the highest volumetric productivity and thus the maximum economic value. The results from this study indicated that at low TS content, maximum volumetric methane productivity was obtained by increasing the S/I ratio to the critical limit without overloading the system; while at high TS content, the optimal methane productivity was affected both the methane yield of unit substrate and the substrate loading.

4. Conclusions

Inoculating SS-AD of yard trimmings with SS-AD digestate or dewatered L-AD effluent obtained comparable methane yields, while reactors inoculated with dewatered effluent showed better stability. For both inocula, an S/I ratio of 1.0 was the critical point beyond which reactors were prone to fail. Reducing TS content to 20% at high S/I ratios increased the cumulative methane yields of yard trimmings by up to 10%, but decreased volumetric methane productivity and reactor stability, thus was not recommended.

Acknowledgement

This material is based upon work that is supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, under award number 2012-10008-20302. The authors would like to thank Mrs. Mary Wicks (Department of Food, Agricultural and Biological Engineering, OSU) for reading through the manuscript and providing useful suggestions.

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392 **Fig. 1** Effect of inoculum source and inoculation ratio on methane yields of yard trimmings:
393 cumulative methane yields using (a) digestate and (b) dewatered effluent as inoculum; daily
394 methane yields using (c) digestate and (d) dewatered effluent as inoculum.

395 **Fig. 2** Effect of inoculum source and inoculation ratio on methane yields of yard trimmings
396 with TS content adjusted to 20%: cumulative methane yields using digestate (a) and
397 dewatered effluent (b) as inoculum; daily methane yields using digestate (c) and dewatered
398 effluent (d) as inoculum.

399 **Fig. 3** Effect of inoculum on daily CO₂ yields of yard trimmings from using (a) digestate as
400 inoculum; (b) dewatered effluent as inoculum; (c) digestate as inoculum with TS content
401 adjusted to 20%; and (d) dewatered effluent as inoculum with TS content adjusted to 20%.

402 **Fig. 4** Volumetric productivity of yard trimmings at different S/I ratios using digestate and
403 dewatered effluent without (a) and with (b) TS adjustment.

404

Fig. 1 Effect of inoculum source and inoculation ratio on methane yields of yard trimmings: cumulative methane yields using (a) digestate and (b) dewatered effluent as inoculum; daily methane yields using (c) digestate and (d) dewatered effluent as inoculum.

Fig. 2 Effect of inoculum source and inoculation ratio on methane yields of yard trimmings with TS content adjusted to 20%: cumulative methane yields using digestate (a) and dewatered effluent (b) as inoculum; daily methane yields using digestate (c) and dewatered effluent (d) as inoculum.

Fig. 3 Effect of inoculum on daily CO₂ yields of yard trimmings from using (a) digestate as inoculum; (b) dewatered effluent as inoculum; (c) digestate as inoculum with TS content adjusted to 20%; and (d) dewatered effluent as inoculum with TS content adjusted to 20%.

(a)

(b)

Fig. 4 Volumetric productivity of yard trimmings at different S/I ratios using digestate and dewatered effluent without (a) and with (b) TS adjustment.

Table 1. Characteristics of feedstock and inoculum

Parameters	Yard trimmings ^b	Wood chips	Lawn grass	Maple leaves	Dewatered Effluent	Digestate
TS (%)	87.7±0.1	88.7±0.0	94.8±0.1	93.8±0.0	17.2±0.1	17.9±0.2
VS (%)	81.1±0.1	84.8±1.2	88.5±0.4	84.5±0.0	9.3±0.0	12.5±0.3
Density (kg/m ³)	152.6±3.0	210.7±4.1	108.8±1.7	138.2±3.3	891.2±23.3	913.7±3.6
Total carbon (%) ^a	46.8±0.2	46.4±0.3	47.8±0.0	46.1±0.3	31.30±0.0	37.07±0.1
Total nitrogen (%) ^a	1.9±0.0	0.6±0.0	3.8±0.0	1.2±0.0	2.76±0.0	2.42±0.1
C/N ratio ^a	24.6±0.1	75.4±2.4	12.4±0.0	41.1±0.3	11.35	14.87
pH	5.0±0.0	4.4±0.0	5.5±0.0	4.5±0.0	8.4±0.1	8.1±0.0
Alkalinity (g CaCO ₃ /kg)	< 0	< 0	1.8±0.1	< 0	15.6±0.4	16.3±0.4
VFAs (g/kg)	ND	ND	ND	ND	10.0±1.4	5.9±0.5
TAN (gN/kg)	ND	ND	ND	ND	4.2±0.3	4.3±0.3
Extractives (%) ^a	23.4±1.6	15.5±0.1	24.9±4.4	29.8±0.2	ND	ND
Cellulose (%) ^a	20.7±0.5	27.5±0.3	23.1±0.8	11.6±0.4	ND	ND
Hemicellulose (%) ^a	14.0±0.4	15.6±0.1	16.4±0.6	9.9±0.4	ND	ND
Lignin (%) ^a	21.0±0.5	26.9±0.4	12.1±0.8	24.0±0.2	ND	ND

^a Based on dry weight of sample; the others are based on wet weight of sample; ^b yard trimining is a mixture of wood chips, lawn grass, and maple leaves at a ratio of 1:1:1, and the data for yard trimming is based on the calculation from its three components; ND = not determined.

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Table 2 Experimental design

TS (%)	S/I ratio based on TS	S/I based on total weight*	S/I based on VS	
			Digestate	Dewatered effluent
20	0.2	0.04	0.26	0.35
22	0.4	0.10	0.51	0.69
24	0.6	0.15	0.77	1.04
26	0.8	0.19	1.03	1.38
28	1.0	0.23	1.28	1.73
30	1.5	0.44	1.93	2.59
35	2.0	0.48	2.57	3.45
20	0.2	0.04	0.26	0.35
20	0.4	0.21	0.51	0.69
20	0.6	0.38	0.77	1.04
20	0.8	0.55	1.03	1.38
20	1.0	0.73	1.28	1.73
20	1.5	1.16	1.93	2.59
20	2.0	1.59	2.57	3.45
Control	0	0	0	0

434 *For the wet basis S/I, the water added for TS adjustment was counted towards the wet weight of the substrate.

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437

Table 3 Initial and final parameters for reactors inoculated by two types of inocula

		pH		Total VFA (gHAc/kg)		Alkalinity (gCaCO ₃ /kg)		VFA/Alkalinity	
	S/I ratio	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Digestate as inoculum									
No TS adjustment	0.2	8.8±0.0	8.7±0.1	3.2±1.7	6.0±0.6	8.2±0.3	13.2±0.3	0.39	0.46
	0.4	8.5±0.0	8.6±0.1	2.5±0.3	5.4±0.6	6.4±0.3	14.2±0.3	0.39	0.38
	0.6	8.5±0.0	8.6±0.1	2.1±0.1	5.4±0.6	4.7±0.3	14.2±0.3	0.46	0.38
	0.8	8.4±0.0	8.6±0.1	2.2±0.3	6.0±0.6	3.8±0.3	12.8±0.2	0.57	0.47
	1.0	8.3±0.0	8.7±0.1	2.2±0.4	6.7±0.5	3.4±0.3	11.6±0.3	0.64	0.58
	1.5	7.9±0.0	5.6±0.0	1.5±0.2	20.6±1.8	2.3±0.8	2.9±0.1	0.65	7.15
	2.0	7.6±0.0	5.7±0.0	2.2±0.0	20.3±1.2	2.3±0.8	3.4±0.4	0.94	6.21
TS = 20%	0.2	8.8±0.0	8.7±0.1	3.2±1.7	6.0±0.6	8.2±0.7	13.2±0.3	0.39	0.46
	0.4	8.6±0.0	8.5±0.1	2.5±0.2	5.3±0.4	6.5±0.2	11.8±0.3	0.38	0.45
	0.6	8.6±0.0	8.3±0.1	2.0±0.3	6.0±0.7	5.1±0.3	11.4±0.2	0.39	0.52
	0.8	8.5±0.0	8.6±0.1	1.9±0.2	4.3±0.1	4.3±0.3	11.5±0.5	0.44	0.37
	1.0	8.5±0.0	7.0±0.4	1.9±0.1	12.9±1.8	3.5±0.3	3.5±0.7	0.56	4.49
	1.5	8.1±0.0	5.5±0.1	0.7±0.0	10.9±1.0	2.2±0.8	2.8±0.4	0.31	3.97
	2.0	7.8±0.0	5.2±0.0	1.1±0.0	11.5±1.2	1.6±0.8	1.6±0.1	0.70	7.16
Dewatered effluent as inoculum									
No TS adjustment	0.2	8.2±0.0	8.3±0.0	2.2±0.2	9.7±1.2	8.0±0.2	13.5±0.4	0.28	0.72
	0.4	8.3±0.0	8.3±0.1	1.5±0.1	9.7±0.6	6.8±0.0	14.2±0.2	0.22	0.69
	0.6	8.3±0.0	8.3±0.0	1.5±0.5	5.4±0.4	6.1±0.0	14.4±0.4	0.33	0.61
	0.8	8.3±0.0	8.4±0.1	1.8±0.0	8.8±0.9	5.2±1.0	14.3±0.1	0.23	0.63
	1.0	7.6±0.0	6.0±0.0	1.5±0.1	20.9±0.9	2.2±0.1	3.6±0.2	0.54	5.95
	1.5	8.0±0.0	6.1±0.0	1.1±0.0	22.3±0.1	2.8±0.2	5.5±0.1	0.64	4.05
	2.0	7.5±0.0	5.8±0.1	1.9±0.2	22.1±1.0	3.4±0.1	4.4±0.2	0.82	5.03
TS = 20%	0.2	8.2±0.0	8.3±0.0	2.2±0.2	9.7±0.7	8.0±0.2	13.5±0.4	0.28	0.72
	0.4	8.2±0.0	8.3±0.0	1.5±0.0	7.8±0.5	6.3±0.1	11.8±0.3	0.23	0.61
	0.6	8.1±0.0	8.3±0.0	1.2±0.0	5.2±0.2	5.7±0.5	11.4±0.2	0.26	0.41
	0.8	8.1±0.0	8.3±0.0	1.2±1.0	6.6±0.3	4.8±0.0	11.5±0.5	0.37	0.62
	1.0	8.0±0.0	8.2±0.1	1.8±0.1	5.4±0.6	4.2±0.1	3.5±0.2	0.35	0.50
	1.5	8.0±0.0	5.7±0.1	1.9±0.2	12.2±0.6	2.8±0.0	2.8±0.5	0.40	5.02
	2.0	7.8±0.0	5.5±0.1	0.3±0.1	11.5±0.1	2.3±0.2	1.6±0.0	0.14	4.78

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