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Maximum organic loading rate for the single-stage wet anaerobic digestion of food waste

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HIGHLIGHTS

- Anaerobic digestion (AD) of food waste (FD) was conducted at high OLR for 225 days.
- High CH_4 yield and high VS reduction were achieved at high OLR simultaneously.
- The cell density in the sludge increased to 15 times that in the original seed sludge.
- The cell density increased during the periods when there was no organic loading.
- The maximum OLR was estimated around 10.5 kg-VS m^{-3} day^{-1} in a single-stage wet AD of FD.

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ABSTRACT

Anaerobic digestion of food waste was conducted at high OLR from 3.7 to 12.9 kg-VS m^{-3} day^{-1} for 225 days. Periods without organic loading were arranged between the each loading period. Stable operation at an OLR of 9.2 kg-VS (15.0 kg-COD) m^{-3} day^{-1} was achieved with a high VS reduction (91.8%) and high methane yield (455 mL g-VS-1). The cell density increased in the periods without organic loading, and reached to 10.9×10^{10} cells mL^{-1} on day 187, which was around 15 times higher than that of the seed sludge. There was a significant correlation between OLR and saturated TSS in the sludge ($y = 17.3e^{0.1679\times}$, $r^2 = 0.996$, $P < 0.05$). A theoretical maximum OLR of 10.5 kg-VS (17.0 kg-COD) m^{-3} day^{-1} was obtained for mesophilic single-stage wet anaerobic digestion that is able to maintain a stable operation with high methane yield and VS reduction.

1. Introduction

Food waste constitutes one of the largest components of the waste stream around the world (HKEPD, 2010; MOE, 2010). In Japan, demand for the reduction or effective utilization of food waste has increased in recent years, following the 2007 revision of the Food Recycling Law that aimed to build a “recycling society”. Food waste is generally incinerated in Japan and the remaining ash is placed in landfills. However, alternative treatment methods are highly desirable, since food waste comprises 30–40% of municipal solid waste, and because it typically has a water content greater than 80%, it requires high amounts of energy to incinerate this waste (MOE, 2010; Sawayama et al., 1997). Anaerobic digestion is a spontaneous process mediated by microorganisms that convert biomass into biogas (a mixture of mainly methane and carbon dioxide) without requiring advanced dewatering or further chemical extraction. In addition, the effluent sludge can be used for a solid soil conditioner or a liquid fertilizer (Dong et al., 2010; Kim et al., 2006). Thus, this treatment represents an attractive alternative to incineration.

In recent years, a number of novel reactor designs, such as two-stage or multiple-stage reactors, with semi-dry and dry sub-types, have been adapted and developed for stable treatment of waste under high organic loading rates (OLRs) (Bolzonella et al., 2003; Dong et al., 2010; Fernández et al., 2008; Forster-Carneiro et al., 2008; Verrier et al., 1987). However, the anaerobic digestion of organic waste generally relies on single-stage systems, which account for more than 95% of Europe’s full-scale plants (Baere et al., 2011; Forster-Carneiro et al., 2008). In the single-stage system, all of the reactions (hydrolysis, acidogenesis, acetogenesis,

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and methanogenesis) take place simultaneously in a single reactor, which permits simpler designs that suffer less frequent technical failures and that have a smaller investment cost (Forster-Carneiro et al., 2008). From these practical perspectives, several new approaches have been tested to improve the efficiency of single-stage reactors, including co-digestion, mesophilic or thermophilic operation, semi-dry digestion (10–20% total solids [TS]), dry digestion (20–40% TS), and the use of anaerobic membrane bioreactors to decouple the solid retention time (SRT) from the hydraulic retention time (HRT) (Cecchi et al., 1991; Climenhaga and Banks, 2008a; Dong et al., 2010; Forster-Carneiro et al., 2008; Heo et al., 2004).

The performance of anaerobic digestion reactors is mainly evaluated based on the OLR (g-volatile solids [VS] m^{-3} day^{-1}), methane yield (mL g-VS^{-1}), and reduction in VS (%). High methane yields of 364–489 mL g-VS^{-1} and a sufficient VS reduction of 83–91% have already been achieved during conventional wet anaerobic digestion (<5% TS)(Cho et al., 1995; Heo et al., 2004; Verrier et al., 1987; Zhang et al., 2007) with a high VS content and high biodegradability (85–90%). In contrast, OLR remains insufficient, at 1–4 kg-VS m^{-3} day^{-1} (Cho et al., 1995; Heo et al., 2004; Verrier et al., 1987; Zhang et al., 2007). One of the main reasons for the low OLR is inhibition of accumulated volatile fatty acids (VFAs) when OLR is too high (Ahring et al., 1995). Anaerobic digestion of readily degradable organic compounds is a delicate balance between the rates of hydrolysis and methanogenesis, because methanogenic bacteria are more sensitive than hydrolytic and acidogenic bacteria to high concentrations of VFAs and the corresponding pH drop. If the rates of hydrolysis and acidogenesis become higher than the rate of methanogenesis as a result of a too-high OLR, the accumulation of VFAs may lead to irreversible acidification of the digester (Pavlostathis and Giraldo-Gomez, 1991).

Unlike in wet anaerobic digestion, semi-dry (10–20% TS) and dry (20–40% TS) anaerobic digestion have been successful at relatively high OLRs of 7–15 g-VS m^{-3} day^{-1} without irreversible acidification, but the methane yield was low (140–314 mL g-VS^{-1}) and VS reduction was also low (31–48%) (Bolzonella et al., 2003; Cecchi et al., 1991; Dong et al., 2010; Vallini et al., 1993). Veeken and Hamelers (1999) pointed out that higher hydrolysis rate limiting occurs at lower moisture contents during digestion because the transport of VFAs from the acidogenic to the methanogenic stages of the digestion can only take place through the liquid phase. Thus, lower moisture contents in the digested sludge can decrease acid production in the liquid phase, resulting in slower acidification and a stable treatment with low methane yield and low VS reduction.

The high solubilization and rapid acid production provided by food waste substrates were originally considered to be appropriate characteristics for anaerobic digestion at high OLR if enough methanogenesis can occur to consume the acids produced during the acidification process so that these acids do not accumulate to excessive levels in the digested sludge. It has generally been assumed that high methanogenesis rates can occur from wet to semi-dry conditions (5–10% TS) in the digested sludge, which can maintain high mass transfer efficiency for the VFAs produced by acidogenesis; under these conditions, operation at a high OLR with high VS reduction and high methane yield could be achieved simultaneously. In the present study, we tested this hypothesis in a continuous laboratory-scale experiment with a range of OLRs (from 3.7 to 12.9 kg-VS m^{-3} day^{-1}) and wet to semi-dry conditions (5–10% TS) in a single-stage continuously stirred tank reactor (CSTR). The CSTR used in the experiment was fed with food waste, and SRT was decoupled from HRT by separation using a centrifuge to increase the total suspended solids (TSS) content and microbial activity in the anaerobic sludge.

2. Methods

2.1. Substrates and seed sludge

The food waste was obtained from a garbage collection company (Ohmura Co. Ltd., Saitama, Japan) and mixed according to the method of Komemoto et al. (2009), who describe how to match the composition of actual food waste in Japan. The food waste substrate consisted of rice (4.0% w/w), noodles (2.5%), bread (1.7%), tea leaves (8.0%), vegetables (53.6%), fruit (24.8%), meat (2.2%), fish (2.7%), and egg shells (0.5%), and was blended using a waste disposal unit (Anaheim, MGF KDF55JK, USA). The total volatile solids (TVS) to TS ratio (92.3%), carbon content (45.0% w/w of TS), and nitrogen content (3.2% w/w of TS) of the simulated food waste were similar to previously reported values of VS/TS (87–97.1%), carbon content (43.7–47.8% w/w of TS), and nitrogen content (2.8–5.2% w/w of TS) (Kim et al., 2006; Lim et al., 2008; Mata-Alvarez et al., 1992; Xu et al., 2011; Zhang et al., 2007). Actively digested sewage sludge slurry from a 6800-m^3 biogas plant (Hokubu Sludge Treatment Center, Yokohama, Japan) that was operating at 36 °C, with a 20- to 25-day retention time, were used as the inoculum (seed) sludge. This sludge was concentrated by sedimentation for 3 days before being used in the experiment. Table 1 summarizes the characteristics of the substrate and the inoculum sludge.

2.2. Anaerobic digestion

Two anaerobic digestion experiments were conducted with different HRTs (8 and 16 days) in single-stage reactors with a working volume of 3000 mL at a mean mesophilic temperature of 37 ± 1 °C. For the mixing condition, the reactor with mixing blade was operated at 60 rpm with a cycle of 1 min on and 10 min off throughout the experiment. The head space of the reactor was flushed with argon gas to maintain anaerobic conditions. A 2-L aluminum gas pack (AAK-2, GL Sciences, Tokyo, Japan) was attached to each reactor for biogas collection. The gas volume was calculated daily based on the downward displacement of water.

Digesters were operated in semi-continuous mode with daily feeding. In order to control the SRT of 60 days, 50-mL of digested sludge sample (1/60th of the total working volume) was taken daily from sampling ports on top of the reactors before substrate loading. Two different HRTs at 8 and 16 days (Reactor 1 and 2) were established by changing the amount of supernatant discharged from the reactors (Fig. 1). To control the HRT, centrifugation (5000 rpm, 10 min) of the digested sludge was conducted and only the supernatant was discharged from both reactors. To keep the working volume constant at 3000 ml, Milli-Q water was added each time substrates were fed to the reactors to replace the discharged supernatant. The amount of daily discharged water was 375 and 187.5 ml for the HRT of 8 and 16 days, respectively. HRT and SRT were calculated as follows:

Table 1
Characteristics of food waste substrate and inoculum (seed) sludge.

Parameter	Units[a]	Food waste	Seed sludge
pH	–	3.77	7.74
Total solids content(TS) %	% of wet weight	10.3	3.81
Total volatile solids content (TVS) %	% of wet weight	9.2	1.7
TVS/TS (%)	–	92.3	60.7
Total COD (TCOD)	g-COD_{cr} L^{-1}	152	27.8
Soluble COD (SCOD)	g-COD_{cr} L^{-1}	–	2.30
Carbon content	% of TS	45.0	19.5
Nitrogen content	% of TS	3.20	5.6

[a] COD_{cr}, chemical oxygen demand.

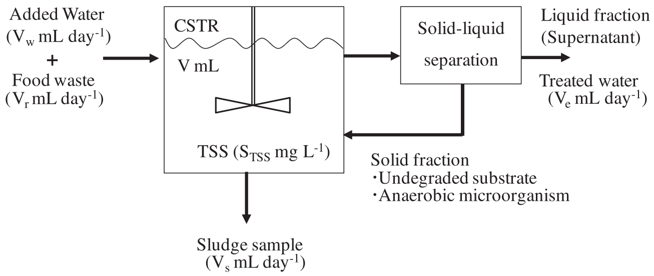

Fig. 1. Schematic diagram of the experimental setup and material flows used to control the SRT and HRT in the laboratory tests.

$$\mathrm{HRT(days)} = \frac{V}{V_w + V_r} = \frac{V}{V_e + V_s} \quad (1)$$

$$\mathrm{SRT(days)} = \frac{V \times S_{TSS}}{V_S \times S_{TSS}} \quad (2)$$

where V (mL) is the reactor's working volume (mL), V_w (mL day^{-1}) is the volume of Milli-Q water added daily, V_r (mL day^{-1}) is the volume of substrate added daily, V_e (mL day^{-1}) is the rate of withdrawal of supernatant discharge obtained from centrifugation, and V_s (mL day^{-1}) is the rate of withdrawal of digested sludge as samples. S_{TSS} (mg L^{-1}) is the total suspended solid (TSS) concentration of the digested sludge. OLR was increased from 3.7 to 12.9 kg-VS m^{-3} day^{-1} (listed as phases 1–6) following the schedule in Table 2. Periods with no organic loading were established between the loading periods (the periods between the six phases in Table 2). To evaluate the repeatability of the digestion performance, the same OLR (7.4 kg-VS m^{-3} day^{-1}) were used in the reactors during phases 3 and 4. Substrate feeding to Reactor 1 (HRT 8 days) was stopped completely by Day 60 due to an irreversible acidification of the digester.

2.3. Enumeration of bacterial cells in the samples

0.1 mL of the sludge suspension was diluted with 0.9 mL of saline water including 4% of paraformaldehyde, then filtered the solution through an Anodisc filter with a 0.02-μm pore size (6809–6002, Whatman PLC, Middlesex, UK), backed by a cellulose membrane filter with 0.22-μm pore size (Millipore Co., Villerica, MA, USA) under a vacuum of 100 mm Hg. One drop of 0.25% SYBR Gold (S11494, Invitrogen Co., Carlsbad, CA, USA) was placed on the Anodisc filter, with the side that retained the bacteria facing upwards, and kept the filter in the dark for 15 min. The stained Anodisc filter was then completely dried and mounted on a glass slide with a drop of antifade reagent (SlowFade Antifade Kit, S-2828, Invitrogen). The filter was then covered with a cover glass. The cell density was then estimated by direct cell counting using an Axioskop 2 Plus epifluorescence microscope (Carl Zeiss, Oberkochen, Germany). 20 fields were counted under blue excitation light, and determined the total count based on the average of these 20 counts (Shibata et al., 2006).

2.4. Experimental parameters and analytical methods

TS, TVS, pH, total organic carbon (TOC), total organic nitrogen (TON), total chemical oxygen demand ($TCOD_{cr}$), soluble chemical oxygen demand ($SCOD_{cr}$), and the VFA content were measured. The TS, TVS, TOC, TON, pH, and $TCOD_{cr}$ of pretreated substrates and digestion samples were measured before filtration using a combusted 0.45-μm glass filter (GC-50, Advantec, Ehime, Japan). The pH of all batch reactors was measured using a pH meter (B-212, Horiba, Kyoto, Japan). TOC and TON were measured by catalytic oxidation using an elemental analyzer (EA1108, Fisons, city, country). $TCOD_{cr}$, $SCOD_{cr}$, and the VFA concentration were quantified in the filtrate in accordance with the standard methods of the American Public Health Association (APHA, 2005). The VFA concentrations (acetic acid, propionic acid, *n*-butyric acid, *i*-butyric acid, *n*-valeric acid, and *i*-valeric acid) were measured using a gas chromatograph (GC-9A, Shimadzu, Kyoto, Japan) equipped with a packed column (Shincarbon A, Shimadzu) and a flame ionization detector. The column temperature was maintained at 140 °C. The temperatures at the injector and detector were maintained at 200 °C. Helium was used at the carrier gas at a flow rate of 50 mL min^{-1}.

The volume of biogas was quantified by the water displacement method. Carbon dioxide and methane concentrations were monitored using a gas chromatograph (GC-2014AT, Shimadzu) equipped with a packed column (Shincarbon ST, Shimadzu) and a thermal conductivity detector. The injector and detector temperatures were maintained at 120 and 260 °C, respectively. The column temperature was increased from 40 to 250 °C. Helium was used as the carrier gas at a flow rate of 40 mL min^{-1}. The volumes of accumulated methane and carbon dioxide were corrected for standard temperature (273.15 K) and pressure (100 kPa) conditions (i.e., STP).

2.5. Estimation of sludge acclimation

Methane yield (mL g-VS^{-1}) can only represent a constant reaction rate when there is no accumulation of intermediary products (Veeken and Hamelers, 1999). To estimate the tendency of sludge acclimation and the change in methane-generation activity, a

Table 2
Operation conditions and schedule for changes in the organic loading rate (OLR), solid retention time (SRT) and hydraulic retention time (HRT) in the two reactor.

Day	OLR: kg-VS m^{-3} day^{-1} (OLR: kg-COD m^{-3} day^{-1})[a]	Reactor 1		Reactor 2	
		SRT (day)	HRT (day)	SRT (day)	HRT (day)
0–40 (Phase 1)	3.7 (6.0)	60	8.0	60	16.0
41–49	0.0	–	–	–	–
50–69[b] (Phase 2)	5.5 (9.0)[b]	60	8.0	60	16.0
70–83	0.0	–	–	–	–
84–110 (Phase 3)	7.4 (12.0)	–	–	60	16.0
114–132	0.0	–	–	–	–
133–148 (Phase 4)	7.4 (12.0)	–	–	60	16.0
149–154	0.0	–	–	–	–
155–187 (Phase 5)	9.2 (15.0)	–	–	60	16.0
188–214	0.0	–	–	–	–
214–225 (Phase 6)	12.9 (21.0)	–	–	60	16.0

[a] VS, volatile solids; COD, chemical oxygen demand.
[b] Substrate feeding to Reactor 1 (HRT 8 days) was stopped completely by day 60 due to an irreversible acidification of the digester.

reaction-rate constant for methane conversion was calculated and used this to define the methane yield from the substrates; then this yield among the OLRs was compared. The methane yield and reaction-rate constant were calculated as follows (Veeken and Hamelers, 1999):

$$M_t = M_{\max} \times (1 - \exp^{-k_c t}) \quad (3)$$

Where M_t represents the methane yield (in mL g-VS^{-1} at STP) at time t (in days), $M_{\max}$ is the maximum methane yield (mL at STP), and k_C is the first-order conversion-rate constant (in days^{-1}).

3. Results and discussion

3.1. The reactor performance: biogas production and VS reduction

Fig. 2a shows the volumetric biogas production rate (L L^{-1} day^{-1}) and VS reduction (%) throughout the study. During phase 1 (OLR = 3.7 kg-VS m^{-3} day^{-1}), Reactor 1 (HRT8) and 2 (HRT16) showed similar biogas production rates and reached a steady state at around 2.7 L L^{-1} day^{-1}, although the rate in Reactor 1 was slightly lower than the rate Reactor 2. During phase 2 (OLR = 5.5 kg-VS m^{-3} day^{-1}), both reactors showed high biogas production rates from the beginning of the organic loading. However, gas production in Reactor 1 decreased rapidly by around Day 55, and gas production stopped completely by Day 60. On the other hand, gas production increased or was maintained in Reactor 2 as OLR was increased to 9.2 kg-VS m^{-3} day^{-1}. The volumetric biogas production rate increased to approximately 2.7, 4.2, 5.8, and 6.6 L L^{-1} day^{-1} as OLR increased to 3.7, 5.5, 7.4, and

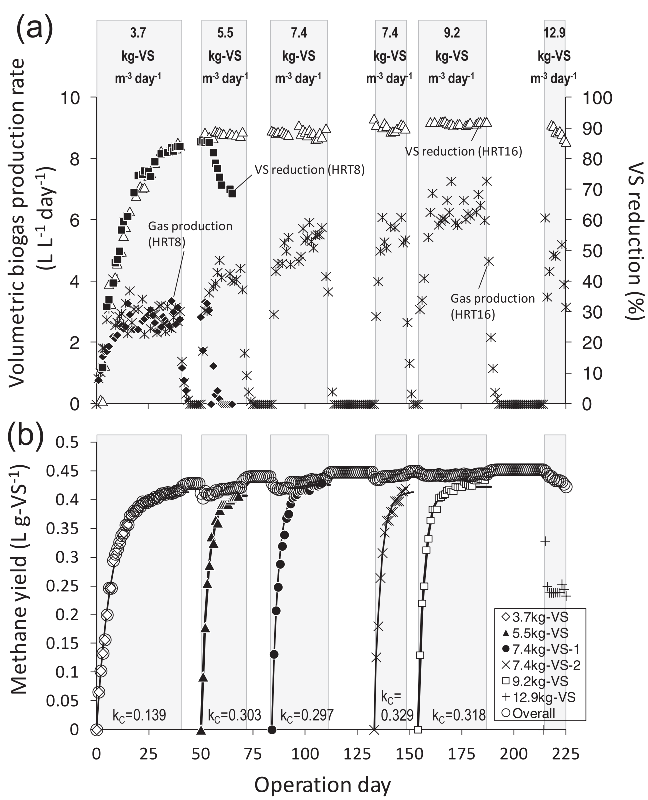

Fig. 2. Time course for the volumetric biogas production rate and the overall VS reduction (%) in each reactor (a). Overall methane yield and the methane yield at each OLR in Reactor 2 (b). The values of the conversion rate constant (k_C) at each OLR were calculated by means of least-squares analysis. Shaded areas represent the six phases shown in Table 2; unshaded areas represent times when the organic loading was temporarily stopped. HRT8 and HRT16, hydraulic retention times of 8 and 16 days, respectively.

9.2 kg-VS m^{-3} day^{-1}, respectively, and was maintained. At the highest OLR (12.9 kg-VS m^{-3} day^{-1}), the volumetric gas production rate decreased below the gas production rate at an OLR of 7.4 kg-VS m^{-3} day^{-1}.

The VS reduction rate, which indicates the treatment efficiency with organic waste as the volatile solids, increased rapidly at the beginning of Phase 1 (with OLR = 3.7 kg-VS m^{-3} day^{-1}), increased gradually at higher OLRs, then maintained an almost steady state after the end of Phase I in both reactors (Fig. 2a). However, in Reactor 1, the VS reduction rate decreased to less than 70% by the last day of Phase 2 (with OLR = 5.5 kg-VS m^{-3} day^{-1}), although the rate in Reactor 2 remained high (more than 90%) until a maximum OLR of 9.2 kg-VS m^{-3} day^{-1} (Phase 5). The VS reduction rate in Reactor 2 decreased during phase 6, with an OLR of 12.9 kg-VS m^{-3} day^{-1}. The overall VS reduction rates were 84.4, 89.0, 90.0, 92.2, and 92.5%, respectively, at the end of phases 1–5 in Reactor 2.

During the digestion of solid organic waste, methane yield is also a key performance index for the reactor's efficiency. Fig. 2b shows the methane yield in Reactor 2 where a stable digestion was maintained without irreversible acidification. In Reactor 2, high methane yield was maintained until an OLR of 9.2 kg-VS m^{-3} day^{-1}; methane yield then decreased at an OLR of 12.9 kg-VS m^{-3} day^{-1} (Fig. 2b). The first-order conversion-rate constant (k_C) at an OLR of 3.7 kg-VS m^{-3} day^{-1} was low (0.139), but increased to 0.303 at an OLR of 5.5 kg-VS m^{-3} day^{-1}. At higher OLRs, k_c vules of around 0.3 were maintined in the phase 2–5 (Fig. 2b). Overall methane yields in Reactor 2 were 417, 421, 444, 455, and 432 mL g-VS^{-1} at the end of the phases with OLRs of 3.7, 5.5, 7.4, 9.2, and 12.9 kg-VS m^{-3} day^{-1}, respectively.

To evaluate the performance of the anaerobic digestion process for the treatment of organic solid waste, three performance indexes are generally used: (1) the OLR (kg-VS m^{-3} day^{-1}), which serves as an index of the processing speed or potential reduction of reactor volume; (2) the methane yield (mL g-VS^{-1}), which serves as an indicator of the efficiency of energy conversion; and (3) VS reduction (%), which provides a measure of the waste reduction potential of the reactor.

In conventional single-stage wet anaerobic digestion, OLRs of 1–4 kg-VS m^{-3} day^{-1} have been commonly used in the treatment of food waste or of the organic fractions of municipal solid waste (Cho et al., 1995; Heo et al., 2004; Mata-Alvarez et al., 1992; Verrier et al., 1987; Zhang et al., 2007). Even in semi-dry and dry anaerobic digestion, OLR seldom exceeds 10 kg-VS m^{-3} day^{-1} (Bolzonella et al., 2003; Cecchi et al., 1991; Dong et al., 2010; Forster-Carneiro et al., 2008; Scherer et al., 2000; Vallini et al., 1993). For the methane yield and VS reduction, however, lower values were typically reported in semi-dry and dry operations (Cecchi et al., 1991; Dong et al., 2010; Forster-Carneiro et al., 2008). In a study of multiple-stage reactors, slightly lower methane yields were noted at high OLR (Cho et al., 1995; Kim et al., 2006; Verrier et al., 1987). In the present study, the reactors maintained high methane yield and VS reduction throughout the experiment, even at the extremely high OLR of 9.2 kg-VS m^{-3} day^{-1}, which was approximately 2–10 times the conventional level during normal operation of a single-stage wet anaerobic digestion. This high OLR of 9.2 kg-VS m^{-3} day^{-1}, which is equivalent to a COD-based OLR of 15 kg-COD m^{-3} day^{-1}, is in the range of OLR values commonly used in an upflow anaerobic sludge blanket (UASB) reactor, which uses granular sludge for high-quality wastewater treatment (Ye et al., 2011).

3.2. The reactor stability in terms of pH, VFA contents, and NH_4^+ and $SCOD_{cr}$ concentrations

At an OLR of 3.7 kg-VS m^{-3} day^{-1}, the pH value remained stable within the optimal range for anaerobic digestion in both reactors, although the pH in Reactor 1 was slightly lower than in Reactor 2 (Fig. 3). In Reactor 1, however, pH decreased sharply when the OLR increased to 5.5 kg-VS m^{-3} day^{-1}. In contrast, the pH in Reactor 2 remained within the optimal pH range until an OLR of 9.2 kg-VS m^{-3} day^{-1}. The total VFA concentration remained low during the first phase in both reactors, with an OLR of 3.7 kg-VS m^{-3} day^{-1}. However, the total VFA content in Reactor 1 increased to 8149 mg L^{-1} on Day 60 after the OLR was increased to 5.5 kg-VS m^{-3} day^{-1}. Unlike in Reactor 1, no serious accumulation of VFAs were observed in Reactor 2 until an OLR of 9.2 kg-VS m^{-3} day^{-1}, although slightly elevated VFA values occurred during the early days of phases 1, 2, and 4 (Fig. 3). To evaluate the effect of overloading of the reactors, the OLR was increased to 12.9 kg-VS m^{-3} day^{-1} during phase 6, and at that OLR, the VFA concentration increased sharply. By the end of this phase, the pH had decreased to 6.46 and the VFA content reached a maximum concentration of 19 210 mg L^{-1}. Both reactors exhibited stable performance in terms of the $SCOD_{cr}$ and NH_4^+ levels in the effluent. The $SCOD_{cr}$ level was generally maintained below 3000 mg/L, except for Reactor 1 during phase 2 and Reactor 2 during phase 6, and similar trends were observed for the effluent NH_4^+ concentration.

To evaluate the performance and process stability of methane fermentation, several factors (e.g., methane yield, pH, VFA concentration, the concentrations of acetic and propionic acids) have been monitored and their practicality has been evaluated for monitoring process stability (Mata-Alvarez et al., 2000). A pH decrease and VFA inhibition of methanogenesis were reported as the most common inhibitory factors in many previous studies (Ahring et al., 1995; Climenhaga and Banks, 2008b). In the present study, different inhibition trends were observed in the two reactors, although VFA accumulation and process inhibition were observed in both reactors. The biogas production in Reactor 1 was completely inhibited by day 60, when the VFA concentration reached 8149 mg L^{-1} (Figs. 2a and 3). On the other hand, the biogas production in Reactor 2 continued even though the VFA concentration reached more than 2 times the value in Reactor 1 by the last day of the study (Figs. 2a and 3). Climenhaga and Banks (2008b) reported similar results in their study, which evaluated the effects of HRT on the performance of anaerobic digestion. They noted that stable methane gas production was maintained with a long HRT of 180 days even though total VFAs accumulated to a level greater than 15 000 mg L^{-1} in their study. However, they did not observe decreased pH when the VFA concentration reached its maximum value. Similarly, a marked decrease of pH in Reactor 1 was observed by day 60, whereas Reactor 2 showed no pH change until the last few days of the study (Fig. 3). This high stability of gas production at high VFA levels suggests that a pH decrease will have a greater inhibition of methanogenesis than an increase in the VFA concentration (Climenhaga and Banks, 2008b; Pullammanappallil et al., 2001).

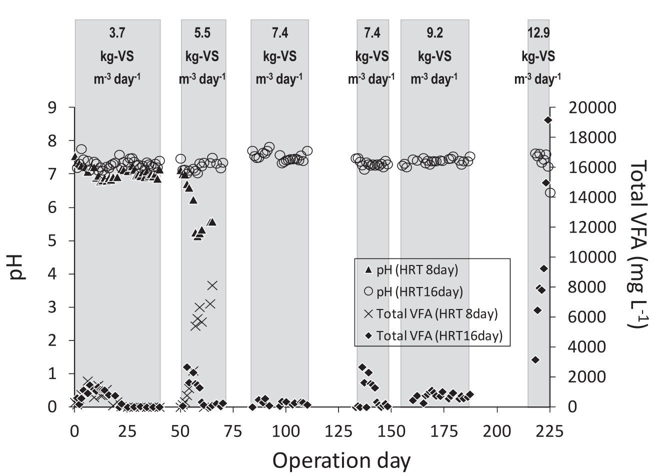

Fig. 3. Time courses for pH and the total volatile fatty acid (VFA) concentration in the two reactors. Shaded areas represent the six phases shown in Table 2; unshaded areas represent times when the organic loading was temporarily stopped. HRT8 and HRT16, hydraulic retention times of 8 and 16 days, respectively.

3.3. Cell density of microorganisms and TSS content in the anaerobic sludge

To estimate the growth and accumulation of anaerobic microorganisms in the digested sludge, cell counts in the sludge were conducted and estimated total cell density for acidogenic and methanogenic bacteria combined, and used this as a key index of sludge stability and activity (Fig. 4a and b). The variations of cell density in both reactors followed similar trends, and increased with increasing OLR. Interestingly, the cell density showed a stepwise and marked increase during phases 1–3, and then showed a gradual increase during the periods of organic loading in phases 4–6 in Reactor 2.

The cell densities in both reactors showed a similar trend during phase 1, with an OLR of 3.7 kg-VS m^{-3} day^{-1}, and were around 2.1×10^{10} cells mL^{-1} at the end of phase 1 (Fig. 4a). After a period with no organic loading between phases 1 and 2, the cell density increased to 2.6 times the value at the end of phase 1, with a level of approximately 6.0×10^{10} cells mL^{-1} in both reactors. This phenomenon was repeated after the period with no organic loading between phases 2 and 3, when the cell density increased to around 9.0×10^{10} cells mL^{-1}, which was about 4.3 times the cell density at the start of the experiment. Inoculum sludge concentrated by sedimentation for 3 days was used under all experimental conditions,

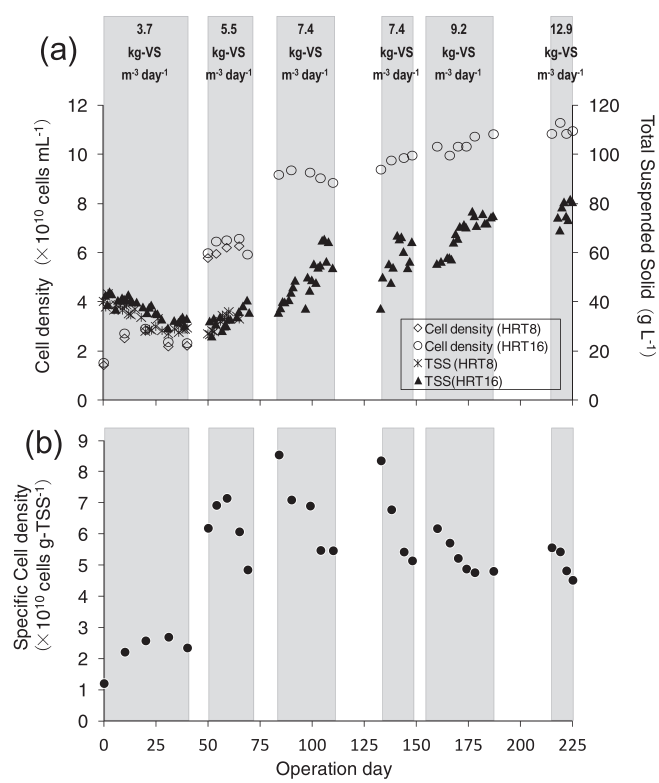

Fig. 4. Time courses for cell density, total suspended solid (TSS) concentration (a) and specific cell density (cells g-TSS^{-1}) in the digested sludge (b). Shaded areas represent the six phases shown in Table 2; unshaded areas represent times when the organic loading was temporarily stopped. HRT8 and HRT16, hydraulic retention times of 8 and 16 days, respectively.

and the original cell density of the seed sludge was 0.75×10^{10} cells mL^{-1} when the seed sludge was collected from the anaerobic digestion plant. The final cell density which was 10.9×10^{10} cells mL^{-1}, which means that cell accumulation was to approximately 15 times the original value in digested seed sludge obtained from the sewage treatment plant.

The TSS concentration decreased during phase 1 and then reached a steady state at around 31 g L^{-1} by the end of the phase. Thereafter, TSS increased gradually with increasing OLR, to a value of 79 g L^{-1} at the end of phase 6, although TSS decreased slightly during the periods with no organic loading between phases 3 and 4 and between phases 4 and 5. TSS saturation occurred at the end of each phase except phases 2 and 6, and the steady-state TSS concentrations at OLRs of 3.7, 5.5, 7.4, and 9.2 kg-VS m^{-3} day^{-1} were approximately 31, 39, 59, and 79 g L^{-1}, respectively.

The VSS and VS values provide an index of the microorganism concentration in the anaerobically digested sludge. However, the concentration of microorganisms depends on the condition of the digested sludge, since the sludge consists of both microbes and undegraded solid organic matter after treatment of a solid organic substrate. In this study, the cell density in the digested sludge increased during the period with no organic loading between phases 1 and 2 (Fig. 4a). The same increase was observed between phases 2 and 3. However, the TSS concentration did not increase during these periods (Fig. 4a). These results showed that only microbe cells increased in the digested sludge during periods with no organic loading. Fig. 4b shows the time course for the specific cell density per unit TSS (cells g-TSS^{-1}). Interestingly, the specific cell density increased during the periods with no organic loading between phases, and high values of specific cell density were observed during the early days of each period with organic loading, except at the start of phase 1. The high values of specific cell density, with values of 6.0×10^{10}–8.5×10^{10} cells g-TSS^{-1} during the early days of each phase, subsequently decreased and reached a similar steady-state value of around 5.0×10^{10} cells g-TSS^{-1} by the end of phases 3, 4, and 5. This result shows that the sludge quality, evaluated based on the specific cell density, was clearly different between phase 1 and subsequent phases. The low value of k_c observed during phase 1 may have resulted from the low specific cell density in this phase (Fig. 2b). In this study, the cell density (cells mL^{-1}) increased with increasing OLR, although the sludge qualities in each phase after phase 2 did not differ greatly in terms of their specific cell density (cells g-TSS^{-1}). This suggests that the increased cell density (cells mL^{-1}) resulted from an increase in the TSS concentration in the digested sludge with increasing OLR, except from phase 1 to phase 2. The stable digestion even at the high OLR of 9.2 kg-VS m^{-3} day^{-1} in the single-stage wet anaerobic digestion may have become possible as a result of this accumulation of microbial cells in the digested sludge.

3.4. The effect of accumulation of microbial cell to the VFA component

Interestingly, different trends in the VFA components in the two reactors were observed (Fig. 5). At VFA concentrations of less than 5000 mg/L, acetic acid and propionic acid accounted for a high percentage of total VFAs in both reactors. However, at VFA concentrations greater than 5000 mg L^{-1}, *n*-butyric acid and *n*-valeric acid accounted for a high proportion of the total VFAs in Reactor 1 (Fig. 5a) but not in Reactor 2 (Fig. 5b). The accumulation of *n*-butyric acid and *n*-valeric acid were reported as typical trends during irreversible acidification, which occurred in Reactor 1 by 60 days (Ahring et al., 1995; Cho et al., 1995; Dong et al., 2010; Izumi et al., 2010). In contrast, high acetic acid and propionic acid proportions were maintained at all total VFA levels in Reactor 2, which had a high cell density in the sludge. For efficient methane production, it is important to have a balance between the reaction rates

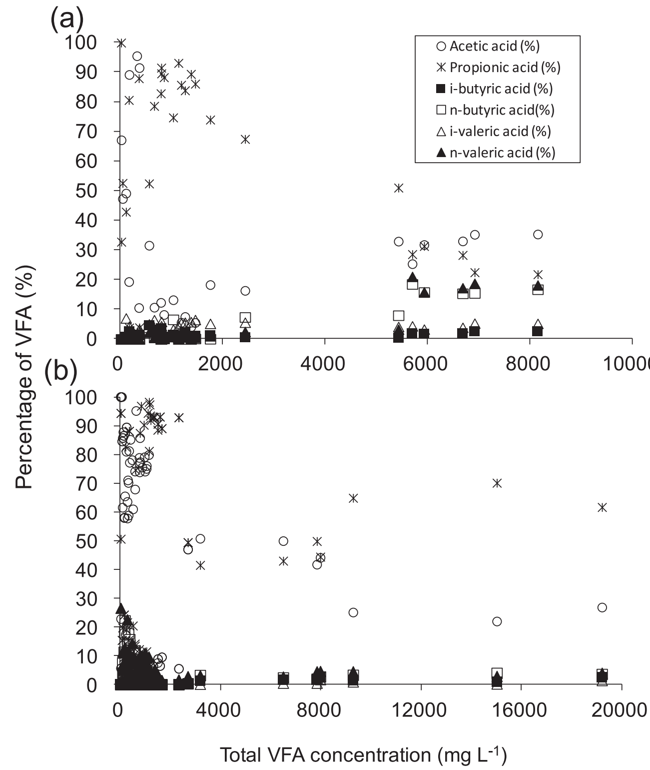

Fig. 5. Changes in the proportions of each VFA as a function of the total VFA concentration in the digested anaerobic sludge. (a) Reactor 1, with HRT = 8 days. (b) Reactor 2, with HRT = 16 days.

during the different steps involved in the anaerobic digestion of complex organic matter (Vavilin et al., 2008). The importance of a balance between acidogenesis and methanogenesis were noted in many previous works (Ahring et al., 1995; Rétfalvi et al., 2011; Vavilin et al., 2008). In Reactor 2, decreases in gas production and in VFA accumulation were observed at the highest OLR of 12.9 kg-VS m^{-3} day^{-1} (Fig. 2a, 3). However, a drastic increase in TSS and the accumulation of longer-chain fatty acids such as *n*-butyric acid and *n*-valeric acid was not observed even at this high OLR. This suggested that high hydrolytic and acidogenic activity were maintained even at high OLR, resulting in increasing acid production and acidification of the digested sludge. The low proportions of the longer fatty acids at higher total VFA concentrations also indicated that high activity by microbes that consume the *n*-butyric acid and *n*-valeric acid could be retained in the digested sludge.

3.5. The performance comparison and the maximum OLR for the single-stage wet anaerobic digestion with high methane yield

The theoretical methane production rate can be defined using the chemical composition of the substrate, and relatively similar values ranging from 0.4 to 0.5 L CH_4 g-VS^{-1} were reported in previous studies of food waste digestion (Cho et al., 1995; Heo et al., 2004). This range is narrow because the chemical composition of food waste does not vary significantly among seasons and countries, despite differences in the food culture (Kim et al., 2006; Lim et al., 2008; Mata-Alvarez et al., 1992; Zhang et al., 2007). A high methane yield were obtained in the single-stage wet anaerobic digestion compared with yields from recently developed digestion systems, such as multiple-stage reactors and semi-dry or dry anaerobic digestion (Table 3). This suggests that the methane yield is not a useful performance index for distinguishing between the

Table 3
The performance and operational conditions on the various types of anaerobic digestion of food waste.

Reactor type	Temp. (°C)	Operated days	TS (TSS) of Sludge (%)	OLR (g-VS L^{-1} day^{-1})	CH_4 yield (mL g-VS^{-1})	VS reduction (%)	Operation	Reference
Single wet CSTR	50	28	–	–	425	–	Batch	Zhang et al. (2007)
Single wet CSTR	50	28	–	–	445	–	Batch	Zhang et al. (2007)
Single wet CSTR	37	25	–	–	472	–	Batch	Cho et al. (1995)
Single wet CSTR	35	40	–	–	489	–	Batch	Heo et al. (2004)
Single wet CSTR	35	90	0.7–1.1	1.68–2.8	478	90–91	Continuous	Mata-Alvarez et al. (1992)
Single wet CSTR	35	–	–	4.4	371	87	Continuous	Verrier et al. (1987)
Single wet CSTR	60	–	–	6.25	364	83	Continuous	Verrier et al. (1987)
Single semi-dry	30	60	16.0	–	273	–	Batch	Dong et al. (2010)
Single semi-dry	30	60	13.5	–	283	–	Batch	Dong et al. (2010)
Single semi-dry	30	60	11.0	–	314	–	Batch	Dong et al. (2010)
Single semi-dry	55	60	20.5	–	220	–	Batch	Forster-Carneiro et al. (2008)
Single semi-dry	50	80	20.1	9.2	230	–	Continuous	Bolzonella et al. (2003)
Single semi-dry	–	–	–	13.5	300	–	Continuous	Vallini et al. (1993)
Single semi-dry	–	–	–	7.6	220	–	Continuous	Scherer et al. (2000)
Single semi-dry	55.0	80	14.16	5.9	262	48	Continuous	Cecchi et al. (1991)
Single semi-dry	54.8	–	9.35	6.9	254	43	Continuous	Cecchi et al. (1991)
Single semi-dry	54.9	–	19.47	9.2	165	34	Continuous	Cecchi et al. (1991)
Single semi-dry	54.0	–	19.09	10.7	140	31	Continuous	Cecchi et al. (1991)
Single semi-dry	54.6	–	17.33	13.5	160	37	Continuous	Cecchi et al. (1991)
2 stage	60	–	–	5.65	420	96	Continuous	Verrier et al. (1987)
2 stage	35	36	0.46	–	330	78	Continuous	Wang et al. (2005)
3 stage	50	30	–	11.1	395	–	Continuous	Kim et al. (2006)
2 stage	37	120	–	1.04	373	90	Continuous	Cho et al. (1995)
2 stage	37	30	–	1.75	367	89.2	Continuous	Cho et al. (1995)
2 stage	37	17	–	3.39	360	89.8	Continuous	Cho et al. (1995)
Single wet CSTR[a]	37	40	3.4 (3.3)	3.7	417	84.4	This study	–
Single wet CSTR[a]	37	69	4.2 (4.1)	5.5	421	89.0	This study	–
Single wet CSTR[a]	37	148	6.7 (6.5)	7.4	444	90.0	This study	–
Single wet CSTR[a]	37	187	7.2 (7.1)	9.2	455	92.2	This study	–
Single wet CSTR[a]	37	225	8.3 (8.1)	12.9	432	92.5	This study	–

[a] Single CSTR with a biomass retention scheme.

efficiency of different reactor types, and that process stability at high OLR and cost and energy reductions are more critical performance indicators for current waste management in full-scale plants.

In multiple-stage reactors, a series of two or more reactors regulates the production of VFAs in a hydrolysis and acidification reactor and the solubilized substrate is then introduced into the next reactor in the series for methane production and recovery (Zhu et al., 2011). One of the main advantages of multiple-stage reactors is that UASB and expanded granular sludge bed (EGSB) reactors have already been used for high-quality wastewater treatment at OLRs of more than 10 kg-COD m^{-3} day^{-1} (Ye et al., 2011). If removal of the suspended solids from the wastewater before loading of the methanogenic reactor could be achieved, effective granulation or the accumulation of microorganisms in the digested sludge are expected in the methanogenic reactor, resulting in increased stability during operation at high OLR. Multiple-stage reactors produced slightly lower methane yields of around 300–400 mL g-VS^{-1} than in single-stage wet reactors (Table 3). In the multiple-stage reactors, dilution water is generally added into the process to enhance solubilization of the substrate before loading it into the methanogenic reactor. This dilution of the substrate increases wastewater volume and decreases COD in the wastewater, resulting in decreased HRT in the methanogenic reactor. Thus, the lower HRT may be related to the slightly lower methane yield in the multiple-stage digestion.

In contrast, with multiple-stage digestion, semi-dry (10–20% TS) and dry (20–35% TS) digestion, which use high-TS sludge without adding water to the substrate, have achieved high OLR and stable digestion. This high performance in operational plants can paradoxically be explained by the low VS reduction and low methane yield of these reactors. A low VS reduction of around 50% and a low methane yield of 140–314 mL CH_4 g-VS^{-1} were reported in previous studies of semi-dry and dry anaerobic digestion (Table 3). Cecchi et al. (1991) reported that the VS reduction decreased with increasing OLR in semi-dry anaerobic digestion, with VS reductions of 48, 43, 34, 31, and 37% at OLRs of 5.9, 6.9, 9.2, 10.7, and 13.5 kg-VS m^{-3} day^{-1}, respectively. Interestingly, although decreased VS reduction and methane yield were observed, VFA accumulation and inhibition of methanogenesis were not observed even at a high OLR of 13.5 kg-VS m^{-3} day^{-1}. This low VS reduction indicates that undegraded organic matter remained in the digested sludge and was not transformed into short-chain fatty acids, since the mass transfer efficiency decreases at high TS contents in the anaerobic sludge, resulting in high stability without VFA inhibition of methanogenic activity. Veeken and Hamelers (1999) noted that the limitations in mass transport at high TS levels will have positive effects by preventing the accumulation of VFAs and the resulting inhibition of methanogenesis. They also proposed that improved performance in dry anaerobic digestion could be achieved by varying the rate of leachate recirculation, which can regulate the hydrolysis of the biowastes without VFA accumulation.

In Reactor 1, with an HRT of 8 days, irreversible acidification occurred during phase 2, at an OLR of 5.5 kg-VS m^{-3} day^{-1}, and gas production was completely inhibited by day 60 (Figs. 2a and 3). This suggests that the large amount of additional water at a shorter HRT accelerates hydrolysis and acidogenesis in the reactor, resulting in irreversible accumulation of VFAs followed by inhibition of methanogenesis. In contrast, the reactor performance was maintained at the longer HRT of 16 days in Reactor 2, which produced high methane yield and high VS reduction throughout the experiment, even at the high OLR of 9.2 kg-VS m^{-3} day^{-1} (Fig. 2a and b). The TSS concentration also increased to 80 g/L, which is intermediate between the values under wet and semi-dry conditions, and

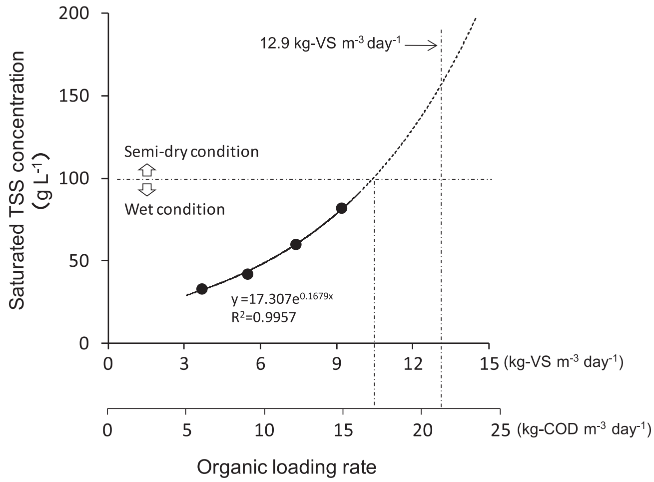

Fig. 6. The relationship between the saturated TSS concentration and the operational organic loading rate.

the cell density in the digested sludge increased to more than 10 times the value in conventional sludge when the OLR increased to 9.2 kg-VS m^{-3} day^{-1} (Fig. 4a). These results indicated that Reactor 2 maintained a high mass transfer efficiency for the digested sludge at a lower sludge TS than under semi-dry or dry conditions, and that high process stability could be achieved by the high microbial density that developed in this reactor.

Based on the results, it is possible to predict the maximum OLR that can be used in the single-stage wet anaerobic digestion of food waste. In a CSTR, the TSS concentration should become saturated and reach a steady state with continuously stable treatment, and the saturated concentration would depend on the biodegradability of the substrate and on the OLR. In this study, the TSS concentrations became saturated and reached a steady state, and the saturated TSS concentration increased with increasing OLR. Fig. 6 shows the relationship between the saturated TSS concentration and OLR (kg-VS m^{-3} day^{-1}). A significant correlation between the two factors ($y = 17.3e^{0.1679x}$, $r^2 = 0.996$, $P < 0.05$) was found. In phase 6, the effect of overloading the reactor by introducing the highest OLR (12.9 kg-VS m^{-3} day^{-1}) into reactor 2 were estimated. Based on this regression equation, and if stable digestion can be achieved at this highest OLR without irreversible acidification, the TSS in the digested sludge will increase to at least 150 g-TSS L^{-1}, which represents semi-dry conditions; thereafter, the mass transfer efficiency, methane yield, and VS reduction will decrease. If sufficient mass transfer efficiency can be maintained in the digested sludge until 100 g-TSS L^{-1}, the maximum OLR would be approximately 10.5 kg-VS m^{-3} day^{-1} (equivalent to 17 kg-COD m^{-3} day^{-1}) in a single-stage wet anaerobic operation with high methane yield and high VS reduction.

4. Conclusions

A stable operation at a high OLR of 9.2 kg-VS (15.0 kg-COD) m^{-3} day^{-1} was achieved with a high methane yield. Microbial growth mainly occurred during periods without organic loading and the cell density increased to 15 times higher than that of the seed sludge. However, saturated TSS increased with increasing OLR, which will decrease the mass transfer efficiency in the sludge, resulting in a decrease in methane yield. A theoretical maximum OLR of 10.5 kg-VS (17.0 kg-COD) m^{-3} day^{-1} that maintains a stable operation with a high methane yield was estimated from the OLR and saturated TSS correlation.

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