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Anaerobic digestion of brewery primary sludge to enhance bioenergy generation: A comparison between low- and high-rate solids treatment and different temperatures

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ABSTRACT

Anaerobic digestion of brewery wastewater solids in the form of primary sludge was investigated for its potential as a source of energy (methane). We operated a low-rate (hydraulic retention time (HRT) = solids retention time (SRT)) continuously stirred anaerobic digester (CSAD) and a high-rate (SRT > HRT) anaerobic sequencing batch reactor (ASBR) in parallel for 250 days. We found that high-rate anaerobic digestion was beneficial for solids-rich waste flows even during a long-term operating period that included a shock load of nonbiodegradable total solids. The ASBR biomass achieved a higher specific methanogenic activity compared to the CSAD biomass (0.257 ± 0.043 vs. 0.088 ± 0.008 g CH_4-COD g^{-1} VSS d^{-1}), which aided in stability during the shock load with total solids. The methane yield for the ASBR was 40–34% higher than for the CSAD (0.306 vs. 0.219 l CH_4 g VS^{-1} fed for days 1–183 and 0.174 vs. 0.130 l CH_4 g VS^{-1} fed for days 184–250, respectively). Finally, we operated an ASBR for an additional 295 days to evaluate the effect of temperature variation on system stability. A stable performance was achieved between the operating temperatures of 22–41 °C.

1. Introduction

Anaerobic digestion (AD) has been an alternative method for the treatment of industrial organic wastewaters for over 40 years (McCarty, 2001). With the rising cost of nonrenewable fuels and political pressure to shift society toward renewable energy, interest in production of heat and/or electricity from biogas (i.e., combined heat and power) has been rekindled. In fact, electricity from biogas (not including landfill gas) increased in Europe by 61.5% from 2006 to 2007, and it has also increased in non European countries (EurObserv'ER, 2008). Specifically, high-rate AD of brewery wastewater has considerably reduced the biochemical oxygen demand (BOD) loading to municipal treatment plants and has produced up to five times the amount of energy required for the entire brewery wastewater treatment process (including post-treatment), offering substantial economic savings (Bocher et al., 2008; Getz et al., 2008; Shao et al., 2008). Compared to conventional (aerobic) treatment, AD requires less energy for its operation, produces less sludge, is more resilient, and offsets nonrenewable boiler fuels (Lettinga, 1995; Speece, 1983). In addition, wastewater from the brewing industry typically has variable pH, high chemical oxygen demand (COD) content, and variable levels of nutrients, making it difficult to treat with traditional aerobic methods (Ince et al., 2001; Yan and Tay, 1996). Indeed, the largest brewer in the US, Anheuser-Busch InBev, Inc., operates anaerobic digesters for wastewater treatment at ten of its 12 breweries in the US (Getz et al., 2008).

Anaerobic bioreactors for soluble wastewaters in the brewery industry are almost exclusively based on high-rate systems that extend the solids retention time (SRT) compared to the hydraulic retention time (HRT) by retaining biomass (Klass, 1984; Sung and Dague, 1995). Examples of high-rate anaerobic digester systems include upflow anaerobic sludge blanket (UASB) (Lettinga et al., 1980), anaerobic baffled reactor (ABR) (Bachman et al., 1985), anaerobic migrating blanket reactor (AMBR) (Angenent and Sung, 2001), and anaerobic sequencing batch reactor (ASBR) (Sung and Dague, 1995) systems. Many breweries utilize a wastewater treatment scheme in which after a screening step, the remaining solids (particle size <1 mm) are fed along with soluble organic components to high-rate upflow anaerobic bioreactors. In this case, these solids are mostly carried through in the effluent along with excess methanogenic biomass (referred to as secondary residuals) because the biodegradation of solids in these high-rate bioreactors

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is low due to the short residence times. In a previously published paper, we digested these secondary residuals in mesophilic (35 °C) and thermophilic (55 °C) CSAD reactors (Bocher et al., 2008). We showed that a mesophilic, low-rate AD system with a minimum HRT of 10 days increased the methane production by up to 8.1% when compared to soluble wastewater treatment alone. In addition, the volatile solids (VS) concentration of the secondary residuals stream was reduced by 43%, greatly reducing the sewer fees that are based on BOD and total suspended solids (TSS) from the anaerobic bioreactor effluent.

Instead of feeding solids to high-rate upflow bioreactors, some breweries employ an alternative wastewater solids management strategy in which primary clarification after the screening step generates a separated-slurry stream with particle size <1 mm (i.e., primary sludge). Because of the presence of particulates, such as yeast cells and (hemi)cellulosic particles (i.e., grain, fines, trub, hops, and rice) in primary sludge, a successful treatment strategy must allow the residence time of the solids to be long enough to provide for biological hydrolysis. Generally, most solids-rich slurries, such as waste activated sludge (WAS, up to ~45 g total solids (TS) l^{-1}), require long residence times and are treated with low-rate CSAD bioreactors. This is because of anticipated problems with long-term TS accumulation in high-rate digesters, which could lower the VS/TS ratio, and thus the biological activity (Wang et al., 2009). However, studies on the anaerobic digestion of solids-rich swine waste (Angenent et al., 2002) and WAS (Chang et al., 1994; Wang et al., 2009) have shown that high-rate treatment in ASBRs can accommodate solids. The ASBR employs perhaps the simplest method of solids retention because in the sequence of steps leading up to substrate addition, biomass is allowed to settle before decanting effluent (Sung and Dague, 1995).

Here, we investigated whether high-rate AD is an advantageous treatment system for a high-solids brewery stream (primary sludge) without excessive long-term TS accumulation. For this reason, we operated a CSAD and an ASBR in parallel while feeding the same waste for 250 days. We also investigated whether it was possible to digest brewery primary sludge under variable feed conditions (we obtained 21 different substrate batches during the course of our operating run with differing levels of COD and TS), while maintaining a stable digester performance. The short-term effect of relatively fast changes in the operating temperature on hydrolysis and methanogenesis in the ASBR was investigated during an additional operating period of 295 days. Finally, in a previous paper we had suggested that secondary residuals would be advantageous to digest compared with primary sludge from breweries because of a lower variability and because of augmentation of methanogens from the high-rate, soluble wastewater bioreactor to the CSAD (Bocher et al., 2008). We, therefore, also compared the methane yields for CSAD systems at 37 °C treating primary sludge (this study) with those treating secondary residuals (Bocher et al., 2008) to gauge what would be the best route of AD treatment of solids for optimal energy recovery.

2. Methods

2.1. Experimental apparatus

Experiments were conducted in two identical laboratory-scale bioreactors; one operated as a CSAD and one operated as an ASBR by employing a continuous and intermittent mixing scheme, respectively. The reactors were constructed of glass (Midrivers Glassblowing, Inc., St. Charles, MO) with a maximum working volume of 5 l and had a water jacket to maintain constant temperatures with an external heating recirculator (PolyScience Model 210, Niles, IL). A mechanical agitator (Model 5vb, EMI, Inc., Clinton, CT) was equipped with a 62-mm diameter axial flow impeller (Lightnin A-310, Rochester, NY) to stir the reactors at ~300 rotations per minute (RPM). After day 250 of the operating period (period II), the mixing in the ASBR was carried out by biogas recirculation with a peristaltic pump (Cole-Parmer, Vernon Hills, IL). Primary sludge was introduced into the reactors manually. To prevent biogas loss during feeding, the decanting/feeding tube extended midway into the reactor contents. A peristaltic pump (Cole-Parmer, Vernon Hills, IL) was used for decanting effluent. The gas collection scheme of each digester system consisted of a foam separation bottle, a pressurized ball used to eliminate air from being suctioned into the digesters during the decanting of effluent, a bubbler to allow visual detection of gas production, a biogas sampling port, and a gas meter (type 1-l, Actaris Meterfabriek, Delft, The Netherlands). We have given detailed reactor schematics previously, in Bocher et al. (2008) (CSAD and ASBR before day 250) and Agler et al. (2008) (ASBR after day 250).

2.2. Reactor operation

We operated CSAD and ASBR systems for 250 days (period I), followed by operation of the ASBR alone for 295 additional days (period II). At the beginning of period I, we inoculated the bioreactors with 1.0 l of blended anaerobic granular biomass from a mesophilic anaerobic upflow bioreactor (i.e., EGSB-biobed system) treating soluble brewery wastewater (Anheuser-Busch InBev, Inc., St. Louis, MO). We allowed 2 weeks for the biomass to acclimate to 37 ± 1 °C and the mixing schedule before feeding. Solids removed in primary clarifiers from the Anheuser-Busch InBev, Inc. brewery in Baldwinsville, NY was received every 2–3 weeks and was allowed to settle further in our lab upon arrival to achieve ~40 g VS l^{-1} (Table 1). Next, the substrate was stored at −20 °C until use. The ASBR was mixed for 1 min every 30 min with a 1-h biomass-settling period before decanting. Thus, the cycle for the ASBR was: instantaneous feeding step, ~23-h reacting step, ~1-h settling step, and a 2-min decanting step after which the cycle was repeated. Because the HRT and the SRT are uncoupled in ASBRs, the SRT is only meaningful at steady state. True steady state is only achieved after long periods of operation and certainly not during the rapid loading increases performed during our start-up. See our description of steady state in the materials and methods section of Bocher et al. (2008). Thus, for purposes of comparison we will use HRT in this paper to describe loading rates. During the start-up period (period I), we first operated with an HRT of 50 days (0.8 g VS l^{-1} d^{-1}) after which we shortened the HRT in a step-wise manner by a factor of 1.25 on days 52, 91, 124, 149, 201, 219, and 236 to achieve a final HRT of 10 days (4.0 g VS l^{-1} d^{-1}) (Fig. 1) during period I. Loading rate increases were made when total volatile fatty acid (VFA) concentrations and gas production rates were stable (Ahring et al., 1995) and when at least a time period of one HRT had passed, except during the 40-day HRT (operated for 39 days) (Fig. 1). We refer in this paper to pseudo steady-state conditions based on these stable performance parameters.

During period II, the short-term effects of relatively fast temperature variations on the performance of the ASBR were observed. Initially, the HRT was maintained at 15 days from day 250–286 after which it was shortened to 12.8 days for most of the remainder of the study (except for a brief increase in HRT to 20 days during days 533–543) (Fig. 3). At the beginning of period II, a 5 °C temperature decrease was made whenever stable biogas production conditions were obtained (Fig. 2). The temperature was decreased from 37 to 32 °C on day 357, to 27 °C on day 371, and to 22 °C on day 392. The reactor temperature was then increased to 27 °C

Table 1
Concentration of soluble and total chemical oxygen demand (SCOD and TCOD), volatile solids (VS), and total solids (TS) for the 21 different substrate batches used in period I and II. For each batch of substrate, VS removal efficiency (VS_{rem}) was calculated by the difference between stable concentrations in the influent and effluent for the CSAD and ASBR reactors.

Period	Substrate batch	Days used	SCOD (g/L)	TCOD (g/L)	VS (g/L)	TS (g/L)	VS_{rem} (CSAD)	VS_{rem} (ASBR)
I	F1	0–47	9.50 ± 1.37 (n = 6)	54.08 ± 13.74 (n = 6)	34.56 ± 1.33 (n = 7)	53.51 ± 1.70 (n = 6)	58.76%	59.60%
	F2	48–77	7.92 ± 0.34 (n = 3)	72.11 ± 1.64 (n = 3)	38.36 ± 3.25 (n = 6)	48.19 ± 3.91 (n = 6)	53.62%	54.69%
	F3	78–112	10.27 ± 0.30 (n = 3)	73.75 ± 1.77 (n = 2)	40.17 ± 0.41 (n = 6)	84.66 ± 0.79 (n = 6)	50.14%	59.30%
	F4	113–138	9.24 ± 1.66 (n = 3)	51.39 ± 9.92 (n = 3)	40.15 ± 1.94 (n = 6)	61.33 ± 2.33 (n = 6)	46.75%	52.20%
	F5	139–158	7.54 ± 2.32 (n = 2)	58.59 ± 9.29 (n = 3)	40.40 ± 1.91 (n = 6)	233.10 ± 12.76 (n = 6)	n/a	48.49%
	F6	159–183	17.73 ± 4.37 (n = 3)	86.67 ± 16.29 (n = 3)	40.83 ± 0.69 (n = 4)	192.86 ± 1.43 (n = 4)	n/a	49.42%
	F7	184–194	9.20 (n = 1)	62.00 (n = 1)	37.98 ± 0.99 (n = 4)	78.40 ± 1.80 (n = 4)	n/a	53.11%
	F8	195–217	7.73 ± 0.92 (n = 3)	65.00 ± 7.94 (n = 3)	42.03 ± 0.57 (n = 3)	81.24 ± 0.71 (n = 3)	35.69%	51.16%
	F9	218–227	6.80 (n = 1)	52.00 (n = 1)	45.21 ± 0.33 (n = 2)	151.85 ± 1.04 (n = 2)	38.98%	52.29%
	F10	228–243	9.80 (n = 1)	67.00 (n = 1)	39.51 ± 0.32 (n = 2)	70.45 ± 0.46 (n = 2)	n/a	n/a
	F11	244–250	2.00 (n = 1)	36.00 (n = 1)	40.20 ± 1.62 (n = 2)	104.77 ± 8.88 (n = 2)	n/a	n/a
II	F12	251–273	15.47 ± 5.33 (n = 3)	110.00 ± 19.80 (n = 2)	38.82 ± 2.35 (n = 3)	57.00 ± 2.60 (n = 3)	–	n/a
	F13	274–318	17.00 ± 8.31 (n = 6)	75.67 ± 16.71 (n = 7)	40.30 ± 2.53 (n = 7)	67.72 ± 4.69 (n = 7)	–	43.00%
	F14	319–343	18.25 ± 4.73 (n = 4)	80.00 ± 4.90 (n = 4)	41.20 ± 0.36 (n = 3)	137.70 ± 0.68 (n = 3)	–	48.35%
	F15	344–377	11.88 ± 3.28 (n = 4)	74.50 ± 15.95 (n = 4)	41.96 ± 3.01 (n = 5)	84.02 ± 6.14 (n = 5)	–	n/a
	F16	378–401	17.00 ± 1.75 (n = 4)	94.50 ± 9.47 (n = 4)	40.47 ± 1.03 (n = 5)	100.23 ± 2.61 (n = 5)	–	24.56%
	F17	402–429	22.00 ± 5.66 (n = 2)	93.35 ± 7.57 (n = 2)	40.36 ± 1.64 (n = 3)	76.20 ± 2.62 (n = 3)	–	24.83%
	F18	430–468	12.43 ± 3.22 (n = 5)	91.66 ± 9.10 (n = 5)	41.36 ± 1.11 (n = 6)	89.50 ± 2.39 (n = 6)	–	57.79%
	F19	469–497	16.96 ± 4.36 (n = 4)	84.95 ± 16.76 (n = 4)	40.00 ± 3.56 (n = 4)	104.48 ± 7.75 (n = 4)	–	37.33%
	F20	498–517	13.24 ± 2.58 (n = 3)	103.49 ± 9.89 (n = 3)	39.46 ± 1.89 (n = 3)	115.34 ± 3.51 (n = 3)	–	n/a
	F21	518–544	28.61 ± 8.00 (n = 3)	92.64 ± 8.46 (n = 3)	41.26 ± 4.27 (n = 3)	83.39 ± 1.80 (n = 3)	–	n/a

n/a: Analysis was not performed during periods of instability (usually defined by high VFA levels).
The number of samples for each average are not uniform and represent the maximum number of replicate measurements carried out on each batch of substrate.

on day 438 and to 37 °C on day 441 until biogas production re-stabilized. During the second part of period II, temperature increases were made on days 490, 497, 504, and 511 to 39, 41, 42, and 43 °C, respectively. Subsequently, a decrease to 42 °C was made on day 522, the HRT was increased to 20 days on day 533 (to re-stabilize the performance), and finally the HRT was returned to 12.8 days (42 °C) on day 544 (Fig. 3).

2.3. Analyses

TS, VS, total VFA (distillation method), soluble and total chemical oxygen demand (SCOD and TCOD) (closed-reflux titrimetric method), and alkalinity (endpoint pH titration) were performed according to *Standard Methods* (Clesceri et al., 1998). The TS, VS, SCOD, and TCOD levels of each feed batch were also measured. Total ammonium (i.e., sum of ammonia and ammonium) was measured using an ion-selective electrode (Model Orion 9512, Thermo Electron Corporation, Beverly, MA). To evaluate the performance of the reactors, the following measurements were performed: (1) daily: pH, biogas production, and room temperature and pressure (to correct biogas production to standard conditions) and (2) weekly for reactor effluent: TS, VS, total VFA, SCOD and TCOD concentrations, alkalinity, and total ammonium. Methane content in the biogas was measured periodically with a gas chromatograph (Series 350, Gow-Mac Instruments, Co., Bethlehem, PA) with a thermal conductivity detector. The GC column was a $4' \times 1/8''$ o.d. 20% DC-200 on Chromosorb P AW-DMCS, 80/100 mesh (Varian, Inc., Palo Alto, CA). The temperatures for the injection port, detector, and column were 50, 115, and 25 °C, respectively. VS removal efficiencies were evaluated as the percentage of the influent VS that was removed in each reactor. The efficiencies were calculated individually for each substrate batch, except for batches used during the 20 and 16-day HRT for the CSAD when VFA levels were elevated, and the 10-day HRT because both reactors remained unstable (Table 1). In addition, we performed specific methanogenic activity tests (after day 67) monthly or bimonthly to evaluate the role of acetoclastic methanogens in the reactors. Specific methanogenic activity tests were adapted from Rinzema et al. (1988), and were performed at the operating temperatures of the sampled biomass. The bottles were prepared in an anaerobic hood. The nutrient solution was prepared according to Zehnder et al. (1980) after a modification (Angenent et al.,

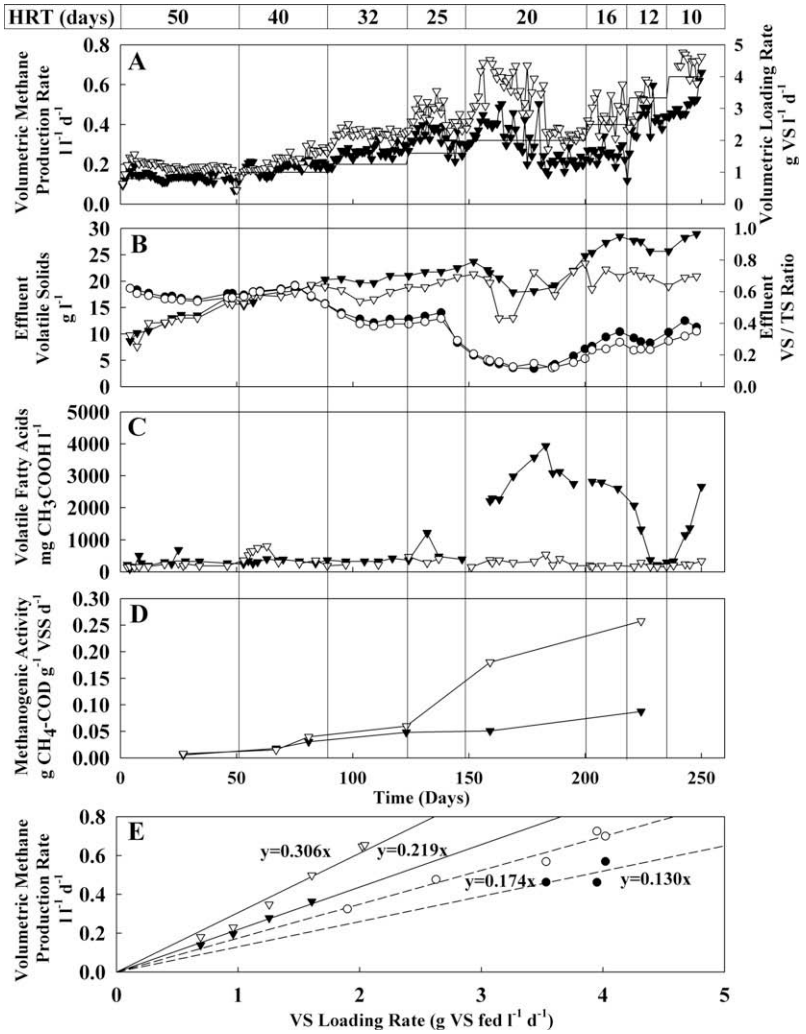

Fig. 1. Operating conditions and performance of the CSAD (filled symbols) and ASBR (open symbols) systems (period I, days 1–250). (A) Volumetric methane production rate. (B) Effluent volatile solids concentration (triangles) and volatile/total solids ratio (circles). (C) Effluent volatile fatty acid concentration. (D) Specific methanogenic activity. (E) Methane yield in l CH_4 g VS^{-1} fed divided into two distinct periods: day 1–183 (triangles) and day 184–250 (circles).

2002). Acetate was added at a concentration of 2 g l^{-1}. Pure nitrogen gas was used for flushing the headspace. Before the methanogenic activity measurement on the second day, we added acetate (an additional 1 g l^{-1}), adjusted the pH under an anaerobic atmosphere, and flushed the headspace again with pure nitrogen. Fiber specimens were viewed under a light microscope (BX41, Olympus, Melville, NY) and digital images were captured with a CCD camera (QImaging, Burnaby, Canada). Openlab 3.5 software (Imporovision Inc., Lexington, MA) was used to digitally capture the images. Fiber specimens were also viewed under a Hitachi S-450 SEM (Hitachi America, Brisbane, CA) at 20 kV accelerating voltage and captured on Polaroid 55P/N film (Polaroid, Minnetonka, MN). The negatives were scanned and inverted with Photoshop 7 (Adobe System, Seattle, WA).

2.4. Statistical analysis

SAS software (Version 9.2, SAS Institute Inc., Cary, NC) was used to analyse the relationship of methane yield to independent variables describing the substrate. We chose to use the "Reg" procedure with the C(p) method for selection of variables (Eq. (1)). The C(p) method finds the least number of independent variables that are able to sufficiently describe the dependent variable. The program selects the proper variables for a model equation by locating a balance between a better fitting model (increased R^2 values) and a minimized total mean square error of the model.

3. Results

3.1. Substrate characteristics and variability

To evaluate the effect of variability of primary sludge on treatment stability, we obtained 21 different batches of substrate from the brewery (F1–F21, Table 1). Even though the VS concentration was maintained at 40 g l^{-1}, the TS concentration changed greatly during the course of the study (Table 1). Much of this variability was caused by the presence of variable amounts of nonbiodegradable diatomaceous-earth filter material (Fig. S1A in the Supporting Information). The average pH for the feed was 5.28 ± 0.62 (n = 16), while the SCOD concentration in the substrate varied substantially from a minimum of ~2 g l^{-1} in F11 to a maximum of ~29 g l^{-1} in F21 (Table 1). TCOD concentrations also varied widely (~36–110 g l^{-1}) throughout the study (Table 1). Using light microscopy and SEM, we observed that the feed contained large quantities of fibrous plant-derived material, which likely made up much of the insoluble (particulate) fraction of COD (Fig. S1A and B). Measured

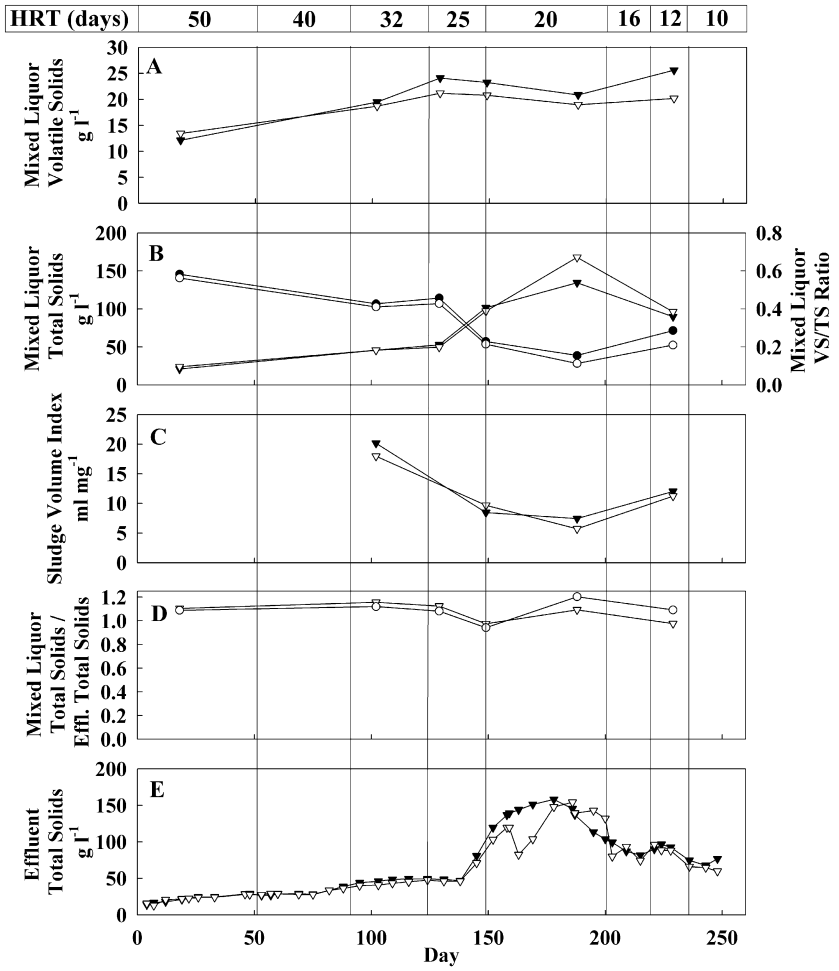

Fig. 2. Mixed liquor and effluent solids characterization of the CSAD (filled symbols) and ASBR (open symbols) systems (Period I, days 0–250). (A) Mixed liquor volatile solids. (B) Mixed liquor total solids (triangles) and mixed liquor VS/TS ratios (circles). (C) Sludge volume index. (D) Mixed liquor solids/effluent solids ratio for ASBR, VS (triangles) and TS (circles). (E) Effluent total solids.

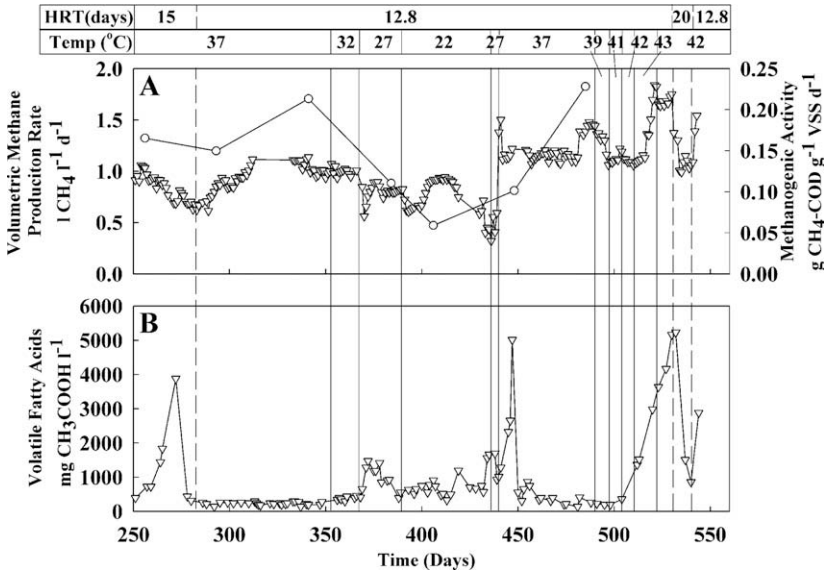

Fig. 3. Operating conditions and performance of the ASBR system (period II, days 251–545). Solid vertical lines correspond to changes in temperature, and dashed vertical lines correspond to changes in HRT. (A) Volumetric methane production (triangles) and specific methanogenic activity (circles). (B) Effluent volatile fatty acid concentration.

levels of total ammonium were consistently low (compared to the effluent) with an average of 166.72 ± 120.57 mg NH_3–N l^{-1} (n = 11) during period I and somewhat higher with an average of 508.36 ± 174.47 mg NH_3–N l^{-1} (n = 10) during period II.

Table 2
Details of reactor performance during operational changes in period I and period II.

	Change	Day	Methane production	VS/TS ratio	VFA levels
Period I (day 1–250): Reactor start-up and decrease in HRT from 50 to 10 days	Initial start-up	1–138	Increase to 0.370 ± 0.031 and 0.487 ± 0.044 l CH_4 l^{-1} d^{-1} (n = 10) for CSAD and ASBR, respectively	Reached stable levels ~0.45	Stable <500 mg CH_3COOH l^{-1}
	F4–F5 (increase in TS, Table 1)	139–158	Decrease to 0.286 ± 0.059 and 0.408 ± 0.031 l CH_4 l^{-1} d^{-1} (n = 5)	Begin to decrease	Begin to increase in CSAD
	F5–F6 (high TS and SCOD, Table 1)	159–183	Local minimum on day 184: 0.150 and 0.275 l CH_4 l^{-1} d^{-1} in CSAD and ASBR, respectively	Minimum 0.11 and 0.12 on days 178 and 186 for CSAD and ASBR, respectively	Increase in CSAD to ~4000 mg CH_3COOH l^{-1}
	After biomass washout	CSAD:159–250 ASBR:184–250	Yield decrease: 0.219–0.130 l CH_4 g^{-1} VS fed and 0.306 to 0.174 l CH_4 g^{-1} VS fed for CSAD and ASBR, respectively		
	Change	Day	Methane production	VFA level	SMA
Period II (day 251–545): Effects of alteration of temperature on ASBR performance and stability	Temperature decrease (37–22)	357–438	Decrease to 0.64 ± 0.02 l CH_4 l^{-1} d^{-1} (n = 8)	Increase to ~500–700 mg CH_3COOH l^{-1}	Decrease from 0.213 ± 0.025 to 0.059 ± 0.012 g CH_4-COD g^{-1} VSS d^{-1}
	Temperature increase (37–39)	490	Increase to 1.50 l CH_4 l^{-1} d^{-1}	Decrease to ~200 mg CH_3COOH l^{-1}	
	F20 (high TS)	498	Decrease to ~1.10 l CH_4 l^{-1} d^{-1}		
	Temperature increase (41–43)	504		Begin to increase sharply	
	F21 (normal TS) and temperature decrease (43–42)	F21: 518; temperature decrease: 522	Increase to ~1.70 l CH_4 l^{-1} d^{-1}	Reached maximum ~5200 mg CH_3COOH l^{-1}	
	HRT to 20 days	533–543	Decrease to ~1.10 l CH_4 l^{-1} d^{-1}	Decrease to <1000 mg CH_3COOH l^{-1}	
	HRT to 12.8 days	544	Increase until end of study	Increase until end of study	

VS/TS, volatile solids/total solids; VFA, volatile fatty acid; HRT, hydraulic retention time; SMA, specific methanogenic activity.

3.2. Period I – Comparison between operating conditions

The CSAD and ASBR were operated similarly (except for mixing duration) over the duration of period I to investigate whether high-rate AD treatment is advantageous compared to low-rate AD treatment in regards to long-term stability and methane yields (i.e., bioenergy recovery). Table 2 summarizes how changes in operating conditions affected the performance characteristics of the reactors. The performance between reactors was compared with data gathered during periods of pseudo steady-state conditions.

3.2.1. Initial start-up period

During the first 138 days of the start-up period (Table 2), the volumetric methane production rate increased in both reactors from 0.134 ± 0.021 and 0.183 ± 0.008 l CH_4 l^{-1} d^{-1} (n = 10) (50-d HRT, F1) to 0.370 ± 0.031 and 0.487 ± 0.044 l CH_4 l^{-1} d^{-1} (n = 10) (25-d HRT, F4) for the CSAD and ASBR, respectively (Fig. 1A), due to higher VS loading rates (shorter HRTs). The decreasing retention times resulted in increasing effluent VS concentrations to 21.76 and 19.85 g l^{-1} (Fig. 1B) and declining VS removal efficiencies to 46.8% and 52.2% in the CSAD and ASBR on day 136 of the operating period, respectively (Table 1). The effluent VS/TS ratio stabilized during this period (days 100–138) (Fig. 1B), while the effluent VFA concentrations were stable and low (<500 mg CH_3COOH l^{-1}) in both reactors (Fig. 1C). On day 159 of the operating period, the specific methanogenic activity in the CSAD was considerably lower than in the ASBR (0.044 ± 0.005 vs. 0.157 ± 0.014 g CH_4-COD g^{-1} VSS day^{-1} for CSAD vs. ASBR, respectively) (Fig. 1D). Throughout the initial start-up period, effluent SCOD levels (Fig. S2A) followed a pattern similar to effluent VFA levels (Fig. 1C), while the effluent TCOD levels in both reactors increased to ~30 g l^{-1} (Fig. S2A). In the same period, alkalinity and total ammonium were slightly lower in the CSAD than in the ASBR (~2000 vs. 2500 mg $CaCO_3$ l^{-1} (Fig. S2B) and 1250 vs. 1500 mg NH_4^+–N l^{-1} (Fig. S2C) for CSAD vs. ASBR, respectively), and on day 150 of the operating period the pH was stable around 7.7 and 7.8 for the CSAD and ASBR, respectively (Fig. S2D). This data clearly shows that the ASBR performed better due to a higher VS destruction than the CSAD, and that both systems showed stable operating performances.

3.2.2. Variable total solids concentrations in the influent

Between days 139–183 of the operating period, both reactors were fed with very high concentrations of TS (~200 g l^{-1} or 20%) while the VS concentration remained constant at ~40 g l^{-1} (F5 and F6, Table 1). This led to considerable accumulation of TS in both systems with mixed liquor TS concentrations in the CSAD and ASBR systems of 134.42 and 167.71 g l^{-1}, respectively (Fig. 2B). Consequently, the effluent VS/TS ratios decreased from 0.47 to 0.43 on day 138 to 0.11 and 0.15 on day 178 of the operating period, respectively (Fig. 1B) with similar mixed liquor VS/TS ratios (Fig. 2B). Since active biomass is virtually inseparable from feed solids, the biomass became crowded with TS (in fact the reactor was packed with solids), which led to active biomass washout from the reactors. Indeed, the mixed liquor VS concentrations in the CSAD and ASBR systems had decreased somewhat, while the mixed liquor TS concentrations had increased greatly by day 180 of the operating period (Fig. 2A and B). In addition, the settleability of the mixed liquor was maximized on day 180 (lowest sludge volume index (Fig. 2C)). However, such settling was not observed in the ASBR (even at the end of the intermittent periods without mixing) due to constant biogas production. Despite slightly larger TS accumulation in the ASBR (the mixed liquor total solids to effluent total solids ratio was 1.2 on day 180, which was the highest recorded (Fig. 2D)), the performance for only the CSAD reactor was impaired with high VFA levels of ~3000 mg l^{-1} throughout the 20-day and 16-day HRT periods (days 147–228) (Fig. 1C). During this period, the volumetric methane production rates for the CSAD first increased due to an increase in the VS loading rate on day 149 (from ~1.6 to 2 g VS l^{-1} day^{-1} (Fig. 1A)), but then started to decrease due to higher VFA concentrations. Conversely, the VFA

concentrations in the ASBR remained low, which indicates that the ASBR was able to maintain stable conditions even with decreasing VS/TS ratios. In fact, the ASBR was able to maintain stable reactor performance despite the increase in VS loading rate, resulting in a considerably elevated volumetric methane production rate (Fig. 1A). We concluded that the approximately three times higher specific methanogenic activity of the ASBR biomass compared to the CSAD biomass enabled the ASBR to maintain lower levels of VFAs and higher volumetric methane production rates during this period even though some active biomass had been washed out. In other words, the higher methanogenic activity of ASBR biomass aided bioreactor stability.

During days 184–217 of the operating period, the TS concentration of ~80 g l^{-1} in the feed (F7 and F8) was within a similar range compared to the initial start-up period (Table 1). This decreased the mixed liquor TS concentrations slowly to ~90 g l^{-1} for the CSAD and ASBR systems (Fig. 2B) because of TS washout (effluent TS concentration were higher than the influent for a couple of weeks after the feed change (Fig. 2E)), indicating that TS accumulation was reversible for this type of substrate for both systems. The effluent VS/TS ratio increased in both reactors from a minimum of 0.11 and 0.12 on days 178 and 186 to 0.42 and 0.35 on days 243 and 248 in the CSAD and ASBR, respectively (Fig. 1B). At the end of the 12-day HRT, the effluent VS concentration for the CSAD was ~25 vs. ~20 g l^{-1} for the ASBR (Fig. 1B), which translated into higher VS removal efficiencies (i.e., hydrolysis) in the ASBR compared to the CSAD (52.3% vs. 39.0% on day 227 of the operating period). On day 224 of the operating period, the specific methanogenic activity for ASBR biomass remained to be nearly three times higher than CSAD biomass (Fig. 1D). When the HRT was shortened from 12 to 10 days on day 237 of the operating period, the VFA concentration in only the CSAD began to increase even though the methane production rate increased in both systems (Fig. 1A and C). Together with rapidly decreasing alkalinity and pH levels, a failure of the CSAD was imminent by day 250 (Fig. S2B and D). We believe that decreasing alkalinity and pH levels, which were observed at an HRT of 10 days (Fig. S2B and D), indicate that the ASBR would have failed also (if we had operated longer). Regardless, from this data it is clear that the ASBR system had superior stability characteristics compared to the CSAD.

3.3. Period II – Temperature variation

The second phase of the study (period II, days 251–545) was performed to ascertain the effect of short-term temperature variations (both lower and higher than 37 °C) on process stability, VS removal efficiency, and volumetric methane production rate in the ASBR system when treating brewery primary sludge. Table 2 presents details of how changes in operating temperature affected reactor performance. After stabilization at an HRT of 15 days (and 37 °C), we decreased the HRT to 12.8 days for which stable methane production levels (1.10 ± 0.04 l CH_4 l^{-1} d^{-1}; $n = 9$) were achieved by day 334 of the operating period (Fig. 3A). Beginning on day 357, the temperature was adjusted incrementally by first decreasing the operating temperatures in a step-wise fashion to a minimum of 22 °C. Second, after a return to pseudo steady-state performances at 37 °C, the operating temperature was increased in a step-wise fashion to a maximum of 43 °C (Fig. 3).

3.3.1. Short-term effects of lower temperatures

The step-wise temperature decrease from 37 to 22 °C resulted in a considerable reduction in the volumetric methane production rate from 1.10 ± 0.04 to 0.64 ± 0.02 l CH_4 l^{-1} d^{-1} (Fig. 3A). The VS removal efficiency also decreased from ~48% to ~25% (shown by increased effluent VS concentrations (Fig. S3E)), indicating that hydrolysis was negatively affected by lowering temperatures. From our specific methanogenic activity data, we observed an approximate four times decrease in activity from 0.213 ± 0.025 to 0.059 ± 0.012 g CH_4-COD g VSS^{-1} d^{-1} at 37 and 22 °C, respectively (Fig. 3A). A considerable long-term accumulation of VFAs was not observed in the temperature range between 37 and 22 °C at a 12.8-day HRT (days 287–438) because hydrolysis remained the rate-limiting step. The fact that methanogenesis kept up with hydrolysis guaranteed stable conditions even though the temperature was decreased. When we increased the temperature from 27 to 37 °C on day 441, the VFA concentrations increased sharply to ~5000 mg CH_3COOH l^{-1} (Fig. 3B) because of a faster raise in hydrolysis compared to methanogenesis (much of the substrate had accumulated in the mixed liquor). However, this imbalance was apparent for less than a week because the volumetric methane production rate stabilized to 1.17 ± 0.05 l CH_4 l^{-1} d^{-1} ($n = 10$) with low VFA concentrations of ~200–300 mg CH_3COOH l^{-1} on day 450 of the operating period (Fig. 3A and B). In addition, the specific methanogenic activity remained at similar levels compared to before the temperature decrease (0.228 ± 0.010 g CH_4-COD g^{-1} VSS d^{-1}; $n = 3$; Fig. 3A). In summary, unstable performances in our digester during step-wise temperature drops from 37 to 22 °C were not observed because hydrolysis remained the rate-limiting step. After restoring the temperature to 37 °C, the volumetric methane production rate recovered almost immediately to the levels that were observed before the temperature drop.

3.3.2. Short-term effects of higher temperatures

The volumetric methane production rates did not change considerably during the temperature increases from 37 to 39 °C and from 39 to 41 °C. Beyond 41 °C, however, methanogenesis became the rate-limiting step instead of hydrolysis because VFA concentrations increased sharply to ~1500 mg CH_3COOH l^{-1} on day 513 of the operating period (Fig. 3B). We observed a sharp increase in methane production to ~1.70 l CH_4 l^{-1} d^{-1} when we began feeding F21, and this was accompanied by a further increase in the VFA concentration. These high VFA concentrations did not decrease when we decreased the temperature to 42 °C. Since methanogens were still active at 42 °C, we increased the HRT to 20 days to recover reactor stability. This was accomplished with a considerable drop in VFA concentrations by day 537 (Fig. 3B). However, shortening the HRT to 12.8 days caused instability to reoccur. From these experiments, we concluded that 41 °C was the upper temperature limit during short-term increases in the operating temperature. In other words, below an operating temperature of 42 °C the hydrolysis step is rate-limiting, which prevented unstable conditions in our system.

4. Discussion

4.1. Solids treatment in ASBR was superior compared to CSAD

The sequence of steps of an ASBR are designed to utilize settling to uncouple the HRT from the SRT, which results in a higher concentration of biomass and a longer time allowed for hydrolysis to occur to escalate the fraction of substrate converted to methane (Sung and Dague, 1995). Indeed, others have shown that achieving high concentrations of biomass in an ASBR due to settling increases degradation and methane yields (Park et al., 2001; Wang et al., 2009). The ASBR in this study produced statistically significantly more methane than the CSAD. However, the biomass concentrations in the ASBR were lower than in the CSAD because of a higher VS destruction and poor settling characteristics of the biomass during continuous biogas production (mixing by bubble formation) in the ASBR. The low settleability of the biomass (TS and VS) explains a somewhat similar solids behavior in both systems during period

Table 3
Estimated sludge retention times (SRT) in the CSAD and ASBR systems for four hydraulic retention times (HRT) periods.

HRT	Mixed liquor sample day	Effluent sample days	SRT_{CSAD}	SRT_{ASBR}	Number of samples	*t*-test *p*-value $SRT_{ASBR} > SRT_{CSTR}$
50	18	19, 22, 26	44.32 ± 3.74	51.34 ± 2.49	3	$p = 0.03$[b]
32	102	104, 109, 118	30.09 ± 1.45	32.98 ± 1.84	3	$p = 0.05$
25	129	131, 138	27.64 ± 1.71	26.61 ± 0.48	2[a]	$p = 0.73$[c]
12.8	229	221, 224, 228	12.41 ± 0.66	13.99 ± 0.73	3	$p = 0.03$[b]

The SRT is calculated based on the inert solids measurement of mixed liquor (g IS in reactor) and effluent (g IS/d). For each data point, only one mixed liquor sample was available.

[a] Only two steady effluent samples were available at the 25-day HRT because of the change to F5 with high-solids during this HRT period.

[b] The SRT of the ASBR reactor is significantly higher than the CSTR ($p < 0.05$).

[c] the SRT of the CSTR is not significantly higher than the ASBR (1–0.73 = 0.27) at $p < 0.05$ significance level.

I, even with high TS concentrations in the feed (Fig. 2). The solids behavior was not exactly identical, but similar, as shown by the estimated SRTs (based on inert solids data), with slightly longer SRTs for the ASBR compared to the CSAD for three HRT periods (50, 32, and 12.8 days). At the 25-day HRT, however, no statistically significant difference between the SRTs in the systems was observed (Table 3). This result is in agreement to the minor delays with TS washout that were observed for the ASBR system compared to the CSAD system after the switch from F6 to F7 (Table 1 and Fig. 2E). However, this minor discrepancy cannot explain why hydrolysis, and therefore the methane yield, was considerably elevated for the ASBR. In addition, the morphology of the mixed liquor over the height of the ASBR and its effluent was very similar (no granules or biomass flocs were observed), and with the low *in situ* settling characteristics, in addition to periodic mixing, it is unlikely that active biomass enrichment in the bottom of the ASBR occurred. Therefore, the only other difference between the CSAD and the ASBR that can explain a superior digester performance is the duration of mixing (continuous vs. intermittent mixing), which already has been identified by others as an important factor for reactor performance and stability (Dague et al., 1970; Griffin et al., 1998; Hansen et al., 1999; McMahon et al., 2001; Stroot et al., 2001). For some of these studies the biomass concentration and SRT was increased due to increased solids settling before decanting effluent, however, Stroot et al. (2001) and McMahon et al. (2001) maintained similar SRTs on purpose to solely identify the effects of mixing. They reported that minimally-mixed digesters demonstrated an improved performance and a much more stable operation than digesters that were continuously and vigorously mixed. They observed that vigorous, continuous mixing inhibited relationships between syntrophs and their methanogenic partners, possibly by disrupting the spatial juxtaposition between these organisms (McMahon et al., 2001; Stroot et al., 2001). Here, the more than three times higher methanogenic activity of ASBR biomass compared to CSAD biomass supports their observation that reduced mixing fosters a well-functioning syntrophic community. The ASBR biomass with a higher methanogenic activity was able to handle the washout of active biomass due to TS crowding, which added stability to the bioreactor. This improved stability due to lower mixing intensities was also found by Stroot et al. (2001) and McMahon et al. (2001).

4.2. Hydrolysis was the rate-limiting step in solids digestion over a large temperature range (22–41 °C)

Because the ASBR displayed good stability in period I even under stressful conditions, we evaluated the effect of temperature on methane yield in period II. At low temperatures, VFA levels increased only slightly even though methane production fell, indicating decreased rates of hydrolysis and methanogenesis. Methanogenic activity measurements confirm that methanogens were less active at lower temperatures (Table 2), yet they were still able to consume VFAs at the rate of production, maintaining stable operation of the digesters. In other words, stability was maintained because hydrolysis remained the rate-limiting step at these lower temperatures. Similarly, Bohn et al. (2007) did not observe that methanogenesis became rate-limiting >18 °C in their study of anaerobic digestion of crop residues. At 42 °C and a 12.8-day HRT, we did observe that methanogenesis became the rate-limiting step, because VFAs accumulated even though methane production had increased, indicating that hydrolyzing microbial guilds increased their rate of reaction more than methanogenic microbial guilds. In addition, when the HRT was increased to 20 days at 42 °C, VFA levels rapidly dropped, demonstrating that hydrolysis was simply outpacing methanogenesis at high temperatures and loading. Here, we observed increased methane production even at temperatures >41 °C, which was also observed by Varel et al. (1980). In that study, workers found that in multiple anaerobic digesters increases in temperature (30–65 °C in 5 °C increments) were followed by increases in methanogenesis. If the rate of hydrolysis for a particular waste outpaces the rate of methanogenesis during inherent temperature variations for full-scale systems, then lowering the loading rate may be necessary to allow microbial guilds within the microbial community to come into balance.

4.3. Substrate characteristics strongly affected rates of hydrolysis in CSAD and ASBR

After the episode of high TS influx had passed during period I (after day 184 of the operating period) the methane yields in *both* reactors were lower for the remainder of the period: a methane yield of 0.219 vs. 0.130 l CH_4/g VS fed was observed for the CSAD, while this was 0.306 vs. 0.174 l CH_4/g VS fed for the ASBR (Fig. 1E). Wang et al. (2009) had observed that accumulation of solids in ASBRs (with well-settling, high-solids substrate) resulted in decreased performance. However, we did not observe permanent solids accumulation and the VS/TS ratios were reversed in both reactors (Table 2). Instead, feed characteristics caused slower hydrolysis and we were able to show this by statistical modeling data gathered during period II. We evaluated which of the parameters: temperature, substrate TS, substrate TCOD, and substrate SCOD affected the methane yield during period II (evaluated when methane production and VFA levels were stable). We found that the statistically significant variables were temperature (T, °C), substrate SCOD (g l^{-1}), and substrate TS (g l^{-1}), explaining 85% of the variation ($r^2 = 0.85$) according to Eq. (1):

$$\text{Yield} = -0.247 + (0.014 \times \text{T}) + (0.016 \times \text{SCOD}) - (0.001 \times \text{TS}) \quad (1)$$

The analysis also showed that temperature alone could only explain 59% of the variation ($r^2 = 0.59$) (see Section 2 for more details on the analysis). Thus, when hydrolysis was the rate-limiting step, methane yield was positively coupled to temperature and feed SCOD concentration and negatively to feed TS levels. It should be noted that the parameters of the equation show the general trends

of the relationship between variables and yield, but a simple equation such as this cannot be used to predict the behavior of a complex anaerobic microbial community. Extension of this modeling effort to period I, thus, shows that a relatively lower SCOD level and a relatively higher TS level after day 195 of the operating period compared to before caused slower hydrolysis, resulting in the lower methane yield. This resulted in two distinct periods with different methane yields within period I (Fig. 1E).

4.4. Primary sludge digestion or secondary residual digestion: what is the best approach?

Here, we have shown that primary sludge from brewery wastewater can be successfully utilized for methane production in low-rate and high-rate anaerobic digesters for solids removal. In addition, we have found that high-rate digesters, such as ASBR systems, are advantageous with regards to methane yields and stability without long-term problems with TS accumulation. We showed in our previously published work that secondary residuals were successfully digested in low-rate anaerobic digesters (Bocher et al., 2008). In that study, we noted that one possible advantage of treating secondary brewery residuals compared to primary sludge was the continuous augmentation of methanogens from the excess biomass of the upflow bioreactor into the CSAD, which sustained a methane yield of 0.21 l CH_4 g^{-1} VS fed at a relatively short minimum HRT of 10 days under mesophilic conditions. Here, with primary sludge we achieved a similar maximum yield of 0.22 l CH_4 g^{-1} VS fed in the CSAD, albeit at a longer minimum HRT of 12.8 days, which supports the hypothesis of benefits due to methanogen augmentation. Based on the production rates of brewery primary sludge and this methane yield, we calculated that a CSAD reactor would produce approximately 5.4% more methane compared to the methane that is generated from existing upflow anaerobic bioreactors that are treating soluble brewery wastewater. In a similar calculation, we had reported that secondary residual digestion by CSAD systems would increase the methane production by 8.1% when compared to the upflow anaerobic bioreactors (Bocher et al., 2008). Thus, secondary residual digestion is more advantageous than treatment of primary sludge in a similar anaerobic treatment system. Because the methane yields and VS removal efficiencies for both substrates were similar, the higher overall methane production for secondary residuals is due to the additional excess biomass quantities compared to primary sludge. We believe that more secondary residuals are available than primary sludge due to the presence of fermentative bacteria and methanogenic granules from the anaerobic treatment system for soluble wastewater in addition to the same material as the primary sludge (i.e., yeast cells from alcohol fermentation and small (hemi)cellulose particles from hops and rice). Because of augmentation of methanogens and the additional solids material it is more advantageous to digest secondary residuals compared to primary sludge for a similar bioreactor configuration.

For primary sludge treatment with high-rate systems, such as ASBRs, we calculated (based on our methane yields) that the anticipated additional methane generation compared to the existing upflow anaerobic bioreactor would be 7.6%. We anticipate the highest additional methane generation, however, with secondary residual treatment in high-rate systems, but unfortunately we have not performed a long-term study to observe the methane yield. Regardless, the brewery is designing a high-rate, high-solids treatment system for secondary residuals. This makes sense, because with secondary residuals removal from the effluent, some excess biomass is incorporated, which translates to lower disposal costs of BOD and TSS to the post-treatment (mostly activated sludge systems operated by cities).

5. Conclusion

In a traditional brewery wastewater treatment scheme, the soluble fraction of brewery wastewater is treated in high-rate anaerobic digester systems, such as the systems that are based on the UASB concept. Whenever possible, the solids fraction of wastewater should also be treated with anaerobic digestion to increase the methane generation and reduce the organic load to post-treatment systems, such as activated sludge systems. Here, we showed that for this type of solids waste, a high-rate system (ASBR) was advantageous compared to a low-rate system (CSAD) in regards to performance and stability. The ASBR did not accumulate considerably more TS during a period of TS shock load than the CSAD. We showed that removed solids (primary sludge) from brewery wastewater can be digested in both CSAD and ASBR systems with a considerable increase in total methane generation compared to soluble wastewater digestion alone.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biortech.2010.03.023.

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