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ORIGINAL PAPER

Anaerobic digestion of secondary residuals from an anaerobic bioreactor at a brewery to enhance bioenergy generation

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Abstract Many beer breweries use high-rate anaerobic digestion (AD) systems to treat their soluble high-strength wastewater. Biogas from these AD systems is used to offset nonrenewable energy utilization in the brewery. With increasing nonrenewable energy costs, interest has mounted to also digest secondary residuals from the high-rate digester effluent, which consists of yeast cells, bacteria, methanogens, and small (hemi)cellulosic particles. Mesophilic (37 °C) and thermophilic (55 °C) lab-scale, low-rate continuously-stirred anaerobic digestion (CSAD) bioreactors were operated for 258 days by feeding secondary residuals at a volatile solids (VS) concentration of $\sim 40\ \mathrm{g\ l^{-1}}$. At a hydraulic retention time (HRT) of 15 days and a VS loading rate of $2.7\ \mathrm{g\ VS\ l^{-1}\ day^{-1}}$, the mesophilic bioreactor showed an average specific volumetric biogas production rate of $0.88\ \mathrm{l\ CH_4\ l^{-1}\ day^{-1}}$ and an effluent VS concentration of $22.2\ \mathrm{g\ VS\ l^{-1}}$ (43.0% VS removal efficiency) while the thermophilic bioreactor displayed similar performances. The overall methane yield for both systems was $0.21\ \mathrm{l\ CH_4\ g^{-1}}$ VS fed and $0.47–0.48\ \mathrm{l\ CH_4\ g^{-1}}$ VS removed. A primary limitation of thermophilic digestion of this protein-rich waste is the inhibition of methanogens due to higher nondissociated (free) ammonia (NH_3) concentrations under similar total ammonium (NH_4^+) concentrations at equilibrium. Since thermophilic AD did not result in advantageous methane production rates or yields, mesophilic AD was, therefore, superior in treating secondary residuals from high-rate AD effluent. An additional digester to convert secondary residuals to methane may increase the total biogas generation at the brewery by 8% compared to just conventional high-rate digestion of brewery wastewater alone.

Keywords Anaerobic digestion · Methane yield · Secondary residuals · Continuously-stirred anaerobic digestion · Bioenergy

Introduction

Mixed-culture anaerobic digestion (AD) is a waste treatment system that meets current demands toward more sustainable environmental practices [12]. It also provides economic benefits by generating a surplus of bioenergy because the produced methane can be used to offset nonrenewable energy at the factory [16]. In comparison, aerobic treatment facilities utilize considerable quantities of nonrenewable energy for aeration. In addition, only one-fifth to one-tenth as much excess biomass is produced per unit of organic substrate converted compared to aerobic treatment processes, such as activated sludge processes. Compared to aerobic processes, AD also decreases the amount of nutrients required and the reactor volume and footprint, making it a more economical and environmentally sustainable treatment [18]. Many negative aspects of AD have now been overcome; for example, the slow start-up time was expedited by using inoculum from existing plants [21]. Because of these advantages over aerobic treatment systems, numerous industries have for over 30 years successfully utilized

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AD to reduce organic pollutants in high-strength waste streams, especially in the brewing industry [16, 38].

Breweries started to build full-scale AD systems for treatment of their high-strength soluble wastewater to offset energy costs during the oil crisis in the 1970s. They remained popular waste treatment systems even during periods of relatively low nonrenewable energy prices, because a considerable reduction in soluble biological oxygen demand (BOD) by the AD systems drastically reduces the sewer costs to municipalities. In 1995, ~25% of economic gain from AD of brewery wastewater treatment came from energy offsets and ~75% from reduced sewer costs. Currently (2007), this allocation has changed due to increasing nonrenewable energy costs with ~50% of economic gain from bionergy generation and ~50% of gain from reduced sewer costs (personal communication, Anheuser-Busch, Inc., St. Louis, MO). As an example, Anheuser-Busch successfully operates high-rate AD systems in nine of their US breweries and offsets ~10% of boiler fuel. The desire, however, is to further save nonrenewable energy costs by converting process wastewater solids with a particle-size <1 mm into methane. Such solids are not degraded in the high-rate systems with relative short HRTs of <24 h, and therefore they leave in the AD effluent stream as secondary residuals. AD of secondary residuals would allow further degradation of BOD in the wastewater stream, reducing sewage costs for secondary biological treatment as well.

Biological hydrolysis in AD is the rate-limiting step for solids conversion to methane [13], and therefore the HRT for nonsoluble organic material treatment is much longer (days) than for easily degradable soluble organic matter (hours). Secondary residuals from the high-rate AD system consist of yeast cells from alcohol fermentation, small (hemi)cellulose particles from hops and rice, and bacteria and methanogens (excess biomass) from the high-rate AD system. To achieve a higher hydrolysis rate when treating solids, a higher shear rate relative to those digesters that treat soluble materials is proposed [25]. A continuously-stirred AD (CSAD) system utilizes mechanical mixers that can provide enhanced shear to break up larger particles when operated at a high rotational speed, potentially aiding in the biological hydrolysis. The use of continuous mixing also promotes substrate-microbe contact (reducing external and internal diffusion limitations), and maintains a more homogeneous pH, temperature, and bulk composition [29]. Mixing intensity, and thus shear rate, has been shown to positively affect the performance of anaerobic digesters treating municipal solid residuals [24, 31]. In a recent study we did not find a gain in performance at higher shear for the treatment of cow manure from a dairy farm. However, long-term stability was improved at high shear rates [15].

In addition to mixing, the operating temperature is a fundamental variable affecting reactor performance, because of improved hydrolysis rates and methane yields due to favorable kinetics at higher temperatures [22, 37]. Therefore, thermophilic AD (55 °C) has been found to improve both biosolids and pathogen destruction over mesophilic AD (35–37 °C) [3, 36]. Thermophilic digesters have also been shown to better maintain high COD removal efficiencies after increases in organic loading than mesophilic digesters [14, 22]. However, compared to mesophilic temperatures, thermophilic temperatures may contribute to higher VFA concentrations in the effluent due to acetate and hydrogen removal inhibition by short-chain VFAs, such as propionate [35]. Thermophilic AD also has greater energy requirements for heating if the wastewater is not already hot. Finally, higher levels of the inhibiting nondissociated ammonia at thermophilic compared to mesophilic temperatures under similarly high concentrations of ammonium species (i.e., the sum of ammonia and ammonium) is a problem for protein-rich wastes [2, 21, 42].

To investigate whether AD of secondary residuals from a high-rate anaerobic bioreactor treating brewery wastewater is feasible, we operated two low-rate AD systems for 258 days. We operated CSAD bioreactors to break up anaerobic granules and other particles that are part of the secondary residuals to support biological hydrolysis. In addition, we investigated if thermophilic conditions would enhance methane yields from secondary residuals over mesophilic conditions. Finally, we have estimated how much additional biogas (in %) can be anticipated compared to just soluble wastewater treatment at a brewery.

Materials and methods

Experimental apparatus

Experiments were conducted in two laboratory-scale glass bioreactors (Midrivers Glassblowing, Inc., St. Charles, MO) with a maximum working volume of 5 l (Fig. 1). The bioreactors had a water jacket to maintain constant temperatures of 35 ± 1 °C/37 ± 1 °C or 55 ± 1 °C with an external heating recirculater (Model 210, PolyScience; 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 continuously stir at 300 rotations per minute (RPM), which resulted in a mixing power of $2\ \mathrm{W\ l^{-1}}$. The rotation velocity was measured using a tachometer (Model 461891, Extech Instruments, Waltham, MA), while the power was measured with a clamp meter (Model 380941, Extech Instruments). Feeding was performed by poring secondary solids through a tube at the top of the bioreactor. This tube extended midway into

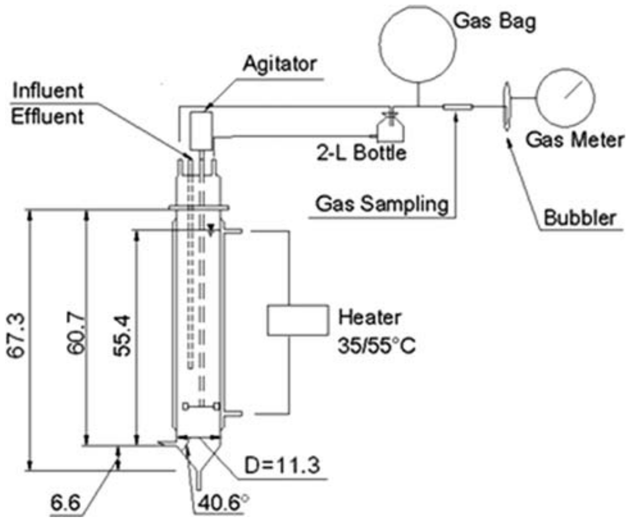

Fig. 1 Schematic of the bioreactor setup

the reactor contents to prevent biogas loss. A peristaltic pump (Cole-Parmer; Vernon Hills, IL) was used for decanting effluent from the same tube. The gas collection system of each digester setup 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 determination of gas production, a biogas sampler, and a gas meter (Model 1 l, Actaris Meterfabriek, Delft, The Netherlands) (Fig. 1).

Reactor operation

We started the reactors with inoculum that was already acclimated to the respective digester temperatures; the mesophilic reactor was inoculated with 1 l of granular residuals from a mesophilic anaerobic upflow bioreactor treating brewery wastewater (Anheuser-Busch, St. Louis, MO); and the thermophilic reactor was inoculated with 1 l of blended residuals from a thermophilic digester treating a combination of primary sludge and waste activated sludge (Western Lake Superior Sanitary District, Duluth, MN). After inoculation, a 24 h acclimation period was allowed before mixing and another 24 h period before feeding. Secondary residuals were received every 1–2 weeks and stored at 4 °C. The feed was prepared by centrifuging the residuals from the brewery until the desired solids concentration of 40 g VS l^{-1} was achieved (4% solids content based on VS: RC−5B; Sorvall Instruments, Ramsey, MN). The residuals were then homogenized in a household blender for ~30 s. The reactors were fed manually every 24 ± 1 h after first removing a similar volume of effluent. The HRT was 50 days (corresponding to a VS loading rate of 0.8 g VS l^{-1} day^{-1}) during the initial operating period and was decreased in a step-wise fashion over the operating time to 10 days (4.0 g VS l^{-1} day^{-1}; Table 1). The HRT was equal to the solids retention time (SRT) due to continuous mixing. Each increase in VS loading rate was made when stable VFA concentrations and steady gas production rates were achieved [1]. A minimum time period of one HRT was allotted, except during the 50-day and 10-day HRT operating conditions. In chemostats or first-order completely stirred tank reactors, at least three or four space times (i.e., SRT periods) are necessary to reach steady state [11]. However, for undefined mixed cultures in AD bioreactors such a time period may not be sufficient to guarantee true steady state because the community composition may not be constant even after a 1 year operating period [4]. For AD systems during start up, steady state is, therefore, mostly based on performance parameters, such as biogas production rates or intermediate concentrations, to circumvent excessive long operating periods [8, 17, 33, 41]. Here we have referred to a stable performance as pseudo steady state biogas production rates and this was achieved when daily biogas production rates were within 10% of their average values after the operating period of at least one HRT/SRT time period.

Physical and chemical analysis

The following reoccurring measurements were performed for the reactor performance: (1) daily: pH, biogas production, room temperature, and pressure (to correct biogas production to standard conditions); (2) biweekly: total solids (TS), VS, and total volatile fatty acid (VFA_t) of the reactor effluent; and (3) weekly: soluble and total chemical oxygen demand (SCOD and TCOD) concentrations, alkalinity of the reactor effluent, and the methane content in the biogas with a gas chromatograph (Series 350, Gow-Mac Instruments, Co., Bethlehem, PA) with a thermal conductivity detector. The TS and VS of the inoculum were measured, while the TS, VS, SCOD, and TCOD levels of each feed batch were measured. TS, VS, VFA_t, SCOD, and TCOD were performed according to *Standard Methods* [6]. Orthophosphate tests (HACH Company, Method 8048, Loveland,

Table 1 Reactor operating conditions for the mesophilic and thermophilic bioreactors and the days of the operating period when changes were made

HRT (days)	50	40	30	20	30	25	20	15	10
VS loading rate (g VS l^{-1} day^{-1})	0.8	1.0	1.3	2.0	1.3	1.6	2.0	2.7	4.0
Mesophilic change (days)	1	22	55	73	111	166	204	231	248
Thermophilic change (days)	1	22	73	91	118	178	204	231	248

CO; equivalent to Standard Method 4500-P E for wastewater) were performed on reactor effluent samples collected from day 146 to the end of the operating period. Total ammonium (i.e., sum of ammonia and ammonium) was measured using an electrode (Model Orion 9512, Thermo Electron Corporation, Beverly, MA). The free ammonia was calculated from the total ammonium and pH data according to Schwarzenbach et al. [27] by using a pKa of 8.95 for 37 °C [7] and a pKa of 8.41 for 55 °C [2].

Results and discussion

The mesophilic and thermophilic bioreactors were fed secondary residuals from a brewery at various HRTs to ascertain the feasibility of treatment using a high shear (2 W l^{-1}). The VS and TS concentration in the influent were 38.8 ± 2.8 g VS l^{-1} and 52.6 ± 4.6 g TS l^{-1} ($n = 38$), respectively, over the operating period of 258 days and the variation in solids levels was due to different characteristics in each batch of residuals (settling and centrifugation was used to thicken the feed). In the influent to the bioreactors, SCOD and TCOD were 10.28 ± 3.54 g l^{-1} ($n = 30$) and 71.96 ± 17.31 g l^{-1} ($n = 24$), respectively, and the free ammonia and total ammonium were 9.1 ± 4.5 mg NH_3-N l^{-1} and 250 ± 145 mg NH_4^+-N l^{-1} ($n = 27$). Despite variations in VS, COD, and ammonia concentration over the operating period, the same influent was fed daily into the mesophilic and thermophilic bioreactors to compare their performance (except during periods of severe instability). Throughout the operating period, methane was produced in both systems and the methane composition in the biogas averaged 62% ± 5% and 60% ± 4% ($n = 22$) in the mesophilic and thermophilic reactors, respectively. Because of protein, DNA, and phospholipids destruction, the levels of nitrogen and phosphorus species were high and no nutrient addition was necessary. For example, the amount of phosphorus present in the mesophilic and thermophilic bioreactor fluctuated from 13.8 to 21.8 mg P l^{-1} and from 13.9 to 24.1 mg P l^{-1}during days 146 to day 230, respectively.

Mesophilic bioreactor performance

The mesophilic bioreactor showed a stable behavior during the first 73 days of the operating period. The volumetric specific biogas production rate (specific biogas production) reached pseudo steady state values of 0.26 l l^{-1} day^{-1} and 0.49 l l^{-1} day^{-1} at the 40- and 30-day HRT, respectively (Fig. 2b). Also, the mesophilic bioreactor had an effluent VS concentration that gradually increased from 13 g l^{-1} on day one to 19 g l^{-1} on day 70 (Fig. 2a). This increase was observed because of incoming solids (and some biomass growth), and without degradation this increase in solids concentration would have been much higher (Fig. 2a). Because of the observed stable performance conditions (e.g., VFA concentrations and biogas production rates), the HRT was further shortened from 30 to 20 days on day 73 of the operating period (Table 1). Despite stable pH levels of ~7.7 and VFA_t levels below 340 mg CH_3COOH l^{-1} (Fig. 2), severe foaming was observed in the mesophilic reactor at day 74, causing the effluent VS to decrease from 19 to 16 g l^{-1} on day 75 of the operating period due to solids entrapment in the foam (Fig. 2a). When foam overwhelmed the gas collection system on day 91, the working volume of the digester was decreased to 4 l (a 20-day HRT was ensured by feeding less) to allow greater headspace. The entrapment of active biomass away from the mixed liquor into the foam resulted in slight increases in the VFA_t

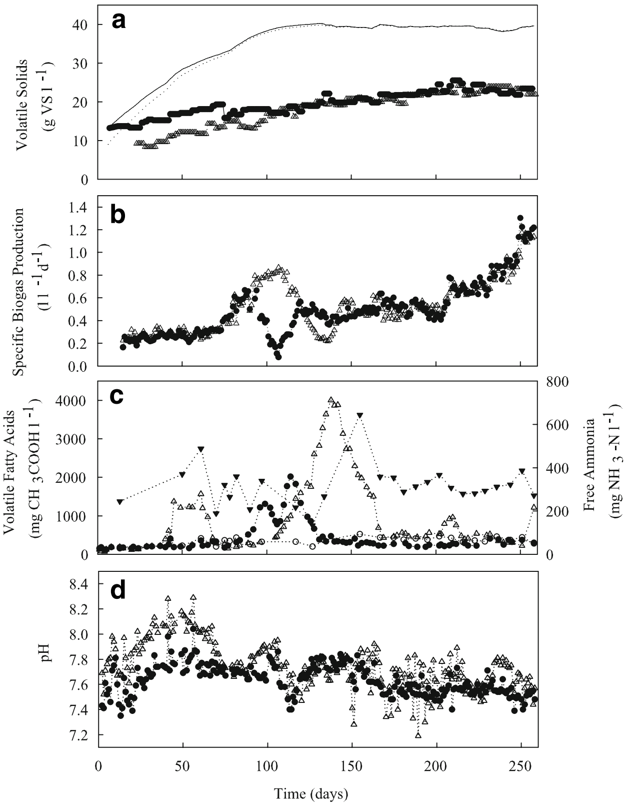

Fig. 2 Bioreactor performance over the operating period: **a** VS concentration in the effluent of the mesophilic bioreactor (*thick line*) and the thermophilic bioreactor (*thin line*) if no degradation had taken place; and VS concentration in the effluent of the mesophilic bioreactor (*filled circle*) and the thermophilic bioreactor (*open triangle*), **b** specific biogas production at standard conditions for the mesophilic bioreactor (*filled circle*) and the thermophilic bioreactor (*open triangle*), **c** volatile fatty acid concentrations in the effluent of the mesophilic bioreactor (*filled circle*) and the thermophilic bioreactor (*open triangle*) and free ammonia in the effluent of the mesophilic bioreactor (*open circle*) and the thermophilic bioreactor (*filled inverted triangle*), and **d** pH levels in the effluent of the mesophilic bioreactor (*filled circle*) and the thermophilic bioreactor (*open triangle*)

levels from days 84–92 of the operating period, resulting in unstable conditions with a rapid drop in volumetric biogas production rates and an increase in VFA_t levels to 1,300 mg $CH_3COOH\ l^{-1}$ on day 99 (Fig. 2c). To restore stable conditions, equal volumes of water and secondary residuals was fed at a longer HRT of 40 days (days 98 and 99 of the operating conditions), and, in addition, the temperature was elevated 2 °C (from 35 to 37 °C) to reduce foaming (day 99). Next, only water was fed at a 20-day HRT, which resulted in stabilization of foam formation (days 100–107). A substantial decrease in specific biogas production rates was the result of foam formation and a reduction in VS loading rates (0.67 $l\ l^{-1}\ day^{-1}$ at day 94–0.08 $l\ l^{-1}\ day^{-1}$ at day 107 of the operating period). To further alleviate the high VFA_t levels in the effluent, the HRT was prolonged to 30 days on day 111 of the operating period (Table 1). Shortly thereafter the VFA_t levels reached a maximum of 2,000 mg $CH_3COOH\ l^{-1}$ on day 114, and then began to quickly decrease, allowing the pH to increase to 7.8 (Fig. 2d). To prevent any future problems, we added a solution of 4.5% HCl and 12.5% H_3PO_4 to the feed to maintain an optimum pH of 7.6 from day 147 to the end of the operating period.

Stable conditions were re-established and we were able to shorten the HRT to 25 days on day 166 (Table 1), which resulted in a pseudo steady state specific biogas production of 0.45 $l\ l^{-1}\ day^{-1}$ (Fig. 2b). Further reductions in HRT resulted in pseudo steady state specific biogas production rates of 0.70 $l\ l^{-1}\ day^{-1}$ and 0.88 $l\ l^{-1}\ day^{-1}$ at a 20- and 15-day HRT, respectively (Fig. 2b). At a 10-day HRT, the specific biogas production averaged 1.18 $l\ l^{-1}\ day^{-1}$ (pseudo steady state was not reached at a 10-day HRT). These values, combined with a continuously low VFA_t level of ~300 mg $CH_3COOH\ l^{-1}$ and a constant VS concentration of 23 g VS l^{-1} showed that the biomass was able to handle increases in solids loading rates well once it was acclimated to the substrate and with proper pH control (from days 204 to the end of the operating period).

Thermophilic bioreactor performance

The thermophilic bioreactor showed more volatility compared to the mesophilic bioreactor, which has also been observed in other studies [10, 26, 28, 34, 39, 40]. The pH levels in the effluent of the thermophilic bioreactor were higher than the mesophilic bioreactor for the fist 60 days of the operating period (Fig. 2d), partly because of higher total ammonium concentrations (2,015 and 1,445 mg NH_4^+-N l^{-1}, respectively) due to higher protein destruction. In combination with the higher pKa levels for the ammonium/ammonia chemical equilibrium at 55 °C compared to 37 °C, free ammonia levels approached 500 mg NH_3-N l^{-1} on day 61 of the operating period (vs. 75 mg NH_3-N l^{-1} for the mesophilic bioreactor; Fig. 2c). Such high free ammonia levels in the thermophilic bioreactor are inhibitory to methanogens [19, 30] and this compromised a stable reactor performance, resulting in increased VFA_t levels of 1,234 mg $CH_3COOH\ l^{-1}$ for days 49–65 of the operating period (Fig. 2c). To reduce free ammonia concentrations and to restore low levels of VFA_t, an acidic solution was added to the influent from day 55 until the end of the operating period to maintain a target pH of 7.6, which is the optimum pH for the acetate-utilizing methanogen *Methanosaeta concilii* [30].

We added a solution of 3% HCl daily from day 55–110, which reduced the pH levels to 7.8, the VFA_t levels to 283 mg $CH_3COOH\ l^{-1}$, and the free ammonia to 189 mg NH_3-N l^{-1} by day 70 of the operating period. When the HRT was decreased from 40 to 30 days on day 73 (Table 1), a considerable increase in specific biogas production occurred (Fig. 2b). The specific biogas production rose again to 0.84 $l\ l^{-1}\ day^{-1}$ when the HRT was further decreased from 30 to 20 days on day 91 (Fig. 2b). However, the pH gradually increased to almost 8.0, and therefore we started to add a 15% HCl solution on day 111 to reduce the volume of 3% HCl solution addition. To decrease the pH to 7.6, enough HCl was added to increase the Cl^- concentration to a calculated level of 2,700 mg $Cl^-\ l^{-1}$ and we speculate that this anion at high concentrations inhibited the methanogens, explaining the sudden drop in biogas production starting on day 113 of the operating period and the subsequent raise in VFA_t concentrations up to 4,000 mg $CH_3COOH\ l^{-1}$ on day 138 (Fig. 2c). With the intention of lowering the VFA_t levels, we prolonged the HRT from 20 to 30 on day 118. The reduction in feeding rate and the inhibition were responsible for a lower specific biogas production (from 0.62 $l\ l^{-1}\ day^{-1}$ at day 118 to 0.22 $l\ l^{-1}\ day^{-1}$ at day 136). To reduce the Cl^- concentration, addition of the HCl solution was terminated from day 126 to day 139. As occurred previously (days 45–61 of the operating period), pH levels increased resulting in much higher free ammonia levels of up to 640 mg NH_3-N l^{-1} at day 155 (an increase in total ammonium concentrations from ~1,500 to ~2,000 mg NH_4^+-N l^{-1} from day 136 to the end of the operating period was partly responsible for the higher free ammonia levels, data not shown). To avoid another spike in the Cl^- concentration while maintaining an optimal pH, we used an acid solution of 4.5% HCl and 12.5% H_3PO_4 from day 139 until the end of the operating period [5].

The re-establishment of acid addition caused a sustainable pH level of ~7.6 from day 167 of the operating period (Fig. 2d). Lowering the pH decreased the free ammonia concentration to 358 mg NH_3-N l^{-1} (on day 167) and the VFA_t level to 430 mg $CH_3COOH\ l^{-1}$ (on day 169, Fig. 2c). The specific biogas production increased to a pseudo steady state average of $0.44\ \mathrm{l\ l^{-1}\ day^{-1}}$ at a 30-day HRT (day 177), and we shortened the HRT to 25 days on day 178 of the operating period (Table 1). The volatile nature of the thermophilic reactor was further exemplified after shortening the HRT again to 20 days (on day 204), which resulted in temporarily higher $\mathrm{VFA_t}$ levels of $960\ \mathrm{mg\ CH_3COOH\ l^{-1}}$ (Fig. 2c). A further decrease in the HRT to 15 days resulted in stable reactor performances and the pseudo steady state specific biogas production rates during this period were 0.50, 0.66, and $0.84\ \mathrm{l\ l^{-1}\ day^{-1}}$ for the 25, 20, and 15-day HRTs, respectively (Fig. 2b). The specific biogas production at the 10-day HRT varied from 1.12 to $1.17\ \mathrm{l\ l^{-1}\ day^{-1}}$, but did not reach pseudo steady state. The last HRT decrease from 15 to 10 day on day 248 caused another increase in $\mathrm{VFA_t}$, possibly signaling forthcoming instability ($1{,}200\ \mathrm{mg\ CH_3COOH\ l^{-1}}$ at the end of the operating conditions).

Methane yields

To calculate potential bioenergy production rates, it is important to report methane yields during periods of pseudo steady state biogas production [32]. We estimated the methane yields by linear regression of the specific methane production rate (corrected for standard temperature and pressure) over the VS loading rate or VS removal rate (Fig. 3). For this plot, only methane production data was used for the final ten days of a given HRT. The influent and effluent solids data collected during this same period of stable reactor performance was used to calculate the average VS removal and loading rates. The methane yield achieved for the mesophilic bioreactor was $0.21\ \mathrm{l\ CH_4\ g^{-1}}$ VS fed ($R^2 = 0.93$) and $0.48\ \mathrm{l\ CH_4\ g^{-1}}$ VS removed ($R^2 = 0.78$). Similar methane yields were found for the thermophilic bioreactor, with methane yields of $0.21\ \mathrm{l\ CH_4\ g^{-1}}$ VS fed ($R^2 = 0.95$) and $0.47\ \mathrm{l\ CH_4\ g^{-1}}$ VS removed ($R^2 = 0.81$) (Fig. 3). Based on this information, we did not find a superior performance of the thermophilic bioreactor when treating secondary residuals from a high-rate anaerobic bioreactor treating brewery wastewater. Other studies have found similar methane yields. For example, El-Hadj et al. [9] studied mesophilic and thermophilic digestion of a mix of primary and secondary sewage sludge and achieved about 50% VS removal with a methane yield of $0.238\ \mathrm{l\ CH_4\ g^{-1}}$ VS fed. Lehtomaki et al. [20], treating manure and crop residue in CSAD systems at solids loading rates similar to this study, achieved 0.213–$0.268\ \mathrm{l\ CH_4\ g^{-1}}$ VS fed.

Large increases in VS loading rate resulted in foaming

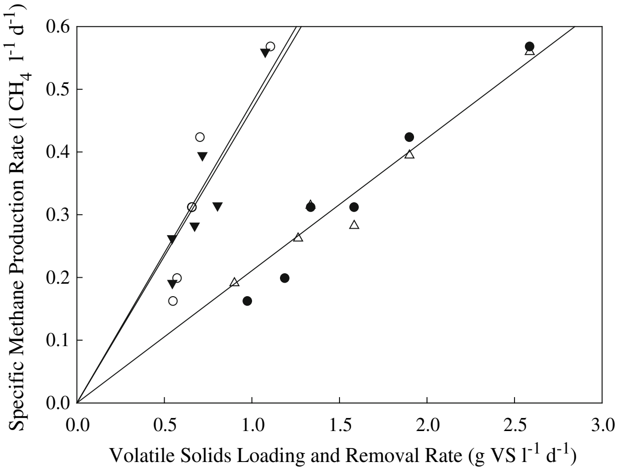

Fig. 3 Specific methane production rate over the VS loading rate for the: mesophilic bioreactor (*filled circle*); and thermophilic bioreactor (*open triangle*); and specific methane production rate over the VS removal rate for the: mesophilic bioreactor (*filled inverted triangle*); and thermophilic bioreactor (*open circle*). The methane yield for the VS fed and VS removed were obtained by linear regression analysis at each pseudo steady state operational period from a 40- to 15-day HRT

Foaming occurred in both bioreactors when the loading rate was increased considerably, although it was much less of a problem in the thermophilic bioreactor. In the mesophilic bioreactor, excessive foam occurred on day 73 when the HRT was decreased from 30 to 20 days (a 50% increase in the VS loading rate). The foam entrapped methanogenic biomass, resulting in lower biogas production and an increase in $\mathrm{VFA_t}$ levels, which led to unstable conditions. A similar 50% increase in the VS loading rate during the shortening of the HRT from 15 to 10 days, caused some foaming in the thermophilic bioreactor. However, foaming subsided after two days. Possible causes of foaming, such as the presence of excessive filamentous bacteria and grease, or insufficient mixing [23] were absent, and therefore the 50% increases in the loading rate were the likely causes of foaming. In a study of three different full-scale AD systems, excessive foaming occurred each time an increase greater than 20% was made to the VS loading rate [23]. These authors concluded that VS overloading was the primary cause. Thus, we recommend increasing the VS loading rate not more than 20% to prevent foaming events during startup of a full-scale AD system for secondary residuals treatment.

Comparison of mesophilic and thermophilic bioreactor performance

Because pseudo steady state conditions were achieved at a 15-day HRT ($2.7\ \mathrm{g\ VS\ l^{-1}\ day^{-1}}$) and not at a 10-day HRT, we compared the performance of both systems at a 15-day HRT. Based on volumetric biogas production rates and $\mathrm{VFA_t}$, TS, and VS concentrations in the effluent, the mesophilic bioreactor slightly outperformed the thermophilic bioreactor at a 15-day HRT, but this was not a significant difference (Table 2). Based on this data and the estimated methane yields, we conclude that the mesophilic and thermophilic bioreactors performed similarly under pseudo steady state conditions. However, the thermophilic bioreactor showed more periods of unstable conditions, because of free ammonia concentrations exceeding 400 mg NH_4^+ l^{-1}, which were inhibiting methanogens. This problem was tackled by maintaining optimum pH levels of 7.6 to maintain free ammonia concentrations of ~325 mg NH_4^+ l^{-1} for the last 100 days of the operating period. In accordance to other reports in the literature, thermophilic digestion of this protein-rich waste is not advisable because of the high total ammonium concentrations and the resulting inhibiting free ammonia concentrations due to the higher temperatures [2, 3].

Tangible advantages from secondary residuals digestion

We used secondary residuals from the brewery rather than primary residuals (i.e., biosolids in nontreated brewery wastewater that passes the 1 mm screens), because of its lower variability in residuals quality (e.g., pH, VS concentration, settleability) and quantity. The high-rate AD system, thus, acts as an equalization system to damp out variability. Another advantage of using secondary residuals from the high-rate AD system is that slow-growing methanogens, which are present in these residuals, are continuously augmenting the low-rate CSAD system. Conventional (nonaugmented) mesophilic CSAD systems are operated at HRTs of ~15 days to prevent washout of slow-growing methanogens. After we obtained stable conditions at a 15-day HRT, we shortened the HRT to 10 days and no decline in performance was noted for the mesophilic CSAD (although time did not permit attainment of pseudo steady state operating conditions). This shows that augmentation of active methanogens from the secondary residuals can sustain a stable performance at a relative short HRT of 10 days even in low-rate CSAD systems for which the SRT and HRT are equal. Anheuser-Busch, Inc. is now designing a bioreactor system to implement secondary residual conversion into their existing bioenergy operations. Such a conversion process will consist of a 10-day HRT CSAD system that is continuously fed (a true completely-stirred tank reactor) rather than batch fed. For the lab-scale study, a continuous feed was not feasible due to solids clogging in the feed pump. The designed system will also include a settler to thicken the secondary residuals to obtain a design HRT of 10 days, and thus to ensure a relative small reactor volume. Based on our determined methane yield of 0.21 l CH_4 g^{-1} VS fed and the theoretical methane yield of 0.35 l CH_4 g^{-1} TCOD removed (for wastewater) and the empirical ratio of 0.13 g VS g^{-1} TCOD for the amount of secondary residuals produced per amount of wastewater fed

Table 2 Performance and effluent data for the mesophilic and thermophilic bioreactors at a 15-day HRT under pseudo steady state conditions

Bioreactor/ performance data	Biogas ($l\,l^{-1}$ day^{-1})	$VS_{removed}$ (%)	VS_{conc} (g VS l^{-1})	TS_{conc} (g TS l^{-1})	$TCOD_{removed}$ (%)	$TCOD_{conc}$ (g COD l^{-1})	$SCOD_{conc}$ (g COD l^{-1})	PH	VFA_t (mg CH_3COOH l^{-1})	Free ammonia (mg NH_3–N l^{-1})
Mesophilic	0.88 ± 0.06	43.03	22.22 ± 0.39	39.24 ± 0.58	45.39	39.30 ± 12.20	2.82 ± 0.69	7.54 ± 0.04	350 ± 61.5	74.1 ± 11.0
Thermophilic	0.84 ± 0.07	41.69	22.74 ± 1.02	38.85 ± 1.39	45.12	39.49 ± 12.21	6.33 ± 0.14	7.71 ± 0.03	360 ± 22.0	321 ± 7.1
n	10	NA	4	4	NA	3	3	10	4	2
P-value	0.15	NA	0.40	0.63	NA	0.98	0.03	$1.1 \cdot 10^{-7}$	0.61	$3.1 \cdot 10^{-3}$

Standard errors (SE) for *n* date points are shown for the average data at pseudo steady state conditions. SE for $VS_{removed}$ and $TCOD_{removed}$ is not given because the influent VS concentration was from a single data point. The *P*-values (student *t* test) for a comparison of the means between the mesophilic and thermophilic bioreactors show a statistical difference when $P < 0.05$ (95% confidence level) to the high-rate AD system (personal communication, Anheuser-Busch, Inc.), an additional methane generation of 8.1% can be expected compared to the amount of methane that is already produced from soluble brewery wastewater treatment alone. Besides offsetting costs for nonrenewable energy by extra methane generation, secondary residuals treatment also reduces sewer costs and costs for caustic usage. The latter savings are anticipated, because total ammonium generation in the CSAD reactors can be utilized in the equalization tanks for soluble brewery wastewater treatment as a source of alkalinity and nutrients.

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