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Anaerobic digestion of whole stillage from dry-grind corn ethanol plant under mesophilic and thermophilic conditions

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ABSTRACT

Anaerobic digestion of whole stillage from a dry-grind corn-based ethanol plant was evaluated by batch and continuous-flow digesters under thermophilic and mesophilic conditions. At whole corn stillage concentrations of 6348 to 50,786 mg total chemical oxygen demand (TCOD)/L, at standard temperature (0 °C) and pressure (1 atm), preliminary biochemical methane potential assays produced 88 ± 8 L (49 ± 5 L CH_4) and 96 ± 19 L (65 ± 14 L CH_4) biogas per L stillage from mesophilic and thermophilic digesters, respectively. Continuous-flow studies for the full-strength stillage (TCOD = 254 g/L) at organic loadings of 4.25, 6.30 and 9.05 g TCOD/L days indicated unstable performance for the thermophilic digester. Among the sludge retention times (SRTs) of 60, 45 and 30 days tested, the mesophilic digestion was successful only at 60 days-SRT which does not represent a practical operation time for a large scale bioethanol plant. Future laboratory studies will focus on different reactor configurations to reduce the SRT needed in the digesters.

1. Introduction

Limited fossil resources are being consumed for growing demands of transportation fuels and production of carbon-containing chemicals. In the world where two-thirds of the petroleum reserves are located in the Middle East, having technologies to develop home-grown alternative energy sources, such as bioethanol from biomass (sugar crops, starch crops, dairy products, cellulosic materials and municipal sludge/solid waste), has enormous economic and strategic advantages. In addition, the increasing CO_2 level in the atmosphere leading to global climate change is forcing policy makers and industries to use alternative energy sources which can mitigate the so-called "greenhouse" effect (Rass-Hansen et al., 2007).

Corn fiber has recently created great interest as a biomass substrate partly due to the many co-products generated from it during bioethanol production and in the US; the majority of fuel ethanol originates from corn (Kim and Dale, 2004). A typical bioethanol plant process (illustrated in Fig. 1) can be subdivided into production of ethanol (milling, hydrolysis, fermentation and distillation) and downstream stillage processing [stillage evaporation, distiller's dried grains with solubles (DDGS) drying]. Smaller scale ethanol plants use a dry milling process in which corn kernels are not separated before fermentation as in the wet milling that requires larger capital cost (Schaefer, 2006). Initially hammer or roller milled corn kernels are mixed with water and cooked. Hydrolysis converts starch to fermentable sugars using enzymes added to the cooked mash. Hydrolyzed substrate is fermented with yeast resulting in production of CO_2 plus ethanol. After CO_2 is scrubbed (to be sold for carbonated beverages), remaining liquid is distilled and dehydrated with molecular sieves to produce ethanol with 95% and 99.95% purities, respectively.

Fermentation residue (after ethanol is removed) is called whole stillage which is centrifuged to produce *wet cake* and thin stillage (liquid portion). The thin stillage is partially (around 50%) recycled as backset to the second stage of the liquefaction process. The remaining thin stillage passes to the evaporators, where it is concentrated. Centrifuged solids are dried and called distiller's dry grain (DDG) and thin stillage is evaporated to syrup and mixed with DDG to produce DDGS (distiller's dried grains with solubles) to be sold as livestock feed.

Stillage handling is one of the major limitations of the corn to ethanol process, since DDGS drying and stillage evaporation account for approximately 30.3% and 16.5% of total energy consumption of a bioethanol plant, respectively (Lurgi, 2010). Each liter of ethanol produced can generate up to 20 L of stillage with TCOD of 100 g/L (Wilkie et al., 2000), and evaporator condensate from thin stillage is the largest wastewater contributor (Schaefer, 2006). Although anaerobic digestion has lower alkalinity and

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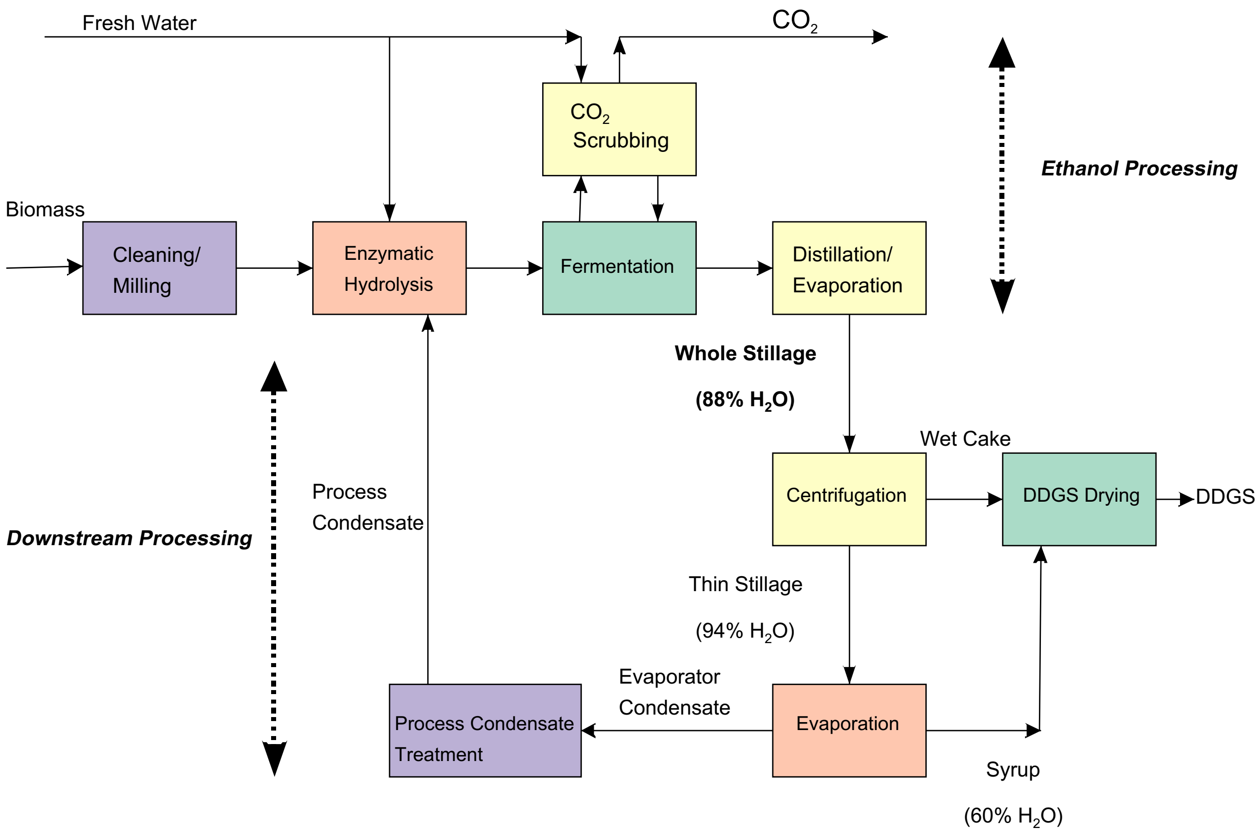

Fig. 1. Schematic of a corn bioethanol plant process (DDGS: distiller's dried grains with solubles).

nutrient requirements compared to aerobic biodegradation and has a great potential to reduce the organic pollution of stillage and to produce methane as fuel for the bioethanol, it has not been applied with the magnitude possible. Previous researchers observed promising performances from mesophilic digestion of thin corn stillage [64,500 mg TCOD/L; 32,200 mg total solids (TS)/L] in both suspended growth and fixed-film systems with a methane yield ranging from 0.25 to 0.37 m^3/kg TCOD removed that could replace 60% of the daily energy requirement of the bioethanol plant (Stover et al., 1984). Two stage (acidogenesis and methanogenesis) mesophilic fluidized bed digesters could also be used for a wet-mill ethanol plant waste mainly due to much lower solid loadings [9028 mg TCOD/L; 2181 mg total suspended solids (TSS)/L] than average (Kothari et al., 1986). One pilot scale upflow anaerobic sludge blanket (UASB) reactor achieving 76% TCOD removal with 0.33 m^3 CH_4/kg TCOD removed was also used for a corn ethanol plant as a stillage pretreatment step before aerobic trickling filters; however influent wastewater TCOD was only 3600 mg/L (Lanting and Gross, 1985). Attached growth systems in which reactor cell tissues are attached to an inert medium, such as rocks, ceramic or plastic to improve bacteria retention can be more suitable to treat stillage since a granular biomass retention system can clog with high TSS leading to biomass washout. Other researchers treated a thin corn stillage (94,000 mg TCOD/L; 61,000 mg TS/L) in a complete-mixed thermophilic digester at hydraulic retention times (HRTs) of 30, 20, 15 and 12 days at corresponding volumetric organic loadings rates (OLRs) of 3.2, 6.1, 6.4 and 7.6 g TCOD/L days (Schaefer, 2006; Schaefer and Sung, 2008). Steady state was achieved at HRTs of 30, 20 and 15 days; however reactors failed after a week of operation at HRT of 12 days with total volatile fatty acids (TVFAs) of 7000 mg/L. Another study reported on thin stillage digestion with a high-rate thermophilic anaerobic digestion system (Agler et al., 2008). At a shorter HRT of 10 days, an OLR of 7.50 g TCOD/L days was achieved with prolonged stable performance compared to Schaefer and Sung (2008).

Mesophilic or thermophilic digestion (single or dual stage) of whole corn stillage has not been studied at the full strength (254 g TCOD/L) and the know-how regarding the reactor design and configuration is not available in the literature. All studies mentioned above attempted to digest only a portion of whole stillage, mostly thin stillage, leaving the majority of the methane potential of the stillage untreated outside the digesters. However, if whole stillage (with a TCOD of 5 times higher than that of thin stillage) can be pumped directly to the anaerobic digesters bypassing the centrifugation, evaporation and drying processes (Fig. 1) and treated in a practical time, it could produce methane more than enough to replace the natural gas in a dry-grind process. Furthermore, the excess methane could be sold to the power grid.

Currently, due to the rich in nutrients (fiber, protein, lipids, and starch) nature of whole stillage, it is more profitable to sell as animal feed. This explains the rationale of previous studies for not using whole stillage for anaerobic digestion. While biogas from thin stillage could be used to dry the whole stillage before it is used as animal feed, if corn to ethanol ramps up, the animal feed market will be quickly saturated. This is already creating problems for ethanol plants located in remote areas with no farming communities nearby. Therefore alternate methods should be developed to handle this renewable resource.

The main objective of this research was to evaluate the primary biodegradation performance [biogas production, TCOD, TS and volatile solids (VS) removal efficiencies] of whole stillage from a corn bioethanol plant in batch and continuous-flow digesters under thermophilic (55 ± 2 °C) and mesophilic (35 ± 2 °C) conditions.

2. Methods

2.1. Corn stillage and inoculum characterization

Prior to the actual start-up, characterization of corn stillage, mesophilic (35 ± 2 °C) and thermophilic (55 ± 2 °C) inocula were completed. Whole corn stillage was obtained from a bioethanol plant in US and kept refrigerated at 4 °C prior to use. Whole stillage was taken from a well-mixed solution and special attention was shown to take representative samples with high TS concentrations. Parameters tested in laboratory conditions for whole stillage, mesophilic and thermophilic inocula were given in Table 1.

Mesophilic inoculum was taken from the effluent line of the anaerobic sludge digesters (at SRT of 15–20 days) treating a mixture of primary sludge (PS) and thickened waste activated sludge (WAS), at Robert O. Pickard Environmental Center (ROPEC) sewage treatment plant located in Gloucester (ON, Canada). ROPEC has preliminary and primary treatment followed by a conventional aerobic activated sludge unit operated at an average SRT of 5 days. At ROPEC, ferric chloride is added to WAS for phosphorous removal prior to thickening. Thickened WAS and PS are blended in a 58:42 (v/v) ratio and undergo mesophilic (35 °C) anaerobic sludge digestion to produce a stabilized biosolids product for disposal. Thermophilic inoculum was taken from the effluent line of the thermophilic sludge digesters at Annacis Island Wastewater Treatment Plant in Vancouver (BC, Canada). The Annacis plant, largest of the five Metro Vancouver treatment plants, contains physical, biological, and chemical treatment units. The preliminary (screening and grit removal) and primary sedimentation is followed by trickling filters and secondary clarifiers. Thickened PS and WAS are mixed and undergo an extended (SRT > 20 days) thermophilic (55 °C) anaerobic process to ensure pathogen reduction. Sludge dewatering is done with centrifuges to 30% total solids before disposal (over 100 tonne of biosolids per day). The methane productions in this study were verified by BMP assays and gas chromatography (GC) injections from the head space of biochemical methane potential (BMP) bottles.

2.2. Batch experiments

BMP assay (Owen et al., 1979; Angelidaki et al., 2009), analogous to the biochemical oxygen demand (BOD) test, is widely used to assess the methane potential of a waste. Anaerobic biodegradability of whole stillage was studied in duplicate under both mesophilic (35 ± 2 °C) and thermophilic (55 ± 2 °C) conditions. Serum bottles (125 mL) with butyl rubber stoppers were used as batch digesters. Whole stillage is diluted to yield TCOD concentrations of 6343, 12,696, 25,393 and 50,786 mg/L, respectively and 40 mL of diluted stillage and 40 mL of inocula were added into the serum bottles. A total of 20 (10 mesophilic, 10 thermophilic) BMP assays were run including duplicates and blanks (inoculum only). The inocula were starved for 2 days prior to their use in the BMP assays. Nitrogen bubbling was applied to batch reactors when stillage and inocula were mixed to prevent exposure to air and reactors were sealed after addition of a mixture containing equal parts of dry sodium bicarbonate ($NaHCO_3$) and dry potassium bicarbonate ($KHCO_3$) to achieve an alkalinity of 4000 mg/L (as $CaCO_3$). Dry forms of $(NH_4)_2SO_4$, KH_2PO_4 were added to stillage to achieve a COD:N:P ratio of 100:5:1 (mass basis). Batch bottles were kept in mesophilic and thermophilic temperature programmable shakers (90 rpm) to keep the bacteria in suspension. Biogas productions from BMP assays were measured daily by inserting a needle attached to a manometer. Gas volume readings were done at the incubation temperatures (35 and 55 °C). Volume determinations were made by allowing the manometer water level to move up and equilibrate between the bottle and atmospheric pressure (~1 atm). The mesophilic and thermophilic biogas volumes were then corrected to STP (0 °C at 1 atmosphere). Total and volatile solids (TS/VS) and total and soluble chemical oxygen demand (TCOD/SCOD) concentrations were measured in the beginning and at the end of the BMP assays to assess the organic removal efficiencies. Similarly, TVFAs, pH and biogas composition (N_2, CH_4, CO_2) were measured two times a week until batch reactors stopped producing biogas.

2.3. Reactor experiments

Upon completion of batch studies, methane potential of corn whole stillage at full strength was further tested in semi-continuous (SC) flow digesters at SRTs of 60, 45 and 30 days. Two Plexiglass™ bench-scale digesters (ID = 14 cm, L = 44 cm, 6 and 3-L total and wet volumes, respectively) with mechanical mixers were constructed. Both digesters were kept in a temperature (33 ± 2 °C) controlled room. The reactor temperature of the thermophilic digester was further elevated to 55 ± 2 °C by a water bath. Digester SRTs were maintained by first removing and then adding a

Table 1
Characteristics of whole corn stillage and inocula used[a].

Parameter	Whole corn stillage	Mesophilic inoculum	Thermophilic inoculum
pH (–)	4.34	7.92	8.5
TS (%, w/w)	12.4 (0.1; 3)[b]	1.9 (0.2; 2)	4.3 (0.0; 2)
VS (%,w/w)	11.5 (0.1; 3)	1.1 (0.2; 2)	2.4 (0.0; 2)
VS/TS (–)	92.5 (0.0; 3)	55.7 (3.4; 2)	55.8 (0.2; 2)
TCOD (mg/L)	253,929 (24,643; 2)	24,143 (143; 2)	45,714 (2857; 2)
SCOD (mg/L) (<0.45 μm)	51,286 (3301; 4)	1191 (18; 4)	18,571 (857; 2)
BOD_5 (mg/L)	66,600	–	–
$SBOD_5$ (mg/L) (<0.45 μm)	15,033	–	–
NH_3–N (mg/L)	5.9 (0.1; 2)	1229 (86; 2)	3761
TKN (mg/L)	5300 (774; 2)	–	–
Total phosphorus (mg/L)	3506 (63; 2)	–	–
Alkalinity[c]	Not detected	4267 (0; 2)	15,167
TVFA (mg/L)[d]	863 (12; 2)	73 (15; 2)	5874 (334; 3)
Acetic acid (mg/L)	631 (2; 2)	63 (12; 2)	3764 (274; 3)
Propionic acid (mg/L)	42 (7; 2)	11 (2; 2)	1741 (129; 3)
Butyric acid (mg/L)	189 (3; 2)	0 (0; 2)	368 (72; 3)

[a] TS, VS: total and volatile solids; TCOD, SCOD: total and soluble (<0.45 μm) chemical oxygen demands; BOD_5, $SBOD_5$: 5 day biochemical and soluble biochemical oxygen demands; TKN: total kjeldahl nitrogen.
[b] Data represent arithmetic mean of replicates (standard deviations; number of data points).
[c] Bicarbonate alkalinity in units of mg l^{-1} as calcium carbonate ($CaCO_3$).
[d] TVFA = summation of acetic, propionic and butyric acids.

constant mixed liquor volume from the sampling ports of the digesters daily with a modified wide mouth 100 mL syringe. Daily biogas productions were measured by Wet Tip Gas Meters connected to bench-scale digesters. For each SRT, when daily biogas productions from digesters were observed to be stable, pH, TCOD, SCOD, TS, VS, TVFAs, NH_3–N, biogas composition and HCO_3^- alkalinity analyses were done biweekly.

2.4. Analysis

All analyses were performed for the samples at room temperature. TS and VS were determined based on standard methods procedure 2540G (APHA, 1995). For supernatant NH_3–N determination, centrifugation [for 20 min at 5856 relative centrifugal force (RCF) in a Dupont instruments Sorvall SS-3 automatic centrifuge] was used and NH_3–N measurements were performed using an ORION Model 95-12 ammonia gas sensing electrode connected to a Fisher Accumet pH meter model 750. The analysis was conducted according to standard methods 4500D (APHA, 1995). Colorimetric COD measurements were performed based on standard methods procedure 5250D (APHA, 1995) with a Coleman Perkin–Elmer spectrophotometer Model 295 at 600 nm light absorbance. Before soluble COD (SCOD) determination, sludge samples were centrifuged (for 20 min at 5856 RCF) and filtered through membrane disc filters with 1.2 μm first and then with 0.45 μm pore sizes. Reactor pH, TVFAs (acetic, propionic and butyric acids) and biogas composition (nitrogen, methane and carbon dioxide percentage) were monitored at the beginning, during (two times a week) and end of BMP assays. TVFAs were measured by injecting supernatants into a HP 5840A GC with glass packed column (Chromatographic Specialties Inc., Brockville, ON, Canada, Chromosorb 101, packing mesh size: 80/100, column length × ID: 304.8 × 0.21 cm) and a flame ionization detector (oven, inlet and outlet temperatures: 180, 250 and 350 °C, respectively, carrier gas flowrate: 25 mL·helium/min) equipped with HP 7672A autosampler (Ackman, 1972). Biogas composition was determined with an HP 5710A GC with metal packed column (Chromatographic Specialties Inc., Brockville, ON, Canada, Porapak T, packing mesh size: 50/80, column length × OD: 304.8 × 0.635 cm) and thermal conductivity detector (oven, inlet and outlet temperatures: 70, 100 and 150 °C, respectively) using 25 mL·helium/min as the carrier gas (van Huyssteen, 1967).

3. Results and discussion

3.1. Batch experiments

Specific cumulative biogas and methane productions from thermophilic and mesophilic digesters corrected at STP are shown in Fig. 2a–d. In these figures, biogas or methane productions attrib-

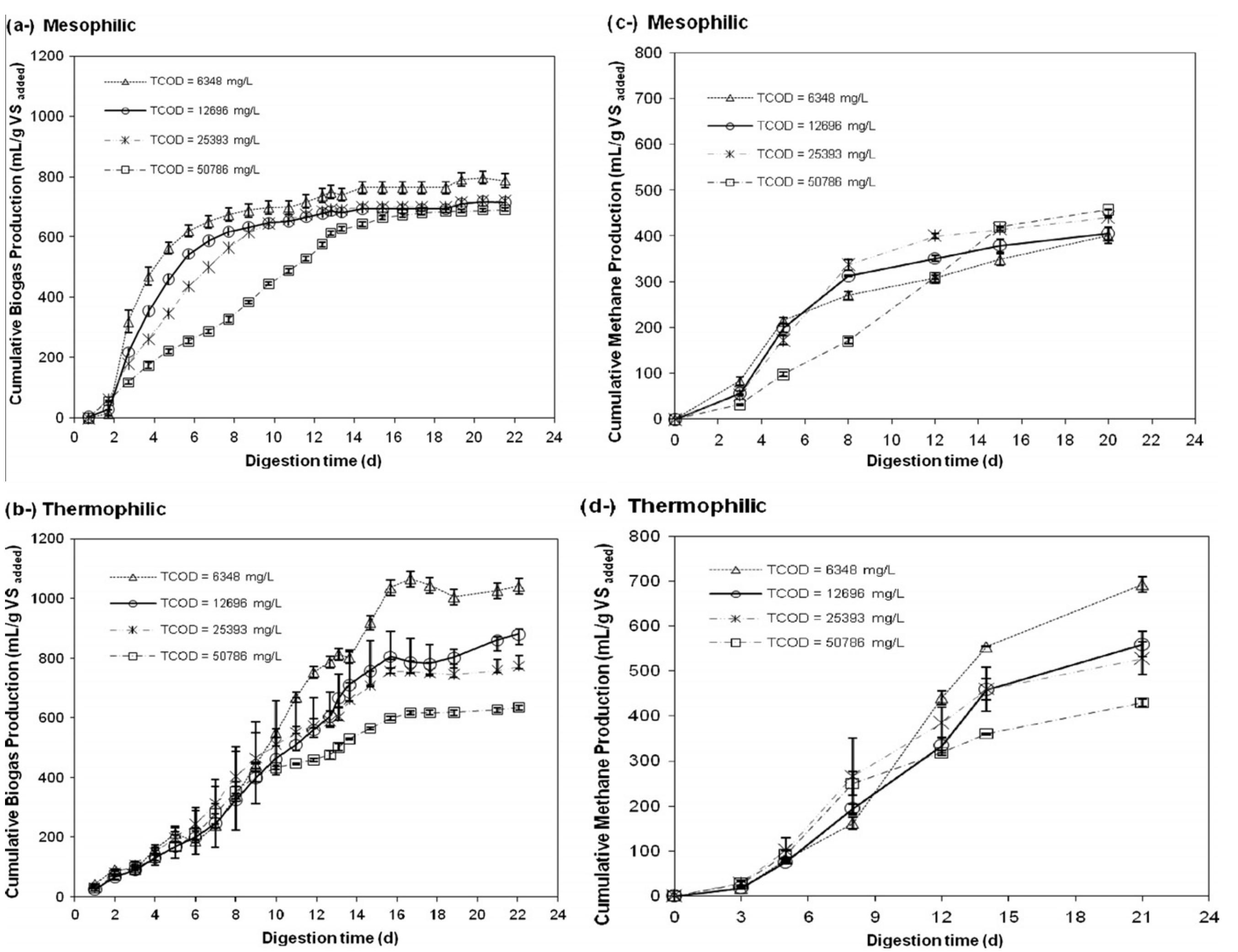

Fig. 2. Specific cumulative biogas and methane productions from BMP assays with mesophilic inoculum (a, c), and thermophilic inoculum (b, d) adjusted at standard temperature (0 °C) and pressure of 1 atm (data represent the mean and error bars represent absolute difference between mean and duplicates, respectively).

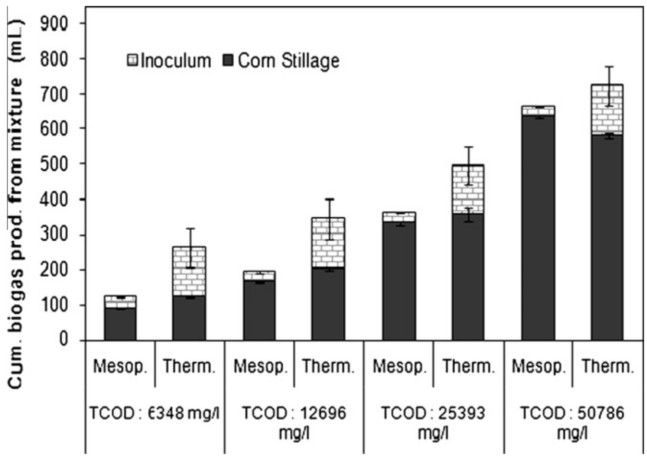

Fig. 3. Contributions of corn stillage and inoculum into cumulative biogas production adjusted at standard temperature (0 °C) and pressure of 1 atm (data represent the mean and error bars represent absolute difference between mean and duplicates, respectively).

uted to mesophilic and thermophilic inocula were subtracted from the total biogas or methane production from the mixtures (also illustrated in Fig. 3) in order to evaluate degradation of whole corn stillage itself. Higher biogas produced by the thermophilic inoculum (Fig. 3) was due to higher TS (4.3% w/w), TCOD (45,714 mg/L) and TVFA (5874 mg/L) concentrations of the thermophilic compared to those of mesophilic inoculum which were 1.9% TS (w/w), 24,143 mg TCOD/L and 73 mg TVFA/L, respectively (Table 1).

Fig. 2a–d indicate that, none of the bottles experienced a significant lag phase at the beginning of the batch test under specific organic loadings of 0.27–4.20 g TCOD/g VS added. At the highest organic loading (50,786 mg TCOD/L; 4.20 g TCOD g/VS added), degradation was complete in 15–16 days; an additional week of digestion did not display any significant changes in COD removal although bottles continued producing small amounts of biogas. At the end of 22 days, bottles with whole corn stillage concentrations of 6348, 12,696, 25,393 and 50,786 mg TCOD/L achieved 94 ± 1%, 86 ± 1%, 87 ± 2%, 83 ± 1% under mesophilic, and 97 ± 3%, 90 ± 6%, 86 ± 0%, 82 ± 0% VS removals under thermophilic conditions, respectively. Similarly, bottles with 6348, 12,696, 25,393 and 50,786 mg TCOD/L concentrations achieved 86 ± 11, 76 ± 11, 88 ± 0, 86 ± 3% under mesophilic, and 73 ± 5, 94 ± 5, 85 ± 2, 89 ± 9% TCOD removals under thermophilic conditions, respectively. As an overall average, mesophilic digestion of corn stillage achieved 88 ± 1% VS and 84 ± 10% TCOD removals while thermophilic removals were 84 ± 10% VS and 85 ± 9% TCOD. It is necessary to emphasize that although higher overall biogas production was observed from thermophilic BMPs (Fig. 3), the additional biogas (brick patterns in Fig. 3) came from the thermophilic inoculum itself. Under both mesophilic and thermophilic conditions, corn stillage produced similar amount of biogas (black columns in Fig. 3) which was consistent with VS and TCOD removals given above. At the end of the 22 days, at STP, bottles with 6348, 12,696, 25,393 and 50,786 mg TCOD/L concentrations produced 96 ± 3, 175 ± 14, 352 ± 16, 636 ± 5 mL final biogas under mesophilic, and 122 ± 2, 203 ± 4, 356 ± 17, 585 ± 7 mL biogas under thermophilic conditions, respectively. These results correspond to specific cumulative biogas yields of 788 ± 24, 715 ± 14, 720 ± 11, 691 ± 5 mL per gram VS added under mesophilic (Fig. 2a), and 1041 ± 22, 881 ± 16, 772 ± 37, and 635 ± 8 mL per gram VS added under thermophilic conditions (Fig. 2b) at 6348, 12,696, 25,393 and 50,786 mg TCOD/L concentrations, respectively.

Biogas composition (percentage of methane, carbondioxide and nitrogen) was also analysed for both mesophilic and thermophilic bottles at the beginning, during (two times a week) and end of BMP assays. Thermophilic reactors had higher biogas methane contents than mesophilic digesters. The results correspond to specific methane yields of 401 ± 17, 406 ± 14, 441 ± 2, 458 ± 0 mL per gram VS added under mesophilic (Fig. 2c), and 693 ± 17, 560 ± 24, 529 ± 37, and 429 ± 8 mL per gram VS added under thermophilic conditions (Fig. 2d) at 6348, 12,696, 25,393 and 50,786 mg TCOD/L concentrations, respectively. In other units, average specific mesophilic and thermophilic methane yields (at STP) were calculated as 0.50 ± 0.04 and 0.60 ± 0.08 L CH_4/g VS removed and 0.23 ± 0.02 and 0.26 ± 0.04 L CH_4/g TCOD removed with concomitant 20% and 13% improvements for thermophilic over mesophilic digestion, respectively. These results are in agreement with thermophilic specific methane yields (0.6–0.7 L CH_4/g VS removed) reported on corn-thin stillage with 6.1% TS concentration (Schaefer and Sung, 2008).

Previous studies indicated that thermophilic bacteria have higher methanogenic activity than mesophilic bacteria (Mladenovska and Ahring, 2000). In this study, at STP, thermophilic bottles achieved slightly higher average biogas productions (96 ± 19 L biogas/L whole stillage) than the mesophilic digesters (88 ± 8 L biogas/L whole stillage). At the end of the 22 days of digestion, the organic removal efficiencies were similar (Table 2). This can be due to the highly biodegradable nature of the substrate (93% of solids are organic; Table 1) under anaerobic conditions and relatively long digestion time (22 days) as well as the sufficient amount of inocula that result in low specific loadings (in a range of 0.27–4.20 g TCOD/g VS added) for bottles. It is important to emphasize that BMP assays are effective and cost efficient way of testing preliminary methane potential of a substance under defined conditions (Owen et al., 1979), but in general do not reflect full-scale systems with continuous-flow feeding pattern.

Table 2
Initial and final organic matter concentrations in BMP assays.

Total chemical oxygen demand (TCOD)				Volatile solids (VS)		
Reactor	Initial (mg/L)	Final (mg/L)	Removal (%)	Initial (%, w/w)	Final (%, w/w)	Removal (%)
Mesophilic bottle						
R1/R1-d[a]	6348 ± 0	857 ± 714	86 ± 11	0.29 ± 0.00	0.02 ± 0.00	94 ± 1
R2/ R2-d	12,696 ± 0	3000 ± 1429	76 ± 11	0.58 ± 0.00	0.08 ± 0.01	86 ± 1
R3/ R3-d	25,393 ± 0	3000 ± 0	88 ± 0	1.15 ± 0.00	0.15 ± 0.03	87 ± 2
R4/ R4-d	50,786 ± 0	7286 ± 1429	83 ± 3	2.30 ± 0.00	0.39 ± 0.02	83 ± 1
Thermophilic bottle						
R1/ R1-d	6348 ± 0	1714 ± 333	73 ± 5	0.29 ± 0.00	0.01 ± 0.01	97 ± 3
R2/ R2-d	12,696 ± 0	810 ± 571	94 ± 5	0.58 ± 0.00	0.06 ± 0.03	90 ± 6
R3/ R3-d	25,393 ± 0	3905 ± 619	85 ± 2	1.15 ± 0.00	0.16 ± 0.00	86 ± 0
R4/ R4-d	50,786 ± 0	5762 ± 2667	89 ± 5	2.30 ± 0.00	0.42 ± 0.00	82 ± 0

[a] d: duplicate bottle.

3.2. Continuous-flow experiments

Bench-scale semi-continuous flow digester studies were also run to evaluate the performances of mesophilic and thermophilic digesters fed with full strength whole stillage (254 g TCOD/L, 12.4% TS w/w) different SRTs. Before feeding the digesters under identical loading conditions, mesophilic and thermophilic inocula solid contents were adjusted to the same level which was 1.4% (w/w) VS. Digestion operation was first started with an SRT of 60 days (OLR of 4.25 g TCOD/L days). Digesters were operated until steady-state conditions were reached and then maintained in this state over a month. Upon completion of this run, the SRT was gradually reduced to 45 and then 30 days (corresponding OLRs of 6.03 and 9.05 g TCOD/L days, respectively).

Figs. 4 and 5 indicate daily biogas productions at STP, and VFA concentrations from both mesophilic and thermophilic digesters at different SRTs, respectively. Generation and consumption of intermediate compounds, such as VFAs, is one of the important indicators regarding the activities of acid and methane producers. Accumulation of TVFAs is usually associated with microbial stress and inhibition of methane producers which may occur under an organic or hydraulic overloading, presence of toxics or temperature fluctuations (Parkin and Owen, 1986). Among these acids, at neutral reactor pH, adverse effects have only been shown for propionate at concentrations higher than 1000 mg/L, while acetic and butyric acids did not have significant toxicity on methanogenic bacteria at concentrations up to 10,000 mg/L (Hobson and Shaw, 1976; Fischer et al., 1984; Marchaim and Krause, 1993). It was interesting to realize from Fig. 5 that under identical substrate loading conditions, propionic acid concentration in the thermophilic digester increased from 300 to 2998 mg/L after 30 days of feeding and stayed at this level, while mesophilic digester had negligible (<220 mg/L) amounts of propionic acid after the first 15 days of acclimation period. In the thermophilic digester, propi-

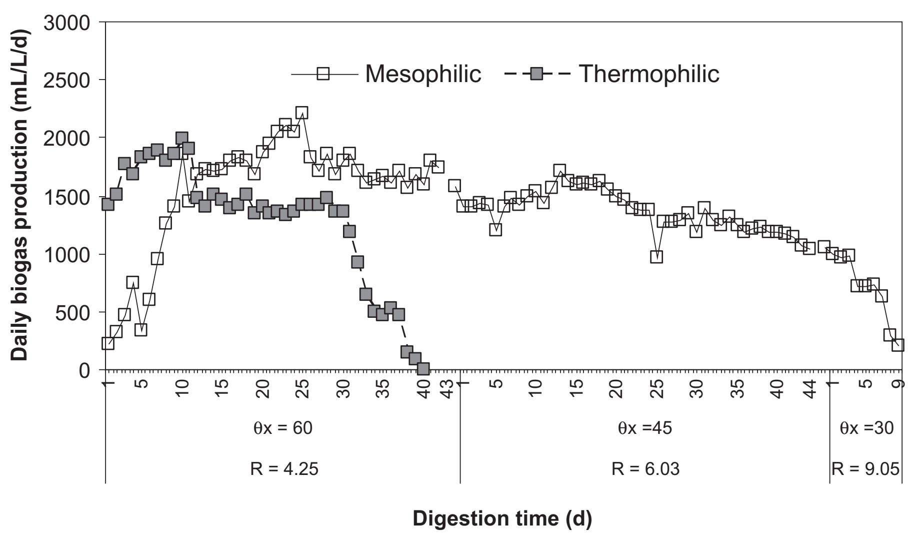

Fig. 4. Daily biogas production from semi-continuous digesters at different sludge retention times adjusted at standard temperature (0 °C) and pressure of 1 atm [θ_x = sludge retention time (days); R = volumetric organic loading rate (g TCOD/L days)].

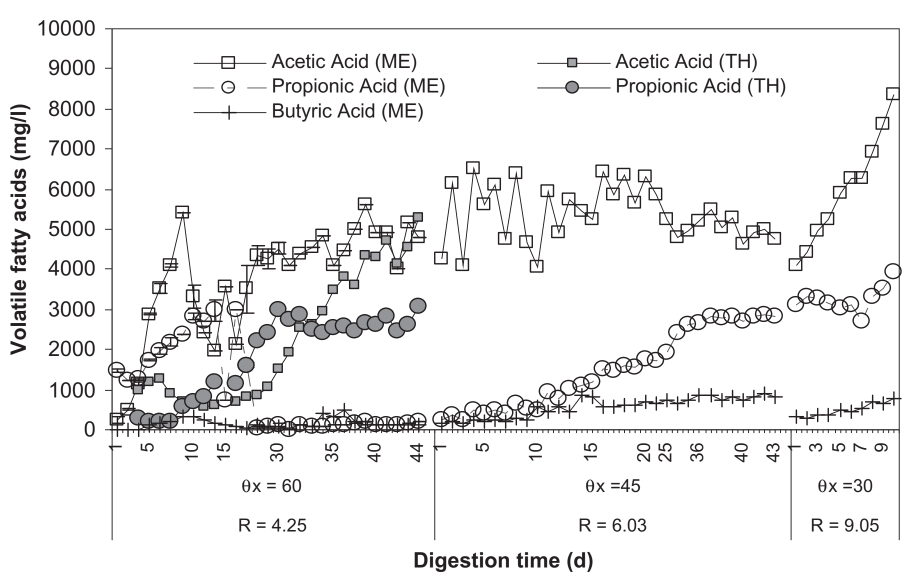

Fig. 5. Volatile fatty acid concentrations of semi-continuous digesters at different sludge retention times [TE = thermophilic (55 ± 2 °C); ME = mesophilic (35 ± 2 °C); θ_x = sludge retention time (days); R = volumetric organic loading rate (g TCOD/L days); negligible level of butyric acids in thermophilic digester are removed from the graph for clarity].

Table 3
Results from semi-continuous digesters at 60, 45 and 30 days sludge retention times (SRTs).

	SRT = 60 days		SRT = 45 days	SRT = 30 days
Parameters	Mesophilic	Thermophilic	Mesophilic	Mesophilic
Feed (whole corn stillage) characteristics				
TCOD (mg/L)	253,929 (24,643; 2)[a]	253,929 (24,643; 2)	271,429	271,429
VS (%, w/w)	11.5 (0.1; 3)	11.5 (0.1; 3)	13.9 (0.1; 2)	13.9 (0.1; 2)
TS (%, w/w)	12.4 (0.1; 3)	12.4 (0.1; 3)	13.0 (0.1; 2)	13.0 (0.1; 2)
Loading conditions for reactors				
[d]OLR (g VS/[b]L days)	1.93	1.93	2.88	4.33
OLR (g TCOD/[b]L days)	4.25	4.25	6.03	9.05
Removal efficiencies				
VS (%)	82.5 (1.6; 6)	86.4 (1.1; 6)	70.2 (5.5; 10)	[c]n/a
TS (%)	76.3 (1.6; 6)	78.5 (1.6; 6)	65.2 (6.0; 10)	n/a
TCOD (%)	85.1 (0.9; 7)	85.7 (1.8; 7)	75.6 (5.8; 11)	n/a
Biogas (mL/L days)	1787 (156; 30)	1578 (192; 16)	1365 (175; 30)	703 (305; 11)
CH_4 (%)	54 (3; 9)	53 (4; 6)	45 (2; 10)	31 (1; 2)
pH in reactors	7.5 (0.1; 31)	7.9 (0.1; 29)	7.2 (0.1; 18)	7.2 (0.4; 10)
[e]Alkalinity (mg $CaCO_3$/L)	9052 (829; 4)	19,749 (6077; 3)	13,188 (601; 4)	14,815 (–; 1)
Acetic acid (mg/L)	4273 (896; 21)	896 (232; 27)	5996 (551; 18)	6006 (1372; 10)
Propionic acid (mg/L)	493 (898; 21)	1281 (841; 27)	2230 (593; 18)	3254 (320; 10)
Butyric acid (mg/L)	151 (116; 21)	92 (95; 27)	739 (96; 18)	504 (167; 10)
[f]TVFA (mg/L)	4918 (453; 21)	2269 (983; 27)	8364 (464; 18)	9763 (1720; 10)
Reactor effluent characteristics				
SCOD (mg/L)	10,048 (822; 6)	10,095 (1280; 6)	23,207 (4950; 10)	28,000 (n/a; 1)
NH_3–N (mg/L)	1891 (208; 4)	1709 (97; 4)	1879 (202; 8)	2746 (n/a; 1)

[a] Data represent arithmetic mean of measurements (standard deviation, number of data points).
[b] Liter of reactor.
[c] n/a = not available.
[d] OLR = Organic loading rate.
[e] Bicarbonate alkalinity in units of mg $CaCO_3$/L.
[f] TVFA = Total volatile fatty acids (summation of acetic, propionic and butyric acids).

onic acid was the dominant fatty acid, indicating that propionic acid utilizers were more sensitive to high organic loadings than acetoclastic methanogens. It is highly possible that dramatic reduction in biogas production from the thermophilic reactor, which has occurred after 27–30 days of feeding (Fig. 4), was due to the high levels (~3000 mg/L) of propionic acids present. The propionic to acetic acid ratio in the thermophilic digester has reached to 2.6 which was much higher than the typical digester failure ratio of 1.4 (Hill et al., 1987). Similarly, when the SRT was further reduced to 45 days (OLR or R of 6.03 g TCOD/L days) in the mesophilic digester, propionic acids gradually increased from negligible to 2400 mg/L (Fig. 5), which has also resulted in gradual reductions of biogas productions (Fig. 4). In both thermophilic (at SRT of 60 days) and mesophilic digesters (at SRT of 30 days), due to the accumulation of propionic acids, pH dropped below the optimum range (<6.5), and external buffer addition for pH control was not successful. Table 3 indicates the organic removals as well as the effluent characteristics of digesters at different SRTs. As it can be seen from Table 3, in addition to TVFAs, NH_3–N concentrations in the mesophilic digesters also increased as OLR was raised from 4.25 and 6.03 to 9.05 g TCOD/L days. At SRT of 60 days, although both mesophilic and thermophilic digesters had similar NH_3–N concentrations (1891 and 1709 mg/L, respectively; Table 3), these total ammonia levels correspond to a free (more toxic) ammonia (NH_3) of ~300 and <50 mg/L for thermophilic (at 55 °C and pH of 7.9) and mesophilic digesters (at 35 °C and pH of 7.5), respectively (Grady et al., 1999). Therefore, it can be postulated that the elevated level of free ammonia (>100 mg NH_3/L digester failure limit) accumulated at 55 °C also accelerated the thermophilic digester failure process at an OLR of 4.25 g TCOD/L days, while the mesophilic digester was still stable.

The fact that the thermophilic digester operating at 55 °C was unable to cope with an OLR of 4.25 g TCOD/L, which was routinely handled by the mesophilic digester suggests that thermophilic bacteria was more sensitive to elevated VFA and NH_3 formations and alkalinity fluctuations which was also reported by other studies (Duff and Kennedy, 1982; Parkin and Owen, 1986). When comparing thermophilic and mesophilic reactor performance under identical substrate loadings, it is important to emphasize that, at different reactor temperatures, morphologically and physiologically different microorganisms may be dominant in the conversion processes (LaPara et al., 2000; Tiago et al., 2004). Similar differences may also exist in acidogenic microorganisms. The poorer performance of thermophilic compared to mesophilic digesters therefore can be attributed to this decreased physiological stability and presence of fewer types of bacteria at elevated temperatures.

4. Conclusions

Preliminary biochemical methane potential tests indicated significant methane potential for whole corn stillage with TCOD ranging from 6348 to 50,786 mg/L under mesophilic (35 °C) and thermophilic (55 °C) conditions. However, when the continuous-flow operation was used for whole corn stillage at full strength (254 g TCOD/L), thermophilic digester was unable to cope with an organic volumetric loading rate of 4.25 g TCOD/L at SRT of 60 days. Among the SRTs tested (60, 45 and 30 days), the mesophilic digester was stable only at 60 days-SRT with a methane yield of 58 L/kg stillage.

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