Bioresource Technology 133 (2013) 206–212

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Pilot scale anaerobic co-digestion of municipal wastewater sludge with biodiesel waste glycerin

Vahid Razaviarani [a], Ian D. Buchanan [a,*], Shahid Malik [b], Hassan Katalambula [b]

[a] Department of Civil and Environmental Engineering, University of Alberta, 9105-116 St. N.W., Edmonton, Alberta, Canada T6G 2W2
[b] Edmonton Waste Management Centre of Excellence, Gold Bar Wastewater Treatment Plant, 10977-50 St. N.W., Edmonton, Alberta, Canada T6A 2E9

HIGHLIGHTS

- Co-digestion was tested in 1200 L control and test anaerobic digesters.
- Six glycerin loading rates were applied to the test digester.
- The digesters' methane production, VS removal and COD removal were compared.
- The maximum upper loading limit that did not cause a process upset was identified.

ARTICLE INFO

Article history:
Received 7 November 2012
Received in revised form 14 January 2013
Accepted 20 January 2013
Available online 29 January 2013

Keywords:
Pilot-scale
Anaerobic co-digestion
MWS
Biodiesel waste glycerin

ABSTRACT

The effect on process performance of adding increasing proportions of biodiesel waste glycerin (BWG) to municipal wastewater sludge (MWS) was studied using two 1300 L pilot-scale digesters under mesophilic conditions at 20 days SRT. The highest proportion of BWG that did not cause a process upset was determined to be 23% and 35% of the total 1.04 kg VS/(m^3 d) and 2.38 kg COD/(m^3 d) loadings, respectively. At this loading, the biogas and methane production rates in the test digester were 1.65 and 1.83 times greater than of those in the control digester which received only MWS, respectively. The COD and VS removal rates at this loading in the test digester were 1.82 and 1.63-fold those of the control digester, respectively. Process instability was observed when the proportion of BWG in the test digester feed was 31% and 46% of the 1.18 kg VS/(m^3 d) and 2.88 kg COD/(m^3 d) loadings, respectively.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Anaerobic digestion is a widely used process for the degradation and stabilization of organic waste due to its environmental and economical benefits. Direct anaerobic treatment of many industrial organic wastes is not practical because the wastes do not provide sufficient buffering capacity or nutrients to ensure stable operation, particularly at small scales. Conversely, municipal wastewater sludge is a reliable source of micro-nutrients and many municipal facilities do not employ all of the capacity available in on-site anaerobic sludge digesters (Schwarzenbeck et al., 2008). Therefore, co-digestion of industrial organic waste with municipal wastewater sludge allows beneficial use of materials that cannot be digested alone.

The biodiesel production industry generates a large amount of waste glycerin representing about 10% (wt) of the initial raw material (Chi et al., 2007). Annual waste glycerin generation increased rapidly after 2006 and is expected to reach 8.8 billion kg annually by 2015 (Ayoub and Abdullah, 2012). This has led to a surplus of waste glycerin and a dramatic decline in crude glycerin price (Yazdani and Gonzalez, 2007). The lack of an economical purification process for waste glycerin (Slinn et al., 2008), together with the variability of its quality have made the marketing of waste glycerin uneconomical (Robra et al., 2010). Therefore, beneficial disposal methods for waste glycerin have been investigated (Ayoub and Abdullah, 2012; Gu and Jerome, 2010).

Several studies have evaluated the benefits of co-digesting waste glycerin with organic wastes such as municipal solid waste (Fountoulakis et al., 2010), manure and energy crops (Holm-Nielsen et al., 2008) and pig manure (Amon et al., 2006; Astals et al., 2012). Most studies have been conducted at lab-scale. Yet, pilot-scale studies which more closely resemble full scale operating conditions are required to assess several operational parameters.

The objectives of this study were (1) to investigate the effects of increasing the proportion of biodiesel waste glycerin (BWG) mixed with municipal wastewater sludge (MWS) on pilot-scale anaerobic digester performance with respect to methane production, total

---

* Corresponding author. Tel.: +1 780 492 0244; fax: +1 780 492 0249.
E-mail address: ian.buchanan@ualberta.ca (I.D. Buchanan).

0960-8524/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2013.01.101

Nomenclature

MWS	municipal wastewater sludge
BWG	biodiesel waste glycerin
$COD_{removed}$	removed chemical oxygen demand (g/L)
COD_{added}	added chemical oxygen demand (g/L)
GPR	Gas production rate (m^3 biogas/m^3 d)
MPR	Methane production rate (m^3 CH_4/m^3 d)
SCOD	soluble chemical oxygen demand
SMP	specific methane production (m^3 CH_4/kg COD_{added}, m^3 CH_4/kg VS_{added})

COD removal, volatile solids destruction, and process stability; and (2) to identify the upper limit of the BWG proportional loading that does not cause a process upset.

2. Methods

2.1. Substrates

Municipal wastewater sludge (MWS) consisting of a 3:1 (v/v) mixture of primary treatment scum and sludge (PS) and thickened waste activated sludge (TWAS), was obtained from the Gold Bar Wastewater Treatment Plant (WWTP) in Edmonton, Alberta, Canada. Biodiesel waste glycerin from canola oil biodiesel production was collected from a biorefinery in Calgary, Alberta, Canada. Digested sludge from a full scale mesophilic anaerobic digester at the Gold Bar Wastewater Treatment Plant was used as the inoculum (seed) for the start-up of the digesters. The characteristics of MWS and BWG varied somewhat during the study as shown in Table 1. The BWG is an organic readily digestible material which had a high pH and alkalinity compared to the MWS. The SCOD/COD ratio indicates the level of the feed solubilization which directly affects the biogas production (Tang et al., 2010). This ratio was approximately 0.98 in the BWG which was almost 14 times higher than that of the MWS.

2.2. Semi-continuous pilot digester

Two 1300 L (1200 L active volume) completely mixed digesters housed in a trailer were received from the King County Wastewater Treatment Division in Washington. The trailer pilot plant was transferred to and set up at the Gold Bar WWTP. The continuously stirred tank reactors (CSTR) were operated in the mesophilic temperature range (36 ± 1 °C) with a solids retention time (SRT) of 20 days. Each digester was initially fed 1200 L of seed sludge and then 60 L of digested material was withdrawn and replaced with the same volume of feed each day (7 days/week) to provide a 20 day SRT. The control digester was fed only municipal wastewater sludge (MWS) while the test digester received the same MWS with BWG as a co-substrate. The organic loading rate was determined on the basis of total COD.

Each digester was heated via an external thermal jacket. The digesters' temperatures were monitored by type J thermocouples. A top-mounted three-bladed digester mixer was operated at a nominal shaft speed of 100 rpm in each digester. A data logger collected and logged the digesters' internal temperatures, volumes of biogas produced, and digester active volumes every 5 min.

2.3. Digester feed and organic loading rate protocols

Initially, the digesters received the same amount and type of feed (MWS) in order to establish their baseline performance. This operating mode was continued for 30 days. Subsequently, the COD loading to the test digester was increased with the addition of BWG to the MWS feed to achieve the desired COD loading, while maintaining the 20-day SRT. Except for the highest loading, each COD loading to the test digester (expressed as a percentage of the control digester's COD loading) was maintained for 30 days (Table 2). The test digester loading rate was increased progressively by adding greater volumes of BWG to eventually reach the maximum nominal COD loading of 180% relative to the control digester COD loading.

Each day, a volume of MWS sufficient to meet the line flushing and feeding requirements for both digesters (approximately 70 L for each digester) was obtained from the on-site sludge blend tanks and transferred to a grinder tank where it was thoroughly mixed prior to being transferred to a feed tank (see Fig. 1). Before feeding, 60 L of digested sludge were drained from the control digester to its effluent tank. Samples were collected from the feed and the effluent tanks for subsequent analysis. The control digester feed line was flushed with the MWS and 60 L of MWS were then

Table 1
Characteristics of municipal wastewater sludge (MWS) and biodiesel waste glycerin (BWG).

Feed	Parameters	Nominal COD loading (%)			
		100	130	150	180
MWS	COD (g/L)	34.1 ± 4.5[a]	37.83 ± 2.32	31.06 ± 1.06	31.50 ± 1.35
	SCOD (g/L)	N/A[b]	2.75 ± 0.25	2.29 ± 0.60	1.60 ± 0.07
	TS (g/L)	26.5 ± 1.4	23.90 ± 1.33	23.15 ± 1.92	22.10 ± 1.15
	VS (g/L)	20.5 ± 1.3	18.05 ± 1.12	16.18 ± 1.06	16.33 ± 0.87
	TA[c] (mg/L)	1520 ± 8.5	1487 ± 8.0	1506 ± 9.2	1500 ± 8.7
	pH	6.0 ± 0.2	5.65 ± 0.22	5.77 ± 0.29	5.71 ± 0.18
BWG	COD (g/L)	N/A	1830 ± 21.21	1707 ± 24.75	1707 ± 24.75
	SCOD (g/L)	N/A	1790 ± 6.36	1678 ± 4.95	1678 ± 4.95
	TS (g/L)	N/A	488 ± 3.64	484 ± 1.06	484 ± 1.06
	VS (g/L)	N/A	426 ± 2.56	442 ± 5.65	442 ± 5.65
	TA[c] (mg/L)	N/A	9454 ± 11.5	9448 ± 9.3	9448 ± 9.3
	pH	N/A	8.39 ± 0.02	8.33±.035	8.33 ± 0.35

[a] Standard deviation.
[b] Not applicable.
[c] Total alkalinity (TA) represented as mg/L $CaCO_3$.

Table 2
Organic loading rate (OLR) at various increments.

Nominal COD loading (%)	OLR (kg VS/m^3 d)		OLR (kg COD/m^3 d)	
	Control	Test	Control	Test
100	1.03 ± 0.10[a]	1.03 ± 0.10	1.71 ± 0.20	1.71 ± 0.20
130	0.90 ± 0.01	1.03 ± 0.01	1.85 ± 0.06	2.34 ± 0.08
150	0.81 ± 0.05	1.04 ± 0.04	1.55 ± 0.06	2.38 ± 0.06
180	0.82 ± 0.04	1.18 ± 0.04	1.58 ± 0.06	2.88 ± 0.11

[a] Standard deviation.

pumped from the feed tank to the control digester to return its active volume of 1200 L. Then, feed tank and control digester feed lines were emptied and flushed with clean water. The volume of MWS in the grinder tank was determined, and a quantity of BWG was added to the grinder tank in order to achieve the required total COD target (130%, 150% or 180% of the control digester feed COD). After thorough mixing, the feed was transferred to the feeding tank. 60 L of digested sludge were drained from the test digester to its effluent tank prior to start feeding. Samples were collected from the feed and effluent tanks for subsequent analysis. The test digester feed line was then flushed with the MWS–BWG mixture and 60 L of the mixture were pumped to the test digester to return its active volume to the 1200 L level. Finally, the test digester feed line and all tanks were emptied and flushed with clean water.

2.4. Analytical methods

A Hewlett Packard 5890 series II gas chromatograph (GC) was used to measure the CH_4 and CO_2 contents in the biogas. The GC was equipped by a Hayesep Q column and a thermal conductivity detector (TCD). Total and soluble chemical oxygen demand (COD, SCOD) in influents and effluents were measured with the closed reflux (5220C) method using HACH DR/4000U spectrophotometer and Orion COD125 thermo reactor. Total solids and volatile solids were measured according to standard methods 2540C and 2540E, respectively. Total alkalinity (TA), partial alkalinity (PA) and pH were measured using Thermix stirrer 120S and ACCUMET AB15 Plus pH meter. The titration end point for partial alkalinity was pH 5.75 and that for total alkalinity was pH 4.30 using the 2320B titration method. All the above measurements were quantified according to the standard methods in triplicate (APHA, 2005). Volatile fatty acids (acetate, propionate, iso-butyrate, *n*-butyrate, iso-valerate and *n*-valerate) in the digester effluents were quantified by a Dionex, ICS-2500 with N_2 as the carrier gas equipped with a self-regenerating suppressor (CSRS® ultra II, 4 mm) and auto sampler AS50 with a 25 µl injection volume. 10 mN NaOH solution was used as the eluent at the ambient temperature with a flow rate of 1.2 ml/min. Samples used for the VFA analysis were all centrifuged at 3030 rpm for 5 min and filtered through a 0.22 µm sterile syringe driven filter (Millex®-GV). Sulfate (SO_4^{2-}) was measured with the Sulfa Ver 4 method using Sulfa Ver reagent and HACH DR/4000U spectrophotometer based on an internally developed method.

3. Results and discussions

The evaluation of reactor performance parameters was based on sampling performed during the final 10 days of each test digester loading period. Comparison of test digester to control digester performance was made on this basis. The measured daily influent and effluent COD concentrations for the control and test digesters throughout the investigation are shown in Fig. S-1 of the Supplementary data. The daily methane production during the final 10 days of each test digester loading period is shown in Fig. S-2 of the Supplemental data. Other measurements that were made daily during the final 10 days of each test digester loading period and presented in the Supplementary data are: the volatile solids concentrations in the influent and effluent of each digester (Fig. S-3); the total and partial alkalinity of each digester effluent (Fig. S-4); and the pH of each digester effluent (Fig. S-5).

3.1. Baseline operation

Baseline operation was conducted to achieve steady state in the two digesters and assess the equivalence of their performance. The mean values and standard deviations of the six parameters monitored during this stage are listed in Table 3. Paired two tailed *t*-tests performed on the data indicated that the parameter means were not significantly different for the two digesters as shown by the *p*-values given in Table 3. This indicates that an equivalent baseline performance level had been established in the digesters.

3.2. Reactor performance

Reactor performance was assessed in terms of COD and VS removal efficiencies and methane production.

3.2.1. COD removal efficiency

The COD removal efficiency is a measurement of organic waste stabilization. The % COD removals are shown in Fig. 2 as are the percentages of the test digester COD loadings due to BWG. At the baseline, the COD removal efficiencies of 61% and 59% were achieved in the control and test digesters, respectively. These

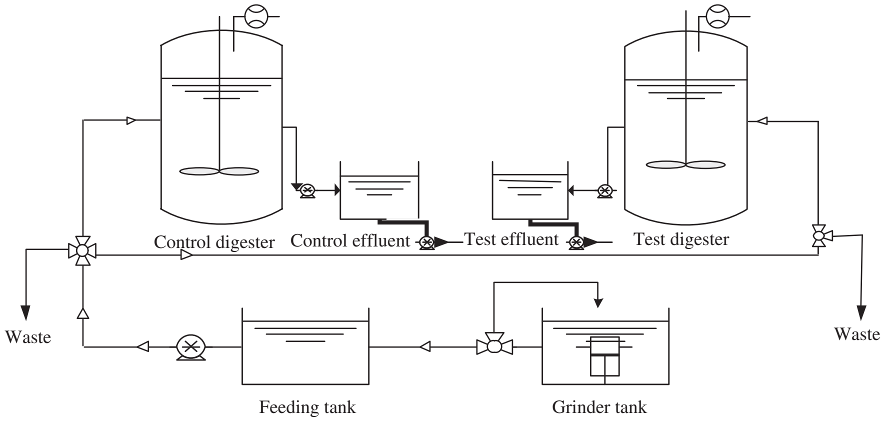

Fig. 1. Schematic of the pilot scale anaerobic digester setup.

Table 3
Comparison of digester performance during baseline operation (100%).

Parameter	Mean value ± standard deviation			*p*-Value
	Feed	Control effluent	Test effluent	
TCOD (g/L)	34.1 ± 4.5[a]	13.4 ± 2.7	14.1 ± 1.1	0.40
VS (g/L)	20.5 ± 1.3	10.8 ± 0.4	11.3 ± 0.4	0.44
Methane production (m^3/d)	N/A[b]	0.63 ± 0.1	0.58 ± 0.1	0.39
pH	6.0 ± 0.2	7.2 ± 0.1	7.2 ± 0.1	0.34
PA (mg CaCO$_3$/L)	N/A	2577 ± 92	2535 ± 85	0.27
TA (mg CaCO$_3$/L)	1520 ± 8.5	3656 ± 139	3599 ± 95	0.30

[a] Standard deviation.
[b] Not applicable.

values represent mean COD removal rates of 1.04 and 1.00 kg COD/(m^3 d) in control and test digesters, respectively. When the test digester COD loading was increased to 130% of the control COD loading its COD removal efficiency increased to 115% of that of the control (Fig. 2). The COD removal rates during this loading period were 1.22 and 1.88 kg COD/(m^3 d) in the control and test digesters, respectively. Taking into account the mean COD loadings of 1.85 and 2.34 kg COD/(m^3 d) to the control and test digesters, respectively, the COD loading that was not removed and would appear in the effluent was 0.63 and 0.46 kg COD/(m^3 d) for the control and test digesters respectively, indicating a superior quality of test digester effluent in terms of COD stabilization.

At the nominal 150% test digester COD loading relative to the control, COD from BWG represented 35% of its total COD loading (Fig. 2). The efficiency of COD removal in the test digester was 1.35 times that of the control digester at this loading. During this period, the COD removal rates in the control and test digesters were 0.93 and 1.69 kg COD/(m^3 d), respectively. The COD values in the control and test digester effluents were 0.62 and 0.69 kg COD/(m^3 d), respectively. These results indicate that the increased loading did not adversely affect the test digester effluent quality in terms of COD and used the digester treatment capacity more effectively. The observed improvement in the COD removal efficiency due to the BWG addition is similar to the results of previous studies. Astals et al. (2012) co-digested pig manure with crude glycerol under mesophilic conditions in a 4 L working-volume reactor. COD removal efficiency was reported to increase by 61% during the co-digestion of a mixture in which COD from BWG amounted to 65% of the total 3.56 kg COD/(m^3 d) loading.

A reduction in COD removal efficiency was noted when the test digester loading was increased to 180% of the control digester's COD loading. Under this condition, the COD due to BWG represented 46% of the test digester's total COD loading (Fig. 2). COD removal efficiency in the test digester declined to only 70% of the control digester's COD removal efficiency. Clearly this loading was not sustainable as it allowed 58% of the applied COD to be released in the digester effluent.

3.2.2. Volatile solids removal efficiency

Volatile solids removal is a major anaerobic digestion performance indicator as it relates to the mass of organic solids destroyed. VS removals as well as the percentages of total VS due to BWG in test digester feed are shown in Fig. 3. The VS removal efficiency in the control and test digesters was not significantly different at the baseline loading (100%) when they achieved average VS removals of 47% and 45%, respectively. These represent VS removal rates of 0.49 and 0.46 kg/(m^3 d) in the control and test digesters, respectively. During the 130% COD loading period when VS from BWG represented 13% of the total 1.03 kg VS/(m^3 d) test digester loading, its VS removal efficiency was 12% greater than that of the control, as shown in Fig. 3. Given the higher test digester VS loading, this corresponds to a 45% greater VS removal rate being achieved in the test digester compared to the control. During this period, the actual VS removal rates were 0.38 and 0.55 kg VS/(m^3 d) in the test and control, respectively. These loading and removal rates indicate that the amounts of VS loading that was not removed and would appear in the effluent were 0.52 and 0.48 kg VS/(m^3 d) for the control and test digesters, respectively. At the test digester loading of 150% COD, when VS from BWG accounted for 23% of the total 1.04 kg VS/(m^3 d) loading, the VS removal rates in the control and test digesters were 0.32 and 0.52 kg/(m^3 d), respectively. This corresponds to a 64% increase in VS removal rate in the test digester relative to the control. These loading and removal rates indicate that the amounts of VS loading that was not removed and would appear in the effluent were 0.49 and 0.52 kg VS/(m^3 d) for the control and test digesters, respectively. Improvement in VS removal due to the addition of waste glycerin is in agreement with previous studies. Astals et al. (2012) reported that BWG addition amounting to 67% to the total 1.9 kg VS/(m^3 d) loading increased the VS removal efficiency up to 107% compared to the digestion of pig manure alone.

Increasing the VS from BWG to 31% of the total 1.18 kg VS/(m^3 d) test digester loading resulted in its VS removal efficiency being approximately 30% lower than that of the control digester (Fig. 3). This indicates that a process upset had occurred in the test digester.

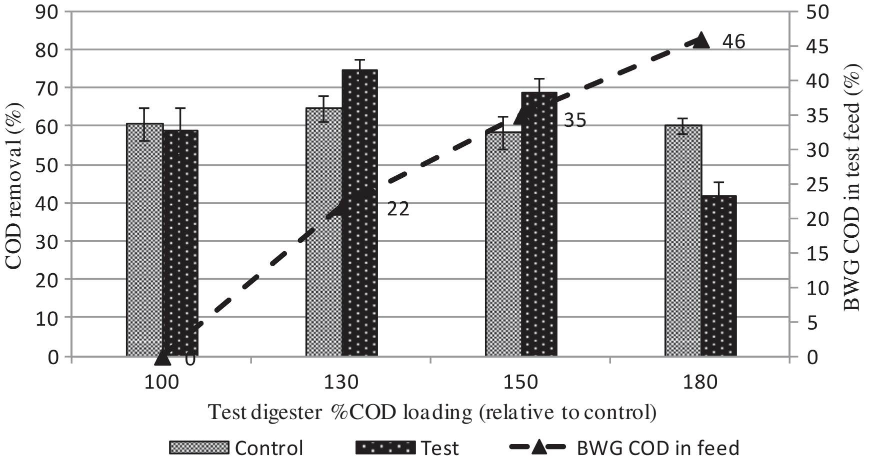

Fig. 2. COD removal efficiency and BWG COD percentage at various loadings.

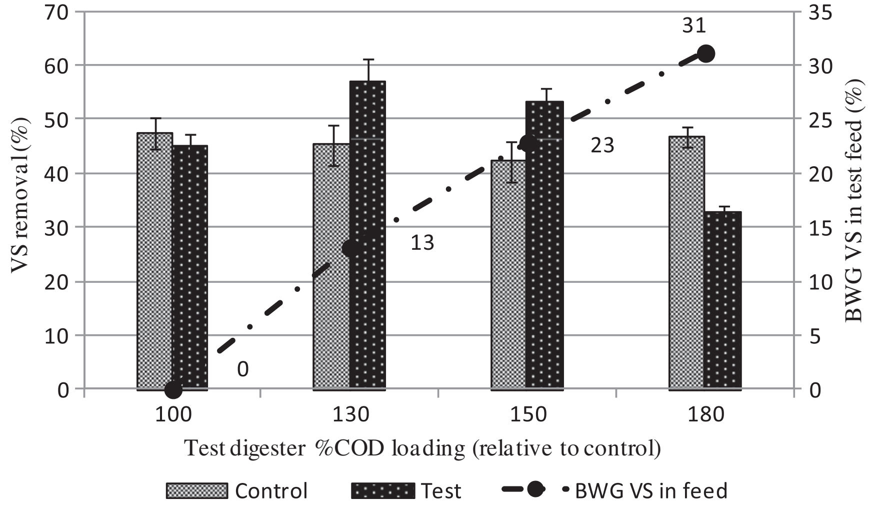

Fig. 3. VS removal efficiency and BWG VS percentage at various loadings.

3.2.3. Biogas and methane production rates

The gas production rate (GPR) and methane production rate (MPR) are two major performance indicators in the anaerobic process. The GPR and MPR, expressed as the volume of biogas or methane produced daily per unit reactor volume, are shown in Fig. 4. At the baseline, no statistically relevant difference was found between the mean GPR or MPR of the test and control digesters when only MWS was fed to both digesters (Table 3). Progressive addition of BWG to the test digester feed increased its GPR as well as MPR relative to the control (Fig. 4). The test digester GPR and MPR increased to 1.45 and 0.93 $m^3/(m^3\,d)$ at its 130% COD loading. These represented 39% and 48% increases in the GPR and MPR relative to the control digester, respectively. As the BWG addition was increased to the 150% COD level in test digester, its GPR and MPR were 65% and 83% greater than those of the control digester. The MPR values in the control and test digesters were 0.47 and 0.86 $m^3/(m^3\,d)$, respectively. Therefore, the addition of BWG enhanced the methane production by 0.39 $m^3/(m^3\,d)$. At this 150% loading, the test digester feed contained 1.1% (v/v) of BWG. Fountoulakis et al. (2010) reported that the addition of 1% (v/v) BWG to sewage sludge increased the MPR in their 3 L anaerobic digester from 0.16 to 0.4 $m^3/(m^3\,d)$. As shown in Fig. 4, when the test digester % COD loading was increased to 180% relative to the control, its GPR and MPR declined dramatically.

3.3. BWG loading and specific methane production

To express how effectively the BWG addition influences the methane production, the specific methane production (SMP) was determined in terms of COD and VS added as shown in Fig. 5. The SMP is defined here as the volume of methane produced daily per unit mass of COD or VS added to the reactor daily. There was no significant difference between control and test digester SMP at the 100% COD loading (Fig. 5). The SMP in terms of COD added to the test and control digesters were also not significantly different during the test digester nominal 130% and 150% COD loadings. However, the SMP in terms of VS added to each digester was 30% greater in the test digester relative to the control at the nominal 130% loading and 40% greater at the 150% loading. The composition of the biogas produced in the control digester remained relatively constant throughout the test period as shown in Table 4. The methane content of biogas produced in the test digester increased from approximately 58% at the 100% loading (no BWG) to a high of 66.5% at the nominal 150% test digester loading (Table 4). Dramatic declines in the test digester SMP values were observed during the 180% loading period with 48% and 34% decreases in the test digester SMP in terms of COD and VS added compared to the control (Fig. 5).

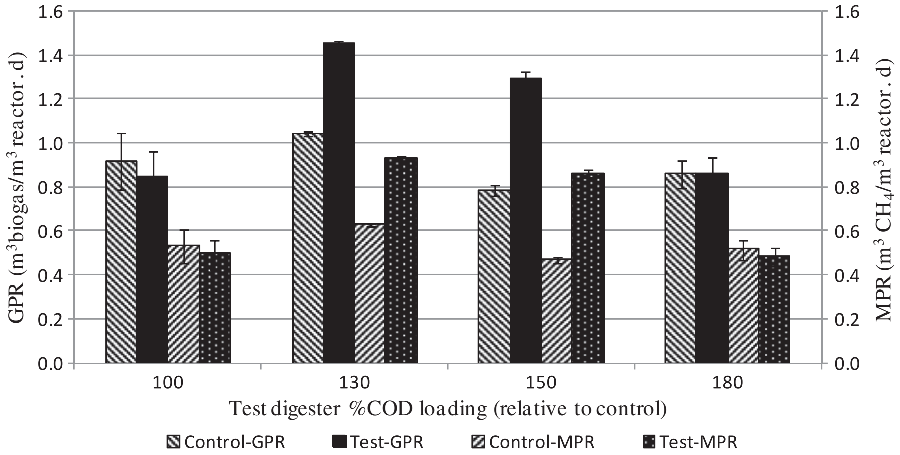

Fig. 4. Gas production rate (GPR) and methane production rate (MPR) at various loadings.

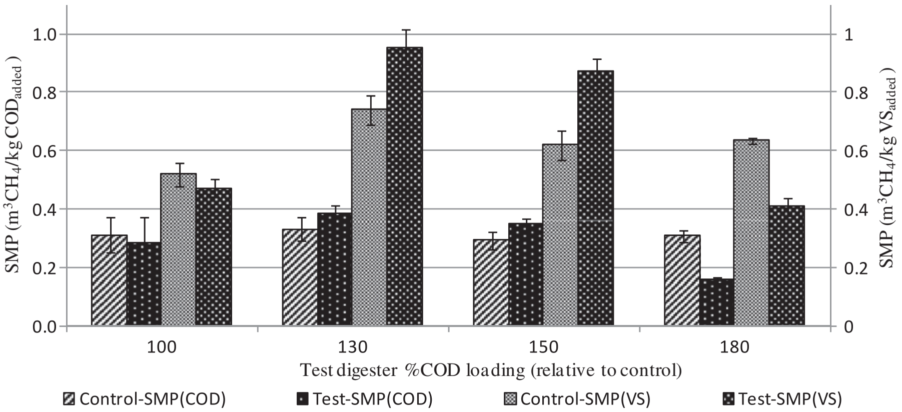

Fig. 5. Specific methane production (SMP) in terms of COD and VS added in various loadings.

Enhancements in biogas and methane production by addition of BWG to organic wastes have been reported by a number of researchers (Castrillon et al., 2011; Fountoulakis et al., 2010; Ma et al., 2008; Robra et al., 2010; Tokumoto and Tanaka, 2012). The improvement in methane production was linked to the increase in the overall degradation of the feed organics (in terms of VS) as solubilization of BWG is significantly higher than that of the MWS (Table 1) and the higher methane potential of BWG.

3.4. Process stability

The process stability was determined through the investigation of sensitive parameters such as pH, partial and total alkalinity, volatile fatty acids (VFA) and SO_4^{2-}. The mean value and standard deviation of the effluents from both reactors are presented in Table 4. The pH value as an indicator of acid–base balance in effluents remained in the optimum range of 6.6–7.8 as a stabilized anaerobic process (Ferrer et al., 2010). During co-digestion, PA and TA in the test digester were always lower than those of in the control digester and the VFA concentrations were generally greater in the test. The VFA/alkalinity ratio remained below 0.03; 10 times lower than the maximum safe values of 0.3 reported by Siles et al. (2010). The ratio of intermediate alkalinity (IA) to partial alkalinity (PA) was reported by Astals et al. (2012) and Ferrer et al. (2010) as a highly sensitive parameter to assess the stability of an anaerobic process. This ratio should remain below 0.4 for stable operation. The IA/PA ratio did not exceed 0.4 in either of digesters until it reached 0.45 in the test digester at the 180% COD nominal loading. A reduction in alkalinity is typically caused by an increase in VFA and CO_2 generation. During the process, VFA and % CO_2 in both digesters remained at acceptable levels but increases were observed at the 180% loading in test (Table 4). These results suggest that a large reduction in PA (below 2500 mg $CaCO_3$/L) in the test digester beginning from the period of 180% loading resulted a considerable decline in the test digester's buffering capacity where the system was exhibiting signs of instability.

It is well-known that the anaerobic digestion process involves interactions and syntrophy among the several groups of bacteria and archaea. Syntrophy and competition between sulfur reducing bacteria (SRB) and methane producing bacteria (MPB) in the reactors is presented in terms of a COD/SO_4^{2-} ratio in Table 4. The predominance of either of SRB or MPB depends on a combination of factors involved in the anaerobic process. At the COD/SO_4^{2-} ratio of 2, while the MPB prevail over the SRB in acetate degradation, the SRB are more dominant in H_2 utilization (O'Reilly and Colleran, 2006). During co-digestion, the COD/SO_4^{2-} ratios were above approximately 4 in both the test and control digesters. O'Reilly and Colleran (2006) also indicated that at a COD/SO_4^{2-} ratio of 4 and above, the MPB are the main population involved in acetate degradation and H_2 utilization. Thus, the MPB were not hampered

Table 4
Characteristics of reactor effluents and emissions.

Parameter		Nominal COD loading (%)			
		100	130	150	180
Control	pH	7.22 ± 0.06[c]	7.28 ± 0.07	7.18 ± 0.03	7.21 ± 0.01
	PA[a] (mg/L)	2577 ± 79	2693 ± 144	2436 ± 98	2372 ± 38
	TA (mg/L)	3656 ± 139	3747 ± 176	3377 ± 135	3256 ± 38
	VFA[b] (mg/L)	9.01 ± 1.29	7.30 ± 1.56	3.48 ± 1.66	5.78 ± N/A[d]
	COD/SO_4^{2-}	5.98 ± N/A	4.72 ± N/A	4.73 ± N/A	3.98 ± N/A
	$\%CH_4$	58 ± 0.75	60 ± 0.84	60 ± 0.74	60 ± 0.63
	$\%CO_2$	42 ± 0.89	40 ± 0.92	39 ± 0.10	39 ± 0.80
Test	pH	7.20 ± 0.07	7.25 ± 0.07	7.18 ± 0.05	7.09 ± 0.05
	PA (mg/L)	2534 ± 85	2492 ± 94	2305 ± 134	2041 ± 123
	TA (mg/L)	3599 ± 95	3522 ± 116	3155 ± 203	2970 ± 150
	VFA (mg/L)	16.03 ± 4.84	4.85 ± 2.9	42.1 ± 8.12	91.09 ± N/A
	COD/SO_4^{2-}	6.59 ± N/A	4.98 ± N/A	5.20 ± N/A	6.29 ± N/A
	$\%CH_4$	58 ± 0.77	64 ± 1.17	66.5 ± 2.02	56 ± 1.68
	$\%CO_2$	42 ± 0.66	36 ± 0.83	33 ± 1.55	43 ± 1.69

[a] Partial alkalinity (PA) and total alkalinity (TA) represented as mg/L $CaCO_3$.
[b] Volatile fatty acids (VFA) represented as mg/L acetic acid.
[c] Standard deviation.
[d] Not available.

by SRB predominance during the process as the COD/SO_4^{2-} ratio were always above 4 in the test.

The accelerated increase in VFA concentration in the test digester and decrease in the biogas CH_4 content suggest that methanogens inhibition occurred at the 180% COD loading. Consequently, as the proportion of the BWG in the feed was increased and reached 1.8% (v/v) of the feed, a reduction in methane production was observed. In a similar study, Fountoulakis et al. (2010) reported that adding 3% (v/v) BWG to sewage sludge resulted in VFA accumulation and process instability. Robra et al. (2010) proposed another scenario for the methanogens inhibition due to the addition of BWG to the cattle slurry in a 4 L CSTR digester at mesophilic conditions. They observed that increasing the addition of BWG from 5% to 10% (wt) in the feed, no significant improvement in biogas production was achieved, although a greater amount of BWG had been fed. This observation was attributed to the high concentrations of methanol and KOH in their BWG which inhibited biocenosis and caused process instability.

3.5. Maximum safe loading rate

The maximum safe loading limit for BWG during co-digestion depends on the characteristics of the primary substrate and the BWG itself. The limit established from the present research, as shown in Figs. 2–5, was found at the 150% nominal COD loading where the COD due to BWG was 35% of the 2.38 kg COD/(m^3 d) loading and the VS from BWG was 23% of the 1.04 kg VS/(m^3 d) loading. This amount of BWG represented 1.1% (v/v) of the feed material.

4. Conclusions

Co-digestion of BWG and MWS at the maximum feasible OLR of 1.1% BWG (v/v) increased the GPR and MPR in test by 65% and 83% compared to the control digester, respectively. At this loading, the test digester COD and VS removal rates were 82% and 63%, greater than those in the control digester, respectively. A considerable decline was observed in the test digester methane production, COD and VS removals when the proportion of COD and VS due to BWG in its feed was increased to 46% and 31% of the 2.88 kg COD/(m^3 d) and 1.18 kg VS/(m^3 d) loadings, respectively.

Acknowledgements

Funding for this project was provided by the Water Environment Research Foundation through a Grant to Camp Dresser & McKee Inc., and a Grant from EPCOR Water Services Inc., and a matching CRD Grant from the Natural Sciences and Engineering Research Council of Canada. The authors wish to thank Ryan Litwinow, Radek Roznicki and Richard Adjei of the Edmonton Waste Management Centre of Excellence; Daryl Seehagel of EPCOR Water Services, Inc.; David Parry and Scott Vandenburgh of CDM, Bob Bucher of the West Point treatment plant in King County WA, and the operations staff of the Gold Bar wastewater treatment plant for their invaluable assistance throughout the project.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2013.01.101.

References

Amon, T., Amon, B., Kryvoruchko, V., Bodiroza, V., Patsch, E., Zollitsch, W., 2006. Optimising methane yield from anaerobic digestion of manure: effects of dairy systems and of glycerine supplementation. Int. Cong. Ser. 1293, 217–220.

APHA, 2005. Standard Methods for the examination of Water and Wastewater, 21st ed. American Public Health Association, Washington, DC, USA.

Astals, S., Nolla-Ardavol, V., Mata-Alvarez, J., 2012. Anaerobic co-digestion of pig manure and crude glycerol at mesophilic conditions: biogas and digestate. Bioresour. Technol. 110, 63–70.

Ayoub, M., Abdullah, A.Z., 2012. Critical review on the current scenario and significance of crude glycerol resulting from biodiesel industry towards more sustainable renewable energy industry. Renewable Sustainable Energy Rev. 16, 2671–2686.

Castrillon, L., Fernandez-Nava, Y., Ormaechea, P., Maranon, E., 2011. Optimization of biogas production from cattle manure by pre-treatment with ultrasound and co-digestion with crude glycerin. Bioresour. Technol. 102, 7845–7849.

Chi, Z., Pyle, D., Wen, Z., Frear, C., Chen, S., 2007. A laboratory study of producing docosahexaenoic acid from biodiesel-waste glycerol by microalgal fermentation. Process Biochem. 42, 1537–1545.

Ferrer, I., Vázquez, F., Font, X., 2010. Long term operation of a thermophilic anaerobic reactor: process stability and efficiency at decreasing sludge retention time. Bioresour. Technol. 101, 2972–2980.

Fountoulakis, M.S., Petousi, I., Manios, T., 2010. Co-digestion of sewage sludge with glycerol to boost biogas production. Waste Manage. 30, 1849–1853.

Gu, Y., Jerome, F., 2010. Glycerol as a sustainable solvent for green chemistry. Green Chem. 12, 1127–1138.

Holm-Nielsen, J.B., Lomborg, C.J., Oleskowicz-Popiel, P., Esbensen, K.H., 2008. On-line near infrared monitoring of glycerol-boosted anaerobic digestion processes: evaluation of process analytical technologies. Biotechnol. Bioeng. 99, 302–313.

Ma, J., Van Wambeke, M., Carballa, M., Verstraete, W., 2008. Improvement of the anaerobic treatment of potato processing wastewater in a UASB reactor by co-digestion with glycerol. Biotechnol. Lett. 30, 861–867.

O'Reilly, C., Colleran, E., 2006. Effect of influent COD/SO4(2−) ratios on mesophilic anaerobic reactor biomass populations: physico-chemical and microbiological properties. FEMS Microbiol. Ecol. 56, 141–153.

Robra, S., Serpa da Cruz, R., de Oliveira, A.M., Neto, J.A.A., Santos, J.V., 2010. Generation of biogas using crude glycerin from biodiesel production as a supplement to cattle slurry. Biomass Bioenergy 34, 1330–1335.

Schwarzenbeck, N., Bomball, E., Pfeiffer, W., 2008. Can a wastewater treatment plant be a powerplant? A case study. Water Sci. Technol. 57, 1555–1561.

Siles, J.A., Martan, M.A., Chica, A.F., Martan, A., 2010. Anaerobic co-digestion of glycerol and wastewater derived from biodiesel manufacturing. Bioresour. Technol. 101, 6315–6321.

Slinn, M., Kendall, K., Mallon, C., Andrews, J., 2008. Steam reforming of biodiesel by-product to make renewable hydrogen. Bioresour. Technol. 99, 5851–5858.

Tang, B., Yu, L., Huang, S., Luo, J., Zhuo, Y., 2010. Energy efficiency of pre-treating excess sewage sludge with microwave irradiation. Bioresour. Technol. 101, 5092–5097.

Tokumoto, H., Tanaka, M., 2012. Novel anaerobic digestion induced by bacterial components for value-added byproducts from high-loading glycerol. Bioresour. Technol. 107, 327–332.

Yazdani, S.S., Gonzalez, R., 2007. Anaerobic fermentation of glycerol: a path to economic viability for the biofuels industry. Curr. Opin. Biotechnol. 18, 213–219.