

Semi-continuous mesophilic anaerobic co-digestion of thermally pretreated scum

Bradley Young, Kevin Kennedy, Robert Delatolla and Ranya Sherif

ABSTRACT

The objective of this study is to investigate the mesophilic, anaerobic, co-digestion of scum with thickened waste activated sludge (TWAS) and primary sludge (PS). Observations of the reactor waste show that higher pretreatment temperatures assist in scum integration and appear to limit coagulation and separation effects associated with digesting fats, oils and greases. The 0.40 kg VS/m³ · d scum loaded reactor with scum pretreated at 70 °C achieved the greatest improvement in biogas production over PS and TWAS co-digestion without scum addition. Based on cell viability analysis of the reactor waste, scum-containing reactors were shown to produce 48 and 39% more biogas per m³ of live cells; signifying that scum addition increased the activity of the microbial population embedded in the waste or caused a microbial shift in the waste towards more active communities.

Key words | anaerobic co-digestion, cell viability analysis, fats, oils and grease (FOG), mesophilic, scum

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INTRODUCTION

Scum is the mass of sewage solids, buoyed up by entrained gas, grease, or other substances that float on the surface of primary clarification units in municipal water resource recovery facilities (MWRRFs), with scum accounting for approximately 4% of the total solids (TS) collected in the entire MWRRF (Outwater 1994). Scum is often collected in troughs at the end of the primary clarification basin, and is currently disposed of by landfilling, incineration or anaerobic digestion. In order to benefit from reuse and the production of biogas, MWRRFs have been anaerobically co-digesting scum. Prior to pumping scum to the anaerobic digestion facility, it is often heated for a set holding time (HT) in a scum concentrator to facilitate transport to the anaerobic digester. The effects of varying scum concentrator temperature and HT operations, and the concomitant effect on biogas generation when scum is anaerobically co-digested with primary and secondary municipal sludge, is not well documented in the literature. Cortell (2008) studied the effects of mesophilic anaerobic

co-digestion of various combinations of fats, oils and grease (FOG) from restaurants with municipal sludge, and demonstrated a 50% increase in biogas production at a volumetric organic loading rate of 0.48 kg VS/m³ · d. Kabouris *et al.* (2008) reported a 100% increase in biogas production when restaurant FOG was mesophilically digested with primary sludge (PS) and thickened waste activated sludge (TWAS) at a 10% increase in volatile solids (VS) loading due to FOG. However, in both of the above studies no heating or other pretreatment of the FOG was described, and the non-pretreated FOG was directly digested as produced.

Heating has been shown to increase the solubility of the various components in FOG and scum, making them more readily available for digestion or in some cases increasing the concentration of components such as long chain fatty acids (LCFAs), which can be acutely inhibitory to methanogenesis (Parkin & Owen 1986; Salminen & Rintala 2002; Gomec 2006). LCFAs have low solubility and

require proper digester mixing to prevent the reformation of surface floating scum in the digester and non-mobile sedimentation (Lyberatos & Skiadas 1999). Methanogenic consortia have also been observed to clump if excess scum is trapped within the system, which inhibits hydrogenotrophic methanogenesis and leads to high hydrogen partial pressures and elevated levels of H_2 in the mixed liquor. If this condition persists it can slow volatile fatty acid (VFA) oxidation leading to accumulation of butyric and propionic acids, which results in acidic reactor conditions and further exacerbates the inhibition of methane production by acetoclastic methanogens as well as physical reactor fouling (Gomec & Speece 2003; Speece 2008; Long *et al.* 2012). Elevated concentrations of organic constituents have also been reported to increase ammonia concentrations within the mixed liquor, which can become inhibitory above 3,000 mg/L NH_3-N during mesophilic anaerobic digestion (Benabdallah *et al.* 2009). Young *et al.* (2013) performed batch biological methane potential (BMP) assays on pretreated scum co-digested in a tri-mixture with TWAS and PS where the pretreatment method was conventional heating at different HTs. Optimizing the pretreatment parameters and scum load showed a maximum increase in gas yield that was 1.6 times greater than the gas yield of the PS and TWAS system per gram of VS added. Interestingly, the authors concluded that the major factor contributing to an increase in biogas yield was the concentration of the scum loading, with lower relative VS loadings of scum demonstrating the greatest biogas yield. Similarly, Kabouris *et al.* (2008) and Wan *et al.* (2011) showed that a high loading of scum potentially increases the LCFA concentration to inhibitory levels and hence reduces the biogas yield. Furthermore, the scum pretreatment temperature has been shown to potentially effect the mixability of the scum in the digester and thus the rate of biogas production (Young *et al.* 2013).

The objective of this study is to quantify biogas production, cell viability of the sludge at various scum loadings, and pretreatment temperatures along with the overall performance of mesophilic anaerobic biodegradation of real municipal scum co-digested with real municipal sludge (PS and TWAS). Specifically, the effects of initial scum loading with municipal sludge and the temperature of pretreatment in the scum concentrator are

investigated using semi-continuous mesophilic biological reactors operated according to a conventional organic loading rate ($1.6 \text{ kg VS/m}^3 \cdot \text{d}$) and conventional hydraulic retention times (15 and 20 days).

METHODS

Waste samples

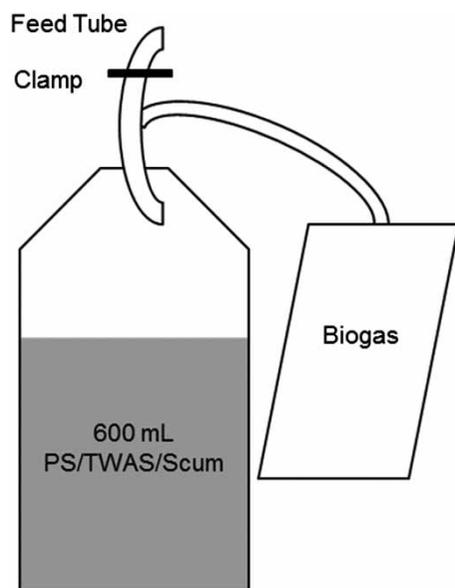
The municipal sludge and scum used in this study was sampled from the Robert O. Pickard Environmental Centre (ROPEC) MWRRF, Ottawa, Ontario, Canada. The facility is an activated sludge, secondary treatment plant that operates at a solids retention time (SRT) that is maintained between 5 and 7 days. The solids management plan consists of four circular mesophilic pancake digesters with gas tube mixing, and two mesophilic cylindrical pseudo egg shaped anaerobic digesters that are mixed hydraulically. Digesters are loaded with a sludge mixture composed of 58% TWAS and 42% PS by volume operating at 35°C and an SRT of 20 days. Active anaerobic digester effluent (inoculum), TWAS, PS, and scum were sampled from the ROPEC MWRRF and stored at 4°C for a maximum of 8 weeks to prevent unwanted and unquantifiable digestion. Samples of scum were collected in large quantities from the beach section of the primary clarifier and were well mixed to alleviate the variability of scum composition (Metcalf & Eddy 2003). The samples were characterized for TS, VS, soluble chemical oxygen demand (SCOD), and total chemical oxygen demand (TCOD) as shown in Table 1.

Laboratory reactors

Semi-continuous 1 L glass Kimax flask reactors with an active volume of 600 mL (Figure 1) were loaded at $1.6 \text{ kg VS/m}^3 \cdot \text{d}$ with varying fractions of TWAS, PS, and scum (Table 2). TWAS and PS fractions were added to the control reactors (PS/TWAS only, no scum added) and the scum loaded reactors at a ratio of 58:42 throughout the entire study. In the case of the scum loaded reactors, a percentage of the PS/TWAS mass measured as VS was simply replaced by scum to maintain a consistent loading rate for all

Table 1 | Characterization of raw waste and thermally pretreated scum ($N = 5$)

Waste	TCOD (mg/L)	SCOD (mg/L)	TS (%)	VS (%)
Inoculum	21,900 ± 3,400	1,110 ± 244	3.0 ± 0.0	1.7 ± 0.0
PS	45,200 ± 3,800	3,090 ± 83	5.5 ± 0.0	4.2 ± 0.1
TWAS	56,200 ± 5,220	2,690 ± 116	5.2 ± 0.2	4.1 ± 0.0
50 °C scum	89,200 ± 8,680	1,390 ± 23	50.7 ± 3.5	48.3 ± 3.5
70 °C scum	134,000 ± 14,200	3,420 ± 49	53.9 ± 1.2	50.9 ± 0.9

**Figure 1** | Schematic of reactors.

reactors. The HT in the scum concentrator, the scum loading, temperatures of the scum in the concentrator and the HRT values used in the study were based on the operational capabilities of conventional treatment plants, ROPEC and the work conducted by Young et al. (2013).

Reactor operation was configured according to two full 2^2 factorial designs at HRTs of 15 and 20 days, with factors being scum loading and temperature in the scum concentrator. A HT value of 48 hours in the scum concentrator and a loading rate of 1.6 kg VS/m³ · d were used for all of the semi-continuous reactors in this study. The scum load applied to the reactors was either a low value of 0.40 gVS/L (25% by mass) or a high value of 0.72 gVS/L (45% by mass). Prior to loading, the scum was either maintained at a temperature of 50 or 70 °C throughout the 48 h HT. The notation used in this study to identify the reactors and the feed mixtures

Table 2 | Semi-continuous reactor configuration and loading

Label	Reactor configuration	Loading rate (kgVS/m ³ · d)
R_{cont}	T:P (0.56:0.41 gVS)	1.6
RL,50	T:P:S (0.42:0.30:0.24 gVS)	1.6
RL,70	T:P:S (0.42:0.30:0.24 gVS)	1.6
RH,50	T:P:S (0.31:0.22:0.43 gVS)	1.6
RH,70	T:P:S (0.31:0.22:0.43 gVS)	1.6

T = TWAS, P = PS, S = scum, L = low scum fraction (0.40 kg · scum · VS/m³ · d), H = high scum fraction (0.72 kg · scum · VS/m³ · d), 50 = temperature of scum concentrator (50 °C), 70 = temperature of scum concentrator (70 °C).

applied to each reactor is shown in Table 2. All selected operating values of the reactors are based on conventional activated sludge systems and thus of potential application for current and future activated sludge MWRRFs.

The reactors were started by adding 600 mL of methanogenic inoculum to each reactor and flushing the reactor with nitrogen gas for 1 minute prior to being sealed with a modified rubber septum and secured with a plastic cap. A 3/8 inch inner diameter glass tube and 1/8 inch inner diameter syringe were inserted through the rubber septum and sealed using silicone sealant. A 2 L Teflon bag was used to collect biogas during digestion. All reactors were contained in a hot room maintained at 35 °C and continuously mixed using an orbital shaker set at 115 rpm throughout the experimental phase.

The reactors were fed daily (once/day) by extracting 40 mL (1/15th of the active volume) and 30 mL (1/20th of the active volume) of waste through the rubber tube for HRTs of 15 and 20 days, respectively. Subsequently, the TWAS, PS, and scum mixtures were added to the reactors in accordance with the ratio of wastes shown in Table 2 to achieve the desired loading rate of 1.6 kg VS/m³ · d in all reactors. During the start-up phase, all reactors were monitored for biogas production, TS, VS and pH. Conditions

were considered steady state when daily biogas production was within $\pm 5\%$ daily fluctuation.

Analytical methods

pH was measured using an XL25 dual channel pH/ion meter equipped with a glass electrode (Fisher Accumet, MA, USA). TS and VS were measured by standard method 2540 B & E (APHA 2005). TCOD and SCOD were measured using TNT 823 and TNT 822 test vials, respectively (HACH, CO, USA). Ammonia was measured using TNT 832 test vials (HACH, CO, USA). SCOD and ammonia samples were centrifuged at 10,000 rpm (RCF of 11,292) for 30 minutes, filtered using a 0.45 μm filter and analyzed using a DR 5000 spectrophotometer (HACH, CO, USA). Alkalinity was measured using standard method 2320 B (APHA 2005). Volatile acids (VAs) were measured by standard titration as outlined by Speece (2008). Biogas production was measured daily by pumping gas that was collected in Teflon bags into a manometer.

Cell viability method

Cell viability analysis was performed using the live/dead viability/cytotoxicity kit L-7013 (Life Technologies, CA, USA) in combination with fluorescent microscopy. The viability kit is comprised of two stains, the first being SYTO 9, a green fluorescent nucleic acid stain that is highly membrane-permeant and the second stain, propidium iodide (PI), being a cell membrane-impermeable stain. A 1 mL sample of effluent from each reactor was centrifuged twice at $250 \times g$ for 10 minutes, with the supernatant being discarded and sterilized water used to resuspend the sample. One hundred and fifty μL of 0.2% dye solution was added to each sample and kept in contact with the sample for 15 minutes. Each sample was centrifuged at $250 \times g$ for 5 seconds to pellet the cells and allow for the supernatant to be discarded. Finally, the cells were re-suspended in 150 μL fresh Hanks' balanced salt solution (HBSS) washing solution and 10 μL of each sample was pipetted onto glass slides and covered. Immediately after covering the glass slide, the cells were observed with a LSM 510/AxiomImager fluorescent microscope (Zeiss, VA, USA). A $\times 63$ oil immersion objective was used to observe the cells, and 20 images

were acquired for analysis per sample. Cell viability analytical quantification of the acquired microscopic images was performed using a Nikon NI Vision Assistant using color threshold analysis according to Delatolla *et al.* (2009).

Statistical methods

All statistical models and results were determined using Minitab 15 statistical software. The design of the experiment was conducted as two 2^2 factorial designs. Analysis of variance was used to determine the statistical significance and interactions of each factor, where a p -value of less than 0.05 is interpreted in this study to be significant for all biogas production and biodegradability measurements.

RESULTS AND DISCUSSION

Reactor start-up

During the start-up and the following steady phase of operation, the loading rates of the reactors were maintained at $1.6 \text{ kg VS/m}^3 \cdot \text{d}$ (Figure 2). All reactors demonstrated a linear increase in biogas production until approximately one full HRT was achieved. After one HRT the biogas production began to stabilize and a pseudo steady state phase is observed until approximately two to three HRTs. After a total of two to three HRTs, steady state conditions were verified in the reactors as the variance of the daily biogas production of the reactors was observed to be $< 5\%$ of the mean on a daily basis (Figure 2). The reactors were operated and characterized at steady conditions for a minimum of an additional HRT. Additionally, at steady state operation, the biogas composition did not significantly change in the reactors and maintained a constant methane fraction of 59–61%.

Characterization of scum loaded reactors

The primary objective of anaerobic digestion is to stabilize sludge for economical and environmentally conscious disposal (Appels *et al.* 2008). Due to the high organic concentration and variable nature of scum, it is paramount to understand the effects of digestion and waste stabilization for scum loaded reactors. Throughout the experimental

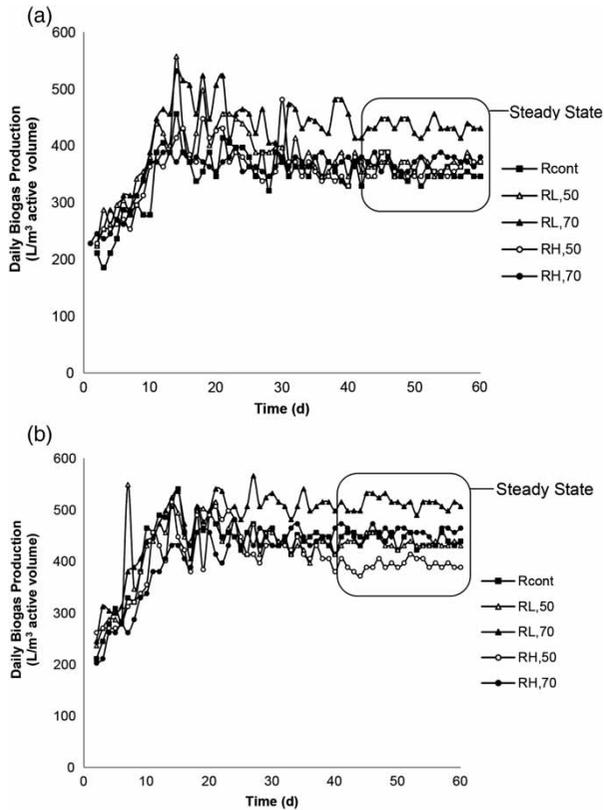


Figure 2 | Daily biogas production of start-up and steady conditions, (a) 15 day HRT, (b) 20 day HRT.

phase, all reactor effluent was measured for pH, VS, TS, TCOD, SCOD, total alkalinity, VA, and $\text{NH}_3\text{-N}$ to monitor the sludge and scum stabilization process as well as the overall health of the anaerobic digestion system.

Stabilization of the sludge with respect to VS destruction showed the scum loaded reactors were all statistically equal to the control (R_{cont}) with an average VS destruction of $60 \pm 3\%$ and $61 \pm 3\%$ for 15 and 20 day HRT, respectively (Figure 3). This signifies that the addition of up to $0.72 \text{ kg VS/m}^3 \cdot \text{d}$ scum did not negatively affect the volatile stabilization of sludge and the organic compounds were adequately digested in the system. Moreover, the VS destruction observed in the lab scale reactors is comparable to the data measured at the full scale ROPEC plant, $57 \pm 1\%$, where the inoculum, PS and TWAS was sampled for this study.

The VA/total alkalinity ratio in well operated digesters is between 0.1 and 0.35, with lower ratios being considered optimal (Balaguer *et al.* 1992; Siles *et al.* 2010). The VA/total alkalinity ratios for all reactors in this study were constant at

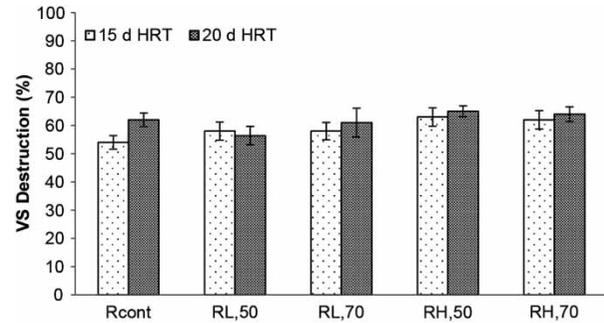


Figure 3 | Average and 95% confidence intervals of VS destruction at steady HRTs of 15 and 20 days.

0.13 ± 0.01 , indicating the scum containing reactors were not at an increased risk of acidification. Additionally, monitoring pH, VA concentration, $\text{NH}_3\text{-N}$, SCOD, TCOD and total alkalinity at both 15 and 20 day HRTs at steady state operation shows that all reactors were operated below inhibitory values and concentrations for acidogenesis and methanogenesis (Chen *et al.* 2008) (Figure 4). Gomez (2006) concluded that pH control is necessary when digesting scum in a continuous system due to rapid hydrolysis and

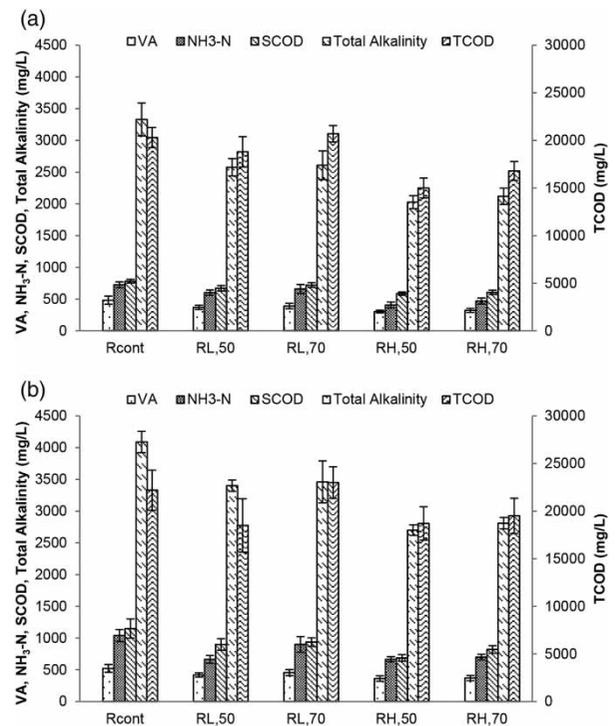


Figure 4 | Average and 95% confidence intervals of steady state effluent concentrations and values at (a) 15 day HRT and (b) 20 day HRT.

acidogenesis of the scum component. Lay *et al.* (1997) showed that the digestion of scum decreased the pH of their system to levels below 5 without the addition of alkalinity, which is significantly below the optimal pH range of 6.7–7.3 for acidogenesis and methanogenesis to occur simultaneously. In the herein presented study, RH, 50 and RH, 70 contained the highest scum fraction and the pH of these reactors was maintained at an average of 7.0 and 7.1 at HRTs of 15 and 20 days, respectively, without additional alkalinity. It is likely the buffering capacity of the TWAS and PS mixture helped maintain pH values within the optimal range for anaerobic digestion, however it should be noted that additional scum is likely to decrease the pH further as the replacement of TWAS and PS with scum will increase the rate of hydrolysis and acidogenesis, while also decreasing the natural buffering capacity. In accordance with the pH measurements, the total alkalinity was lowest in the highest scum loaded RH reactors, supporting the significance of not overloading a continuous system with scum.

TCOD, SCOD, $\text{NH}_3\text{-N}$, and VA were in the expected range of a MWRRF anaerobic digestion facility and are comparable to the full scale ROPEC plant operation. All reactor values demonstrated that the laboratory scale anaerobic reactors were operated under comparable conditions to the ROPEC full scale plant and were maintained in a healthy state throughout the study. This suggests that scum loading in accordance to the operating conditions of this study would not adversely affect current solid waste management practiced by MWRRFs.

Biogas production

At steady state operation, the biogas production for R_{cont} and all scum containing reactors was measured to determine the effects of scum loading as well as conventional thermal pretreatment. Operating at a 15 day HRT, RL, 70 produced the highest daily biogas production, a 24% improvement over R_{cont} (Figure 5). RL,50 and RH,70 produced slight, but significant improvements in biogas production over R_{cont} while the biogas production of RH,50 was not statistically different than R_{cont} . At a 20 day HRT operation, RL, 70 produced the greatest average daily biogas production, with a 16% improvement over R_{cont} (Figure 5). RH, 70 produced a

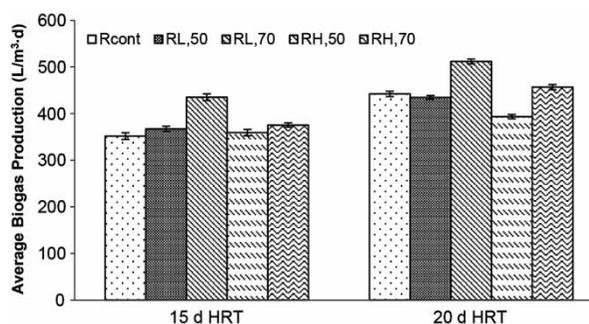


Figure 5 | Average and 95% confidence intervals of daily biogas production at steady state.

slight yet significant increase in biogas production, whereas the biogas produced by RL,50 was statistically equal to R_{cont} , and RH, 50 produced statistically less daily biogas than R_{cont} . Similar to the 15 day HRT operation, reactors fed with scum from the 70 °C concentrator produced the largest increase in biogas production.

Increased biogas yields in RL, 70 and RH, 70 may be attributed to an increased SCOD load provided by the heated scum fraction. Conventional thermal heating at 70 as compared to 50 °C increased the SCOD load from $1,390 \pm 23$ to $3,420 \pm 49$ mg/L (Table 1), providing a more readily accessible substrate for digestion. The higher heat also appeared to allow the scum to be more workable and easily entrained within the anaerobic system thereby increasing the quantity of the scum available for microbial degradation. In congruence with the added biogas production, the ability of the scum to become more entrained decreased the rate at which the scum blanket formed in the anaerobic digester, allowing for longer operation between cleaning cycles.

Observations of the reactors loaded at low scum loadings of $0.40 \text{ kg} \cdot \text{VS} \cdot \text{scum} / \text{m}^3 \cdot \text{d}$ demonstrate that the scum remains well mixed within the system, whereas a higher scum loading of $0.72 \text{ kg} \cdot \text{VS} \cdot \text{scum} / \text{m}^3 \cdot \text{d}$ showed scum separating from the mixed liquor and remaining at the top of the reactors for both 15 and 20 day HRT operation. This phenomenon separated portions of the scum from the active volume of the reactor and hindered the digestion processes. Analysis of the VS destruction and average daily biogas production data corroborate the visible observations. The VS stabilization data show that VS destruction does not correlate to the biogas production for

the scums-containing reactors, as the reactors with the highest biogas production do not correspond to the reactors with the greatest VS destruction. Particularly, the least biogas produced per VS consumed occurred in the high scum containing reactors, RH. This phenomenon may be a combined effect of the COD/VS ratio and the sampling method used to collect the waste from the reactors.

The TCOD:VS ratio is lower in scum as compared to PS and TWAS (Table 1). Therefore, normalizing the loading rate on a VS basis provided less TCOD in the scum containing reactors. It is possible the benefits of incorporating scum in the RL reactors are negated in the RH reactors by a greater reduction in TCOD; thus explaining the decrease in VS without a significant increase in biogas production. Additionally, samples collected for VS measurements were extracted from the centroid of the reactors after vigorous mixing. However, scum remained partially separated from the waste in the high scum loaded reactors (congealed and floating at the top of the reactors) and as such was not sampled and thus not included in the VS measurements. Since scum is highly organic, its absence in the VS sample may account for the higher VS destruction measurements. At full scale operation, it is likely the coagulated floating mass observed at high scum loading rates may also generate a scum blanket and become inhibitory to the system, and ultimately require additional cleaning (Massart *et al.* 2008). Although a lower loading rate of $0.40 \text{ kg} \cdot \text{VS} \cdot \text{scum} / \text{m}^3 \cdot \text{d}$ was observed in this study to reduce the formation of a scum blanket, this problem may also be alleviated with advanced mixing.

All the reactors exhibit a decrease in biogas production in comparison to R_{cont} as the HRT is increased from 15 to 20 days (Figure 6). In examining this decrease in biogas production at an HRT of 20 days, as compared to an HRT of 15 days, it is important to recall that a constant loading rate of $1.6 \text{ kg VS} / \text{m}^3 \cdot \text{d}$ was maintained in each reactor. Thus, the 20 day HRT experiments provided a longer digestion period to consume the same mass of organic material. Young *et al.* (2013) proposed that during continuous digestion, scum fractions would have preferential degradation and yield a higher initial biogas production. However, given a longer period of time the ultimate biogas production would produce an improvement, but at a smaller yield. For each of the reactors operated in this study this hypothesis

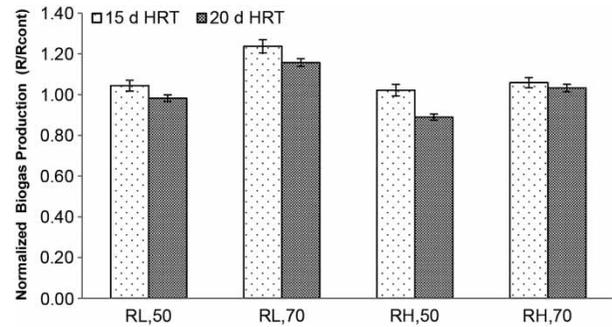


Figure 6 | Average and 95% confidence intervals of daily biogas normalized to R_{cont} .

was confirmed, as the 15 day HRT experiment produced a greater positive differential of biogas production normalized with the control as compared to the 20 day HRT experiments (Figure 6). This signifies scum and other FOG substrates may be utilized in lower HRT systems as a means to generate electricity quickly and efficiently. A practical end use may be at semi-continuous farm anaerobic digesters, which would benefit greatly from the stabilization of the substrate and additional energy production.

A 2^2 factorial experimental design allows for comparison of scum loading and scum pretreatment effects on biogas production as well as their interactions. All of the experiments (15 and 20 day HRT) demonstrate a direct increase in biogas production with an increase in scum pretreatment temperature (Figure 7). Also, an increase in scum loading at both 15 and 20 day HRTs shows a significant effect on biogas production (Figure 7). There appears to be an interaction effect between the scum pretreatment temperature and scum load on daily biogas production, which supports the interaction observed between scum loading and pretreatment temperature in the mesophilic BMP assays produced by Young *et al.* (2013). Heating scum to 50°C softened the substrate to a malleable material; however, integration within the waste did not appear to be enhanced. This potentially reduced the contact between the scum and the microbial consortia embedded in the waste, as the scum remained in a semi-solid phase which likely restricted its access to the microbial community. At 70°C , the scum appeared to be nearly liquefied and was able to integrate into the digester efficiently without clumping or separation. This potentially led to greater contact between the scum and the microbial consortia, which

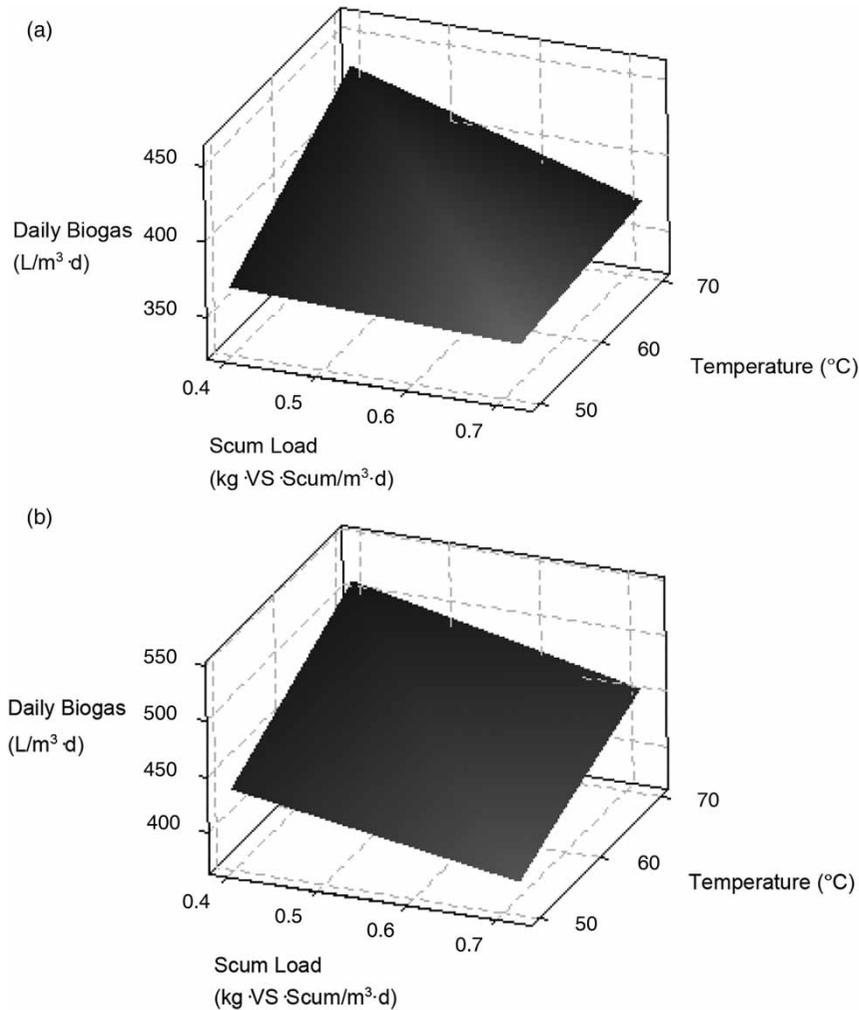


Figure 7 | Surface plot of steady state, average daily biogas production, scum load and pretreatment temperature at (a) 15 day HRT and (b) 20 day HRT.

likely enabled biodegradation of the scum and subsequently lead to the enhanced production of methane.

Equations (1) and (2) were produced to define the surface responses shown in Figure 7, and to quantify the effects and interactions of the scum concentration and temperature of the scum concentrator on biogas production:

$$BP_{15} = 383.25 - 17.75L_{scum} + 20.25T - 13.75L_{scum}T \quad (1)$$

$$BP_{20} = 449 - 24L_{scum} + 35T \quad (2)$$

where BP_{15} is the expected daily biogas production at 15 day HRT ($L/m^3 \cdot d$); BP_{20} is the expected daily biogas production

at 20 day HRT ($L/m^3 \cdot d$); L_{scum} is the coded scum load (1 high, -1 low); T is the coded temperature within the scum concentrator (1 high, -1 low).

The effects of both scum loading and temperature of the scum concentrator, independently, on biogas production were determined to be significant at HRTs of 15 and 20 days. The interaction between scum concentration and temperature in the scum concentrator was also determined to be significant at an HRT of 15 days; however this interaction was not significant at an HRT of 20 days. This finding suggests that given a sufficiently long HRT, the advantages of pretreatment are less beneficial and that other less energy intensive methods to transport scum to the anaerobic digester may be considered in the place of

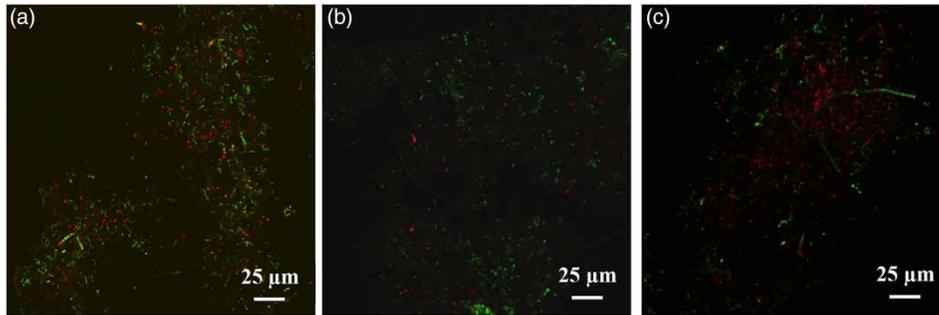


Figure 8 | Viability images of steady state reactor waste at 20 day HRT, (a) R_{cont} , (b) RL,70 and (c) RL, 50. The full colour version of this figure is available in the online version of this paper, at <http://dx.doi.org/10.2166/wqjrc.2016.024>.

heating. It also suggests that if the HRT was decreased, then the interaction between the scum loading and the pretreatment temperature would have a larger effect. However, based on the range of operation defined by current regulatory HRT and organic loading rate of anaerobic digesters in the United States and Canada, the scum concentration and scum treatment temperature produce significant independent and interactive effects. The regression values (R^2) of Equations (1) and (2) with respect to the experimentally measured biogas yield were 84 and 94% for the scum loaded reactors at HRTs of 15 and 20 days. Solving Equation (1) for zero scum load generated average daily biogas production values that were within 7% and 2% of the experimental results observed for R_{cont} at HRTs of 15 and 20 days, respectively.

Microbial viability results

Microbial viability analysis was performed on the R_{cont} , RL, 50, and RL, 70 reactors operating at a 20 day HRT. The two lower scum loaded reactors in the study were chosen for viability analysis based on their improved biogas production compared to the higher loaded reactors. R_{cont} was used as the baseline condition. Examples of viability microscopic images acquired at a magnification of $\times 63$ are shown in Figure 8. Visual inspection indicates that the control and RL, 70 have a greater live percentage of biomass (illuminated in green) relative to dead percentage of biomass (illuminated in red) as compared to RL, 50. (The full colour version of this figure is available in the online version of this paper, at <http://dx.doi.org/10.2166/wqjrc.2016.024>.)

Particularly, the biomass present in R_{cont} was composed of $72 \pm 5\%$ viable cells compared to $66 \pm 8\%$ and $56 \pm 8\%$ for RL, 70 and RL, 50, respectively (Figure 9). As TCOD, SCOD and VA measured in R_{cont} were statistically equal to those of RL, 70 and RL, 50 (Figure 4(b)) the difference in viable cell concentrations may be directly associated with adding scum as an additional substrate. R_{cont} produced statistically the same amount of biogas daily as RL, 50 (Figure 5), however the cell viability results show significantly more viable cells exist in the R_{cont} reactor. Concomitantly, RL, 70 produced significantly more biogas, 16% than R_{cont} , but the two reactors have statistically the same fraction of live cells.

The number of live cells per m^3 of waste was calculated to interpret the reactors, response to scum at a microbial level (Figure 10). R_{cont} produced $11,700 \pm 1,900$ L of biogas/ m^3 of live cells \cdot d, whereas RL, 70 and RL, 50 produced $17,300 \pm 2,000$ L and $16,200 \pm 2,200$ L of biogas/ m^3 of live cells \cdot d, respectively. This indicates that either the tri-mixture of TWAS, PS and scum embeds a similar

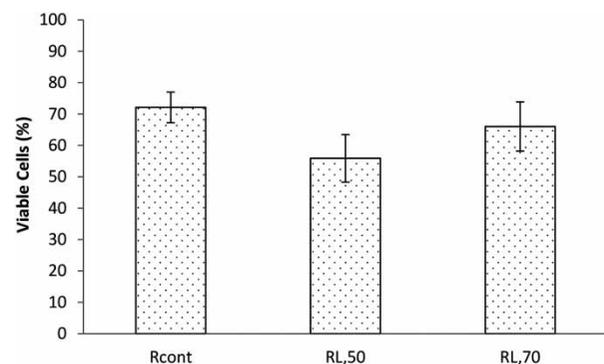


Figure 9 | Average and 95% confidence intervals of cell viability at 20 day HRT.

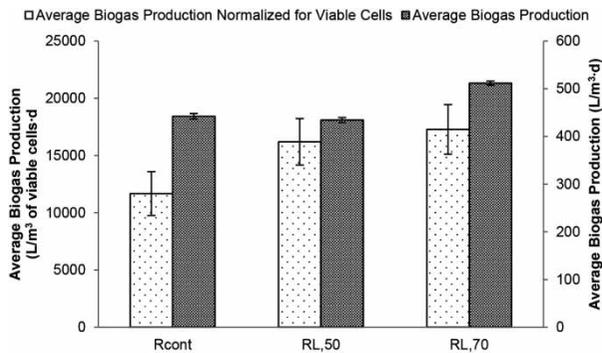


Figure 10 | Average and 95% confidence intervals of biogas production normalized for live cell fraction at 20 day HRT.

microbial population in the waste that is more active than the reactor loaded with only TWAS and PS, or that scum loading caused a shift in microbial population towards more active communities. The cells present in scum-containing reactors appear to produce 48% more biogas per m^3 of live cells than the cells present in the R_{cont} reactor.

CONCLUSIONS

The goal of this study was to investigate the effects of scum loading and scum pretreatment temperature on mesophilic anaerobic co-digestion of scum with PS and TWAS at two distinct HRTs of 15 and 20 days. Semi-continuous reactors were fed with PS, TWAS and scum collected from a full-scale MWRRF, ROPEC, in Ontario, Canada. Reactors fed at an overall loading rate of $1.6 \text{ kg VS} / \text{m}^3 \cdot \text{d}$ and specific scum loadings of 0.40 and $0.72 \text{ kg} \cdot \text{VS} \cdot \text{scum} / \text{m}^3 \cdot \text{d}$ at HRTs of 15 and 20 days all demonstrated TCOD, SCOD, $\text{NH}_3\text{-N}$ and VA concentrations within the healthy range of conventional anaerobic digestion.

Scum loading rates and thermal pretreatment of scum both independently showed a significant effect on biogas production. Reactors fed at lower scum loading rates of $0.40 \text{ kg} \cdot \text{VS} \cdot \text{scum} / \text{m}^3 \cdot \text{d}$ and fed with scum pretreated at higher temperatures of 70°C demonstrated the greatest improvement in biogas production compared to reactors fed without scum; increases in biogas production of 24% and 16% were measured at 15 and 20 day HRTs, respectively. Higher observed rates of biogas production were measured for all scum loaded reactors at 15 day HRT

compared to 20 day HRT, signifying that scum is readily biodegradable and potentially a preferential substrate for digestion. Finally, cell viability analysis indicated that the scum loaded reactors produced more biogas per volume of live cells compared to the control reactor without scum.

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