Comparison of single-stage and two-stage thermophilic anaerobic digestion of SS-OFMSW during the start-up phase

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Abstract The accumulation of volatile fatty acids during the anaerobic digestion of biodegradable substrate such as Source Sorted Organic Fraction of Municipal Solid Waste (SS-OFMSW) is associated with an inhibitory effect on methane producing biochemical pathways. While inconclusive at times, the usage of two-stage digesters has been argued as a potential solution for this issue. In this study single-stage vs. two-stage thermophilic digesters, fed with SS-OFMSW, were examined during the startup phase while increasing the loading rate from 0.5 to ~2 g VS.L⁻¹d⁻¹. While both systems exhibited a stable performance, with neutral pH and low intermediate-to-partial alkalinity, the two-stage digester exhibited better effluent quality with 79 and 57% lower average total chemical oxygen demand (TCOD) and soluble COD (SCOD), respectively. Also, upon reaching steady-state conditions, the two-stage system showed a superior performance with an overall methane content of 54%, compared to an average of 45% in the single-stage system.

Graphical Abstract

Novelty Statement

Despite a large body of literature addressing the benefits of two-stage anaerobic digestion, compared to conventional one-stage digestion, the results are inconclusive and even contradictory often because of differences in operating conditions and type of feed. Most studies comparing single- with two-stage digesters have been reported under mesophilic temperatures, mostly during steady-state operation. With the growing interest in maximizing energy generation from municipal solid waste, thermophilic applications become justifiable. Given the importance of the start-up phase in defining the future performance of a digester, this study compares one- with two-stage digestion of SS-OFMSW during startup, under thermophilic conditions.

Keywords Thermophilic anaerobic digestion; single-stage vs. two-stage; SS-OFMSW

Introduction

The growing pressure towards effective disposal of the Organic Fraction of Municipal Solid Waste (OFMSW) coupled with the rising interest in carbon neutral energy, has encouraged research on clean energy from biomass and organic waste. Source-Sorted OFMSW (SS-OFMSW), with its high moisture content and biodegradability, is a good candidate for anaerobic digestion (AD) offering the advantage of producing methane and soil conditioners [1, 37, 38]. However, with its low carbon-to-nitrogen (C/N) ratio, AD of OFMSW is associated with a low stability due to the accumulation of intermediate byproducts such as volatile fatty acids (VFA) [2]. This constraint is more pronounced under thermophilic temperatures (50-55°C) due to faster kinetics compared to mesophilic systems (30-40°C) leading to an even higher accumulation of VFA, especially with highly biodegradable wastes such as OFMSW [3].

The separation of the acidogenic and methanogenic phases of the AD process has been promoted in some studies to alleviate the accumulation of metabolites by buffering the loading rate and organic matter in the first stage allowing a healthier methanogenesis in the second stage [4, 5]. As such the AD process is separated into two stages with the first stage encompassing the fermentative hydrolytic, the acidogenic and the acetogenic bacteria, while the methanogenic archaea can be separated in a second stage due to their lower resilience, tolerance to upsets, and growth rate [4]. The two-stage digesters can reportedly achieve higher methane generation and chemical oxygen demand (COD) removal [2, 6], lower VFA accumulation [7], improved functional stability for waste with poor cellulose such as OFMSW [8], better resistance to loading fluctuation and more tolerance of higher loading rates [5, 8, 9], as well as reduced retention time requirements [10]. In contrast, other studies showed that a well-designed and adequately operated single-stage continuous stirred tank reactor (CSTR) can perform similar or better than two-stage systems [11, 12]. Furthermore, the separation of the hydrolysis/acidogenesis and methanogenesis phases (or increasing the distance between acidogens and methanogens) is argued to hamper the syntrophic associations and prevent the transfer of hydrogen between both groups of species, thus negatively affecting the synergy between the “producing” and “consuming” microorganisms and increasing the accumulation of propionate and butyrate, both being methanogenic inhibitors [13, 14, 15, 16]. Also, it has been shown that one-stage system is preferable for the treatment of fruit and vegetable waste with 33% higher energy yield compared to two-stage system. The lower energy yield in the two-stage system was attributed to energy and COD loss in the hydrolysis reactor [5].

Most reported two-stage AD systems were operated under mesophilic temperatures. Various digester configurations (CSTR, upflow anaerobic sludge blanket (UASB) and upflow sludge bed) were adopted [2, 4, 7],

with diversified types of feed, including: a combination of food waste and fruit and vegetable waste [17], synthetic substrate [18], high cellulosic substrate [19], a combination of swine manure and market bio-waste [16], corn stalk [20], Food waste [5, 21] as well as municipal waste with and without hydrothermal pretreatment [22]. Also, two-stage systems with thermophilic methanogenic phase [23] or thermophilic acidogenic phase [24] and temperature phased stages [25] have been examined. Recently, Micolucci et al. [26] compared the performance of single- and double-stage thermophilic reactors treating food waste, under steady-state and transient conditions. The results showed higher biogas generation ($0.88 \text{ m}^3/\text{kg VS}$) in the double-stage system compared to the single-stage digester (to $0.75 \text{ m}^3/\text{kg VS}$), as well as 17% higher removal efficiency. Despite the extensive literature comparing one- and two-stage anaerobic digestion, comparisons during the startup phase of systems fed with SS-OFMSW, under thermophilic temperatures, are limited to non-existent. Given the importance of the start-up phase [3] and the inconsistency regarding the effectiveness of the two-stage design, this work targets a comparative analysis of the single-stage versus the two-stage design treating SS-OFMSW under thermophilic conditions during the startup of a CSTR. The assessment contrasts the performance (in terms of biogas and methane generation), treatment efficiency (organic solid reduction) and stability (alkalinity and VFA) of both systems.

Materials and Methods

Feed preparation and characteristics

The waste was collected over two weeks from 10 households to ensure a varied and representative sample. The feed was ground, mixed, homogenized and stored in 150 ml cups at -20°C . Prior to use, the samples were thawed and diluted with distilled water to reach the required volume. The purpose of early collection and storage of waste samples is to reduce fluctuations in substrate composition. The average feed's characteristics, with negligible variation, are presented in Table 1 and are comparable to literature reported values with COD = $292,000 \text{ mg/l}$ [11]; total solids (TS)= 22.61 % and C/N= 11.5 [21].

Table 1 Physico-chemical characteristics of SS-OFMSW

Parameter	Average Value	Literature reported Values
TS (%)	21 ± 0.2	18.6 – 52.5 [21, 37, 39, 40]
Volatile solids (VS, %)	20 ± 0.2	32 – 96 [37, 39]
VS (%TS)	95 ± 0.2	60 – 97 [37,39, 40]
Total COD (TCOD), mg/L	389,172	292,000 – 630,000 [11, 40]
Soluble COD (SCOD), mg/L	13,540	10,400 – 119,000 [39,40]
Ammonia-N, mg/L	30	18 [40]
Partial Alkalinity, mg of CaCO ₃ /L	0	0 [40]

Experimental procedures

The single-stage digestion was carried out in a bench-scale digester (9 L working volume, Bioflo 110, New Brunswick Scientific Co.) and the two-stage digestion was conducted in two digesters connected in series: B1 and B2 (9 L in total, 4.5 L working volume each, Anaerobic Digester W8, Armfield Ltd.) (Fig. 1). Both systems were inoculated with digestate collected from a stable single-stage digester operating at similar conditions (fed with SS-OFMSW at 55°C) and running at an organic loading rate (OLR) = 2 g VS.L⁻¹.d⁻¹ for over a year. A similar approach of seeding single- and two-stage systems with digestate from the same single-stage digester was reported [6]. All three digesters were inoculated with 50% of their working volume (4.5 L in digester A and 2.25 L in digesters B1 and B2, respectively). After seeding, distilled water was added to all digesters till the final working volume was reached. The pH of digester B1 was adjusted on day 1 to be between 5 and 6 with HCl (5M), whereas digester A and B2 had the desired pH 7. The digesters were not fed until the third day after seeding. Physico-chemical parameters and gas monitoring started on day 16 after inoculation.

During the first 30 days, all digesters started at low loading rates of 0.3-0.5 g VS.L⁻¹.d⁻¹. The weight of the waste fed to the digesters was progressively increased to about 110 g to both systems (single- and two-stage), with the same total digester volume of 9L for A, and for B1 and B2 combined (4.5 L each). Around day 90, the OLR reached 2.0 g VS.L⁻¹.d⁻¹ in digester A (HRT = 30 days) and maintained steady till the end of the experiment. Simultaneously, the OLR in B1 was raised to 4.2 g VS.L⁻¹.d⁻¹ (HRT 10 days). Only a portion of B1 digestate was fed into B2 digester to achieve an OLR (2.4 g VS.L⁻¹.d⁻¹) close to that of the single-stage digester, while maintaining an HRT of 17 days. Accordingly, the operational conditions were maintained closely similar in the one-stage and two-stage systems: OLR = 2.0 and 2.4 g VS.L⁻¹.d⁻¹, HRT = 30 and 27 days, respectively. The startup period was divided into three consecutive durations according to the loading pattern and rate: Run 1 (Steady low rate), Run 2 (Incremental rate), and Run 3 (Steady high rate).

Fig. 1 Experimental Layout: (A) single-stage system; (B) two-stage system with
B1=acidogenic reactor and B2= methanogenic reactor

Monitoring

The temperature of the digesters was maintained at $55 \pm 1^\circ\text{C}$ and monitored using thermostatically controlled electric heating jackets connected to built-in temperature probes. The pH was monitored with an immersed probe and adjusted through a manually operated peristaltic pump connected to a NaOH (5N) solution or a HCl (5N) solution depending on the reactor. The biogas composition (methane and carbon dioxide) was monitored once or twice per day using a dual wavelength infrared cell with reference channels (GEM-2000 monitor, Keison Products, UK). Knowing that the startup of digesters seeded with non-acclimated inocula is often unstable, hydrogen accumulation is expected [3]. Yet, given its relatively low proportion compared to CH_4 and CO_2 , hydrogen was not monitored in this study.

Physico-chemical parameters were monitored on a weekly basis with samples collected from discarded digestate prior to feeding. A portion of the sample was centrifuged at 13000 rpm for 20-40 minutes depending on the quality of the sample using a Thermo Scientific Sorvall ST16 Centrifuge and then filtered using Whatman microfiber filter 47mm (pore size: $1.5 \mu\text{m}$), the filtered portion constituted the soluble fraction. SCOD and TCOD analyses were carried out using the modification of Standard Methods 5220D procedure [36] through photometric measurements using COD Digestion Vials, High Range Plus, 0 to 15,000 mg/L. Total, dissolved and volatile

solids (TS, VS, TDS and VDS) were determined using Standard Methods for the analysis of water and wastewater [30]. Suspended solids were calculated by subtracting the volatile part of the total solids. Ammonia content was determined by spectrophotometry using High Range Ammonia Nitrogen by the AmVer™ Salicylate Test 'N Tube™ Method. Both COD and ammonia testing were conducted using a HACH DR/2010 Spectrophotometer. Total and partial alkalinity were determined by titration with HCl (0.2 N) to pH 4.3 and 5.75, respectively using a Thermo Scientific Orion 3 STAR benchtop pH, whereas the intermediate alkalinity was calculated as the difference between the two parameters.

Results and Discussion

Biogas Composition

In the single-stage digester (A), methane content was constant at $33\pm1\%$ during the first run (i.e. Run 1), decreasing from 32% to 19% during the second week of run 2 which was paralleled by an increase in the carbon-dioxide-to-methane (CO_2/CH_4) ratio indicating methanogenic distress (Fig. 2a). This can be attributed to the OLR increase during the start of run 2 which could have caused an initial shock to the methanogenic community. The CO_2/CH_4 ratio kept increasing throughout the rest of the experiment to reach an average of 0.6 during run 3. In comparison, CO_2/CH_4 ratio in B2 (the methanogenic phase of the two-stage system) started at high levels of 0.50 and dropped to about 0.25 by the end of the experiment indicating a healthy methanogenic activity at higher loading rates (Fig. 2b), evident by the high methane content ranging between 54 and 62% (and reaching 70% on some days).

Fig. 2 Loading rate and CO_2 -to- CH_4 ratio: (a) Digester A; (b) Digester B2*
* The CO_2/CH_4 was based on combined biogas generated from B1 and B2

Alkalinity and pH

As a process indicator, pH is dependent on the concentration of free VFA and the overall alkalinity of the reactor [27]. The intermediate-to-partial alkalinity (IA/PA) ratio is important as a representation of VFA alkalinity [28]; therefore, its increase is an indication of a surge in free VFA and thus a distress in the biochemical pathway. The average IA/PA ratio in A and B2 was below 0.3, the recommended threshold for such digesters when total alkalinity is between 4000 and 8000 mg $\text{CaCO}_3\cdot\text{L}^{-1}$ [29]. Despite starting off at a high IA/PA of 0.6, the IA/PA ratio in reactor A gradually decreased to below 0.2 by the start of run 2 and remained so during this run (Fig. 3a). Digester B2 produced an initial IA/PA ratio that was even higher than digester A, and persisted until week 5 (Fig. 3b). This could have inhibited the methanogenic community leading to the high CO_2/CH_4 ratio observed in B2 (Fig. 2b). Beyond week 5, the IA/PA ratio in B2 decreased gradually to as low as 0.1.

Fig. 3 Alkalinity concentrations and IA/PA ratio for digesters A (a) and B2 (b)

The pH in digesters A and B2, was not affected by the initial high concentration of VFA in both systems due to temporary compensation by the high buffering capacity presented by the total alkalinity (TA). The pH range in both digesters remained high throughout the experiment, varying within the acceptable range of 7.4 and 7.9 [9] and eliminating the need for pH control. The TA was relatively stable and remained above 4000 and 3500 mg $\text{CaCO}_3\cdot\text{L}^{-1}$ for digesters A and B2, respectively – an acceptable limit according to Ganesh et al. [5]. However,

the TA did decrease by 41% (from 6800 to 4000 mg $\text{CaCO}_3\cdot\text{L}^{-1}$) in digester A and 32% (from 5300 to 3600 mg $\text{CaCO}_3\cdot\text{L}^{-1}$) in digester B2, between weeks 11 and 12, when the OLR was increased by 26%. This was followed by a doubling in the IA/PA ratio (from 0.15 to 0.37 in A and from 0.05 to 0.12 in B2), indicating a system's upset due to increased substrate loading. Nevertheless, this shock subsided quickly and the reactors recovered with an average IA/PA ratio of 0.28 ± 0.05 and 0.10 ± 0.02 , and an average TA of 5320 ± 273 mg and 5809 ± 563 mg of $\text{CaCO}_3\cdot\text{L}^{-1}$ in digesters A and B2, respectively.

Digestate characteristics

Upon increasing the OLR at the start of run 2, ammonia concentrations in digester A spiked from 0.6 to 1.0 $\text{g}\cdot\text{L}^{-1}$ on week 7, then decreased in week 8, only to slowly increase again till reaching an average of 1.2 $\text{g}\cdot\text{L}^{-1}$ during run 3 (Fig. 4a) – slightly higher than the reported inhibition threshold of 1.0 $\text{g}\cdot\text{L}^{-1}$ for a similar waste type and process design [30]. In comparison, ammonia levels in digester B2 remained above the 1.0 $\text{g}\cdot\text{L}^{-1}$ threshold until week 9 of run 2, when they dropped to a reasonable level of 0.2 $\text{g}\cdot\text{L}^{-1}$ (Fig. 4b). This decrease led to an enhanced degradation of intermediate metabolites resulting in an increase in TA (3740 to 5282 mg $\text{CaCO}_3\cdot\text{L}^{-1}$) [8]. The drop in ammonia was followed by a subsequent gradual increase which paralleled the increase in OLR, to a final value of 0.7 $\text{g}\cdot\text{L}^{-1}$ during the last week.

Fig. 4 Ammonia concentrations, TS of the liquor and VS removal in digester A (a) and B (b)

The average TCOD and SCOD concentrations in reactor A effluent were about 10.5 g.L⁻¹ (11.9 g.L⁻¹ during Run 3) and 4.2 g.L⁻¹ (5.7 g.L⁻¹ during Run 3), respectively, which are consistent with reported values [7] and indicate proper use of the hydrolytic products by methanogens. In comparison, the results of digester B2, during run 3, were even lower with an average TCOD and SCOD of 5.1 g.L⁻¹ and 1.2 g.L⁻¹, respectively, indicating a better degradation of organic components. Similarly, total and suspended solids were lower in B2, with TS = 1.02% in A vs. 0.81% in B2; and TSS = 0.52% in A vs. 0.14% in B2 (Fig. 5). The lower removal rates in digester A during run 3 can be explained by the rise in ammonia levels during this run coupled with the high alkalinity of the system which could hinder bacterial functions at the end of the startup [32]. Similarly, both systems underwent good degradation of organic matter and resulted in a high removal of VS. During the last week of the experiment, VS removal reached 92% in the two-stage system and 86% in the single-stage system, both comparable to reported results (88% in Ward et al. [31]; 83% in Verrier et al. [32]).

The concentration of the organic substrate in B1 liquor was the highest of all three digesters (TS% = 1.09%, VS% = 0.7%, TSS = 0.43%, TCOD = 21.2 g.L⁻¹) with SCOD reaching three folds of the single-stage system (12.6 g.L⁻¹ compared to 4.2 g.L⁻¹ in A). This can be attributed to the high activity of fermenters and the absence of methanogenic activity to utilize hydrolyzed products. Whereas the organic substrate in reactor B2 was the lowest among all three digesters (TCOD = 5 g.L⁻¹, SCOD = 1.5 g.L⁻¹, TSS = 0.13%, TS = 0.64%, VS = 0.29%) arguably because most of the hydrolysis and degradation of larger polymers was achieved in the first stage, and acetate is consumed by methanogens in the second stage [5] (Fig. 5). It is worth noting that prior to the decrease in ammonia concentration during week 9, the VS reduction in the B2 effluent was low, averaging at 43%, and improved significantly to an average of 90% afterwards, exceeding the results of digester A. This is expected in two-stage digesters, where organic matter degradation is superior than in single-stage digesters, due to fermenters thriving in optimal acidic environments [6].

Fig. 5 Effluent characteristics for digesters A, B1 and B2

Impact of stage separation

Considering the difference in pH and operational parameters imposed in the two-stage compartments, a difference in the composition, abundance and activity of some microbial communities of B1 and B2 is expected [33]. This is likely to lead to a superior hydrolysis and better destruction of organic matter due to ideal acidic conditions provided to the hydrolytic and acidogenic communities [6]. In fact, the final percentage of total and suspended solids in the two-stage effluent was lower than the single-stage system (Fig. 5). Similarly, the removal of TCOD and SCOD was 52% and 64%, respectively, higher than the single-stage system. These findings are consistent with those reported by Liu et al. [34], Massanet-Nicolau et al. [35], Micolucci et al. [26], and Nasr et al. [6] but inconsistent with the findings of Nair et al. [18] where the lower organic matter degradation in two-stage digesters was attributed to a decrease in the protozoan population. Also, the results do not concur with those of Shen et al. [17] and Park et al. [9] who reported insignificant differences in COD removal between both systems.

During the early stages, methane concentration in the two-stage system was low, possibly because the starting loading rate was low and the seed (retrieved from a single-stage digester) was not originally acclimated to the conditions in the methanogenic digester (B2). Yet, upon reaching steady loading in Run 3, the overall methane concentration in the total biogas produced from the two-stage system became constantly higher (Fig. 6). Thus, it can be concluded that separating the digestion phases in the two-stage system and increasing the distance between syntrophic bacteria did not hinder methanogenesis [13, 14, 15, 16]. Also, the abundance of hydrolyzed substrates and easily fermentable organic matter, transferred from the acidogenic reactor, does not seem to cause a serious shock to the methane producing community in the methanogenic reactor [16]. This agrees with the findings of Li et al. [20] whereby the two-phase mesophilic digesters exhibited a better performance, compared to single-stage digesters, in treating hydrothermally pretreated MSW. Yet, the observations in this study contradict the findings of Massanet-Nicolau et al. [35] and Shen et al. [17] who reported better performance in single-stage systems treating wheat feed pellets and food waste, respectively, under mesophilic conditions and Schievano et al. [16] reporting practically similar performance in energy generation of the two systems.

Fig. 6 Daily CH₄ content: (a) Digester A; (b) Digester B*
** Considering combined biogas generated from B1 and B2*

Conclusion

Single- and two-stage anaerobic digestion of SS-OFMSW were compared during thermophilic start-up at $OLR \leq 2 \text{ gVS.l}^{-1}.\text{d}^{-1}$. The digesters were monitored for biogas composition, COD removal, IA/PA ratio and ammonia concentration. The superior performance of the two-stage system treating SS-OFMSW, at thermophilic temperatures, was demonstrated with: (a) better effluent quality, with 79% and 57% lower average TCOD and SCOD, respectively, and (b) higher methane content of 54%, compared to 45% in single-stage digestion, upon reaching steady feeding.

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