Sustainable Energy & Fuels

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Journal:	*Sustainable Energy & Fuels*
Manuscript ID	SE-ART-08-2019-000667.R1
Article Type:	Paper
Date Submitted by the Author:	01-Oct-2019
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ARTICLE

Anaerobic digestion of synthetic food waste-cardboard mixtures in a semi-continuous two-stage system

Received 00th XXXXX 20xx,
Accepted 00th XXXXX 20xx

DOI: 10.1039/x0xx00000x

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A two-stage anaerobic digestion system consisting of a continuously-stirred tank reactor and upflow anaerobic sludge blanket (CSTR-UASB) in series was evaluated for semi-continuous digestion of food waste and corrugated cardboard mixtures. CSTR organic loading rates (OLRs) were 8 to 32 g chemical oxygen demand (COD) L^{-1} d^{-1} with varying mixture ratios. The CSTR VFA yield reached a maximum of 24% (COD basis) at 8 g COD L^{-1} d^{-1} and 65% food waste / 35% corrugated cardboard. The UASB methane yield decreased from >90% (at CSTR OLRs of 8 to 16 g COD L^{-1} d^{-1} and all mixture ratios) to 75% (at 32 g COD L^{-1} d^{-1}). The greatest methane production was achieved at a CSTR OLR of 16 g COD L^{-1} d^{-1} and 65% food waste / 35% corrugated cardboard, and the UASB remained operable in all testing phases. While the CSTR-UASB system was able to accommodate changes in feed composition and OLR, both CSTR and UASB microbial communities diverged in response to these imposed changes. This study provides new insights about the simultaneous digestion of cardboard and food waste, major components of municipal solid waste.

Introduction

Food waste and corrugated cardboard are main organic components of municipal solid waste.[1] Food waste, paper, and paperboard also represent major components (21.6 and 14.3%, respectively) of the municipal solid waste discarded in landfills.[2] Likewise, food waste and corrugated cardboard are substantial organic components of military base waste, comprising about 15.5-24.6% and 9.3-16.2%, respectively, of solid waste in US Army bases.[3] Solid waste from US military bases includes a large organic fraction which can pose an environmental and health risk, especially in remote and austere facilities where recycling and other waste management technology may be unavailable.[4]

Much of the cardboard is used for food packaging,[4] and may be comingled with food waste, limiting its recycling. Anaerobic digestion is an environmentally responsible technology to stabilize and reduce the volume of organic wastes while producing biogas fuel.[5] Because anaerobic digestion is a mature and versatile technology that can process high-moisture wastes like food waste as well as drier lignocellulosic wastes like corrugated cardboard, it may be a good option for remote military bases, disaster zones, or refugee camps where no other waste treatment is available.

Anaerobic digestion of corrugated cardboard and synthetic food waste has been investigated previously under laboratory conditions in batch mode.[6] Corrugated cardboard had long lag phases, slow methane production rates, and incomplete conversion to methane, while food waste exhibited higher methane production rates and ultimate yields. However, high food waste loading led to volatile fatty acid (VFA) accumulation and inhibition; therefore, both wastes are challenging anaerobic substrates.

Codigestion of food waste with corrugated cardboard may help overcome these difficulties by supplying a more balanced nutrient mixture and diluting inhibitory intermediates such as VFAs [7]. For example, Zhang, et al. [8] observed more stable methane production from continuous codigestion of food waste with card packaging compared to monodigestion of food waste. In dry anaerobic digestion (20-30% initial total solids loading) of food waste-cardboard mixtures, Capson-Tojo, et al. [9] found that cardboard diluted VFA accumulation and supplemented methane yields under high loading.

Conversely, no synergistic effect was observed in a batch anaerobic digestion study of synthetic military food waste and corrugated cardboard mixtures; specific methanogenic activities and methane yields both increased monotonically with the proportion of food waste, and corrugated cardboard did not buffer against VFA accumulation.[6] It was suggested that the lack of synergy resulted in part from the batch mode of the experiments. Corrugated cardboard assays showed lag phases of 40-60 h while food waste assays produced methane immediately; as such, the corrugated cardboard would have been less bioavailable at the peak time of the food waste digestion rate, limiting substrate interactions. Zhou, et al. [10] examined batch digestion of food waste-corn stover mixtures and observed gaps between the peak methane production rates associated with each substrate. Decreasing the recalcitrance of corn stover by pretreating it shortened these gaps. If a similar asynchrony occurred in a food waste-corrugated cardboard system, then continuous operation could help mitigate this by maintaining a microbial community already adapted to corrugated cardboard hydrolysis.

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Details about experimental section and further description of the results are available in the Electronic Supplementary Information (ESI). See DOI: 10.1039/x0xx00000x

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Two-stage anaerobic digestion is another strategy that provides process stability by separating the acidogenesis and methanogenesis steps into different reactors.[11, 12] This allows greater accumulation of VFAs in the first stage and maintains non-inhibitory pH levels for methanogens in the second stage. A two-stage system suits both readily-degradable substrates like food waste as well as lignocellulosic substrates with low intrinsic buffering capacities like corrugated cardboard;[13] the first stage better tolerates VFA accumulation and low pH. Additionally since hydrolysis is the rate limiting step of cellulose degradation,[14] a two stage system could provide a better adaptation of the microbial community and lower pH in the first stage hydrolysis reactor could potentially accelerate corrugated cardboard degradation. Various multi-stage anaerobic processes successfully degraded food waste,[15-18] lignocellulose,[19, 20] and mixed substrates such as the organic fraction of municipal solid waste.[21, 22]

A continuous two-stage anaerobic digestion system commonly consists of an acidogenic continuously-stirred tank reactor (CSTR) followed by a methanogenic upflow anaerobic sludge blanket (UASB) reactor. CSTRs are relatively simple to design and operate, and are commonly used to treat solid wastes such as food waste and corrugated cardboard.[23] CSTRs can experience microbial washout if the dilution rate exceeds the maximum biomass growth rate, but acidogens can better keep up with fast dilution rates compared to methanogens.[5] UASBs prevent methanogen washout by decoupling the sludge retention time (SRT) from the hydraulic retention time (HRT) using a dense granular sludge.[5, 24] UASBs are well-established technology for treating high-strength wastewaters, but work poorly for substrates containing large solid particles;[23] as such, a CSTR is suited for hydrolysis and acidogenesis of a high-solid raw feedstock, while a UASB offers conditions for efficient acetogenesis and methanogenesis of the resultant organic acids.

The present study evaluates a system in which food waste and corrugated cardboard were fed into a CSTR with military base wastewater (~~ww~~, as a non-potable water source) on a semi-continuous basis. The acidified slurry was then clarified, adjusted to a near-neutral pH, and fed to a UASB. This arrangement potentially allows for higher food waste loading in an acid-phase digester while taking advantage of the high methanogenic efficiency of UASBs. The system was tested at a bench scale at different organic loading rates (OLRs) and feed compositions. HRT and other operating conditions were kept constant to minimize the number of experimental variables. Sludge from both reactors was collected for molecular analysis to assess the effects of OLR and food waste-corrugated cardboard ratios on microbial community structure. This study quantifies the performance of a CSTR-UASB system digesting food waste and corrugated cardboard at varying mixture ratios and CSTR OLRs of 8-32 g chemical oxygen demand (COD) L^{-1} d^{-1}; this will help determine whether a CSTR-UASB design can overcome the challenges of food waste and corrugated cardboard as co-substrates.

Results and discussion

1. Two-stage reactor system performance

1.1. Continuously-stirred tank reactor

The CSTR-UASB was operated for 298 d from the start of Phase I through the end of Phase V. Fig. 1 shows COD balances for the CSTR (A) and UASB (B), and Table 1 shows process performance parameters at quasi-steady state. Electronic supplementary information (ESI) section S1 discusses transient data. The VFA concentration indicates the efficiency of acidogenesis in the CSTR. VFA yield more than doubled from Phase I to Phase II (See Table 3 for phase description), when the food waste fraction was increased from 35% to 65% at an OLR of 8 g COD L^{-1} d^{-1}, then decreased in Phases III-IV when the OLR was increased to 16 g COD L^{-1} d^{-1}, and decreased further in Phase V when the OLR increased to 32 g COD L^{-1} d^{-1}. Higher VFA yields might be expected when the feed was 65% food waste (Phases II and III) because of the readily-degradable nature of food waste. Although the increase from Phase I to Phase II follows this expectation, the subsequent decrease in Phase III does not. This indicates food waste contributes to greater VFA yields at lower loading rates but leads to inhibition at higher loading rates. In all phases, propionate and valerate were the most common VFAs. Other species comprised less than 1% of recovered COD, with the exception of butyrate in phase I (average 1.8%). Accumulation of propionate is often an indicator of acidification, and may result from high loading of glucose[25] (a likely monomer of both food waste and cardboard).

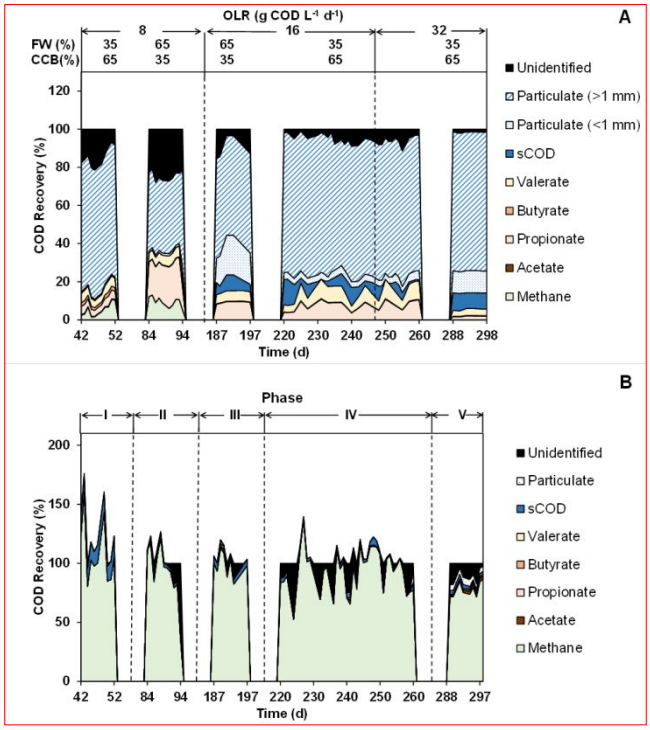

Fig. 1: COD balances over the quasi-steady state periods for the CSTR (A) and UASB (B). Each type of COD measured was normalized by the COD in the influent of the corresponding stage and presented as a percentage (%). Influent COD to the CSTR depended on the OLR at each phase, while influent COD to the UASB depended on the liquid effluent from the CSTR (including VFAs, non-VFA sCOD, and small particulate COD). See Table 3 for phase description. FW: Food Waste; CCB: Corrugated cardboard.

Methane comprised only 5% of COD recovery from the CSTR in Phase I and 10% in Phase II, and became negligible after the OLR was increased from 8 to 16 g COD L^{-1} d^{-1}. The CSTR pH was below 5 for all phases, so the low methane yield is consistent with acidic inhibition of methanogenesis. Methanogens experience inhibition at pH values below 6.2, while acidogens have an optimum range of 5.5-6.5;[5] thus, methanogenesis would be completely inhibited and acidogenesis could be partly inhibited at these pH levels.

Large particulate solids accounted for the largest CSTR effluent COD fraction (38.7-73.5%) in all phases. This fraction was highest in Phases I, IV, and V, when the feed contained 65% corrugated

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cardboard. This trend makes sense considering the recalcitrance of corrugated cardboard and the short HRT. Pommier, et al. [26] observed that paper and cardboard retained their physical shape after batch AD for 85 d; even though the biodegradable COD had been removed, the particles remained large. Additionally, Noike, Endo, Chang, Yaguchi and Matsumoto [14] observed that slower growing cellulolytic microbes were washed out before glucose degraders with decreasing HRT. Therefore, at the short HRT of 2 d in the CSTR, corrugated cardboard structure would remain intact, cellulolytic populations would be relatively low, and a higher fraction of corrugated cardboard fed would result in a higher fraction of large particulate solids recovered. Additionally, since large particulate fractions exceeded the corrugated cardboard fraction in the influent, COD from sorbed soluble organic matter and attached microbial biomass may also have been removed with the corrugated cardboard. Batch AD of particulate solids recovered from the CSTR in Phase III (methodology described in ESI section S5) showed that anaerobic sludge could convert over 90% of COD to methane after 360 h, but had a lag phase of about 100 h. This indicates the particulate solids remained undigested because the HRT was too short, not because the substrates were not digestible.

Small particulate solids constituted small fractions of outgoing CSTR COD (0.8-3.5%) in Phases I, II, and IV (OLR 8 g COD L^{-1} d^{-1}, 35% and 65% food waste, and OLR 16 g COD L^{-1} d^{-1}, 35% food waste, respectively), but were more substantial (11.2-18.5%) in Phases III and V (OLR 16 g COD L^{-1} d^{-1}, 65% food waste, and OLR 32 g COD L^{-1} d^{-1}, 35% food waste, respectively). Non-VFA soluble COD increased from 1.9-2.5% in Phases I-II to 5.3-8.6% in Phases III-V, when the OLR increased from 8 to 16 g COD L^{-1} d^{-1}. The higher fractions of both small particulate COD and non-VFA soluble COD in Phases III-V could indicate decreased hydrolysis (whereby particulate COD would be converted to soluble COD) and acidogenesis (whereby non-VFA soluble compounds would be converted to VFAs) at higher OLRs. Several studies have observed or suggested that hydrolysis and acidogenesis may be inhibited by VFA accumulation and low pH.[27-33] This was attributed to sub-optimal pH for extracellular hydrolytic enzyme function[34] and equilibration of VFAs to their undissociated forms, which can diffuse into bacterial cells and hinder cell function.[35] Since VFA concentrations in an acidogenic reactor exceed those of a functioning single-stage digester, these inhibitory effects may be more prominent.

Ammonia concentrations remained low during the experiment, increasing from 145.8 to 272.8 mg NH_3-N L^{-1} between Phases I and II (OLR 8 g COD L^{-1} d^{-1}, 35% and 65% food waste, respectively), then dropping below 5 mg NH_3-N L^{-1} in Phases III-V (OLR 16 g COD L^{-1} d^{-1}, 65% and 35% food waste, and OLR 32 g COD L^{-1} d^{-1}, 35% food waste respectively). The increase in ammonia from Phase I to II was roughly proportional to the increase in food waste provided. Ammonia in AD systems comes primarily from degradation of organic nitrogen-containing feedstocks or sludge,[5], and food waste contains much more nitrogen than corrugated cardboard. The decrease in total ammonia after Phase II suggests the nitrogen in food waste was released to a lesser extent at OLRs greater than 8 g COD L^{-1} d^{-1}. This could have contributed to nitrogen deficiency at these higher OLRs, which may partly explain the poor conversion achieved. However, ammonia itself was likely not inhibitory because concentrations were well below ~~reported~~ inhibitory levels of 1,400-14,000 mg NH_3-N L^{-1} reported for AD,[36] and the fraction of relatively toxic free ammonia is very low at the measured CSTR pH levels.[5] Additionally, acidogenic bacteria have been demonstrated to be less sensitive to ammoniacal nitrogen inhibition than methanogens.[37, 38]

1.2. Upflow anaerobic sludge blanket

The UASB COD balance in Fig. 1B shows the UASB performance varied less between phases than the CSTR. Methane accounted for the majority of COD recovery. The spikes in methane recovery result from the combined noise in methane measurements and in influent COD measurements; since the normalized methane recovery depends on the COD inflow rate, regular fluctuations in both measurements affected its calculation.

Quasi-steady state methane yields remained above 90% and other forms of COD remained below 8% during Phases I-IV, indicating almost complete conversion of influent COD to methane. During Phase V, methane yield dropped to 77.8%, while VFA and particulate COD increased likely to the higher load fed into the reactor. The UASB pH remained fairly stable (7.4-7.8) and well within the operable range for anaerobic digestion.[5] Alkalinity increased from Phase I-III, then decreased slightly in phases IV-V. It remained within the typical range of 1,000-5,000 mg L^{-1} as $CaCO_3$, indicating suitable buffering capacity in the UASB.[5] Since methanogens are sensitive to pH changes, buffering capacity is important to maintain microbial activity and resist potential acidification by VFA accumulation. Because of this dynamic between VFA concentration and alkalinity, the VFA/Alk ratio better quantifies reactor stability. This value was very low during Phases I-IV, and rose to 0.20 in Phase V; this is well below 0.35, the usual criterion for healthy anaerobic digestion.[39] However, the increase during Phase V may indicate the UASB would become unstable if the OLR were increased further.

The total ammonia concentration in the UASB effluent was 19-25 mg NH_3-N L^{-1} in Phases I, III, and IV; it rose to 54.1 in Phase II and dropped to 2.0 mg NH_3-N L^{-1} in Phase V. These were several orders of magnitude below previously reported inhibitory concentrations of 1,400-14,000 mg NH_3-N L^{-1} for methanogenic communities.[36] No strong trends were observed, though the low concentration in Phase V could have caused inhibition due to nitrogen deficiency. Decreases in ammonia between the CSTR and the UASB in Phases I and II may stem from incorporation of nitrogen into the cell biomass of the UASB. An increase in ammonia between CSTR and UASB, as observed in Phases III-V, suggests organic nitrogen remaining in the CSTR effluent is transformed to ammonia in the UASB. Overall, the UASB was able to convert COD loads from the CSTR into methane and produce an aqueous effluent with only 7-12% of the screened influent COD concentration.

1.3. Total system

The COD balance of the total system is also shown in Table 1. The majority of the influent COD was recovered as large particulate solids from the CSTR in all phases. This indicates poor disintegration and hydrolysis of solids, leading to less COD to methane conversion in the UASB. The UASB performed efficiently; over 77% of the COD from the screened CSTR effluent was converted to methane. COD recovery from the UASB effluent as VFA and non-VFA sCOD was under 2% in all phases, indicating efficient water quality improvement. Methane yield was higher in Phases II and III, when the substrate was 65% food waste, which supports the possibility that most of the COD converted to methane in the UASB comes from food waste. Overall system performance appears to depend primarily on the CSTR, since the CSTR COD balance responded more directly to operating conditions while the UASB outputs were more consistent between phases.

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Table 1: Summary of quasi-steady state performance parameters and COD balances for CSTR and UASB.

	Phase				
Parameter [a]	I	II	III	IV	V
CSTR					
OLR (g COD $L_{reactor}^{-1}$ d^{-1})	8	8	16	16	32
Food waste / Corrugated cardboard (% COD supplied)	35 / 65	65 / 35	65 / 35	35 / 65	35 / 65
Methane	5.2 (3.29) [b]	9.7 (2.14)	0.0 (0.00)	0.0 (0.01)	0.0 (0.00)
VFA	10.9 (1.75)	25.3 (2.09)	14.9 (0.52)	14.6 (4.09)	5.3 (0.61)
Non-VFA sCOD	2.5 (0.99)	1.9 (0.77)	6 (1.91)	5.3 (3.63)	8.6 (0.42)
Particulate (< 1 mm)	0.8 (0.04)	1.2 (0.19)	18.5 (2.02)	3.5 (0.47)	11.2 (0.89)
Particulate (> 1 mm)	68.1 (0.70)	38.7 (1.50)	52.1 (0.01)	71.2 (2.35)	72.7 (0.01)
Undetermined	15.0 (5.06)	25.1 (2.28)	8.5 (4.32)	5.4 (2.45)	2.2 (1.52)
pH	4.5 (0.28)	3.9 (0.02)	3.5 (0.15)	3.8 (0.22)	3.8 (0.04)
Total ammonia nitrogen (mg N L^{-1})*	145.8 (77.76)	272.8 (94.08)	3.3 (0.68)	4.1 (1.45)	1.6 (0.97)
UASB					
OLR (g COD $L_{reactor}^{-1}$ d^{-1})	1.8 (0.22)	4.0 (0.33)	12.2 (1.86)	7.9 (1.26)	15.9 (1.20)
Methane	105.1 (27.23)	99.6 (14.44)	97.8 (10.31)	92.6 (17.90)	77.8 (5.40)
VFA	0.1 (0.01)	0.7 (1.45)	1.6 (2.46)	0.7 (2.33)	4.0 (1.09)
Non-VFA sCOD	7.9 (1.07)	2.7 (1.8)	4.1 (1.9)	3.0 (1.61)	2.7 (0.83)
Particulate	N/A [c]	N/A	N/A	N/A	5.7 (1.26)
Undetermined	1.6 (3.43)	6.0 (7.63)	2.5 (4.98)	9.2 (12.29)	9.8 (5.41)
pH	7.8 (0.18)	7.6 (0.20)	7.7 (0.36)	7.5 (0.10)	7.4 (0.21)
Alkalinity (mg $CaCO_3$ L^{-1})	1,791.5 (100.09)	2,314.5 (647.78)	4,048.5 (544.94)	3,344.7 (326.65)	3,226.1 (200.26)
VFA/Alk (dimensionless)	0.00013	0.061	0.050	0.0027	0.20
Total ammonia nitrogen (mg N L^{-1})*	25.1 (1.73)	54.1 (19.67)	24.2 (12.32)	19.2 (6.22)	2.0 (2.48)
Total system					
OLR (g COD $L_{reactor}^{-1}$ d^{-1})	5.3	5.3	10.7	10.7	21.3
Methane	16.6 (3.06)	29.1 (2.41)	37.4 (9.59)	23 (12.16)	19.4 (3.23)
VFA	0.0 (0.00)	1.0 (1.21)	0.6 (2.28)	0.0 (0.17)	1.0 (0.65)
Non-VFA sCOD	0.9 (0.12)	0.7 (0.30)	1.6 (1.77)	0.7 (1.08)	0.7 (0.50)
Suspended particulate	N/A	N/A	N/A	N/A	1.4 (0.75)
Screened particulate (> 1 mm)	68.1 (0.7)	38.7 (1.5)	52.1 (0)	71.2 (2.35)	72.7 (0)
Specific CH_4 yield (L g VS^{-1})	0.078	0.139	0.179	0.108	0.091
Volumetric CH_4 yield (L $L_{reactor}^{-1}$ d^{-1})	0.311	0.543	1.397	0.857	1.446

[a] Units are percent COD recovery unless otherwise specified

*The pH of both CSTR and UASB was always below 9.25 (The pKa of the NH_4^+/NH_3 pair[40])

Table 1 also shows specific methane yields (SMY, L g VS^{-1}) and methane production rates (MPR, L L^{-1} d^{-1}). SMY followed the same trend as COD recovery as methane; higher yields were achieved in Phases II and III. However, even the highest observed SMY (0.179 L g VS^{-1} in Phase III) fell below reported yields from various configurations of continuous anaerobic digestion of municipal solid waste, which were typically between 0.2 and 0.5 L g VS^{-1}.[41, 42] MPR increased with OLR and with food waste fraction; rates only exceeded 1 L L^{-1} d^{-1} when food waste loading was over 10 g COD L^{-1} d^{-1}, in Phases III and V. Previously published values vary from 0.39 to 3.2 L L^{-1} d^{-1}; results from this experiment mostly fall within this range.[41] Thus, while this system did not extract energy from food

waste and corrugated cardboard as efficiently as other continuous municipal solid waste digesters, its adaptability to high OLRs allowed it to achieve reasonable yields per unit reactor volume.

Overall, the reactors remained operable and achieved stable yields. Corrugated cardboard was not well degraded despite acidogenic CSTR conditions, and system methane yields primarily depended on substrate solubilization in the CSTR; as such, mixing food waste and corrugated cardboard was not beneficial. Efforts for further improvement and investigation should focus on the CSTR, e.g test longer SRT or higher operation temperatures. While a large portion of solids was left undegraded, any waste volume reduction is beneficial where no environmentally-conscious waste management is available. The potential for soluble COD sorbed to particulate solids to leave the system prematurely also makes codigestion of food waste and corrugated cardboard disadvantageous. In practice, corrugated cardboard loading should be limited; if it must be added as part of a comingled dining facility waste stream, the CSTR may require a longer HRT to achieve greater conversion. Solids recycling could also improve performance by retaining more active biomass and allowing for a longer SRT by decoupling it from HRT. Pretreatment is a commonly proposed strategy to improve degradability, though it may be unfeasible in an austere environment because of the additional material and equipment requirements. Food waste loading should still be limited to control VFA accumulation, and a secondary digester to hold and stabilize residual solids and waste sludge is advisable. Other potential difficulties may have arisen from nitrogen deficiency or micronutrient deficiency; even though the medium was supplemented with trace elements and micronutrients, cells might still suffer deficits due to mass transfer limitation.[43]

1.4. Quasi-steady state influent-effluent correlations

Fig. 2A shows several linear correlations performed to investigate relationships between influent and effluent COD components in the CSTR. CCB has been observed to degrade slowly and incompletely, while FW is readily hydrolyzed and converted to VFA.[6, 26] Thus, the un-solubilized solids separated from the CSTR effluent would likely derive mostly from CCB, while VFA would probably come mostly from FW components. Large particulate solids recovered from the CSTR effluent were well-correlated with CCB in the influent, both on percent (Fig. 2A-I) and non-normalized (Fig. 2A-II) bases. However, the VFA yield in the CSTR did not correlate as well with FW (Fig. 2A-III). When this relationship was plotted on a non-normalized basis, it appears that VFA was correlated with FW at low loading rates, but conversion did not increase accordingly at higher loading rates (Fig. 2A-IV). Although CCB is a consistent predictor of large particulate COD, too-high loading of FW appears to have a negative effect on conversion to VFAs.

Correlations between UASB influent and effluent components are shown in Fig. 2B. The methane yield was poorly correlated with the VFA fraction of total COD entering the UASB on normalized (Fig. 2B-I) and non-normalized bases (Fig. 2B-II), poorly correlated with the soluble fraction (sCOD) on a normalized basis (Fig. 2B-III), and fairly well correlated with sCOD on a non-normalized basis (Fig. 2B-IV). The generally poor correlations indicate that despite its designation as an acetogenic-methanogenic reactor, the methane yields did not depend directly on the VFA or solubilized compounds. Thus, the microbial community in the UASB maintained enough metabolic diversity to hydrolyze and convert both small particulate solids and sCOD to methane.

Analogous correlations for the combined two-stage system between methane yield and FW supplied on normalized (Fig. 2C-I) and non-normalized (Fig. 2C-II) bases are shown below. Methane was recovered mainly from the UASB, so large particulate solid COD did not contribute considerably to the methane yield. Given the results of the CSTR above, it follows that most of the COD that was converted to methane originated from FW. These variables showed fair correlations and overall suggest that the system is most efficient when fed a larger fraction of FW.

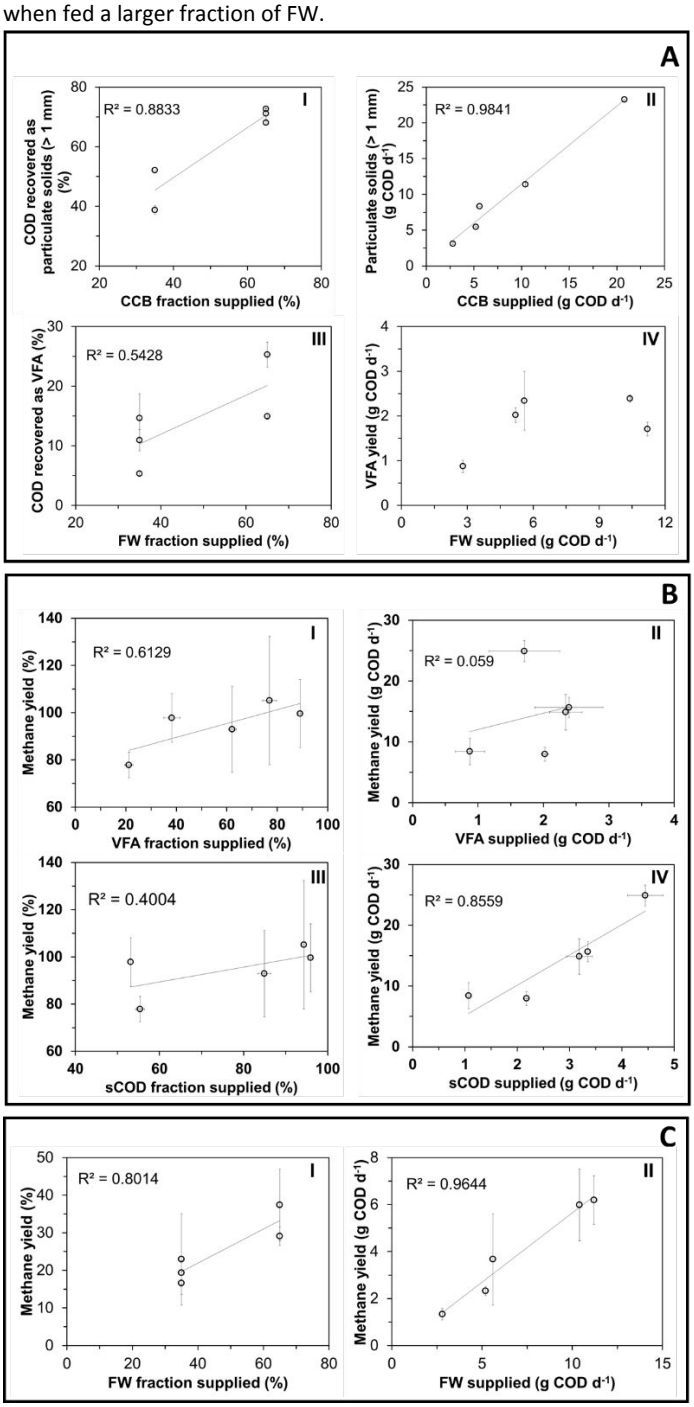

Fig. 2. Linear correlations between influent and effluent COD flows in the CSTR (A), UASB (B), and the total system (C).

2. Microbial community structure

Microbial communities of the CSTR and UASB were analyzed at each phase to survey the effects of changing operational conditions. Fig. 3 shows the relative abundances of bacterial and archaeal orders by reactor and phase. CSTR samples tested negative for archaea, so

only bacteria data are available (Fig. 3A). *Clostridiales* and *Bifidobacteriales* were the most abundant orders. Both fluctuated widely: *Clostridiales* comprised 40-60% of bacteria in Phases I, II, and IV, and 5-6% in Phases III and V, while *Bifidobacteriales* comprised 18-19% in Phases I and II, 60% in Phase III, and 33-37% in Phases IV and V. *Bacillales* and *Lactobacillales* both fluctuated below 12% in Phases I-IV, and increased to 20-26% in Phase V. *Erysipelotrichales* comprised 8% in Phase I and under 0.1% in subsequent phases. *Coriobacteriales* doubled from 8% to 16% between Phases I and II, then fell below 2%. *Pseudomonadales* comprised 13% in Phase II, 9% in Phase V, and less than 3% in Phases I, III, and IV. Other orders had relative abundances less than 5% over the whole trial.

Members of the phyla *Firmicutes* (including *Clostridiales*, *Bacillales*, *Lactobacillales*, and *Erysipelotrichales*), *Actinobacteria* (including *Bifidobacteriales* and *Coriobacteriales*), and *Proteobacteria* (including *Pseudomonadales* and *Xanthomonadales*) are commonly found in single-stage and acidogenic anaerobic reactors.[44-47] These clades encompass a wide range of diversity, including many fermenters, acetogens, and hydrolytic bacteria [48, 49]. Interestingly, *Clostridiales* appeared to have lower relative abundances in phases with higher food waste loading. Members of the most abundant genus, *Clostridium*, sometimes perform cellulolytic roles.[48] If *Clostridium* populations were adapted to corrugated cardboard degradation in the CSTR, high food waste loading could have led to food waste degraders growing quickly and outcompeting them. Overall, the most prominent orders of fermenters in the CSTR (potentially-cellulolytic *Clostridiales* and carbohydrate-fermenting *Bifidobacteriales*) seemed to shift according to different loading conditions.

Archaeal profiles in the UASB (Fig. 3B) remained generally the same over the course of the experiment: *Methanosarcinales* and *Methanobacteriales* together accounted for over 80% of archaea in every phase, and other orders accounted for less than 10% each. The main deviation was in Phase II, when the relative abundance of *Methanosarcinales* decreased to 44% and the relative abundance of *Methanobacteriales* increased to 47% (compared to 54-68% and 27-29%, respectively, in the other phases). The fraction of *Methanomicrobiales* also increased from 2-4% in Phases I-IV to 10% in Phase V.

Methanosarcinales are primarily acetate utilizers, while *Methanobacteriales* and *Methanomicrobiales* are often autotrophic. [50] Thus, acetoclastic and hydrogenotrophic methanogens all exist in significant fractions in every phase. *Methanosarcinales* are often abundant in multi-stage digestion systems.[46, 47, 51] This makes sense because acetate is typically a major component of acidogenic reactor effluent or leachate. Interestingly, *Methanosarcinales* were represented almost exclusively by the genus *Methanosaeta*, with *Methanosarcina* comprising less than 1% of archaea in every sample. *Methanosaeta* have been associated with granulation and lower acetate concentrations,[49, 52] so their dominance in this system may be due to the sludge morphology and VFA concentrations below 1 g COD L^{-1} in the UASB. Supaphol, Jenkins, Intomo, Waite and O'Donnell [47] also observed higher abundances of *Methanosaeta* compared to *Methanosarcina*, which they attributed to the acetate oxidizing activity of *Arcobacter*. The bacterial orders *Clostridiales* and *Thermotogales* contain acetate oxidizers that could contribute to a similar effect.[53] Overall, the archaeal community remained relatively similar between phases. Since the UASB in this study performed fairly consistently over the course of the experiment, the stable community profile seems reasonable.

Contrastingly, the UASB bacterial profiles changed visibly over time (Fig. 3A). The main trend showed *Thermotogales* falling from almost 50% of the community in Phase I to 6% in Phase V. *Bacteroidales* increased from 5% in Phase I to 24% in Phase V. *Spirochaetales* and *Clostridiales* followed similar patterns; they were 2-4% in Phases I-II, 5-7% in Phases III-IV, and 11-14% in Phase V. *Nitrospirales* increased from 2% in Phase I to 10-14% in Phases II-IV, then decreased again to 5%, while *Anaerolineales* increased from 8-11% in Phases I-II to 16-19% in Phases III-IV, then decreased again to 12%. Other orders had relative abundances below 5% over the whole trial.

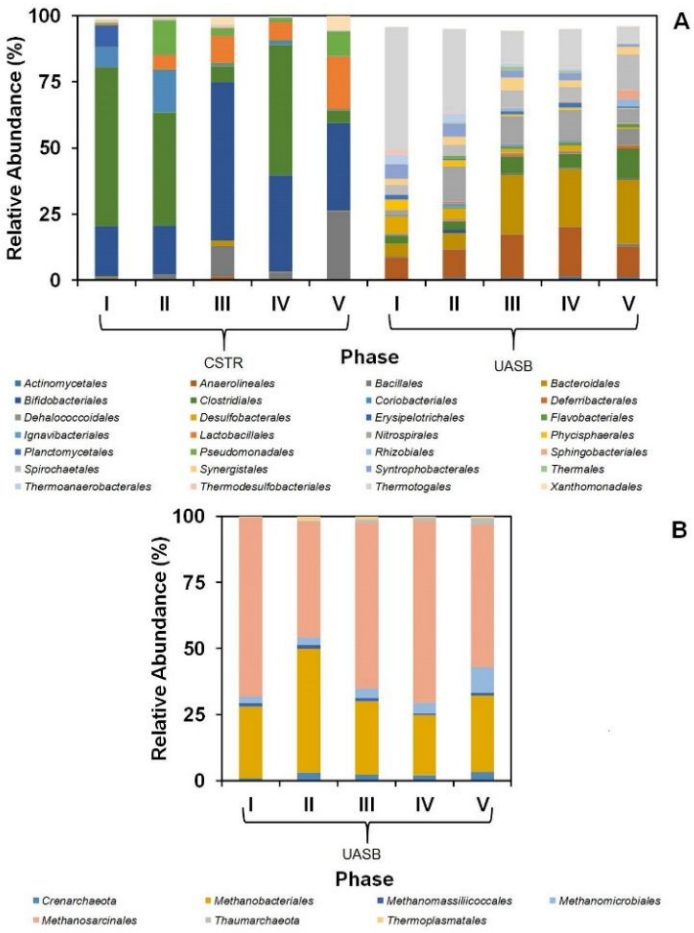

Fig. 3: Relative abundances of bacterial (A) and archaeal (B) orders in the CSTR and UASB at each phase. Archaea were not detected in the CSTR.

Members of *Thermotogales* have been observed in several methanogenic systems.[48, 49, 51] These bacteria often produce polysaccharolytic enzymes and degrade organic compounds to acetate, hydrogen, and carbon dioxide.[48-50] While these are important metabolic functions in anaerobic digestion, they are not unique to *Thermotogales*. *Thermotogales* are typically thermophilic, so their population decline may indicate a gradual shift or maturation due to mesophilic conditions in the UASB rather than adaptation to specific conditions at each phase. The increases in relative abundance of other prominent orders may simply reflect the decline in *Thermotogales*. The other abundant orders contain many species that ferment carbohydrates or proteins to organic acids. The diversity of acidogens indicates that despite being a methanogenic reactor, the UASB continued to digest more complex organic compounds as supported by correlations in Fig. 2. In fact, the bacterial community in the UASB was more similar to that of a single-phase digester (dominated by classes *Clostridia*, *Bacilli*, and

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Bacteroidetes) than that of a second stage in a two-stage system (dominated by *Clostridia* and *Thermotogae*) as determined by Merlino, Rizzi, Schievano, Tenca, Scaglia, Oberti, Adani and Daffonchio [51] This likely contributed to the system's adaptability to different operating conditions.

Altogether, the results display a notorious difference between the microbial consortia of the CSTR and UASB, even though they started with the same inoculum. The archaeal population in the CSTR shrank, which is consistent with previous observations of sludge acidification.[54] The short SRT of 2 d and the high OLRs in the CSTR likely selected for a fast-growing and acid-tolerant population. Decoupling SRT from HRT in the UASB and feeding a less complex and lower-strength influent selected for biofilm-forming species with less hydrolytic activity. The gentler conditions in the UASB likely led to greater diversity than in the acidogenic CSTR, which agrees with previous results from two-stage systems.[46, 55]

Conclusions

A two-stage CSTR-UASB anaerobic system was evaluated for continuous codigestion of military food waste and corrugated cardboard. After continuous operation over five operating conditions, the bench-scale system achieved 5-25% VFA yields in the CSTR and stable methane yields over 77% in the UASB. Molecular analysis showed the archaeal population in the CSTR dropped below detection and the bacterial population exhibited changes with respect to phase, while the archaeal population in the UASB remained fairly stable and the bacterial population shifted gradually. These differences likely arise from the highly selective (low-pH and low-SRT) conditions in the CSTR compared to relatively non-selective conditions in the UASB. Overall, the system handled changes in feed composition and OLR; however, food waste and corrugated cardboard were not synergetic during codigestion. Corrugated cardboard loading should be limited since it does not contribute much to methane production under these conditions. The results of this study provide new insights to evaluate the anaerobic digestion in remote locations where the treatment of food waste and corrugated cardboard is necessary.

Experimental

3. Substrates and inoculum

Synthetic food waste was prepared according to a formulation provided by Air Force scientists (personal communication, Dr. Robert Diltz, Air Force Civil Engineer Center (AFCEC/CXAE), Tyndall AFB, FL) using the following food products (% w/w, wet basis): canned pork and beans (35.6), potato flakes (7.6), and white bread (56.9). Components were homogenized in a blender and mixed, and the mixture ratios were kept constant over the whole experiment. This mixture contains starchy foods and no fresh produce to mimic transportable, calorie-dense military rations more closely than other institutional or household food waste.

Corrugated cardboard was obtained from South Dakota School of Mines and Technology campus waste, ground in a blender, and sieved to recover a fibrous powder with particle diameters between 0.35 and 2.00 mm.

Synthetic wastewater ~~ww~~ was prepared according to Organisation for Economic Co-operation and Development specifications (Table S3).[56] $NaHCO_3$ was added to provide 100 mg L^{-1} of alkalinity as $CaCO_3$, which approximates the intermediate range for wastewater ~~ww~~[57], and 1 mL L^{-1} of a trace element solution was added to supply micronutrients. The trace element solution contained (mg L^{-1}): H_3BO_3 (50), $FeCl_2{\cdot}4H_2O$ (2000), $ZnCl_2$ (50), $MnCl_2{\cdot}4H_2O$ (50), $(NH_4)6Mo_7O_{24}{\cdot}4H_2O$ (50), $AlCl_3{\cdot}6H_2O$ (90), $CoCl_2{\cdot}6H_2O$ (2000), $NiCl_2{\cdot}6H_2O$ (50), $CuCl_2{\cdot}2H_2O$ (30), $NaSeO_3{\cdot}5H_2O$ (100), EDTA (1000), resazurin (200) and 36% HCl (1 mL L^{-1}).

The anaerobic inoculum used in this study was obtained from an industrial UASB fed with brewery wastewater ~~ww~~ (Fort Collins, CO) and stored at 4 ˚C. The sludge had specific acetoclastic and hydrogenotrophic methanogenic activities of 114.2 and 1,649.7 mg COD-CH_4 g VSS^{-1} d^{-1}, respectively. Average sludge granule diameter was 716.5 µm, and coarse particles settled in DI water with a minimum velocity of 0.35 cm s^{-1}. Relevant methods are described in section S5 of the ESI.

Table 2 shows the properties of food waste, corrugated cardboard, wastewater ~~ww~~, and granular anaerobic inoculum, which were discussed previously.[6]

Table 2. Characterization of substrates and anaerobic granular sludge.

Parameter	Food waste	Corrugated cardboard	Sludge	Wastewater ~~ww~~
TS (% wb) [b]	51.5 (1.68) [c]	94.0 (0.26)	8.6 (0.40)	ND
VS	95.3 (0.08)	95.0 (0.25)	8.1 (0.02)	ND
COD (g g^{-1} wb)	0.7 (0.10)	1.2 (0.18)	ND	3.43×10^{-4}
Carbon	44.0	46.1	ND	ND
Nitrogen	2.6	0.1	ND	ND
Phosphorus	0.2	0.0	ND	ND
Cellulose	ND [d]	52.8 (0.07)	ND	ND
Hemicellulose	ND	13.2 (0.52)	ND	ND
Lignin	ND	22.2 (2.85)	ND	ND

[a] Units are % w/w dry basis unless otherwise noted; [b] wb: wet basis; [c] Values in parentheses are standard deviations; and [d] ND: not determined

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3.1. Military base waste composition

Several reports on US military base solid waste composition were surveyed to estimate yields of food waste and corrugated cardboard.[3, 4] Any values which fell outside the first and third quartiles of the collective data set were discarded. Averaging the remaining data gave a ratio of 35% food waste / 65% corrugated cardboard (COD basis). This was taken to be the standard case and the basis of the experimental design. This type of data is scarce and survey techniques may vary between studies. Additionally, the waste profile of a base depends on its size, location, tactical purpose, and maturity. Therefore, the feed composition was alternated between 35% food waste / 65% corrugated cardboard and 65% food waste / 35% corrugated cardboard.

3.2. Reactor system operation

3.2.1. Continuously-stirred tank reactor

Fig. 4 shows a schematic of the system. The CSTR was a 3 L fermentor (Applikon Biotechnology B.V.; Delft, The Netherlands) with a 1 L working volume. It was agitated continuously at 150 rpm and maintained at 35 ˚C with a heating jacket. The head plate included a plastic inlet-outlet tube with an inner diameter of 0.95 cm to accommodate large solids. The reactor was seeded with 5 g VSS L^{-1} of sludge. Feeding and effluent removal were performed manually on a daily basis with a peristaltic pump (Cole Palmer Master Flex Model 77521-40 Console drive with Model 77200-62 Easy-Load II pump head) through the inlet-outlet tube, using graduated cylinders to measure volumes of effluent decanted and influent prepared. A constant dilution rate of 0.5 d^{-1} was maintained over all phases. The influent mixture contained food waste and corrugated cardboard diluted in synthetic waste water. Biogas was collected using water displacement and corresponding methane yields were determined daily using gas chromatography. The volume of methane was calculated according to Eq. 1 and then converted to methane mass using the Ideal Gas Law assuming 1 atm and 25 °C.

$$CH_4\,Volume\,(mL) = \left(\frac{CH_4(\%)}{100}\right) * Biogas\,Volume\,(mL) \qquad \text{Eq. 1}$$

The CSTR system was acclimated for six months before the experimental trial. During this period, the CSTR was fed with 35% food waste / 65% corrugated cardboard at a HRT of 2 d and OLRs of 1, 2, and 4 g COD L^{-1} d^{-1}. A short CSTR HRT was purposely selected to determine if the acidic conditions produced by the rapid degradation of food waste would be conducive to faster cardboard degradation. For simplicity, the COD content of the wastewater ~~ww~~ was neglected in OLR calculations, since the concentration was very low compared to solid substrate loading (Table 2). ~~Methane yields and~~ pH was monitored daily. A pH below 5 and a decrease in methane yield indicated acidification.

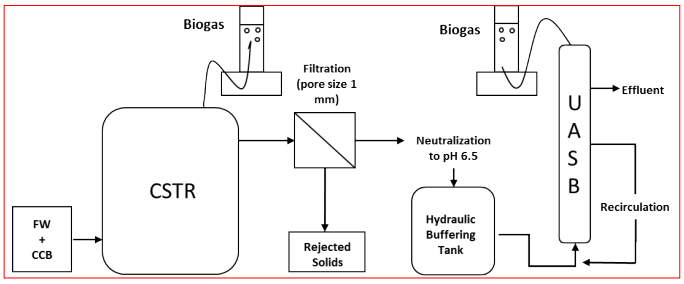

Fig. 4 Schematic of system operation. FW: food waste; CCB: corrugated cardboard; CSTR: continuously-stirred tank reactor; UASB: upflow anaerobic sludge blanket

3.2.2. Upflow anaerobic sludge blanket

The UASB was custom-blown by ChemGlass (Vineland, NJ) and had a working volume of 0.5 L, an inner diameter of 75 mm, and a working volume height of 125 mm. It operated inside of an incubator set to 35 ˚C. The reactor was seeded with 10 g VSS L^{-1} of sludge. Influent was fed into the UASB at 0.5 L d^{-1} from a hydraulic buffering tank continuously mixed and cooled with ice to prevent microbial growth, while effluent was removed by overflow. A peristaltic pump recirculated continuously to increase the upflow velocity to 1.5 m h^{-1}. Biogas production was measured using water displacement.

Before the start of the experiment, the UASB was acclimated to a solution of synthetic wastewater ~~ww~~ spiked with a 5:3:2 mixture (COD basis) of acetic, propionic, and butyric acids and adjusted to a pH of 6.5 with 1.25 g $NaHCO_3$ L^{-1} and dropwise additions of 10 M NaOH. The concentration of VFAs was adjusted to achieve OLRs of 1, 2.5, 5, 7.5, and 10 g COD L^{-1} d^{-1} at a constant HRT of 1 d. The reactor maintained a pH over 7.0 and achieved VFA conversion efficiencies over 90%, indicating a stable methanogenic population.

3.3. Experimental methods

The reactors were placed in series after the acclimation period. CSTR effluent was filtered with a mesh strainer (pore size approximately 1 mm) and neutralized by adding $NaHCO_3$ to increase the total concentration to 1.25 g $NaHCO_3$ L^{-1}, then the pH was raised to 6.5 with 10 M NaOH. The neutralized effluent was poured into the hydraulic buffering tank and fed continuously into the UASB.

To study the effects of feed composition and OLR on system performance, each of these variables was varied one-by-one in sequence. Table 3 lists the experimental conditions tested. HRTs were held constant at 2 d for the CSTR and 1 d for the UASB. The system was operated for at least 20 d at each loading condition to reach a quasi-steady state. Gas production and pH were measured daily; VFA and sCOD concentrations were measured thrice weekly; and alkalinity, ammonia, particulate solids, and biogas methane content were measured weekly for both reactors.

3.4. Analytical methods

Total solids (TS) and volatile solids (VS) were determined gravimetrically, ammonia was measured using an electrode (Thermo Scientific Orion; Waltham, MA), alkalinity was quantified by titration,

and COD was measured using a closed-reflux colorimetric method, all according to Standard Methods.[58] One day per week, screened solids from the CSTR were dried at 105 ˚C for 48 h and weighed to measure the flow of solids. The average COD content of the digested solids was 1.25 g COD g TS^{-1}. Methane contents of reactor headspaces and VFA concentrations were determined using a gas chromatograph (Agilent 6890; Santa Clara, CA) with a flame ionization detector (GC-FID); GC-FID methodology is described by Asato et al.[6] Liquid digestate samples collected thrice-weekly from both reactors were centrifuged at 13,000 rpm for 10 min and analyzed for soluble COD (sCOD) and C2-C5 VFAs. Total COD (tCOD) of the filtered CSTR effluent was measured to quantify small particulate solids escaping the screening step. UASB effluent contained negligible non-soluble COD, so tCOD was not measured until Phase V.

Biomass samples collected at the end of each phase were centrifuged at 14,000 x *g* for 10 min, and DNA was extracted from 500 mg of the pelleted solids using FastDNA SPIN kit for Soil (MP Biomedicals; Santa Ana, CA) per manufacturer instructions. Molecular analysis was performed by MR DNA (Shallowater, TX) using 16S-based tag-encoded FLX amplicon pyrosequencing (TEFAP) [59]. PCR was performed with a HotStarTaq Plus Master Mix Kit (Qiagen; Valencia, CA) to amplify the 16S rRNA sequences, using the bacterial primer 27F and the archaeal primer 349F. The amplification was performed in a single step under the following conditions: 94 ˚C for 3 min (one cycle); 94 ˚C for 30 sec, 53 ˚C for 40 sec, and 72 ˚C for 1 min (28 cycles); and 72 ˚C for 5 min (final elongation). PCR products were purified with Agencourt Ampure beads (Agencourt Bioscience Corporation; Danvers, MA), then sequenced using Roche 454 FLX titanium instruments and reagents (Roche; Basel, Switzerland) per manufacturer specifications.

Taxonomic analysis was conducted using the MR DNA (Shallowater, TX) proprietary analysis pipeline. Barcodes, primers, fragments under 200 bp, sequences with ambiguous bases, and sequences with homopolymer runs over 6 bp were culled. The remaining sequences were denoised and clustered into operational taxonomic units (OTUs) with a criterion of 97% similarity. Singleton sequences and chimeras were removed. OTUs were identified by comparison with GreenGenes, RDP-II, and NCBI databases [60-62] via BLASTn, and then compiled into taxonomic levels. Relative abundances were calculated as percentages of total counts in a sample. Taxa comprising less than 1% of all samples were removed.

3.5. Data analysis

COD balances for the CSTR were performed by normalizing daily flows of methane, VFA, and complex COD by the influent COD. Methane and VFA were converted to COD bases. VFA concentrations were subtracted from sCOD measurements to obtain fractions of non-VFA sCOD. Particulate solids <1 mm were determined by subtracting sCOD from tCOD. Flows of particulate solids >1 mm were calculated by converting the screened solids measurements to a COD basis. COD balances for the UASB were performed similarly, except the influent COD was calculated from the measured tCOD content of the CSTR effluent (including VFA, non-VFA sCOD, and particulate solids <1 mm). Methane, VFA, and non-VFA sCOD flows were determined as described above and normalized by the feed OLR.

Table 3. Experimental conditions during different phases.

Phase	CSTR OLR (g COD L^{-1} d^{-1})	Food waste (% COD)	Corrugated cardboard (% COD)
I	8	35	65
II	8	65	35
III	16	65	35
IV	16	35	65
V	32	35	65

[a]HRT in the CSTR was constant at 2 d, and HRT in the UASB was constant at 1

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This material is based upon work supported by the Air Force Civil Engineer Center (AFCEC), Tyndall AFB, FL [Contract No. FA4819-14-C-0004].

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