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To cite this article: Q Y Wang *et al* 2018 *IOP Conf. Ser.: Earth Environ. Sci.* **186** 012042

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Anaerobic co-digestion of wastes from fruit processing and activated sludge reactor in juice production industry

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Abstract. Anaerobic co-digestion of wastes from fruit processing wastes (FPW) and activated sludge reactor (WAS) were studied in a semi-continuous digester at 30-32 °C during 193 days. The best results of biogas yield and volumetric biogas production were 635.4 L·kg⁻¹ total volatile solids (TVS) fed and 473.9 L·m⁻³·d⁻¹, respectively, obtained when FPW:WAS ratio applied was 1:1 and retention time was 40 days. Biogas yield and volumetric biogas production increased 210% and 357%, respectively, when FPW:WAS ratio was changed from 0:1 to 1:1; and hydrolysis was also improved since TVS removal efficiency achieved nearly 75%. The microbiological analysis of samples taken at day 70, 135 and 168 supported these results with increased FPW:WAS ratio. Both the relative abundance of methanogens and hydrolytic bacteria were observed to increase significantly; in the case of hydrolysis, the percentage of *Chloroflexi* relative abundance increased from 17% at day 70 to 50% at day 168.

1. Introduction

Apple juice has been an important beverage product across the world in recent decades. Large quantities of wastewater and fruit processing wastes (FPW) produced by the fruit juice industry should be disposed. The wastewater can be treated by biological and chemical processes, but extra care also needs to be taken in the disposal of the waste activated sludge (WAS) from the own industrial wastewater treatment plants (WWTP). Previous work showed that WAS can be co-digested with fruit seeds and skin screened from the industrial wastewater in lab-scale reactors,[1] however, so far there is still a lack of studies over co-digestion of FPW and WAS in pilot- or full-scale digesters. The current method for the industrial disposal of FPW and WAS is by landfill or composting. Since these methods have adverse impacts, such as environmental pollution, waste of biological resources and energy, a promising alternative can be applying anaerobic digestion.

However, there are some disadvantages of using one-phase anaerobic digesters for a substrate alone, like WAS or FPW. On the one hand, it should be considered that concentrated nitric acid is used in the juicing process for pickling, thus, being discharged into aerobic sludge tanks. Therefore, reduction of nitrate in the excess WAS discharged into the anaerobic digester can lead to an accumulation of ammonium and consequently, inhibit the methanogens. On the other hand, the high carbohydrate content of FPW can lead to the rapid production of volatile fatty acids (VFA) that can accumulate, resulting in a rapid pH decrease and inhibition of methanogenic activity. In order to reduce the effect



of acidification and inhibition of microorganisms, different types of wastes are often mixed for co-digestion.[2] Co-digestion is a conventional method to eliminate possible toxic inhibition from intermediates produced during the processes, which therefore, may result in increased digestion capacity and methane production. Substrates with large amounts of easily biodegradable materials can be more easily digested together with another substrate with low carbon/nitrogen (C/N) ratio and high alkalinity.[3] In addition, co-digestion can reduce the impact of seasonal fluctuation of feedstock and keep the digester stable.

In the present study, semi-continuous pilot-scale digestion of WAS alone was firstly investigated and then, the effect of FPW supplementation was explored. The rates of biogas production and total volatile solid (TVS) removal were analyzed to evaluate the possible optimization of the operational parameters. The microbial communities at different digestion stages were also compared to evaluate their development during the process of co-digestion.

2. Material and methods

2.1 Feedstock and inoculum

Both FPW and WAS were collected from fresh fruit juice company. And the inoculum was obtained from a mesophilic upflow anaerobic sludge bed (UASB) reactor treating the fruit-processing wastewater at the same company. The pilot digester was filled with the inoculum sludge (100 L containing $72 \text{ kgVSS} \cdot \text{m}^{-3}$) resulting in a concentration of $9 \text{ kgVSS} \cdot \text{m}^{-3}$.

2.2 Bioreactor design and operating protocol

The experiments were carried out in a stainless steel digester system with a working volume of 0.8 m^3 . The digester was surrounded by insulated cotton fastened by aluminum sheet to maintain the temperature. Integrated control system of automatic heating and recirculation pump was applied in the whole process. pH was monitored on line, without agent addition.

Table 1. Characteristics of reactor influent wastes and operating factors.

Stage Parameter	1	2	3	4	5	6	7
Period (days)	1-27	28-58	59-75	76-92	93-135	136-168	169-193
FPW:WAS (in TS)	0:1	0:1	0:1	0.11:1	0.33:1	1:1	1:1
Daily Feeding Volume (DFV) ($\text{L} \cdot \text{day}^{-1}$)	10	15	20	15	20	20	26
Retention Time (RT) (days)	80	53	40	53	40	40	32
pH		7.07		6.10	5.47		4.98
COD ($\text{mg} \cdot \text{L}^{-1}$)		28,560		30,704	33,920		36,215
Total Solids (TS) ($\text{g} \cdot \text{L}^{-1}$)		41.9		42.8	40.3		41.5
TVS ($\text{g} \cdot \text{L}^{-1}$)		22.3		24.1	26.6		30.9
$\text{NH}_4^+ \text{-N}$ ($\text{mg} \cdot \text{L}^{-1}$)		229.0		221.1	184.3		139.5
C/N		9.2		10.0	11.5		15.5

The experimental period was divided in two phases and seven stages (Table 1). The FPW:WAS ratio of each stage was based on TS-of the sludges and the mixing of both in the feedstock tank was calculated to result in mass proportions according to the established stages. The proportions were measured by the correspondent volumes of the two sludges considering their different TS concentrations, resulting in seven DFV. The pH, nitrogen ammonium and biogas volume were measured daily. VFA levels, alkalinity, TS and TVS of the effluent were measured every two days.

2.3 Microbial molecular ecology

Approximately 0.5 g centrifuged sludge samples were collected in triplicate to analyze the taxonomic composition and relative abundance. Genomic deoxyribonucleic acid (DNA) were extracted and

amplified and purified PCR products of the archaeal and bacterial 16S rRNA genes were subsequently sequenced on a MiSeq sequencing platform (Illumina Inc, California, USA).

3. Results and discussion

3.1 Reactor operation results

The anaerobic pilot-scale digester was operated for over 6 months (193 days); from the reactor start-up period until the end, despite the short term of each of the seven stages, the reactor operation showed apparent stability and good performance. VFA levels ranged from 2.63 to 5.45 mmol·L⁻¹ and alkalinity ranged from 27.76 to 55.99 mmol·L⁻¹, thus, providing high buffering capacity for digestion system. The results of biogas yield and volumetric biogas production (VBP) during the experimental period are shown in Figure 1. They are correlated with organic loading rate (OLR), FWP:WAS ratios and RT applied in the different stages, together with pH and VFA/alkalinity ratios (α) of the effluent.

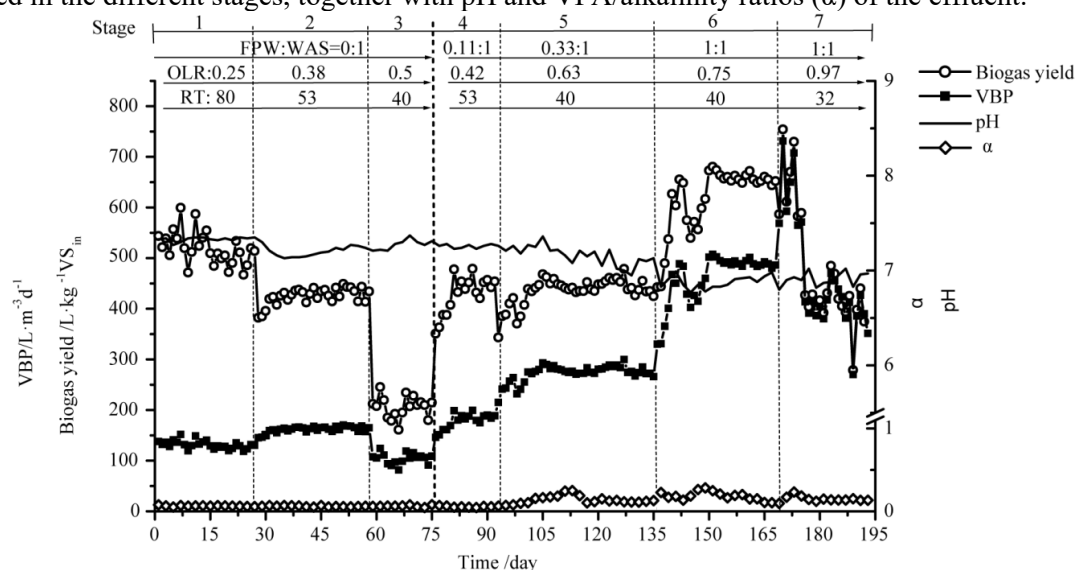


Figure 1. Results of biogas yield (L·kg⁻¹ TVS) and VBP (L·m⁻³·d⁻¹), and pH and VFA/alkalinity ratio (α) of the effluent, as a function of the FWP:WAS ratio, OLR (kg TVS·m⁻³·d⁻¹) and RT (day) in Phase I (stages 1 to 3) and Phase II (stages 4 to 7) of the experimental period.

In Phase I, during the two first stages of digestion of WAS alone, increasing the OLR from 0.25 to 0.38 kg TVS·m⁻³·d⁻¹, VBP increased by 24%, from 131.5 to 163.1 L·m⁻³·d⁻¹. In the case of biogas yield, the increase of OLR resulted in direct decrease from stage 1 to 3 (519.1 to 204.7 L·kg⁻¹ TVS). These values of biogas yield are not as high as found in some previous studies with co-digestion of mixed fruit and vegetable wastes. [4] However, it can still be considered as an important expected result, since digestion of WAS alone is, in fact, considered as of low biogas production, generally lower than 200 L·kg⁻¹ TVS. [5] In Phase II, the co-digestion of FPW and WAS was performed from day 76 to 193 (stages 4 to 7), and both VBP and biogas yield increased until stage 6 when applied OLR and RT were 0.75 kg TVS·m⁻³·d⁻¹ and 40 days, respectively. The biogas yield was significantly improved from 433.7 L·kg⁻¹ TVS to 635.4 L·kg⁻¹ TVS, respectively, with RT decreasing from 53 to 40 days (stage 4 to 6).

The good performance of the digester in the stages 4 to 6 even at high OLR can be explained firstly, by the use of appropriate C/N ratios (10.0 to 15.5), corresponding to ratios of FWP:WAS from 0.11:1 to 1:1, respectively (Table 1). Feedstock with low C/N ratio could cause accumulation of too much ammonia, increasing pH and resulting in inhibition of methanogens in the reactor. The co-substrate mixture with suitable C/N ratio was benefit for the improvement of biogas yield and in accordance with applying higher OLR. The highest biogas yield was achieved in stage 6 when the C/N ratio was

15.5 This occurrence was similar to that of Mshandete who observed the highest methane yield, obtained in the co-digestion of fish waste and sisal pulp, when the C/N ratio was 16. [6] Secondly, an increase of the TVS/TS ratio of the mixed wastes could be beneficial for process improvement; apparently, the organic fraction of FWP is more easily degradable than that of WAS.

The pH and VFA/alkalinity ratio (α) fluctuations inside the digester showed that the co-digestion system was well buffered. Changing the composition of the feedstock or increasing the values of OLR from stage 1 to stage 7, did not result in great variation of the effluent pH. The values were maintained between 6.89 and 7.32. Because of the addition of FPW, the range of α in the co-digestion process, especially in stages 5, 6 and 7, was clearly different from those of the stages with the digestion of WAS alone. The ratio VFA/alkalinity (α) is a sensitive parameter which is more appropriate than pH as a parameter to monitor the buffering capacity [7], and should be maintained between 0.1 and 0.35 for steady operation. Although the influent pH decreased more sharply during the days 76 to 193 (6.10 to 4.98), the ratio α of the effluent was still below 0.3, indicating that the degradation process was stable without the risk of acidification.

The results of TVS removal efficiency correlated with the RT is shown in Figure 2.

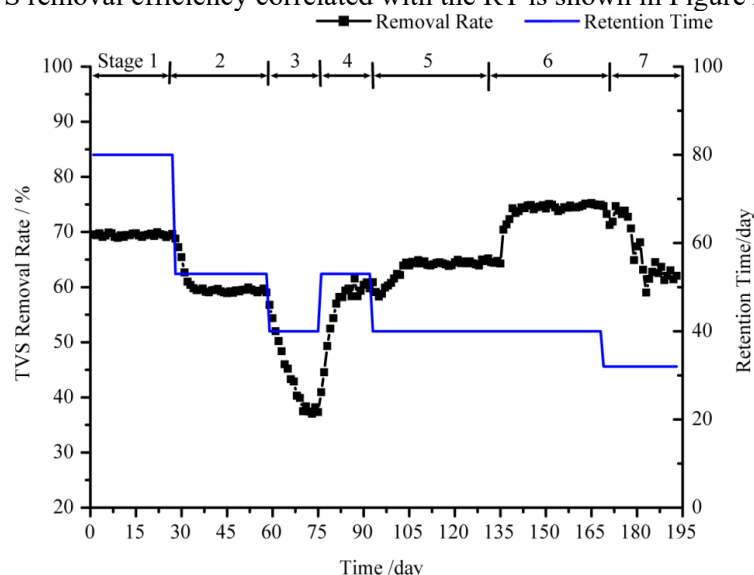


Figure 2. Results of TVS removal efficiency (%) correlated with the retention time (day) in the different stages of the experimental period.

In Phase I, the average TVS removal efficiency reached 69% and 59% at a RT of 80 (stage 1) and 53 days (stage 2), respectively. Decreasing RT, the efficiency decreased significantly, but the concentrations of soluble COD and VFA in stage 3 were still low, at levels of no more than $1000 \text{ mg} \cdot \text{L}^{-1}$ and $3.0 \text{ mmol} \cdot \text{L}^{-1}$, respectively. In Phase II, the efficiency varied during the co-digestion stages (4 to 7) when both sludges were fed at different ratios. In stage 4, when the applied ratio was 0.11 to 1, the efficiency increased to 58% compared with the 38% removal efficiency of stage 3. This increase could also have occurred because in stage 4 the OLR was lower ($0.42 \text{ kg TVS} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$) and RT was higher (53 days) than those of stage 3 ($0.50 \text{ kg TVS} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$ and 40 days, respectively). However, when the FWP:WAS ratio was increased 0.33:1 in stage 5 with a higher OLR of $0.63 \text{ kg TVS} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$ and RT lowered to 40 days, the efficiency increased to 65%. In stage 6, when the ratio was increased even more to 1:1, and applying an OLR of $0.75 \text{ kg TVS} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$ with RT maintained at 40 day, the efficiency reached 75%. Therefore, it is clear that the addition of FPW enhanced the hydrolytic rate of the organic solid wastes and led to an improved TVS removal efficiency in the digester. However, keeping the same feeding ratio of 1:1, but decreasing the retention time to 32 days in stage 7, with a high OLR of $0.97 \text{ kg TVS} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$, the removal efficiency decreased to approximately 62%. This was most likely due to insufficient time for the hydrolysis of the feeding, especially because of the high proportion of WAS.

3.2 Statistical comparisons of microbial communities

The digester sludge samples were collected and analyzed to identify the microbial community at day 70 (Phase I, stage 3), day 135 (Phase II, stage 5) and day 168 (Phase II, stage 6). All the three stages were under a RT of 40 days. In general, the Shannon Index was negatively correlated with the FPW/WAS ratios, which were 4.42, 4.85 and 5.49 at a FPW/WAS ratio of 0:1, 0.33:1 and 1:1, respectively. Therefore, the addition of FPW was beneficial for the increase of microbial community diversity.

As the digestion progressed, *Methanosaeta* became the dominant methanogens at the genus level, with their relative abundance increasing from 28.99% at day 70 to 43.76% and day 168 (Table 2). *Methanosaeta* were the predominant acetoclastic methanogens as their substrate constant was lower than that of *Methanosarcina*, and they could benefit from the low VFA concentration during the digestion process.[8] This illustrated that the conversion of acetic acid was the main pathway for methane production. The proportion of archaea relative to the total number of microbes (the sum of archaea and bacteria) was 3.01% at day 70, increasing to 5.63% at day 135 and 7.93% at day 168; these increases corresponded with a remarkable increase in biogas production (Figure 1).

Table 2. Taxonomic compositions of methanogens at the genus level and relative abundance in the sludge samples.

Methanogens genus FPW: WAS	Relative Abundance (%)		
	Day 70 (0:1)*	Day 135 (0.33:1)	Day 168 (1:1)
<i>Methanolinea</i>	29.55	26.66	14.71
<i>Methanobacterium</i>	11.87	10.92	4.42
<i>Methanosaeta</i>	28.99	34.14	43.76
<i>Methanosarcina</i>	9.51	6.18	1.3
<i>Methanoregula</i>	9.52	12.65	17.69
<i>Methanomassiliicoccus</i>	5.53	4.77	9.18
other	4.42	3.89	7.6

*FPW:WAS ratio

The results in Table 3 show that *Chloroflexi*, *Bacteroidetes*, *Proteobacteria* and *Firmicutes* were the four major phyla found in bacterial communities. At the genus level, *Sulfurovum* (Phylum *Proteobacteria*), which are autotrophic denitrifiers that improve sulfur and nitrogen removal rather than organic solid hydrolysis, made up 46.54% of all bacteria at day 70. In Phase I, during the digestion of WAS alone the concentration of nitrate and the production of sulfide were high during the process; therefore, the competitive effect of *Sulfurovum* on the carbon source led to a reduction in the decomposition of organic compounds into acetate, causing a decrease in the yield of biogas (stage 3, Figure 2). At day 168, *Sulfurovum* declined to 8.32%, while *Leptolinea* and unclassified *Anaerolineaceae* (phylum *Chloroflexi*) increased to 10.03% and 36.99% of the total bacterial population, respectively. The increase of *Chloroflexi* from 17.32% to 49.95% improved the hydrolysis reaction, which resulted in an increase of the TVS removal rate (stage 6, Figure 2).

Table 3. Taxonomic compositions of bacterial communities at the phylum and genus levels and relative abundance for sequences retrieved from sludge samples (only genera with relative abundances higher than 0.5% in at least one sample are listed).

Phylum	Genus	Relative abundance (%)		
		Day 70	Day 135	Day 168
<i>Proteobacteria</i>	<i>Sulfurovum</i>	46.54	37.14	8.32
	<i>Acidovorax</i>	1.47	1.91	2.84
	<i>Pseudomonas</i>	0.13	0.09	2.38
	<i>Psychrobacter</i>	0.26	0.09	1.14
	<i>Arcobacter</i>	0.00	0.01	1.17

	<i>Rhodobacter</i>	0.38	0.67	0.39
<i>Bacteroidetes</i>	<i>vadinBC27</i>	3.23	1.99	1.42
	<i>Owenweeksia</i>	1.07	0.45	0.45
	<i>Paludibacter</i>	0.71	0.37	0.69
<i>Chloroflexi</i>	<i>Leptolinea</i>	1.95	3.20	10.03
	<i>Longilinea</i>	0.79	1.26	3.72
	<i>Anaerolinea</i>	0.42	0.68	1.22
	<i>Sedimentibacter</i>	1.24	1.38	0.98
	Unclassified	12.87	20.20	36.99
	<i>Anaerolineaceae</i>			
<i>Firmicutes</i>	<i>Cryptanaerobacter</i>	0.34	0.74	1.28
	<i>Syntrophomonas</i>	0.26	0.35	0.8
Others		6.68	8.82	9.26

4. Conclusions

The co-digestion of WAS and FPW can overcome the low biodegradability and the low C/N ratio of WAS, and has potential application in the recycling of fruit juice production wastes. The WAS digestion performance was improved by adding FPW at total OLR up to $0.75 \text{ kgVS} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$, with TVS removal efficiency of 74.56%. The optimum operating conditions at all stages, in terms of efficiency and stability, were found at a volumetric ratio of 1:1 (FPW/WAS). The volumetric biogas production and biogas yield under these conditions reached $473.9 \text{ L} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$ and $635.4 \text{ L} \cdot \text{kg}^{-1} \text{TVS}_{\text{in}}$, respectively, corresponding to a retention time of 40 days. When the FPW/WAS ratio was changed from 0:1 to 1:1, the hydrolytic efficiency improved and the TVS removal efficiency increased from 43.83% to 74.56%, which can be attributed to an increase number of *Chloroflexi* and decrease in bacteria of the genus *Sulfurovum*. The biogas production efficiency was not inhibited by the slight increase of VFA from acetogenesis, due to an increase in the relative abundance of *Methanosaeta* and other archaea in the microbial community.

Acknowledgements

This work was supported by the Science and Technology Innovative Program of Shaanxi Province (2011KTZB03-03-01) and Key Research and Development Program of Shaanxi Province of China (2017GY-168)

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