## Global Warming -2020: Extended Abstract Title: Optimization of Anaerobic Co-digestion of Multiple Feedstocks for Biomethane Recovery

Anahita Rabii<sup>1\*</sup>, Saad Aldin<sup>1</sup>, Yaser Dahman<sup>2</sup>, Elsayed Elbeshbishy<sup>1</sup>

<sup>1</sup>Civil Engineering Department, Ryerson University, 350 Victoria St., Toronto, Ontario, Canada M5B 2K3,

<sup>2</sup>Chemical Engineering Department, Ryerson University, 350 Victoria St., Toronto, Ontario, Canada M5B 2K3

\* Corresponding Author: anarabii@ryerson.ca

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#### **Abstract**

Anaerobic co-digestion of organic waste has attracted attention as a promising technology for waste management and biogas recovery. Several parameters need to be considered for the proper operation of this technology including the feedstock selection and their ratios. This research was aimed to investigate the influence of mixing and lipids: proteins: carbohydrates ratios on biomethane production in anaerobic co-digestion of thickened waste activated sludge (TWAS), manure and source separated organics (SSO). The digestion reactors operated in batch mode under hemophilic condition. The results showed that the maximum methane yield was 356 mL CH<sub>4</sub>/g COD<sub>added</sub> corresponding to TWAS: manure: SSO mixing ratio of 2:4:4 and lipids: proteins: carbohydrate ratio of 1: 3.5: 18.5. In comparison, 134, 299, and 332 mL CH<sub>4</sub>/g COD<sub>added</sub> were obtained by mono digestion of TWAS, manure, and SSO. The trend of the methane yield variations in response to the COD: N and to the lipids: proteins ratios relatively conform to each other excluding some of the ratios. On the contrary, the methane yields demonstrated different responses to the ratios of lipids: carbohydrates and proteins: carbohydrates compared to COD: N ratios. Synergistic effect increased the methane yield by 19% in co-digestion of TWAS/manure/SSO.

Keywords: Biomethane Potential, Manure, Thickened Waste Activated Sludge, Mixture Ratio

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## INTRODUCTION

Anaerobic Co-digestion (AnCoD) involves the simultaneous anaerobic digestion (AD) of two or more organic waste feedstocks. Conventionally, anaerobic digestion was a single substrate and single purpose treatment. Recently it has been indicated by applying various substrates at the same time more process stability is achieved. The usage of co- substrates usually improves the biogas yields from anaerobic digester due to positive interaction established in the digestion medium and the supply of missing nutrients by the co-substrates (Kangle et al., 2012; Mata-Alvarez et al., 2011). Anaerobic co-digestion offers several benefits including: improved nutrient balance and digestion, possible gate fees for waste treatment, additional biogas collection, and additional fertilizer i.e. soil conditioner (Elbeshbishy and Nakhla, 2012; Viotti et al., 2004).

The main goal of anaerobic co-digestion is to increase biogas mainly biomethane for heat and electricity. A range of feedstocks can be co-digested at suitable blend ratio to maintain optimum condition required for metabolic activity and improved biogas production for heat and electricity. Feedstocks characterized by higher C:N ratio (>50) such as rice and wheat straws, corn stalks, seaweed and algae can be co-digested by the feedstocks of lower C/N ratio for instance pig manure, poultry manure, food and kitchen wastes to achieve nutrient balance and to avoid the inhibitions which leads to system instability and reduced biogas production as a result of unsuited C:N ratio (Hagos et al., 2017; R et al., 2017; Sosnowski et al., 2003).

In order to attain an improved co-digestion process, some precautions and suitable procedures are necessary. There may be requirements for supplementary digester equipment depending on the size of the operation, quality of waste, and characteristics of the wastes to be co-digested. Mainly, precautions or supplementary equipment would be required for: homogenization and mixing of co-substrates, delivery of the waste, prevention of excessive foaming and scum layer formation, and removal of sediments from the digester.

Multiple aspects are considered when applying AnCoD, cost of transporting the co substrate from the generation point to the AD plant seems to be the most common consideration, the selection of the best co-substrate and blend ratio in order to enhance synergism, dilute disruptive compounds, optimization of methane production and digestate quality, are also important consideration plants evaluate when using the AnCoD (Divya et al., 2015; Mata-Alvarez et al., 2014). The existing work aims to investigate the impact of feedstocks mixing ratio and lipids: proteins: carbohydrates ratio on anaerobic co-digestion of TWAS, manure and SSO.

# MATERIAL AND METHODS

## Substrates and inoculum

TWAS and inoculum were obtained from Ashbridges Bay Wastewater Treatment Plant. The Ashbridges Bay Wastewater Treatment Plant is the main sewage treatment facility in the city of Toronto, Ontario which treats 818000 m<sup>3</sup>/d of wastewater using conventional activated sludge system. Cow manure was collected from a manure pit of a dairy farm located in Newmarket, Ontario. Manure slurry was prepared by addition and homogenization of cow manure with deionized distilled water using a blender. The reactors were fed with the manure slurry immediately after preparation and fibrous solids separation. Source separated organics were collected from Disco Road Organic Processing Facility, City of Toronto, Ontario. The SSO

samples were homogenized before feeding the digesters. Different combinations of the feedstock mixtures were used as feed to digesters. The characteristics of the feed in each digester with are summarized in Table 1.

# **Experimental set up**

This research investigated the influence of feedstocks mixing ratios and their relashonship with the lipids: proteins: carbohydrates ratios on biomethane production in anaerobic co-digestion of TWAS/manure/SSO using biomethane potential assay (BMP). The BMP was conducted according to the procedures described in the literature (Elbeshbishy and Nakhla 2012). The food to microorganism ratio (F/M) is one of the main parameters in anaerobic assays (Angelidaki et al., 2009; Martinez-Jimenez et al., 2017). F/M ratio was kept around 2 and was calculated by dividing the g total COD (TCOD) of the substrate to the g VSS of the inoulum (Pellera and Gidarakos, 2016).

Manure slurry with SSO and TWAS was fed to the reactors in different combinations in triplicates. Control reactors containing TWAS, manure, and SSO individually were also used in triplicates. The characteristics of the feed in each digester with different mixing ratios of the substrates for the average of three measurement of each parameter are summarized in table 1.

The batch reactors operated in working volume of 200 ml in mesophilic condition at 37 <sup>°C</sup> in a pH range of 7-7.4 for 72 days. Gas volume and composition in the headspace of the reactors were monitored and recorded daily using a gas-tight micro syringe and TRACE 1310 Thermoscientific Gas Chromatograph. The analysis of total solids (TS), volatile solids (VS), total suspended solids (TSS) and volatile suspended solids (VSS) were measure according to the Standard Methods for the Examination of Water and Wastewater (APHA, 2005). pH was measured using Accument AB15 pH meter. Chemical oxygen demand (COD), alkalinity, ammonia, nitrite, nitrate, total nitrogen (TN), and total soluble nitrogen (TSN) were measured by colorimetric method using DR3900 Hach Spectrometer and the procedures outlined by Hach.

#### **Extended** Abstract

		TWAS	Manure	SSO	T*/M**/SSO 8/1/1	T/M/SSO 1/8/1	T/M/SSO 1/1/8	T/M/SSO 5/2.5/2.5	T/M/SSO 2.5/5/2.5	T/M/SSO 2.5/2.5/5	T/M/SSO 4/4/2	T/M/SSO 2/4/4	T/M/SSO 4/2/4
Parameters	Units	Mixture (1)	Mixture (2)	Mixture (3)	Mixture (4)	Mixture (5)	Mixture (6)	Mixture (7)	Mixture (8)	Mixture (9)	Mixture (10)	Mixture (11)	Mixture (12)
TCOD	g/L	40	100	109	53	95	101	72	87	89	78	71	79
SCOD	g/L	1.4	42	41	9	38	37	21	32	31	26	25	25
TSS	g/L	31	52	62	37	51	58	44	50	52	46	42	48
VSS	g/L	26.5	45.4	47.0	30.4	43.7	44.8	36.3	41.1	41.5	38.2	33.2	38.5
TS	g/L	38.9	67.8	67.0	44.6	64.8	64.3	53.2	60.4	60.2	56.1	48.1	55.9
VS	g/L	35.2	55.6	49.6	38.6	52.9	48.7	43.9	49.0	47.5	46.2	38.0	45.0
Ammonia	g/L	0.2	0.0	1.3	0.3	0.2	1.1	0.4	0.4	0.7	0.4	0.6	0.6
рН	-	7.0	7.0	7.2	7.1	7.0	7.0	7.1	7.1	7.1	7.0	7.0	7.1
Alkalinity	g CaCO <sub>3</sub> /L	1.9	5.2	6.2	2.7	5.0	5.7	3.8	4.6	4.9	4.1	3.9	4.3
TN	g/L	2.8	2.1	4.0	2.8	2.4	3.7	2.9	2.7	3.2	2.7	2.6	3.1
TSN	g/L	0.4	0.1	1.0	0.4	0.2	0.8	0.5	0.4	0.6	0.4	0.5	0.6
Total carbs	g/L	1.5	27.8	14.1	5.4	23.8	14.2	11.3	17.8	14.4	14.6	11.5	11.8
Total proteins	g/L	3.9	5.8	2.4	3.9	5.2	2.9	4.0	4.5	3.6	4.3	2.9	3.7
Total lipids	g/L	0.4	1.4	1.5	0.6	1.3	1.4	0.9	1.2	1.2	1.0	0.9	1.0

Table 1. Characteristics of feed to digesters with different mixing ratios of TWAS/ manure/SSO

## **RESULTS AND DISCUSSION**

## Anaerobic substrate biodegradation/ Biomethane production

Operation of the digesters carried on until no significant amount of biogas was produced. Fig.1 shows the profile of the cumulative biomethane production versus time during the co-digestion of TWAS, manure and SSO including the control rectors. Among the control digesters, SSO produced the most cumulative methane while TWAS resulted in the lowest amount of cumulative methane production. SSO alone produced more cumulative methane than manure alone in the control reactors. All co-digesters produced more biomethane than the control reactors containing only TWAS.

SSO alone produced 13% more methane than manure alone. The amounts of ultimate CH<sub>4</sub> obtained by single digestion of SSO and manure were higher than that of TWAS alone by 3.2 and 2.9 fold. Figure 1 shows cumulative methane production during the digestion period. As illustrated in figure 1, all of the co-digesters generated higher amounts of CH<sub>4</sub> than the control reactors containing only manure. The reactors with the mix of the three feedstocks at the ratios of 8/1/1 and 5/2.5/2.5 produced more methane than TWAS alone however, they did not show any improvement in comparison with single digestion of manure and SSO. Other combinations produced more methane than TWAS and SSO alone, although only the three of them with the mixing ratios of 2.5/2.5/5, 4/2/2, and 4/2/4 resulted in higher cumulative CH<sub>4</sub> production comparing to all of the control reactors. The maximum cumulative methane production of 1424 mL corresponded to the TWAS/manure/SSO mixing ratio of 2/4/4 and 1:4:15 ratio of lipids: proteins: carbohydrates.

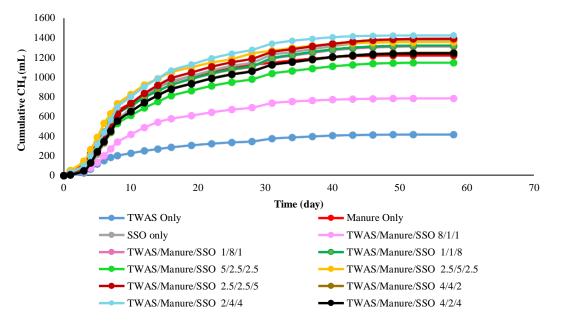


Figure 1. Cumulative methane production for different mixing ratios of TWAS/manure/SSO

## Cumulative methane yields

Figure 2 shows the cumulative methane yields of the digesters with different units including mLCH<sub>4</sub>/g TCOD added, mLCH<sub>4</sub>/g VSS added, and mLCH<sub>4</sub>/mL substrate added are presented in. The cumulative methane yield per mass COD of substrate added were 134, 299, and 332 mLCH<sub>4</sub>/g TCOD added for the control reactors digesting only TWAS, manure, and SSO, respectively. As shown in Figure 2, the lowest and the highest yield corresponded to mono digestion of TWAS and TWAS/manure/SSO mixing ratio of 2/4/4, respectively. The amounts of the biomethane yields ranged from 228 to 356 mLCH<sub>4</sub>/g TCOD added in the co-digesters. CH<sub>4</sub> yield increased by 165%, 19% and 7% compared to single digestion of TWAS, manure, and SSO, respectively.

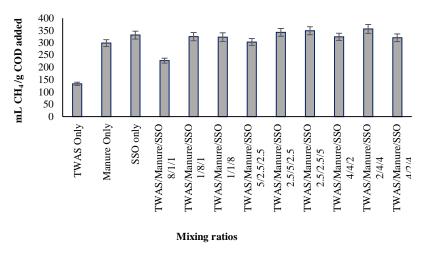


Figure 2. Methane yields per unit mass TCOD added at different mixing ratio of TWAS and manure

The reason would be that the balance of the nutrient with the microbial communities was not necessarily ideal for methanogenic populations in the reactors containing the mix of the three feedstocks at all of the mixing ratios.

## Synergistic effect

In anaerobic digestion, generation of biomethane occurs due to a syntrophic metabolism between methanogenic microbial communities which consists of bacteria and archaea (Viotti et al., 2004). It is verified that both communities of bacteria and archaea are present in AnCoD systems. An improvement in the synergy and diverse microbial consortia is obtained when applying co-digestion of multiple feedstocks (Zamanzadeh et al., 2017). The synergistic effect of co-digestion can be estimated as an additional methane production (mL) for co-substrates over the weighted average of the methane production of individual substrates (Parra-Orobio et al., 2016). Synergistic effect was obtained by calculating the percentage of additional methane yield achieved in co-digestion by dividing the measured yields, over the weighted average of the methane yield of individual substrates per unit volume of substrate added. Synergistic effect was calculated using Eq. 1.

**Extended** Abstract

Weighted MP= MP<sub>manure</sub> \* 
$$P_{manure}$$
 + MP<sub>TWAS</sub> \*  $P_{TWAS}$  + MP<sub>SSO</sub> \*  $P_{SSO}$  Eq. (1)

Where weighted MP is the weighted average of methane production for co-substrates (mLCH<sub>4</sub>);  $MP_{manure}$ ,  $MP_{TWAS}$ , and  $MP_{SSO}$  are the experimental methane yield (mLCH<sub>4</sub>/mL substrate added) for manure, TWAS and SSO; and  $P_{manure}$  and  $P_{TWAS}$ , and  $P_{SSO}$  are the volume (mL) of manure, TWAS, and SSO in the substrates mixture, respectively. When the percentage difference between experimental methane production for the mixtures and the calculated weighted average of methane production was positive, the synergistic effect could exist.

Figure 3 shows the percentage improvement of biomethane production due to the synergistic impact. As demonstrated in Fig 3, in the ternary co-digestion of TWAS/manure/SSO, the most synergetic impact corresponds to the the mixing ratio of 2/4/4. No significant improvement due to synergy was observed at the mixing ratio of 1/1/8. The maximum synergistic effect that was achieved by this experiment was 19 % which was lower than the result obtained by some of digesters in the binary co-digestion.

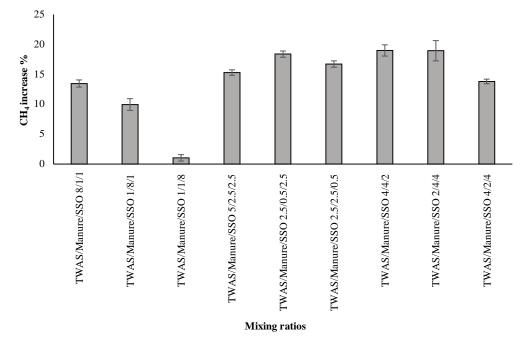


Figure 3. Percentage of CH4 increase due to synergistic effect at different mixing ratios of manure and SSO

#### **COD:N and Lipids: Proteins: Carbohydrates ratios**

Table 2 presents the COD:N ratios, lipids: proteins: carbohydrates ratios, the ultimate methane production and the methane yield per unit mass of COD added at different mixing ratios of the feedstocks. The COD:N ratios of TWAS, manure and SSO were 15, 47, and 27 respectively. For the co-digesters, COD:N varied from 19 to 40. The lipids: proteins: carbohydrates ratios were 1:10:4, 1:4:20, and 1:1.6:9 for TWAS, manure, and SSO respectively. Among them SSO had the most ultimate methane production and methane yield corresponding to 1373 mL and 332 mL CH<sub>4</sub>/g COD added. As shown in Table 2, the minimum ultimate methane production and the methane yield occurred at TWAS mono digestion corresponding to the COD:N ratio of 15 and *9 th International Summit on Global Warming & Environmental Science Volume 7 Issue 1 (2020) Aug 10-11, 2020* 

lipids: proteins: carbohydrates ratio of 1:10:4. On the other side, the maximum ultimate methane production and yields occurred at the mixing ratios of 2:4:4 corresponding to the COD:N ratio of 28 and lipids: proteins: carbohydrates ratio of 1:3:12.

Digester code	TWAS: Manure: SSO (V/V)	COD:N	Feedstock ratio codes	Lipids: Proteins: Carbohydrates	Ultimate CH <sub>4</sub> (mL)	mLCH4/g TCOD added
TWAS Only	1:0:0	15	AA	1:10:4	417	134
Manure Only	0:1:0	47	CC	1:4:20	1218	299
SSO only	0:0:1	27	BB	1:1.6:9	1373	332
T/M/SSO 8/1/1	8:1:1	19	F	1:6.5:9	784	228
T/M/SSO 1/8/1	1:8:1	40	G	1:4:19	1311	325
T/M/SSO 1/1/8	1:1:8	28	Н	1:2:10	1320	324
T/M/SSO 5/2.5/2.5	5:2.5:2.5	25	Ι	1:4:12	1146	304
T/M/SSO 2.5/5/2.5	2.5:5:2.5	32	J	1:3.8:15.5	1355	343
T/M/SSO 2.5/2.5/5	2.5:2.5:5	28	K	1:3:12	1390	350
T/M/SSO 4/4/2	4:4:2	28	L	1:4:15	1248	324
T/M/SSO 2/4/4	2:4:4	28	М	1:3:12	1424	356
T/M/SSO 4/2/4	4:2:4	26	Ν	1:3.7:11.8	1241	321

Table 2. Ultimate CH<sub>4</sub> and yield at different ratios of the substrates, COD:N, and lipids:proteins:carbohydrates

The matrix plot in Figure 4 shows the relationship of COD:N, proteins: lipids, carbohydrates: lipids, and carbohydrates: proteins ratios with the ultimate methane production (mL) and with the yield (mg CH<sub>4</sub>/g TCOD added). The responses of the ultimate methane production and the methane yield to the different ratios is illustrated in figures 4. a) and 4. b), respectively.

As shown in Figures 4. a) and 4. b), the trends of variations of methane versus COD:N, and lipids: proteins were similar. However, lipids: carbohydrates and proteins: carbohydrates did not conform to COD:N ratio. As illustrated in the Figures 4. a), the minimum ultimate methane production corresponds to the minimum COD:N ratio and the minimum lipids: proteins ratio while it corresponded to the maximum lipids: carbohydrates and maximum protein: carbohydrates ratios.

The trend of the ultimate methane production variations in response to the COD:N and to the lipids: proteins ratios relatively conform to each other excluding some of the ratios. The ultimate methane production was higher at the COD:N ratios between 26 and 47 and at the lipids: proteins ratios between 0.23 and 0.62. On the contrary, the increase of the lipids: carbohydrates and proteins: carbohydrates ratios, reduced the ultimate methane production. The maximum methane production occurred at the minimum lipids: carbohydrates and proteins: carbohydrates ratios of 0.05 and 0.17, respectively. In contrast the minimum ultimate methane production corresponded to the maximum lipids: carbohydrates ratios of 0.26 and 2.55, respectively. The lipids: carbohydrates ratios above 0.1 and proteins: carbohydrates ratios above 0.3 resulted in significant decrease in the ultimate methane production. Similar trend was observed for the methane yield in response to the COD:N, lipids: proteins and lipids: carbohydrates, and proteins: carbohydrates ratios.

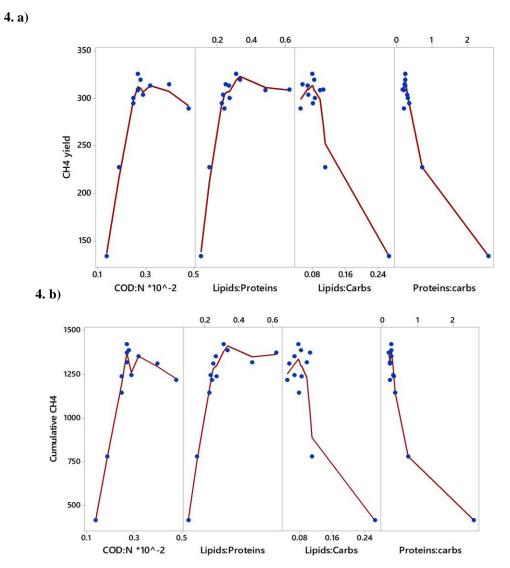


Figure 4. Matrix plot for: a. ultimate CH<sub>4</sub> and b. CH<sub>4</sub> yield at different COD/N and Lipids: Proteins, Lipids: Carbohydrates, and Proteins: Carbohydrates Ratios

#### CONCLUSIONS

Anaerobic co-digestion of TWAS with manure and SSO is advantageous over conventional mono digestion of the single feedstock. Among co-digestion of the three feedstocks at different ternary combinations, the maximum ultimate methane production and yield occurred at TWAS: manure: SSO mixing ratio of 2:4:4 and lipids: proteins: carbohydrate ratio of 1: 3.5: 18.5. The trend of the ultimate methane production variations in response to the COD:N and to the lipids: proteins ratios relatively conform to each other excluding some of the ratios. The ultimate methane production was higher at the COD:N ratios between 26 and 47 and at the lipids: proteins ratios between 0.23 and 0.62. On the contrary, the increase of the lipids: carbohydrates and proteins: carbohydrates ratios, reduced the ultimate methane production. The maximum methane production and yield were 1424 mL 356 mL CH<sub>4</sub>/g COD added, respectively. Synergistic effect improved methane yield in co-digestion of TWAS, manure, and SSO. The most synergistic effect was 19% in co-digestion of TWAS/manure/SSO.

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