

Performance of mixing ratios on the volume of biogas in a batch reactor

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Available online at: www.isca.in, www.isca.me

Received 8th January 2019, revised 2nd April 2019, accepted 20th May 2019

Abstract

Sustainable technologies to reduce and eliminate biological waste into a useful form of energy is anaerobic therapy (AD). But anaerobic digestion has some drawbacks in the case of the mono-system (mono-digestion); therefore, it is preferable to use the participation in digestion (ACoD) of different wastes to improve the performance of digestion of wastes. In this study, the work was divided into two stages, the first stage included anaerobic co-digestion of food waste and sewage at different ratios based on weight while the second stage was performed based on the best mixing ratios in the first stage based on volume. Moreover, a batch reactor was utilized for co-fermentation under mesophilic temperature with hydraulic retention times 21 days for each stage. The results indicated that the pure food waste and co-substrates with higher food waste content increased biogas production compared to sludge digestion in both stages. Furthermore, the biogas yield increased at ratios (1:0, 7:3) (FW > SS) compared to mono sludge (low organic content), also all parameters value has found have a positive effect compared with mono sludge.

Keywords: Biogas generation, inoculum, parameters, anaerobic batch fermentation, mixing ratios.

Introduction

The history of anaerobic digestion dates back to 2100 years by the anaerobic digestion of animal manure in many countries, notably China and India¹. The initial anaerobic plant was recorded to have been built in Bombay, India in 1850. Anaerobic digestion arrived in the UK in 1894 In Exeter, England, lamps were supplied with biogas extracted from treatment plants for municipal and municipal water². The usage of this technology with the major purpose to decrease and stabilize solid biowaste acquired and the introduction of sludge systems in the midst of after 1900 in the last century. To now, the anaerobic system of sludge is as yet a criterion application for new activated sludge plants. The anaerobic co-digestion is the AD of several substrates, is a typical choice to take control the drawbacks of single-fermentation^{3,4}. Because of the great advantage of biomass gas, where the use of joint digestion of the product of anaerobic digestion of municipal bio-waste (MBW) is the best way to get rid of these blocks of waste. Resulting in reducing the toxic component, increasing the load (MBW), strengthening equilibrium in nutrients, developing the cooperative effect of micro-microbes and thus improving the output of biogas in the form of net methane⁵⁻⁹. A number of different experiments have concluded that it can digest the additives of agricultural and domestic waste and treat it in a sustainable and efficient manner^{3,4,10-12}. The common digestion of different organic substrates has an effective effect on the efficiency of methane production compared to single waste as in the mixed mixture in different ratios (1: 1.1: 2.2: 1) Solid waste: sewage sludge¹³. One of the different planning to promote the implementation of anaerobic fermentation is the co-fermentation

of sewage sludge (SS) with other bio-wastes as it increments bio-fermentation organic material and supplies a feedstock with a best C/N ratio^{14,15}. AD as a mono substrate may suffering different damping involving piling up of VFA¹⁶. According to the previous study, the researchers found the best nutrition ratios of fun / s with a fixed vaccine that could contribute to the enhancement of biogas production it was (1: 0, 1: 1, 2: 1)¹⁷.

Estimated the rendering of anaerobic digesters utilizing a mixture of apple waste and swine manure. This admixture improved the biogas product by approximately 18% and 49% at mesophilic temperatures, compared to the utilize of swine manure only¹⁸. The more appealing way seems to be the anaerobic co-digestion of sludge with food waste (FW) or with another kind of biodegradable waste generated. The advantage of the sludge and biowastes co-fermentation include better biogas product, higher loading of biodegradable organic matter, improvement equilibrium of nutrients, etc¹⁹. Biogas production from sludge between 0.25-0.4 m³/K. But Co-fermentation of sewage with FW can improve the biogas yield between 0.4 and 0.6 m³/Kg^{20,21}. In the former study have been pointed, rice straw was mixed with SS (sewage sludge) under co-fermentation at various proportions (2:1, 0:1, 1:2 and 0:3, 1:4%), rice (organic waste) to (SS) based on weight, results indicated that the (SS) with rice improved the ratio (C/N) and then rose biogas yield at mixing ratios (4:1,2:1)²². Generally, these outcomes expose the high possibility of co-fermentation FW with SS to promote biogas production compared than (SS) alone²³. The impact of the substrate to inoculum ratio ((S/I ratio)) on an anaerobic system in a batch mode of the organic food waste (OFW) is very influential in order to get optimal

treatment²⁴⁻²⁶. The purpose of this study is the investigated the optimal ratio mixture of food waste and sludge and feed size of inoculum size and nutrients.

Materials and methods

The review went for assessing biogas potential of food waste under co-digestion with sewage sludge. The sewage sludge tests together with the microbial inoculum were given by the wastewater treatment plant of (Seventh sewage plant of Chengdu, China), while food waste was collected from the company in Sichuan, China (Bowen biotechnology com., LT). The substrates were collected two times, at each stage, fresh materials were collected.

Pretreatment: The underlying material was homogenized in a blender for 20 seconds to expel greater portions, for example, bread scraps, rice grains, maize globules and so on. A size lessening and the subsequent augmentation of the accessible particular surface account for an enhanced organic digestion of the substrate²⁷.

Batch reactor setup: The size of each reactor was 1liter (1000mL). These anaerobic tanks were created of high-density glass. Batch tanks (reactors) consisted of tanks the transport the extracted gas to a glass bottle containing water (the gas collector) via a tube (P.V.C). Thus, the volume of gas was calculated by the displaced liquid. These reactors were sealed with a rubber seal containing two holes. A glass tube was installed in the first hole for the purpose of taking the samples to be examined and the other opening is for the purpose of passing the gas into the water flask. These reactors were placed in the water bath at 37°C and left fermented for 21 days of hydraulic retention. Biogas collected in the head container were measured using a daily water harvesting technique at 7 pm also handshaking of the reactors were performed twice a day during the three stages in order of mass transfer of nutrients, metabolites, and microorganisms within the anaerobic reactors²⁸.

In the first and second stages, biogas tests were completed for every single substrate and also for the blended substrates to detect the ideal ratio of FW mixing with sludge for anaerobic digestion (FW: SS). The biogas scale was performed using water displacement. The mixing ratio between the substrate and the inoculum (1:2) was 300: 600 depending on the weight at the first stage, while the ratio was (1:1) 300:300 in the second stage depending on volume (Table-1, 2).

Analytical methods: Liquid stage description was undertaken before and after anaerobic fermentation²⁹ through the analytical of pH, total solids (TS), volatile solids (VS), EC according to standard methods³⁰. The pH was set utilizing pH meter (Jenway 3510 PH meter), while electronic conductivity was determined by using (DDS-307A device). The volume of gas was measured by water displacement³¹. The oil ratio was measured by the weight method with solvents.

Table-1: Different ratios from FW, SS with fixed inoculum for the first stage depending on the weight.

FW: SS	Ratio/g	Inoculum
1:0	300:0	600
0:1	0:300	600
3:7	90:210	600
7:3	210:90	600
4:1	240:60	600
1:4	60:240	600
1:1	150:150	600

Table-2: Different ratios from FW, SS with fixed inoculum for the second stage depending on the volume.

FW: SS	Ratio/l	Inoculum
1:0	300:0	300
0:1	0:300	300
3:7	90:210	300
7:3	210:90	300
1:1	240:60	300

Raito: FW: SS

Results and discussion

Analysis of biofuel outputting: The readings of biogas yield was presented within 21 days of hydraulic retention (HR) as described in Figures-1,2 for the first and second stages. The percentage of mixing was between food and sewage, with the constancy of the percentage of inoculating as mentioned in (Tables-1, 2). Each ratio started to generate biogas from the total first day as stated (Tables-3, 4), the results pointed out that the food waste produced the maximum biogas, while the sludge produced the lower. Where the value of the production of gas from waste food "FW" and sludge "SS" was (3306.5 ml, 391 ml) respectively during 21-day co-digestion for 1st stage and (0:1"2628.66ml, 461.33ml) for 2nd stage.

Characteristics of substrates before fermentation: All the ratios in all stages were stable as shown in Tables-5,6 which suggests a good possibility for biological digestion, also the pH ratio is stable where it ranged from (6 - 6.3) for food waste, (7.9) for SS for all materials and is a reasonable rate of digestion stability, as well as the rate of conductivity stable, where it amounted to approximately (5.2-5.1) mS/cm for food waste and

(890 μ S/cm) for sludge, which is a characteristic of Chinese food and sewage, respectively, in addition to the proportion of oil amounted to (11-5)%, a natural proportion compared to waste

food for many countries. The volatile solids of the food waste were high and were considered to be an appropriate proportion compared to the percentage of the other ratios.

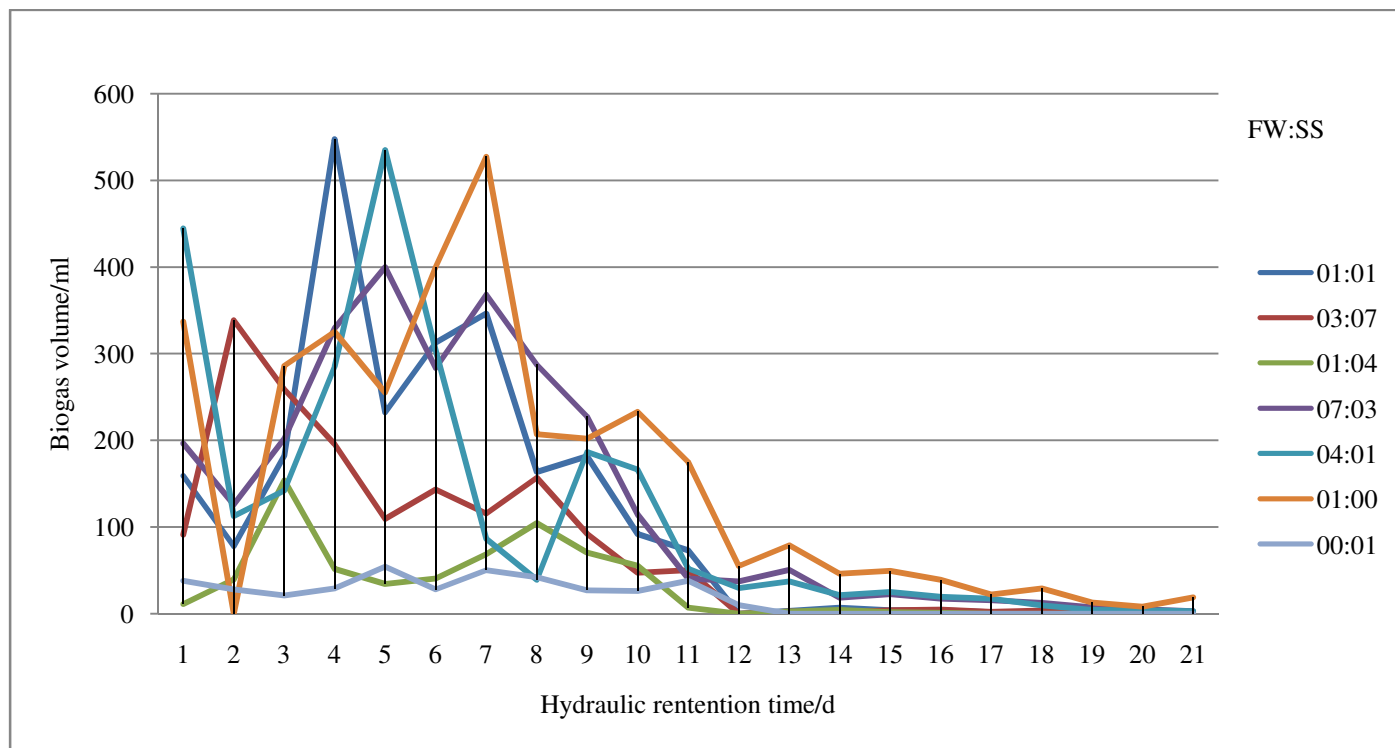


Figure-1: Through the above diagram showing the 1st stage of production of biogas during 21 days of fermentation of the common and individual materials.

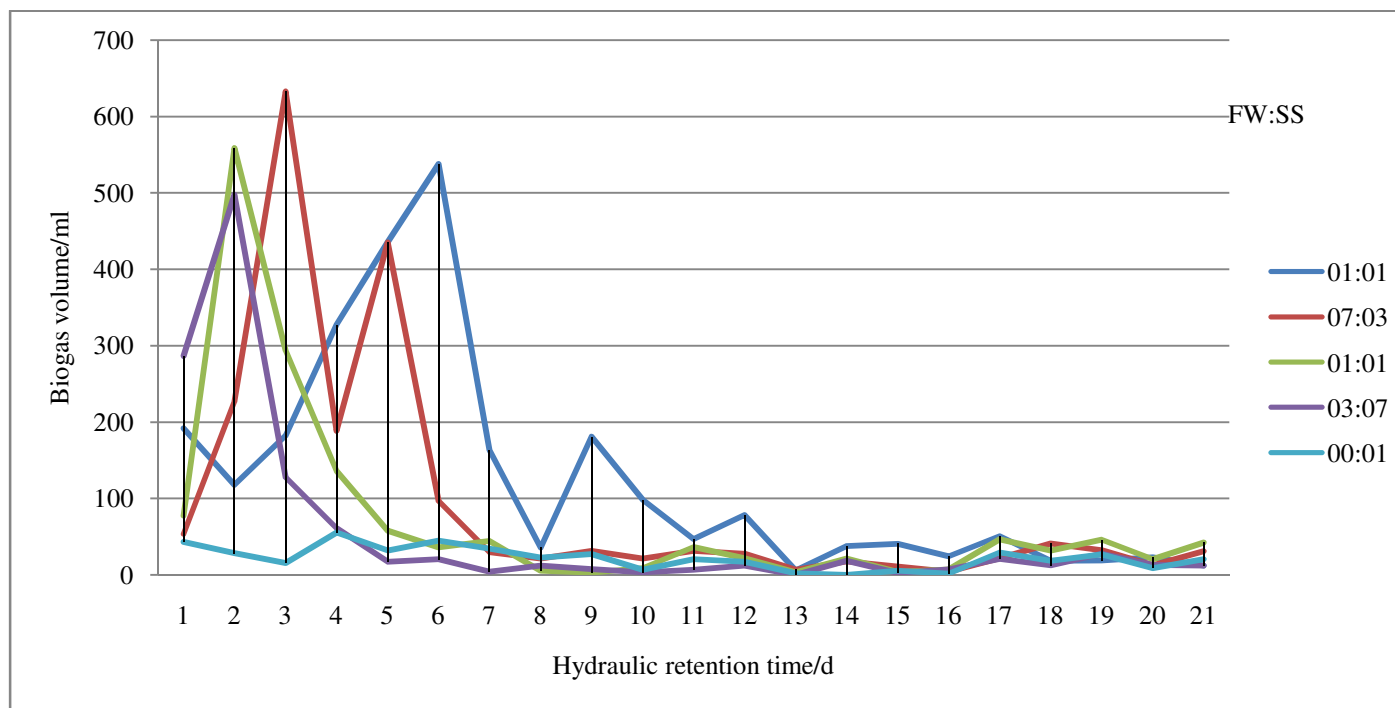


Figure-2: Through the above diagram showing the 2nd stage of production of biogas during 21 days of fermentation of the common and individual materials.

Table-3: It shows the values of biogas production in the 1st stage from different ratios during 21 days from hydraulic retention time.

FW: SS	Inoculum	Biogas generation /ml
1:0	300 g	3306.5
0:1	300 g	391
1:1	300 g	2394.83
3:7	300 g	1623.83
7:3	300 g	2765
4:1	300 g	2525
1:4	300 g	647.03

Table-4: It shows the values of biogas production in the 2nd stage from different ratios during 21 days from hydraulic retention time.

FW: SS	Inoculum	Biogas generation /ml
1:0	300 g	2628.66
0:1	300 g	461.33
1:1	300 g	1502.66
3:7	300 g	1170.33
7:3	300 g	1977

Table-5: Characterized by substrates for the first stage.

Parameters	FW	SS
pH	6.2	7.8
TS	11%	0.57
VS	9%	22
EC	5.22 Ms/cm	-
Oil content	10%	-

Table-6: Characterized by substrates for the second stage.

Parameters			FW:SS		
	1:0	0:1	1:1	3:7	7:3
pH	6.2	7.8	6.1	6	6.1
TS	12.44	1.37	5.80	4.27	6.19
VS	11.52	0.893	5.36	3.69	6.51

Conductivity and oil content for food waste and septic were measured separately and not mixed, 5.21mS/cm, 5 % respectively.

Characteristics of substrates after fermentation: After fermentation for the results show that the ratio of the volatile solids is stable except in the ratio contained high sludge because the percentage of the Sludge is greater than the food ratio, so there is instability in the reduction of volatile solids, because of the biodiversity present in the fermented mixture shown in (Tables-7, 8). Also, the TS, for food waste alone is high because the concentration of solids in the FW alone is greater than that of the common mixture³². The results for PH also indicated that the digestion goes well when the pH rates from 6.1 to 6.5 in all stages, though the better pH rate is between 6.8 and 7.2³³. The analysis of the conductivity was guided in setting the "salt content" in the substrates. The fixed value for the conductivity ratio is 1, the outcomes of the conductivity ($P < 1$) ranged from (3.43mS/c), (1.36mS) for the food waste and to the sludge respectively in first stage, while (3.51, 1.31) for "FW, SS" in second stage respectively, reason is attributed high EC index in ratio (1:0) waste due to the hypothesis that the profiles of substrates in the production of biogas listed of the food rich in "metals" added to the waste of food, but it did not adversely affect the interaction.

Discussion: The increase in cumulative gas production in the ratios (1: 0, 4: 1.1: 1, 7: 3), (1: 0, 1: 1, 7: 3) as shown in Figures-3 and 4 in the first and second stages respectively. Because the increase in food waste in the reactor leads to the production of higher gas compared to the high proportion of the feed because, infact, the decomposition of high-SS is a step restricting the rate of anaerobic bio-degradation^{23,33}. In high rate anaerobic co-fermentation of food waste, the optimum "C/N" ratio for biogas production with no reverse impact on the implementation was found to be in the good range, so the high rate of macro and micro-nutrients in sewage sludge can cause a deficiency of the ratio C/N which are low of sewage sludge^{34,35}. But the volume of gas in the first cycle is higher than the second cycle, due to the percentage of the substrate: Inoculate(S:I) in the first cycle, the ratio of the substrate to the inoculate was (2:1) while in the second cycle it was (1:1). The enhancing of the biogas production in the first stage due to the inoculate was higher than substrate²⁸.

In the first stage, as for the ratios of (4:1, 7:3, 1:1) gas production was almost not bad during the whole fermentation.

At the end of fermentation, these ratios produced (2525ml, 2765ml, 2394.83ml), respectively except (1:4 and 3:7) where the quantities were given below the quantities above where the results were (647.03ml, 1623.83ml) respectively, and the reason is attributed to that the acid phase of the fermentation mixture in statuses of (1:4 and 3:7), the yield of biogas produced reduces in the whole stage. When the percentage of SS is greater than the proportion of food waste, it also leads to an increase in bacteria and "C/N"³⁶. Because of the higher ratio of SS (1:4, 3:7) led to a lower rate of biogas, the result explains the reality that the hydrolysis of SS is the rate-shorten stage on the anaerobic bio-fermentation³⁷.

Therefore, the proportion of domestic food waste "FW" within the co-fermentation, can affect the generation of biogas significantly, that is, the high proportion of "FW", can lead to more generation of biogas during fermentation. This is perhaps due to kitchen waste "KW" includes additional organic material which could be possessed usefulness of by microbe readily. In the same time, the higher rate of sludge to food waste SS< FW can be caused in the late or inhibition of the gas production. The promotion of biogas has been observed with the stability of the ratio of "carbon to nitrogen" in the joint digestion of organic substrates with low nitrogen content, oil lead to increase the high production of biogas volume also decreasing problems joined with the gathering of "VFAs". The ratio of volatile solids at (1:4) is lower because the content of the SS is greater than the FW compared to the other ratios, where the value of volatile solids is 94% compared to (1:0, 3:7: 7:3, 4:1, 1:4) and (0:1 99.15%, 99.4%, 99.4%, 99.2%, and 99.7%) respectively.

In the second stage, the mixing rate was at this stage the FW and the SS with the stability of the inoculum and depending on the volume where the ratio of mixing between the substrates and the inoculum "1:1" as mentioned previously in Table-2. In the wake of considering the rate of biogas, it was watched that FW + SS at a proportion of (7:3) gives the most elevated gas creation (1977ml) for the mixture. Biogas generation from FW alone in the proportion 1:0 was (2628.6ml). The ability to get rid of "VS" was (7.86% during the ratio 1:0) and (4.83% with 7:3). By monitoring the gas production on a daily basis, it was noticed that the gas production started a little on the first day, except for

(3:7), where the gas production was large, reaching (286ml) due to last time, the "pH" value was somewhat higher, which was not suitable for the outgrowth and efficiency of "anaerobic microorganism" but after 4 days it turns into positive through some activity in anaerobic microbial.

Gas production in the ratio of pure food waste was the highest value, with (24% and 34%) of the whole process for the first and second stages, respectively as shown in Figures-5,6; compared to the other mixing percentages (3:7, 0:1, 1:4) due to sludge alone or larger than the food waste in the case of joint digestion this procedure leads to a higher fat content in the substrates that contain large proportions of sludge and lead to inhibition³⁸. As for the percentage of food waste alone "1: 0" gave the highest gas production during fermentation during 21 days, compared with the results.

This mentions that co-fermentation more than one substrates indigestion is not an ensure to achieve higher biogas output than the single food waste. Other proof the ratio of solids and volatile solids were large and stable compared to other g-mixing ratios. Therefore, the results in both phases show that (1:0) which recorded the highest gas percentage, but the volume of gas in the first cycle is greater than the second here shows that the effect of the amount of inoculum can raise or reduce the production of biogas³⁹. However, this stage was characterized by the stability of its characteristics such as the PH, totally solid, volatile solid, the proportion of salts and oil content as well as the quantities of gas in recent days has been stable. By fermentation, during the first week, we found that the common mixing of "sewage and food waste "in proportions (1:0, 7:3,1:1) had the highest gas volume of (1955.6ml, 1663.6ml, and 1159.6ml) respectively while (0:1, 3:7) was the least. The SS+FW with the inoculate "I+FW" also contributed to the stability of the biogas generation in the fermented reaction. The increase in the inoculum to the substrates has a positive effect on the increase of biogas, which was observed in the first stage compared to the second stage⁴⁰⁻⁴². Thus, when the ratio of substrates is less than the vaccine will be caused negatively affect the efficiency of the process and lead to the accumulation of fats and salts and increase the rates of microorganisms with nutrient depletion and then discourages the system⁴³.

Table-7: Characterized by the substrates after fermentation for 1st stage.

Parameters	FW: SS						
	1:0	0:1	1:1	3:7	7:3	1:4	4:1
pH	6.2	7.9	6.2	6.2	6.2	6.1	6.1
TS	5.466	0.0608	3.138	2.453	4.162	1.907	1.907
VS	99.15	99.71	99.46	99.42	98.22	94.80	99.21
EC Ms/cm	3.51	1.31	2.57	2.30	2.37	2.27	4.09

Table-8: Characterized by the substrates after fermentation for 2nd stage.

Parameters	FW: SS				
	1:0	0:1	1:1	3:7	7:3
pH	6.2	7.9	6.2	6.2	6.2
TS	5.466	0.0608	3.138	2.453	4.162
VS	7.86	0.40	2.91	2.44	4.83
EC Ms/cm	3.43	1.31	2.57	2.30	2.37

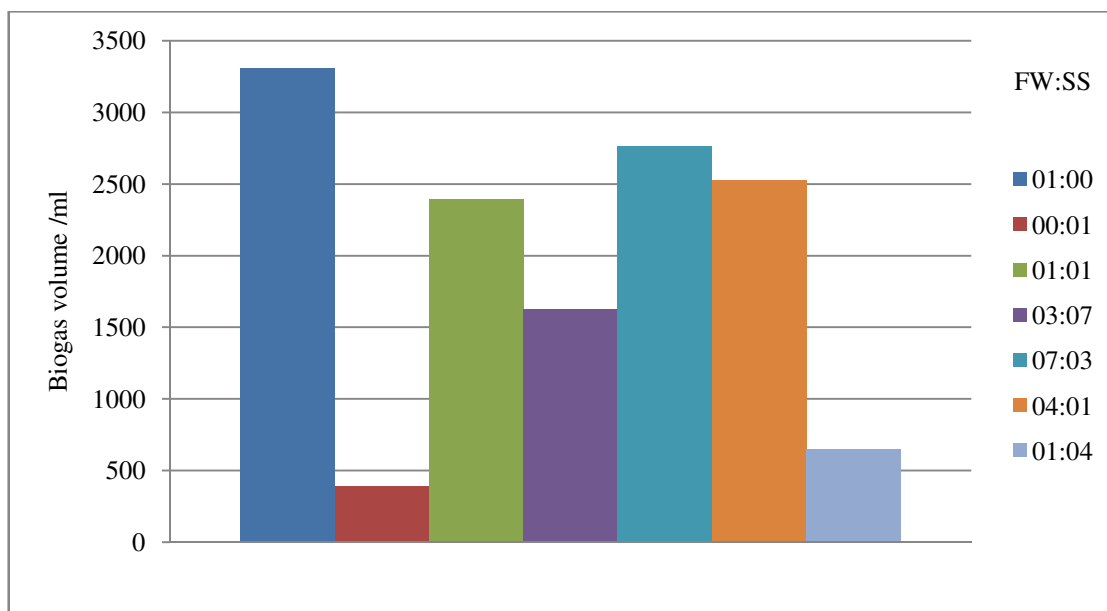


Figure-3: Total biogas production in 1st stage with inoculum ratio 600g.

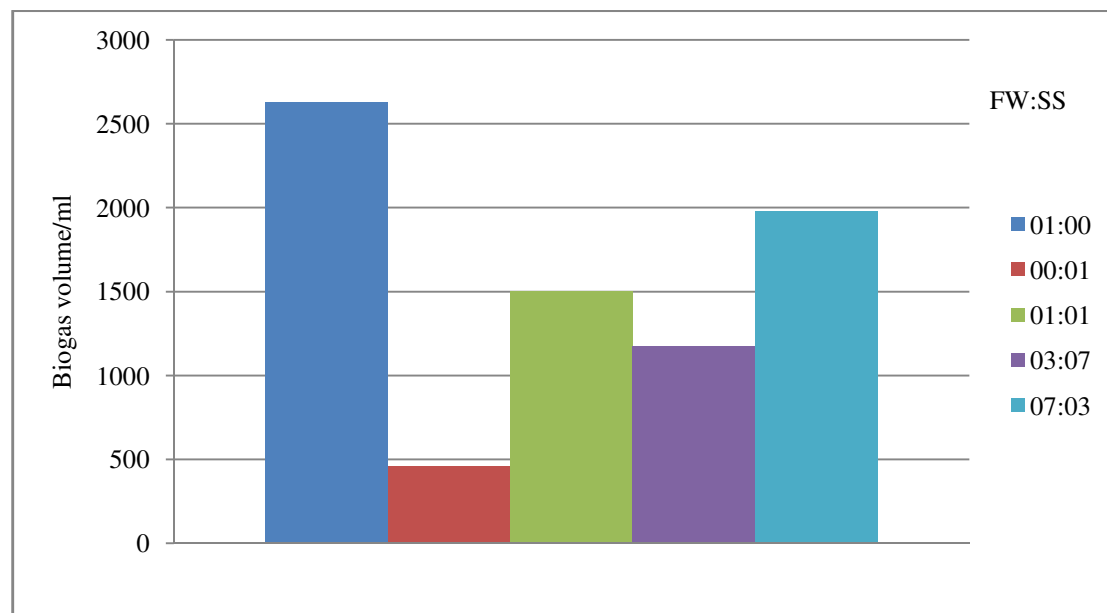


Figure-4: Total biogas production in 2nd stage with inoculum ratio 300ml.

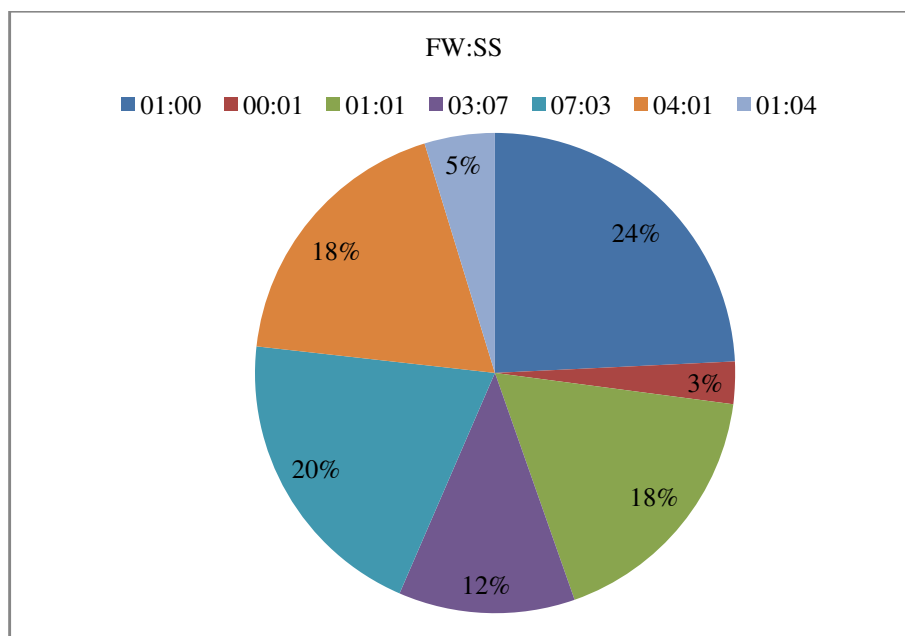


Figure-5: Shows the ratio of the volume of gas to each mixing ratio of organic substrates during the 21 day fermentation period of the first stage.

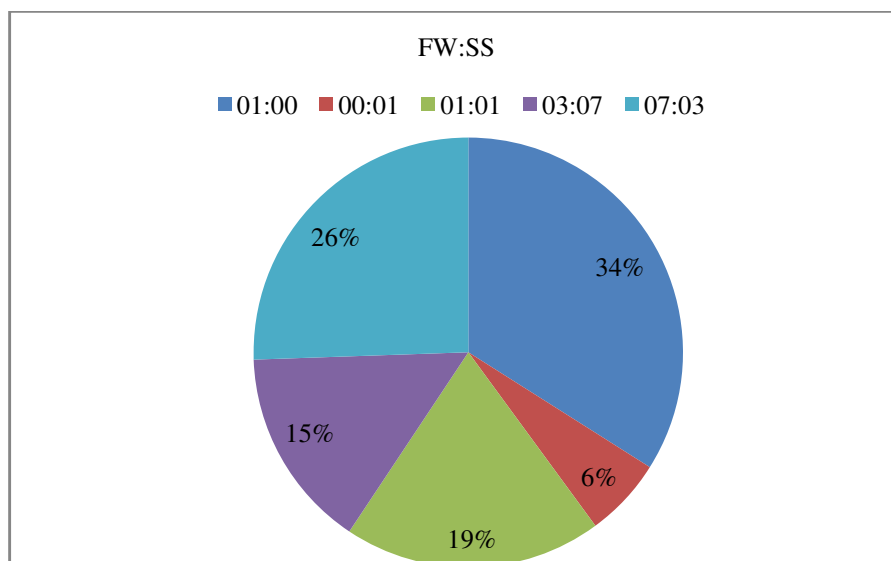


Figure-6: Shows the ratio of the volume of gas to each mixing ratio of organic substrates during the 21 day fermentation period of the second stage.

Abbreviations: AD: Anaerobic digestion, SS: Sewage sludge, FW: SS: Food waste: Sewage sludge, ESS: Excesses sewage sludge, KW: Kitchen waste, OW: Organic waste, ACoD: Anaerobic co-digestion, DS: Domestic sludge, S/I: Substrate/Inoculum, WAS: Waste activated sludge, C/N: Carbon/nitrogen, OFW: Organic food waste, MBW: municipal bio waste.

Conclusion

The mixing of substrates with suitable proportions of various organic wastes has led to an increase in the production of biogas. The outcomes in both stages showed that the co-

digestion of pure food and other mixing ratios with a high amount of food waste especially in ratios (1:0, 7:3) enhanced biogas production compared to sludge digestion. Due to the sludge contains high concentrations of microbial, fats, with low organic content, could cause the lack of nutrients in the digestion medium, the C/N ratio, and inhibition of biogas generation. The addition of a high amount of food waste led increased C/N, nutrients, and organic content. Due to food waste have the composition of 78% carbohydrates, 6.5% protein, and low lipid ratios. Thus, the co-fermentation of SS with FW in the case of a higher FW than SS is an attractive choice. It can be

useful in promoting biogas production. The volume of gas in the first stage is greater than the second here shows that the effect of the amount of inoculum can raise or reduce the production of biogas.

Acknowledgments

I gratefully acknowledge to the Southwest Jiaotong University, Chengdu Province, China and all thanks to the Department of Environmental Engineering Laboratory for efforts in the sampling, analysis, and providing of equipment.

References

1. Maragkaki A., Fountoulakis M., Gypakis A., Kyriakou A., Lasaridi K. and Manios T. (2017). Pilot-scale anaerobic co-digestion of sewage sludge with agro-industrial by-products for increased biogas production of existing digesters at wastewater treatment plants. *Waste management*, 59, 362-370.
2. Gunaseelan V.N. (1997). Anaerobic digestion of biomass for methane production: a review. *Biomass and Bioenergy*, 13(1-2), 83-114.
3. Martín-González L., Colturato L., Font X. and Vicent T. (2010). Anaerobic co-digestion of the organic fraction of municipal solid waste with FOG waste from a sewage treatment plant: recovering a wasted methane potential and enhancing the biogas yield. *Waste management*, 30(10), 1854-1859.
4. Zhang H., Gu J., Sun W., Gao H. and Wang X. (2012). Effects of different rations of materials on biogas production, VFA and the activity of dehydrogenase during anaerobic process. *Journal of Agro-Environment Science*, 31(2), 422-427.
5. Dai X., Duan N., Dong B. and Dai L. (2013). High-solids anaerobic co-digestion of sewage sludge and food waste in comparison with mono digestions: stability and performance. *Waste management*, 33(2), 308-316.
6. Angelidaki I., Ellegaard L. and Ahring B.K. (2003). Applications of the anaerobic digestion process *Biomethanation II*, Springer, 1-33.
7. Gamble K.J., Houser J.B., Hambourger M.S. and Hoepfl M.C. (2014). Anaerobic Digestion from the Laboratory to the Field: An Experimental Study into the Scalability of Anaerobic Digestion. Appalachian State University.
8. Tafdrup S. (1994). Centralized biogas plants combine agricultural and environmental benefits with energy production. *Water Science and Technology*, 30(12), 133.
9. Mata-Alvarez J., Dosta J., Macé S. and Astals S. (2011). Codigestion of solid wastes: a review of its uses and perspectives including modeling. *Critical reviews in biotechnology*, 31(2), 99-111.
10. Murto M., Björnsson L. and Mattiasson B. (2004). Impact of food industrial waste on anaerobic co-digestion of sewage sludge and pig manure. *Journal of environmental management*, 70(2), 101-107.
11. Xiao-jiao W., Gai-he Y., Yong-zhong F., Guang-xin R., Xin-hui H. and Zi-lin S. (2011). Anaerobic co-digestion effects of manure and straw and analysis of influencing factors. *Journal of Agro-Environment Science*, 30(12), 2594-2601.
12. Veenstra S. (2000). Wastewater treatment I. Delft: International Institute for Infrastructure. *Hydraulics and Environmental Engineering (IHE Delft)*.
13. Purwantoro D., Nayono S.E. and Hidayah R. (2012). Anaerobic treatment of septic tanks' sludge within the frame of integrated water resource management.
14. Part C.F.R. (1995). 503," Standards for the Use or Disposal of Sewage Sludge. Code of Federal Regulations.
15. Khalid A., Arshad M., Anjum M., Mahmood T. and Dawson L. (2011). The anaerobic digestion of solid organic waste. *Waste management*, 31(8), 1737-1744.
16. Clemens J., Trimborn M., Weiland P. and Amon B. (2006). Mitigation of greenhouse gas emissions by anaerobic digestion of cattle slurry. *Agriculture, ecosystems & environment*, 112(2-3), 171-177.
17. Amon T., Amon B., Kryvoruchko V., Bodiroza V., Pötsch E. and Zollitsch W. (2006). Optimising methane yield from anaerobic digestion of manure: effects of dairy systems and of glycerine supplementation. Paper presented at the International Congress Series.
18. Das A. and Mondal C. (2016). Biogas production from Co-digestion of substrates: A Review. *International Research Journal of Environment Sciences*, 5(1), 49-57.
19. Ağdağ O.N. and Sponza D.T. (2007). Co-digestion of mixed industrial sludge with municipal solid wastes in anaerobic simulated landfilling bioreactors. *Journal of Hazardous Materials*, 140(1-2), 75-85.
20. Cristancho D.E. and Arellano A.V. (2006). Study of the operational conditions for anaerobic digestion of urban solid wastes. *Waste management*, 26(5), 546-556.
21. Hills D.J. (1979). Effects of carbon: nitrogen ratio on anaerobic digestion of dairy manure. *Agricultural wastes*, 1(4), 267-278.
22. Fischer J.R., Iannotti E. and Fulhage C. (1983). Production of methane gas from combinations of wheat straw and swine manure. *Transactions of the ASAE*, 26(2), 546-548.
23. Brown D. and Li Y. (2013). Solid state anaerobic co-digestion of yard waste and food waste for biogas production. *Bioresource technology*, 127, 275-280.
24. Tchobanoglous G., Burton F.L. and Stensel H. (1991). Wastewater engineering. *Management*, 7, 1-4.

25. Zhu H., Stadnyk A., Béland M. and Seto P. (2008). Co-production of hydrogen and methane from potato waste using a two-stage anaerobic digestion process. *Bioresource technology*, 99(11), 5078-5084.
26. Iacovidou E., Ohandja D.-G. and Voulvoulis N. (2012). Food waste co-digestion with sewage sludge—realising its potential in the UK. *Journal of environmental management*, 112, 267-274.
27. Carrère H., Dumas C., Battimelli A., Batstone D., Delgenès J., Steyer J. and Ferrer I. (2010). Pretreatment methods to improve sludge anaerobic degradability: a review. *Journal of Hazardous Materials*, 183(1-3), 1-15.
28. Ahring B.K. (2003). Perspectives for anaerobic digestion *Biomethanation I*, Springer, 1-30.
29. Ahring B. and Westermann P. (1983). Toxicity of heavy metals to thermophilic anaerobic digestion. *European journal of applied microbiology and biotechnology*, 17(6), 365-370.
30. Association A.P.H., Association A.W.W., Federation W.P.C. and Federation W.E. (1915). Standard methods for the examination of water and wastewater. American Public Health Association, 2.
31. Bunsen R. (1857). Gasometry: comprising the leading physical and chemical properties of gases. Walton & Maberly.
32. Solli L., Bergersen O., Sørheim R. and Briseid T. (2014). Effects of a gradually increased load of fish waste silage in co-digestion with cow manure on methane production. *Waste management*, 34(8), 1553-1559.
33. Xu S.Y., Karthikeyan O.P., Selvam A. and Wong J.W. (2012). Effect of inoculum to substrate ratio on the hydrolysis and acidification of food waste in leach bed reactor. *Bioresource technology*, 126, 425-430.
34. Cervantes F.J., Pavlostathis S.G. and van Haandel A. (2006). Advanced biological treatment processes for industrial wastewaters. IWA publishing.
35. Wang Y., Wang D., Yang Q., Zeng G. and Li X. (2017). Wastewater opportunities for denitrifying anaerobic methane oxidation. *Trends in biotechnology*, 35(9), 799-802.
36. Chen D., Guo Y., Huang R., Lu Q. and Huang J. (2010). Pretreatment by ultra-high pressure explosion with homogenizer facilitates cellulase digestion of sugarcane bagasses. *Bioresource technology*, 101(14), 5592-5600.
37. Eiroa M., Costa J., Alves M., Kennes C. and Veiga M.C. (2012). Evaluation of the biomethane potential of solid fish waste. *Waste management*, 32(7), 1347-1352.
38. Kayhanian M. and Tchobanoglous G. (1992). Computation of C/N ratios for various organic fractions. *BioCycle (USA)*.
39. Kayhanian M. and Tchobanoglous G. (1992). Computation of C/N ratios for various organic fractions. *BioCycle (USA)*.
40. Hu J. (2013). Anaerobic digestion of sludge from brackish aquaculture recirculation system: CSTR performance, analysis of methane potential and phosphatase. struvite crystallization.
41. Kameswari K.S.B., Kalyanaraman C., Porselvam S. and Thanasekaran K. (2012). Optimization of inoculum to substrate ratio for bio-energy generation in co-digestion of tannery solid wastes. *Clean Technologies and Environmental Policy*, 14(2), 241-250.
42. Lawal A., Dzivama A. and Wasinda M. (2016). Effect of inoculum to substrate ratio on biogas production of sheep paunch manure. *Research in Agricultural Engineering*, 62(1), 8-14.
43. Hashimoto A.G. (1989). Effect of inoculum/substrate ratio on methane yield and production rate from straw. *Biological wastes*, 28(4), 247-255.
44. Alzate M.E., Muñoz R., Rogalla F., Fdz-Polanco F. and Pérez-Elvira S.I. (2012). Biochemical methane potential of microalgae: influence of substrate to inoculum ratio, biomass concentration and pretreatment. *Bioresource technology*, 123, 488-494.