Effects of substrate to inoculum ratio on Phosphorus Recovery from Different Composition of Food Waste using Anaerobic Batch Digestion

R Selaman¹ and N Wid¹,²
¹Faculty of Science and Natural Resources, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia.
²Water Research Unit, Faculty of Science and Natural Resources, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah.

E-mail:newati@ums.edu.my

Abstract. Anaerobic digestion is a process by which microorganisms break down biodegradable material in the absence of oxygen. The process involves hydrolysis, acidogenesis, acetogenesis and methanogenesis stages. Anaerobic digestion of food waste has been widely investigated for biogas recovery but limited study was performed on phosphorus recovery. Substrate to inoculum ratio is a very crucial parameter in anaerobic digestion in an attempt to recover phosphorus as it highly influences the production of organic acids during acidogenesis. Therefore, this study was carried out to investigate phosphorus recovery at different ratio of substrate to inoculum, where substrates was fixed to ratio 1.0 while inoculum ratio varied to 1.5, 2.0, 2.5, 3.0, 3.5 throughout the digestion process. The main substrate used in the anaerobic digestion was food waste which was segregated into different composition namely carbohydrates rich food waste, fiber rich food waste and protein rich food waste. The phosphorus recovery was performed using anaerobic batch digester at mesophilic (35±1°C) condition and pH= 6.0 for 15 days. Semi treated palm oil mill effluent (POME) was used as the inoculum to boost up the anaerobic digestion. The results indicate that substrate to inoculum ratio (1.0 : 2.0) was the optimum ratio to recover phosphorus, where protein rich food waste shows the phosphorus recovery was about 40.8%, followed by carbohydrate rich food waste and fibre rich food waste with 32.8% and 26.2%, respectively. This study is very important in resources recovery from wastes as it provides information on a new strategies for phosphorus recovery from food waste.

1. Introduction

Food waste (FW) is one of major waste that produced globally [1]. In Malaysia, most of the FW was dumped onto the landfill, consequently lead to the air and water pollution. By way of releasing the greenhouse gases (GHGs) to the surrounding and runoff of the existed nutrients to the river which can cause the eutrophication phenomenon [2-3]. While semi-treated palm oil mill effluent (POME) was the waste that be treated in open pond. This was one alternatives method that be used in most palm oil mill for reducing the nutrients contents, before it runoff to the near river after a period of time (40 to 60 days fermentation. Besides that, it was considered as the cheap method to manage the waste. Semi-treated POME considered as the primary sludge, which claims contained of anaerobic bacteria, where the process of AD can be eventually occur. The process will produce the methane gas (CH₄) which can be
use in generating the electricity in the palm oil mill [4-5]. Nevertheless, this actually contributed in releasing the GHGs to surrounding as it treated in open ponds.

Anaerobic digestion (AD) is a biological process that be used in treated organic waste and at the same time can recovery the valuable of nutrients such as: phosphorus (P)[6]. The process was involved four stages which were hydrolysis, acidogenis, acedogenesis and methanogenesis [7-8]. In determination of phosphorus recovery, the stages that need to be put meticulous attention was only the hydrolysis and acidogenesis, as the process will lead to the formation of volatile fatty acids (VFAs), thus influence the removal of P from the substrate cell (Figure 1) [9].

![Figure 1. Biochemical process in AD zone.](image)

Heading towards to increase the P recovery, substrate to inoculum ratio as one of the factor need to be considerate in AD process. Previous study stated, the purpose of mixing within the digester is to improve the contact between substrates and microorganisms [10]. Moreover, it also claims that mixing also can improve the bacteria’s population to obtain nutrients [11-12]. Additionally, reports by Dong et al. (2015) points out substrate to inoculum ratio in AD process, was also depends on the types of substrate and inoculum used. Different of both, will give different results of analysis. Due of it, this study was focus on the optimization of the inoculum ratio in order to achieve the highest of P recovery and optimum time from the digestion of FW composition (CRFW, FRFW and PRFW) and semi treated POME was acts as the inoculum.

2. Materials and Methods

2.1. Sample collection and preparation

FW composition was collected from a malay restaurant after lunch time which located near Kota Kinabalu City. All the FW was segregated into carbohydrates rich food waste (CRFW), fiber rich food waste (FRFW) and protein rich food waste (PRFW). The FWs was crushed to a mean size about 3-5mm using an electrical food grinder for facilitate the digestion effectively. Then, FWs composition were put in the freezer at 4°C for before used [8]. While, semi-treated POME collected form Lumadan mill beaufort, Sabah and it was put in an incubator at temperature 35°C (± 1).
### Table 1: Chemical characteristics of raw FWs composition.

| Elements                  | CRFW        | FRFW        | PRFW        |
|---------------------------|-------------|-------------|-------------|
| Carbon                    | 2650.08 ± 3.12 | 2711.11 ± 0.87 | 2752.19 ± 2.20 |
| Nitrogen                  | 174.09 ± 0.31  | 185.61 ± 0.92  | 240.56 ± 4.05  |
| Total volatile fatty acids| 431.90 ± 3.12 | 390.56 ± 5.86  | 415.43 ± 0.99  |
| Phosphate                 | 106.18 ± 2.07  | 147.46 ± 3.42  | 114.30 ± 0.98  |
| Potassium                 | 4230.98 ± 3.09 | 4097.67 ± 2.10 | 3987.56 ± 4.87 |

#### 2.2 Materials and Methods

Spectrometric method is a technique used to identify the concentration of orthophosphate (PO$_4^{3-}$). Phosphate ion readily react with ammonium molybdate in the presence of suitable reducing agents to form a blue coloured complex. The reactive phosphate determined by UV spectrophotometer at 880nm wavelength. The mixed reagent used were Ascorbic acid, C$_6$H$_8$O$_6$, Ammonium molybate, (NH$_4$)$_6$MO$_7$O$_{24}$.4H$_2$O, Hydrochloric Acid, HCl and Potassium antimonyl tartrate trihydrate (C$_8$H$_4$K$_2$O$_{12}$Sb$_2$).3H$_2$O [3].

#### 2.3 Operation startup for AD (FW composition and POME)

The anaerobic digestion experimental was done using the batch reactor which represented by duran bottle with a volume of 1 L. Fifteen reactors were used in this study with differ in substrate to inocula ratio. The digestion was done for 15 days at 35°C (± 1) mesophilic condition. The pH value was fixed to 6.0 consider the optimum values during the preliminary analysis. Any reduction or increasing of pH throughout the test will be controlled using 1 M NaOH and 1 M HCl. Any gas was released once in three days to avoid explosion during the test.

#### 3. Results and Discussion

Figure 2 shows the results from CRFW experiment in determined P recovery values. From the data, it shows ratio substrates to inocula (1.0 :2.0) (CRFW-POME-SI-2.0) indicates the highest of P with 4111.14 mg/L (±0.03). Followed by CRFW-POME-SI-1.5 (1.0:1.5) and CRFW-POME-SI-2.5 (1.0:2.5) with P values 3476.82 mg/L (±0.05) and 2590.88 mg/L (±0.03) respectively. Reactor CRFW-POME-SI-3.0 (1.0 :3.0) and CRFW-POME-SI-3.5 (1.0:3.5), recovery lower P compared others ratio with values 1970.82 mg/L (±0.02) and 1450.76 mg/L (±0.09). Optimum P recovery were at range 9 to 12 day of AD.
Figure 2. AD of CRFW (substrates) and POME (Inoculum) in 15 days digestion time in different ratio of inoculum.

While Figure 3 represented data from the FRFW analysis. The results show the FRFW-POME-SI-2.0 reactor (1.0 : 2.0) recovery optimum P with values 3304.01 mg/L (±0.05). Then, FRFW-POME-SI-1.5 (1.0 : 1.5) and FRFW-POME-SI-2.5 (1.0:2.5) with values 2755.10 mg/L (±0.02) and 1760.60 mg/L (±0.04) respectively. The data from FRFW-POME-SI-3.0 (1.0 : 3.0) and FRFW-POME-SI-3.5 (1.0 : 3.5) batch reactors shows the lower of P recovery with values 1604.88 mg/L (±0.02) and 1485.82 mg/L (±0.01) respectively. The optimum day digestion were at range 8 to 10 days.

Figure 3. AD of FRFW (substrates) and POME (Inoculum) in 15 days digestion time in different ratio of inoculum

Figure 4 presents the results obtained from the PRFW analysis. Reactor PRFW-POME-SI-2.0 (1.0 : 2.0) shows the highest of P values with 5115.58 mg/L (±0.04). Next, by reactor PRFW-POME-SI-1.5 (1.0 : 1.5) and PRFW-POME-SI-2.5 (1.0 : 2.5) with values 4417.88 mg/L (±0.07) and 3692.10 mg/L (±0.01) respectively. Followed by reactor PRFW-POME-SI-3.0 (1.0 : 3.0) and PRFW-POME-SI-3.5 (1.0 : 3.5) with values 3822.48 mg/L (±0.01) and 3712.98 mg/L (± 0.07).
Based on the current studies above, P recovery was increase at all ratio of substrates to inoculum at early days of digestion. Then, it gradually decreased after achieved the P optimum. The statistical results show that all substrates to inoculum ratio was significantly different from each other (p<0.05). In this study, substrate (1.0) to inoculum ratio (2.0) shows the highest of P recover compared other inoculum ratio. This explained, an appropriate ratio of inoculum in AD process could contributed in higher the P recovery [12]. In this study, semi-treated POME was used as the inoculum although commonly it can be considered as the substrate [11]. Previous study had claimed that semi-treated POME physical state was slurry and it was also contained of acidogenic bacterias, thus can used in boost up the P recovery [4]. Hence, this eventually indicates the highest value of P recovery from AD of FWs composition and semi-treated POME could be due to the synergetic effects between both which directly influenced the highest value of P. As stated by Selaman, (2016), AD of different substrate in one medium could build up the synergetic effects as the substrates supply any possible missing nutrients in the process. This convince the suitability between FWs composition and semi-treated POME in recovering of P. Besides that, the data shows greater ratio of semi-treated POME (2.5, 3.0, and 3.5), recovering the lower of P compared ratio (2.0). It is possibly due to the accumulation of total volatile fatty acids (TVFAs) which eventually explain the decreasing of P yield at certain time. Similar observations were also noted by Dong et al. (2015), where the formation of TVFAs was occur at early stages of AD process. Then, its turn to accumulate after several day of fermentations due to the higher of its formation. Optimum of P recovery examined at day 5 to 12 for all data. This significantly shows the acidogenesis process was occur after day 4 of AD [11-12].
The biochemical process on how the P was produces in AD of FWs composition shown in Figure 1. While, Figure 5 provides the results obtained from the analysis of FWs composition (1.0) and inoculum (2.0) ratio. Comparing of the results, it can be seen that PRFW recovery the highest of P compared by CRFW and FRFW with values 40.8%. While CRFW and FRFW values 32.8% and 26.2% respectively. Surprisingly, PRFW recovery the highest values of P, though the initial of reactive P values was lower compared other composition which been reported in previous study by Selaman, (2016). In a related report had mention that PRFW contained the better of carbon ratio that will effected the product recovery during AD process [8][9]. So, this explained the highest value of P recovery. In contrast with FRFW, the lignin contained was harder to break down, thus effect the P removal from the substrate cell by the bacterias.

The current results indicates the acidogenesis stages was optimum examined at the day 10 of AD for all FWs composition. Several studies had claimed the short time of AD was appropriate in recovering the highest of P as the TVFAs formation was occur during the early time of digestion [7][12]. This highlight optimum value of P recovery can be achieved in day 10 of AD between FWs composition and semi-treated POME.

4. Conclusion

This study suggested an optimization of substrate to inoculum ratio (1.0: 2.0) was adequate for increasing the highest P recovery throughout the AD process for all FW composition. PRFW shows the highest of P recovery about 40.8% which due to the better carbon and nitrogen ratio in the substrates. While, optimum digestion time for all substrate was on day 10. This predicts the highest of TVFAs production during the period of AD process which influence the P removal from the substrate cells. Eventually, the data shows all FW composition (CRFW, FRFW and PRFW). contained P that can be recovery through AD process.

5. References

[1] Solid Waste & Public Cleansing Management (SWcorp), 2015. Taklimat Pengurusan 3R di Negeri Johor and Pengkomposan Takakura, Slaid, Johor Darul Takzim.
[2] Mohd Dinie, M.S & Mashitah, M.D. 2012. Municipal solid waste management in Malaysia :Current Practices, Challenges and prospect. Journal technology, 4(6): 12-18.
[3] NAHRIM. 2005. A Desktop Study in the Status of Lakes Eutrophication in Malaysia. Malaysia. August.
[4] Mohd Nizar, K., Abdul Jamil, Z., Isharuddin, M.I., Hamdan, J., Wan Muhd Nazri, W.M. & Muhd, K. 2016. The potential treated palm oil mill effluent (POME) sludge as an organic fertilizer.
[5] Abduralahman N.Y., Rosli Y.M. & Azhari, N.H. 2013. The performance evaluation of anaerobic methods for palm oil mill effluent (POME) treatment, A review 87-105.

[6] Sulkayan, S., Tanish, N. & Jeffrey, B. 2015. Nitrogen and phosphorus recovery from wastewater. Water Pollution, 1 155-166.

[7] Kangle, K.M., Kore, S.V., Kore. V.S.& Kulkarni G.S. 2012. Recent trends in anaerobic codigestion: A review. Department of environmental science and technology, 2 210-219.

[8] Shen, D. Yin. J., Yu, X., Wang, M., Long, Y., Shentu, J. & Chen, T. 2017. Acidogenic fermentation characteristics of different types of protein rich substrates in food waste to produce volatile fatty acids. Bioresource Technology, 3 125-132.

[9] Kun, W., Jun, Y. Dondsheng, S. & Na, L. 2014. Anaerobic digestion of food waste for volatile fatty acids (VFAs) production with different types of inoculums : Effect of pH. Bioresource Technology, 161 395-401.

[10] Amha, YM., Pooja, S., Jewis, L., Matt, G. & Adam, L.S. 2017. Elucidating microbial community adaption to anaerobic co-digestion of fats, oils, grease and food waste. Water Research, 123 277-289.

[11] Hidalgo, D. & Marroquin, J. 2014. Effects of inoculum source and co-digestion strategies on anaerobic digestion of residues generated in the treatment of waste vegetables oils. Journal of Environmental Management, 14 17-22.

[12] Braguglia., Agata, G., Andrea, G. & Pamela, P. 2017. Anaerobic bioconversion of food waste into energy. Bioresource Technology, In Press. 2017.

Acknowledgements
The authors would like to express sincere gratitude to the Universiti Malaysia Sabah for the financial support and lab assistants at Faculty of Science and Natural Resources, Universiti Malaysia Sabah.