BORNEO AKADEMIKA (BA)

Volume 8 (1), June 2024 (1-7)

e-ISSN: 2735-2250

Journal home page:
https://borneoakademika.uitm.edu.my/

THE CHARACTERISATION OF PROTEIN-RICH FOOD WASTE AND POND SLUDGE FOR BIOMETHANE RECOVERY

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Received Date: 6 February 2024
Accepted Date: 10 May 2024
Published Date: 7 June 2024

ABSTRACT

Biomethane is a non-renewable energy source that can be produced through the decomposition of biomass. Biomethane has comparable properties to natural gas and, thus, can be transported and stored in the available facilities and infrastructure. Currently, anaerobic digestion (AD) is one of the most favourable techniques that can be used to recover biomethane, as it is a simple and low-cost technology. Protein-rich food waste (PRFW) is one of the food waste compositions that is produced abundantly, and pond sludge (PS) is made of organic materials that enter the ponds, and sink to the bottom, and decompose. Both are claimed to contain high carbon values. Therefore, this study was carried out to determine the physical and biological properties of both substrates and identify the biomethane recovery value through single and mixed digestion. The AD process was performed at mesophilic conditions 35^{o}C (±1) and a pH range of 6.8 to 7.2 for 30 days. The TS values for PRFW and PS were 34.16% (± 0.08) and 40.23% (± 0.06), respectively. While the VS values for PRFW and PS were 88.56% (± 0.04) and 84.30% (± 0.06), respectively. Both substrates also show the availability of facultative anaerobic bacteria (FAB). Hence, this shows the suitability of both substrates to undergo the AD process. Results for biomethane recovery found that mixing digestion between PRFW and PS recovered the highest amount of biomethane with 120.3 mL (±0.05), and the optimum time for digestion was on day 18. While single digestion of PS and PRFW recovered biomethane with 95.0 mL (±0.12) and 79.3 mL (±0.21) on day 21 respectively. The better synergistic effect within FAB in mixing substrates eventually influences the highest biomethane removal from the substrate in a short period of time. Moreover, this study also provides information on new strategies for recovering biomethane.

Keywords: Biomethane recovery; Total Solid, Volatile Solid; Protein-Rich Food Waste; Pond Sludge

Introduction

Food waste (FW) is a primary contributor to climate change, with approximately 1.3 billion tons of food wasted annually. This amount corresponds to 30% of the total food produced for human consumption and to 8–10% of global greenhouse gas emissions (GHG) associated with FW (Xu et al.,2019). According to Vidal-Antich et al. *(*2022), PRFW, as its most valuable component, plays a major role in the overall situation. Approximately 100 million tons of PRFW are generated annually, with municipal solid waste (MSW) containing protein-rich compounds, leading to inadequate waste treatment capacity. Global poultry meat production reached 19.71 million tons in 2019, with 20% of it being wasted, causing greenhouse gas emissions (GHGs). PS is a sludge that is made up of various organic materials that end up at the bottom of a pond and are mixed with some of the various inorganic materials. Sludge disposal methods such as incineration and landfilling are generally the methods that have been used worldwide for sludge disposal. According to Gorka et al. (2018), this method is practical, indicating sludge as a non-toxic compound. Nevertheless, this approach could lead to the formation of undesirable mud and affect the water source by contaminating chemicals such as aluminium (Al) and iron (Fe). Recent evidence suggests that PS can be used as substrates for recovering biogas gas, as it contains organic compounds and the existence of facultative anaerobic bacteria (FAB) (Ebrahimi Nik et al*.,* 2018; Selaman et al*.,* 2023). Due to this, the alternative approach that can be used in the treatment of both PRFW and PS is anaerobic digestion (AD) techniques (Hedge and Trabold., 2018). Besides that, AD is also considered an environmentally friendly process as it can be used not just in treatment but also in recovering biomethane (Meegoda et al*.,* 2018; Kim et al*.,* 2019). Biomethane can be used as a renewable energy source for heating purposes and as vehicle fuel when compressed like natural gas. Therefore, this study aims to identify the characteristics of PRFW and PS collected from cafeterias and ponds at the UiTM Sabah Branch, Kota Kinabalu Campus, while also focusing on the potential of biomethane recovery of both substrates through an AD technique.

Methodology

Sample Collection and Preparation

Raw PRFW was collected after lunch hour from the Manukan cafe located in the UiTM Kota Kinabalu Campus. PRFW, which mainly contained seafood and meat, were grounded to a small size of about 3-5 mm before being analyzed. The purpose is to facilitate the AD efficiency process. Pond sludge (PS) was collected from a pond at UiTM Kota Kinabalu, Kampus. It was kept in an anaerobic state at a temperature of 35ºC (±1) in an incubator (Figure 1).

a **b**

Figure 1: Protein-Rich Food waste (PRFW) (a) and Pond Sludge (PS) (b)

Determination of physical and biological properties of PRFW and PS

The standard procedure for determining the physical and biological properties of the raw materials was done according to Selaman and Wid, (2016). The purpose of this study was to identify whether the samples could be used to undergo the AD process.

a. Determination of Total Solid (TS)

Firstly, an empty crucible was weighted using an analytical balance. Then, the crucible was filled with a PRFW sample and then weighted. All the weight was recorded. Next, the PRFW sample with the crucible was placed in an oven and heated at 105^{o}C. This process was done for 24 hours. Next, the PRFW sample was placed in a desiccator to prevent the re-absorption of moisture by the sample. To increase the reliability of the measurement, PRFW samples were tested in duplicate. The total solid (TS) was calculated using Equation 1. This procedure was repeated for PS.

$$TS\ (\%) = [(A-B) / (C-B)] \times 100\% \quad \text{(Eq.1)}$$

TS is referred to as Total Solid (%)
A = crucible weight + dry sample weight (g)
B = crucible weight (g)
C= crucible weight + wet sample weight (g)

b. Determination of Volatile Solid (VS)

To determine the VS, a PRFW sample from section (a) was placed in a muffle furnace at 550 ^{o}C for a total of 4 hours. Then, the sample was placed into a desiccator and weighted (APHA, 2010). Once the procedure was completed, the percentage of VS was calculated using Equation 3.2. This procedure was repeated for PS.

$$VS\ (\%) = [(A-C)/ (A-B)] \times 100\% \quad \text{(Eq.2)}$$

VS is referred to as Volatile solid (%)
A = crucible weight + dry sample weight (g)
B = crucible weight (g)
C = crucible weight + ash sample weight (g)

c. Determination of pH

The ratio of the PRFW sample to distilled water was set at 1:10 (w/v). The PRFW sample was placed in a bottle and shaken by using an orbital shaker at a speed of130 rpm for 24 hours. In order to determine the pH, the FW sample was filtered using a vacuum filter, and the liquid part was taken to determine the pH. The pH was measured by using a pH meter. This procedure was repeated for PS.

d. Determination of total FAB

Total amount of FAB in the PRFW sample was determined by the anaerobic plate count method following the procedure in the AOAC Official method (990.12). Stock solution was prepared by weighing 34.0 g of potassium dihydrogen phosphate (KH_2PO_4) and then dissolved in distilled water in a 1.0 L volumetric flask until the calibration mark. The solution was adjusted to pH 7.2 by 1.0 M of NaOH. Next, buffered water was prepared by diluting 1.25 mL of stock solution in a 1.0 L volumetric flask until calibration mark and then put into the autoclave at 121^{o}C for 15 minutes. After that, the 10 g PRFW sample was homogenized with the buffered samples and placed onto a plate count agar. Then, the plate was incubated at 35°C (± 1°C) for 48 hours. The colonies were counted as shown in Equation 3. The procedure was repeated for PS.

CFU/g = (Number of colonies x dilution factor) / volume plated (Eq.3)

Operation start-up for AD process

The experimental work was performed using a Duran bottle with a working volume of 400 mL. Digestion tests were performed in an incubator. The sample to distilled water ratio was fixed at 2.0:1.0. While the temperature was set up at mesophilic conditions 35°C (±1). The Duran bottle was charged with PRFW and PS, and pH was controlled from 6.8 to 7.2 by using 1.0 M HCl and 1.0 M NaOH. The digestion time for each digester was set up for 30 days. During the experiment, the gas was released once a day. The determination of gas recovery was done using the water displacement technique (Selaman et al*.,* 2023). The volume of gas recovery was calculated using Equation 4 and Equation 5. This procedure was repeated by mixing samples of PRFW and PS.

Volume of gas recovery (mL) = Volume of distilled water displaced (mL) (Eq.4)

Biomethane recovery = 70% of the volume of gas recovery. (Eq.5)

Data Analyses

Data obtained from the experiments were analyzed in the IBM SPSS Statistic 22.0 statistical software package. For the summary statistics, the results were given as mean ± standard deviation. The appropriateness of the normal distribution of the data was assessed with Shapiro-Wilk normality test. For the comparison of different parameters applications, one way-ANOVA, and for multiple comparison test, Student-Newman-Keuls test were used. $P < 0.05$ was considered as the statistical significant value.

Results and Discussion

Physical and biological properties of PRFW and PS

Table 1 shows that the TS values of PRFW and PS from this study were 34.16% (±0.08) and 40.23% (±0.06), respectively. This value eventually showed that PRFW and PS had a high content of dissolved solids, which could contribute to biomethane recovery. As reported by Adjovu et al. (2023), TS values of more than 30.00% suggest high TS, and therefore, this would point out that both substrates contain high TS. Besides that, Meegoda et al*.* (2018) reported that a high TS indicates that the waste is not suitable for landfilling as it can undergo the AD process in an open space and will consequently contribute to the release of GHGs. A high TS value also represents a high volume of waste; thus, it will need more space for landfilling. The present TS value from other study results showed a difference from the studies by Abd Hammid et al., (2019) and Mrosso et al., (2023) (Table 1). The difference in the value could be due to the different types and compositions of FW used in the studies.

The VS values for the PRFW and PS were 88.56% (±0.04) and 84.30% (±0.06), respectively. The value was not different from other studies (Table 1). This eventually indicates that the PRFW and PS contained high organic content. According to Wid et al*.* (2017), waste that contains a high VS value, which is about 70 -100% is suitable to be treated under the AD process, which could produce high biomethane gas.

Table 1: Physical and biological properties data for current and previous studies.

References	TS (%)	VS (%)	pH	FAB (CFU/g)
PRFW (This study)	34.16 ± 0.08	**88.56 ± 0.04**	**3.67±0.12**	**7.80 x10^2**
PS (This study)	**40.23 ± 0.06**	**84.30 ± 0.06**	**6.83 ± 0.04**	**6.26 x 10^5**
Abd Hammid et al. (2019)	**17.18 ± 0.00**	**85.56 ± 0.00**	**5.61 ± 0.00**	**none**
Andrade et al. (2020)	**none**	**none**	**4.00 ±0.00**	**5.61 x 10^2**
Mrosso et al. (2023)	**36.20±2.34**	**none**	**96.36± 1.73**	**none**

The pH value of PRFW in this study was 3.67 (±0.12), which is an acidic state. This shows the presence of organic acid in PRFW, and its existence could come from the food preservation process. According to Firmo et al. (2022), the meat industry usually uses organic acids or salts during the meat preservation process to avoid meat spoilage by bacteria. Besides that, the amount of organic acid or salt used depends on the duration of food storage. This could be the reason why the pH values from other studies (Abd Hammid et al., 2019) are different (Table 1). Besides that, the values reveal that PRFW contains organic acids, which are obviously appropriate to be used as a carbon source and are likely beneficial for the AD process. While the PS pH value was in the alkaline state, which was 6.83 (±0.04). Although the values of PRFW and PS are different, during the AD process, both will be mixed, which could increase the pH to the approximate value of 6.8 to 7.2. So this could reduce the use of chemicals in controlling the pH.

The data in Table 1 also shows the availability of facultative anaerobic bacteria (FAB) in the raw substrates of PRFW and PS, with values of 6.26 x 10^5 CFU/g and 7.80 x 10^4 CFU/g, respectively. Besides that, the results are different from the study by Andrade et al. (2020). Different values of total FAB might be due to the different types of substrates used in this study. Besides that, one of the factors that caused PS to contain a high amount of FAB is that it was collected in a pond that already contained existing FAB. Besides that, Wolfgang Buckel, (2021) mentioned that the amount of total FAB contained in each substrate would depend on the conditions of the substrates, such as pH and temperature. Each species of FAB has its own characteristics, range of pH value and optimum temperature in which it grows and reproduces the best (Maria Cecilia et al., 2022). So, it can be assumed that FAB in each substrate of PRFW and PS will reproduce, and the amount will increase during the experiment as long as they meet the appropriate pH and temperature.

Biomethane recovery in AD in single and mixing of AD

Figure 2 shows the results from single and mixed AD. The results showed that the mixing substrates between PRFW and PS contributed to the highest biomethane recovery value with 120.3 mL (±0.05). The graph showed that biomethane gradually increased starting from day 1 until day 18 of digestion and then significantly decreased until day 30 of digestion. This was followed by single substrates PS with a biomethane recovery value of 95.0 mL (±0.12). During the digestion process, biomethane was produced from the substrate, slightly increasing from day 1 until day 21; and then continuing to decrease until day 30. On a single substrate, the PRFW value was 79.3 mL (±0.21). The highest biomethane recovery was shown at day 21 and slightly decreased until day 30 of digestion. The accumulation of acid would cause a decline in biomethane production for all reactors. A higher value of acid production could lead to instability in digestion performance and methanogenesis bacteria activity (Mohammed et al., 2021; Abraham et al., 2023).

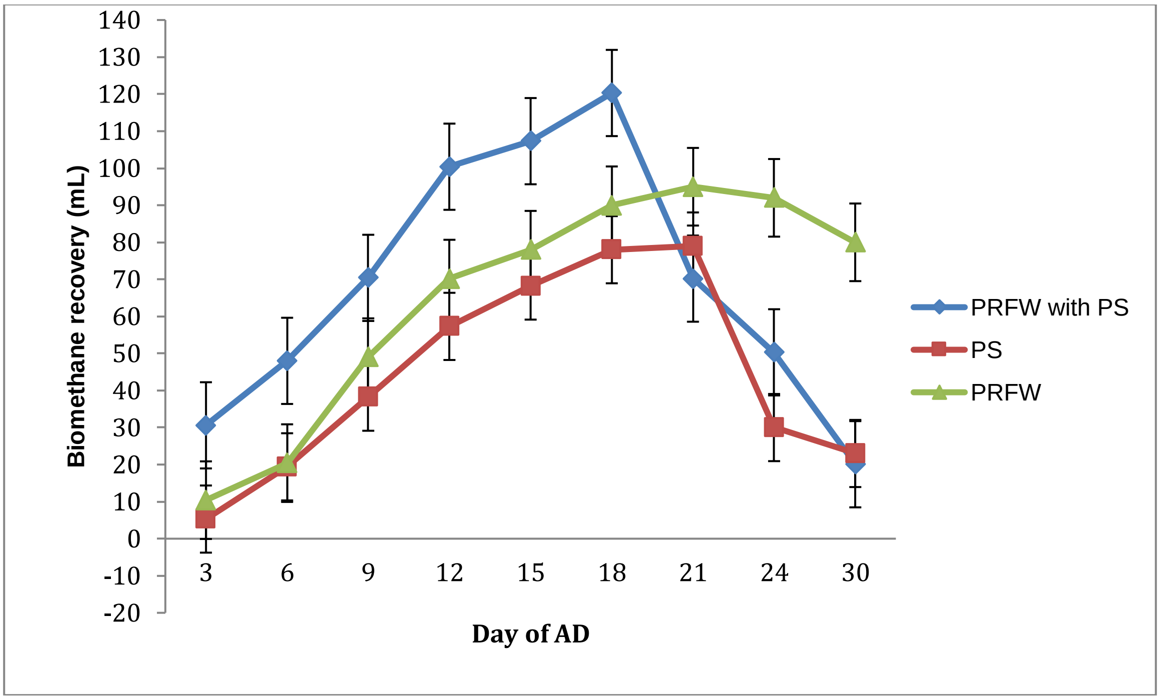

Figure 2: AD substrates (PRFW, PS and PRFW with PS) at 30 days of digestion time

The data also show that the mixing substrates of PRFW and PS eventually produced the highest value of biomethane recovery, which could be due to the combination of both producing better synergistic effects between both FABs during the AD process, which in turn helped in enhancing biomethane production from the substrates (Mohammad Kelif et a*l.,* 2022). Besides that, Rabii et al. (2019) also mentioned that mixing substrates in one AD process could help in increasing the efficiency of the process as the co-substrates will help to supply the missing nutrients in the digestion medium. This finding suggests that a mixture of PRFW and PS can greatly increase the AD product. Interestingly, the results also showed that the optimal days of biomethane recovery during the digestion process for all mixing substrates became shorter as compared to single substrate digestion. This finding showed that the appropriate composition of organic matter in one reactor would affect the growth and performance of bacteria in increasing biomethane recovery.

Conclusions

Physical and biological properties of PRFW and PS show the suitability of both substrates to undergo AD process for biomethane recovery. The mixing substrates between PRFW and PS show the highest value of biomethane recovery with 120.3 mL (±0.05) and the optimum time was shorter (Day 18) compared with single digestion of PRFW and PS. This may be due to the fact that mixing digestion in the AD process could promote better synergistic effects in the digestion medium as the co-substrates supplied the missing nutrients.

Acknowledgement

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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