INFLUENCES OF WASTE TO INOCULUM RATIOS AND TEMPERATURE ON BIOGAS GENERATION OF ORGANIC SOLID WASTE FROM MARKET

Anh Tuan Ta and Sandhya Babel*

School of Biochemical Engineering and Technology, Sirindhorn International Institute of Technology, Thammasat University, Pathum Thani, Thailand
**Email: sandhya@siit.tu.ac.th*

ABSTRACT

Biochemical methane potential (BMP) assay is popularly applied for evaluating the anaerobic digestibility of organic matters. The assay is easy to conduct, inexpensive, and repeatable to make comparisons of the anaerobic digestion and potential biogas production between various materials. The results provide by the BMP assay are valuable to investigate anaerobic biodegradation potential of organic substrates and to advance the design and operation of full-scale anaerobic digesters. In this study, BMP of mixed vegetable wastes generated from Talaad Thai market (Thailand's largest integrated agricultural wholesale market) was evaluated in order to analyze extent and rate of their conversion to methane. Different feedstock to inoculum ratios (F/I) in the range of 0.5 to 5.0 g VS of feedstock/ g VS of inoculum were investigated to identify the optimum ratio for the conversion rates. Furthermore, the study also explored the influence of temperature conditions (i.e. ambient: 30 ± 4°C and constant temperature 37°C) on the anaerobic conversion rates. The results revealed that the F/I ratio of 0.5 g VS/ g VS and temperature of 37°C was the optimum condition to obtain the maximum conversion rates in term of methane production, with the achievement of net methane yield were 341 ml methane/ g VS. However, at the waste/inoculum ratios of 2.0, 3.0, 4.0, and 5.0 g VS / g VS, higher hydrogen production was observed for both temperature conditions. Moreover, the study also pointed out the important role of temperature on the biogas, methane and hydrogen production of the mixed vegetable wastes. The results achieved from the BMP assays provide the extent and rates of conversion of vegetable solid wastes and also suggests optimum conditions to conduct further studies on the anaerobic digestion system using BIOCEL system.

Keywords: Anaerobic digestion, biogas, biomethane potential, renewable energy, vegetable waste.

INTRODUCTION

As one of the top agricultural producing countries, Thailand produces over 15 million tons of fruits and vegetables annually [1]. Correspondingly, large amounts of fruit and vegetable waste are generated, and the disposal of these wastes is costly, both financially and environmentally. However, these solid waste are a potential clean energy sources when they are converted to methane, through the anaerobic process [2]. Anaerobic digestion (AD) is a biological conversion process in the absence of external electron acceptor like oxygen. In the process, organic carbon is converted to its final form of oxidation and reduction state (CO_2, CH_4) [3]. The AD process, not only offers benefits by treating the solid waste, but also yields the biogas for power generation, and when possible the digestate can be used as fertilizer in agriculture. Before starting a full-scale AD reactor, biochemical methane potential (BMP) assays should be investigated. BMP assays analyze which types of substrates, have the highest biomethane potential among various substrates. The assays also determine the optimum ratios between substrates and inoculums; and evaluate the anaerobic biodegradation rate of substrates or retention time required for complete the digestion [4]. For the last 20 years, although various BMP methods have been formulated, their procedures are very similar, the only difference between BMP assays is the biomethane production from the inoculum. There are two methods to determine the biomethane production of the inoculum: the blank assay [2, 4, 5] and pre-incubated inoculum [4, 6, 7]. In the blank method, the biomethane production from the inoculum (i.e. with water or medium only) is deducted from the biomethane production achieved in the assays with the substrate [2-4, 7-9]. For the pre-incubated inoculum method, the biomethane gas, initially presented in the inoculum, must be first depleted. Then it is used for the assays, which the process is maintained until no significant biomethane production is detected. [4, 6].

BMP assay values are sensitive to several parameters such as temperature, pH, solid waste particles size, and feedstock to inoculum ratios. Temperature has a direct effect on survival and growth of bacteria and their metabolic activities [10]. Theoretically, on the AD process, the temperature only affects the digestion rates and not on the bio-digestion of a substrate. However, in very-low-temperature range (<20°C), the degradation rates can be decreased significantly and this lowers the biogas achievement than that of optimum temperature [3]. The amount of inoculum added or feedstock to inoculum ratio (F/I) also plays a significant role in the BMP assays. The F/I ratio only influences theoretically on the kinetics, and not the biomethane yield, which only depends on the organic component [4]. However, the assays may be toxic when the F/I ratio is too high while too low F/I may inhibit the production of the necessary enzyme for the AD. This ratio also affects the lag phase, which is shorter for low ratios [11]. Each feedstock has the optimum F/I ratio, depending on the amount of volatile fatty acid (VFA) produced, and the capability to buffer the medium of the ammonium produced from the proteins hydrolysis process. A small amount of inoculum is expected due to the internal biogas production can affect the results. But when the F/I ratios increase, it can lead to overloads due to the high VFA concentration. According to Hashimoto [12] and Labatut et al. [8], the F/I ratio should be higher than 2 g VS feedstock/g VS inoculum as digesting wheat straw and cow manure at concentrations of 10 – 40 g VS/L and higher than 3 g VS/L, respectively. For recalcitrant wastes (woody substrates and municipal wastes), the optimum biomethane production rate in the BMP assays was achieved at the F/I ratio of 0.5 g VS feedstock/g VS inoculum [13]. For kitchen waste, Neves et al. [5] concluded that when the buffer capacity is lower than 2 mg $NaHCO_3$/g COD, the F/I ratio was more important than the inoculum activity; the acidification occurred and limited the methanization rate with F/I ratios higher than 0.5 g VS/g VS. With maize waste, four different F/I ratios of 0.3, 0.5, 0.6, 1 g VS/g VS showed the optimum methane production rate, the high initial feedstock load gave higher maximum specific biomethane production rate but a lower maximum methane production per load [14].

The primary objective of the study was to evaluate the influences of the feedstock to inoculum ratio F/I in the range of 0.5 to 5.0 g VS of feedstock /g VS of inoculum on the BMP assay. Moreover, the study also explored the effects of temperature conditions (i.e. ambient: 30 ± 4°C and constant temperature 37°C) on the anaerobic conversion rates of mixed vegetable wastes to methane using BMP assay.

METHODOLOGY

Feedstock and inoculum

Organic solid waste from the vegetable market in Talaad Thai market, located in Thailand, was used as the feedstock. The feedstock was cut and ground in a blender to give a fraction with particle size around 2 – 3 mm.

The granular sludge from the up-flow sludge blanket reactor (UASB), Pathum Thani Brewery Co., Ltd. Thailand was used as the inoculum.

Biochemical methane potential assays

In this study, BMP assays were conducted as the blank assay method, and the procedure followed was based on the principles described by Owen et al. [9], and revised by others [13, 15]. The BMP assays were conducted in 120 ml glass serum bottles. The anaerobic bio-digestion and methane production of the feedstock was investigated over the following range of F/I ratios: 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0 g VS feedstock /g VS of inoculum. The mass of the feedstock and inoculum were calculated based on the VS of the feedstock and inoculum. The headspace of reactors was flushed with pure nitrogen gas at 5-10 psi for a period of 5 min and capped tightly with rubber stoppers. In order to determine the influence of temperature on the methanization rate, two temperature conditions (i.e. ambient: 30 ± 4°C and constant temperature 37°C) were applied for all the F/I ratios. For the constant temperature condition, reactors were set in an incubator; while the ambient temperature condition, reactors were placed outside laboratory under the ambient condition. A blank reactor was also set up in the same conditions, and the biogas/methane production from the blank assay was deducted from the biogas/methane production obtained in the substrate assays to determine these net values prior to data analysis. All BMP assays were conducted with duplicate reactors and the average results are presented.

Analytical methods

Elemental composition of the feedstock and inoculum was analyzed by a Thermo Finnigan CHNS Flash EA 1112 elemental analyzer following the manufacturer's standard procedures. Total solid (TS), volatile solid (VS) of feedstock and inoculum were determined gravimetrically following EPA method 1684 [16]. The pH of the feedstock and inoculum was analyzed by EPA method 9045D [17]. For alkalinity, substrate and inoculum were extracted with water as shown in ASTM method D3987 – 12 [18], and the extracted solution was measured for alkalinity following EPA method D1067 – 16 [19].

Biogas measurement and gas composition: The biogas production was measured by water displacement at room temperature [20, 21]. The compositions of biogas (methane, hydrogen and carbon dioxide) were analyzed by a gas chromatograph (PerkinElmer, USA) equipped with a thermal conductivity detector (TCD) and fitted with a Porapak Q, 50/80 mesh column. Helium gas was used as the carrier gas at a flow rate of 25 mL/min. The operating temperatures of column, detector, and injector were 45, 100, and 100ºC, respectively.

RESULTS AND DISCUSSION

Characteristics of feedstock and inoculum

The characteristics of feedstock and inoculum are presented in Table 1. In this study, the feedstock was the mixed tropical vegetable waste, including cabbages, Chinese cabbage, cauliflower leaves, water spinach, lettuce, etc. The VS of the mixed vegetable waste was very high at 79.31% of TS, therefore it should be easily degraded in the AD process. The pH of the mixed vegetable waste and inoculum in the study were 6.5 and 7.83, respectively. The required pH for fermentative bacteria was in a range of 4.0–8.5 [22]. From the elemental composition, the C/N ratio of the mixed vegetable waste and granular sludge were about 10 and 5.2, respectively. According to Divya et al. [23], the suitable C/N ratio for the AD process is in the range 10 to 35.

Table 1 Feedstock and inoculum characteristics

Parameter	Units	Vegetable waste[a]	Granular sludge[a]
Total solid (TS)	(%)	9.60 ± 0.27	8.05 ± 0.03
Volatile solid (VS)	(%)	79.31 ± 0.99	89.36 ± 0.12
pH	-	6.5 ± 0.20	7.83 ± 0.10
Alkalinity	mg $CaCO_3$/L	5690 ± 590	1512 ± 314
Elemental composition[b]			
Nitrogen (N)	(%)	3.82	8.83
Carbon (C)	(%)	37.91	45.99
Hydrogen (H)	(%)	7.45	7.25
Sulphur (S)	(%)	ND	0.70

[a] Values represents the average ± SD of 5 samples; [b] CHNS analysis of dry samples.

Effect of F/I ratio and temperature on biogas production

Figure 1 shows the net biogas generation curves changing with time at different F/I ratios at constant temperature (37°C) and ambient temperature. At all F/I ratios, a rapid initial biogas production (no lag time) was observed. Except for the F/I=0.5 g VS feedstock/ g VS inoculum, biogas production increased with increasing F/I (i.e. the biogas production was proportional to the VS load applied). After 53 days of digestion time, the largest volume of biogas production of 552 mL was observed at the F/I of 5.0 g VS feedstock/ g VS inoculum. At the F/I ratios of 0.5 g VS/ g VS, peaking volume of biogas production was 414 mL with only 0.5 g VS of feedstock was added into the reactor.

The biogas production at the constant temperature (37°C) was generally higher than that of the ambient temperature at all F/I ratios except F/I of 4.0 and 5.0 g VS feedstock/ g VS inoculum. The differences in biogas production between the constant temperature and ambient temperature were 31%, 37%, 13%, 11%, and 9% for F/I of 0.5, 0.75, 1.5, 2.0, and 3.0 g VS feedstock/ g VS inoculum, respectively. However, at the F/I ratios of 4.0 and 5.0 g VS feedstock/ g VS inoculum, the biogas production under the ambient condition is higher than that of constant temperature, and the difference is about 5%.

In addition, Figure 1 also depicts the optimum retention time (i.e. biogas generation time) of each F/I ratio. At F/I ratios of 0.75, 1.0, and 1.5 g VS feedstock/ g VS inoculum, the maximum volume of biogas production was achieved after 12 days of digestion time. While F/I ratios of 2.0 and 3.0 g VS feedstock/ g VS inoculum reached the maximum of biogas production after 25 and 30 days of digestion time, respectively. However, at F/I ratios of 0.5, 4.0, and 5.0 biogas is still being generated after 53 days of the experiment. The results are very important because they determine the optimum retention time of the feedstock when designing a pilot or full-scale anaerobic digestion reactors.

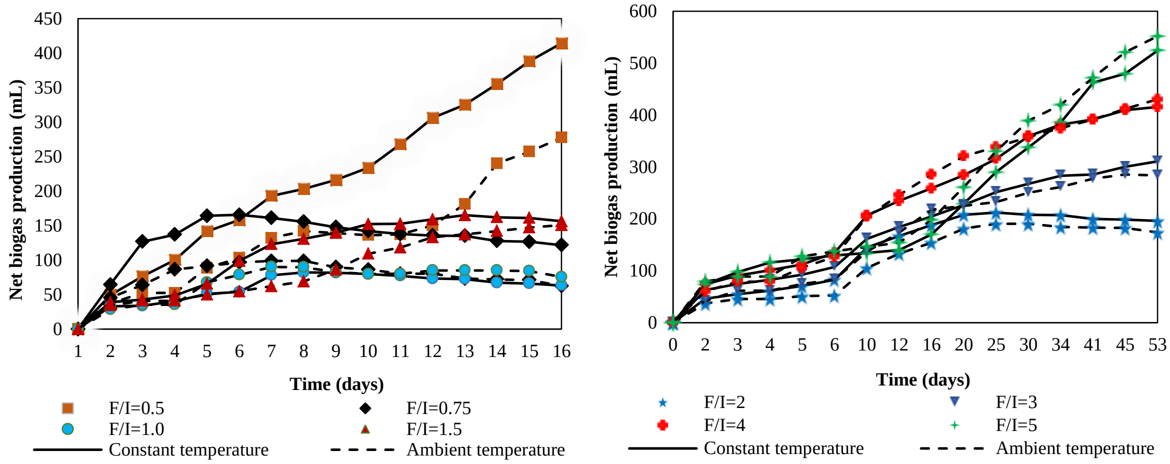

Figure 1 Biogas production at the different F/I ratios.

Effect of F/I ratio and temperature on methane production

Figure 2 presents net methane generation curves changing with time at F/I of 0.5 g VS feedstock/ g VS inoculum at constant temperature (37°C) and ambient temperature. After 53 days of digestion time, the net methane production was 170 ml under the constant temperate 37°C, and 92 mL under the ambient temperature. It means the percentages of methane gas at F/I=0.5 g VS feedstock/ g VS inoculum were 41%, and 33% of the biogas under constant and ambient temperate, respectively. The result gave percentages of methane in the biogas lower than that found in a batch experiment with maize waste carried out by Raposo et al. [14] who obtained a methane percentage of 53% using similar F/I ratio at 37°C.

Figure 3 shows the net methane yield of feedstock (i.e. methane gas generated per gram of feedstock added into the reactor) at F/I=0.5 g VS feedstock/ g VS inoculum, during 53 days of digestion time. The results indicated the significant role of temperature on methane production yield. At the constant temperature (37ºC), the net methane yield of feedstock was higher than 46% compare to that yield at ambient temperature. The results are comparable to the methane yields that obtained by Gunaseelan et al [2] in the BMP experiments from 23 samples of vegetable wastes that ranged from 190 to 410 mL methane/ g VS added.

However, the methane conversion efficiency was very low for the other F/I ratios, after 53 days of experiment time. Even though high volumes of biogas were obtained, methane production of all F/I ratios is very low. At constant and ambient temperature condition, the methane production after 53 days of digestion time at the F/I ratios of 0.75, 1.5, 2.0, 3.0, 4.0, and 5.0 g VS feedstock/ g VS inoculum were, respectively: 6.2 and 4.0 mL, 4.4 and 0.3 mL, 0.7 and 0.4 mL, 1.4 and 0.5 mL, 1.0 and 1.0 mL, 1.1 and 1.0, 0.5 and 0 mL. The low efficiency can be explained as follows. When the F/I ratio is increased, it can lead to overloads due to volatile fatty acids (VFAs) accumulation. VFAs accumulation at high organic loading decreases pH and inhibit of methanogenesis [5, 22]. Thus, in this study, a higher F/I ratio than 0.5 g VS feedstock/ g VS inoculum, the activity of methanogens microbial was restricted by the acidification of environment. From the results, it can be concluded that the optimum ratio of the mixed vegetable waste and granular sludge as inoculum was 0.5 g VS/ g VS, without adding any buffer and medium solutions. At the higher F/I ratios, external alkalinity sources as buffer solutions are necessary to maintain the suitable pH range for methanogenic bacteria during the fermentative process.

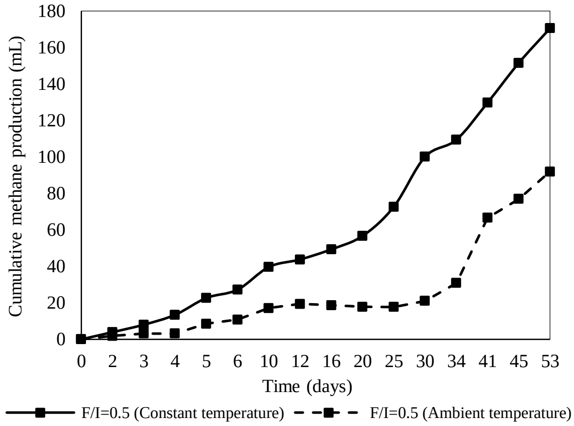

Figure 2 Net methane production at F/I = 0.5.

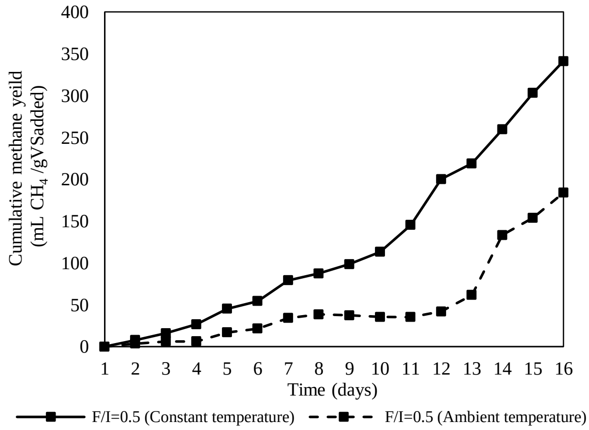

Figure 3 Net methane yield at F/I = 0.5.

Effect of F/I ratio and temperature on hydrogen production

Theoretically, the BMP assays are only conducted to determine the biomethane potential from biodegradable organic matters. However, when the experiments were operated, significant amounts of hydrogen gas were also detected. As the principle of anaerobic digestion, in the acetogenesis process, VFAs are converted into acetate, hydrogen gas, and carbon dioxide. This conversion is governed by the action of obligate hydrogen producing acetogenic bacteria, which are considered as acetogenins and acidogenic. The latter occurs under syntrophy phenomena. After this step, hydrogen will be converted to methane gas by methanogenic bacteria through hydrogenotrophic methanogenesis process. However, at the F/I ratios higher than 2.0 g VS feedstock/ g VS inoculum, the VFAs was accumulated at high organic loading and inhibited activities of methanogenic bacteria. Thus, in the study, the anaerobic digestion was stopped at acetogenesis process, and large amounts of hydrogen gas were obtained.

High amounts of hydrogen were found at F/I ratios in a range of 2.0, 3.0, 4.0, and 5.0 g VS feedstock/ g VS inoculum (Figure 4). The hydrogen generation started after 6 days of digestion time and lasted for 40 days. The hydrogen production also increased with increasing F/I ratio (i.e. the hydrogen biogas production was proportional to the VS added). At the F/I ratios of 2.0 and 3.0, the hydrogen production at constant temperature (37°C) was higher than that of ambient temperature. However, for the F/I ratios of 4.0 and 5.0 g VS feedstock/ g VS inoculum, the achievement of hydrogen production under ambient temperature condition was higher than that of the constant temperature condition.

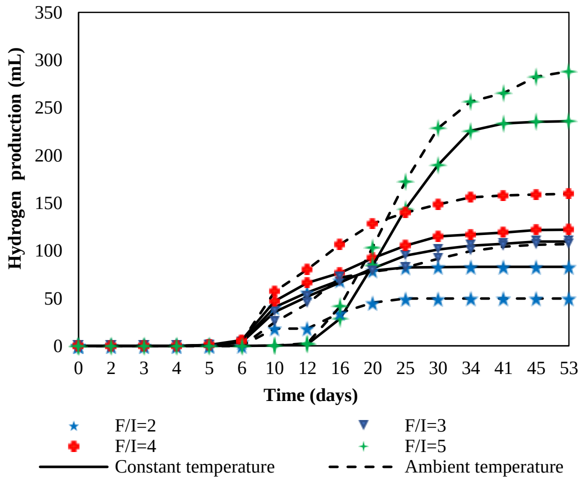

Fig. 4 Hydrogen production at the different F/I ratio.

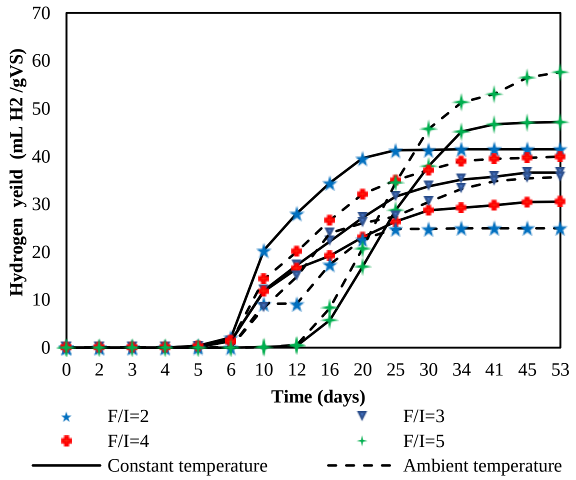

Fig. 5 Hydrogen yield at the different F/I ratios.

CONCLUSION

The mixed vegetable waste generated from the Talaad Thai market, Thailand has low TS (9.60%) and high VS (79.31% of TS), thus it is easily degraded in the anaerobic digestion process. The findings of this study clearly prove that the waste to inoculum ratio F/I has a significant influence on the biogas and methane production of the mixed vegetable waste. Without adding any medium or buffer solutions, after 53 days of digestion time, the F/I of 5.0 g VS feedstock/ g VS inoculum gave the highest biogas production (552 mL), while the F/I of 0.5 g VS yielded the highest methane production (188 mL). Moreover, temperature also plays an important role in the BMP assays. The biogas and methane production at the constant temperature (37°C) was generally higher than that of the ambient temperature at all F/I ratios except F/I of 4.0 and 5.0 g VS feedstock/ g VS inoculum. The optimum F/I ratio for anaerobic conversion rates of the mixed vegetable wastes to methane was 0.5 g VS/ g VS. On the other hand, the constant temperature (37°C) was found to be the optimum temperature condition. At the optimum conditions the net methane production and yield were 414 mL and 341 mL /g VS of feedstock. In addition, the study also found that at F/I ratios higher than 2.0 g VS feedstock/ g VS inoculum generated high amounts of hydrogen production, due to the inhibition of methanogenesis under high concentration of VFAs. Therefore, at the higher F/I ratios, external alkalinity sources as buffer solutions are necessary to maintain the suitable pH range for methanogenic bacteria during the fermentative process.

REFERENCES

[1] Buara, P. & Kumcha, U., (2006). Fruit and Vegetables production in Thailand. Food and Agriculture Organization.

[2] Gunaseelan, V.N., (2004). Biochemical methane potential of fruits and vegetable solid waste feedstocks. *Biomass and Bioenergy*, *26*(4), 389-399.

[3]Angelidaki, I. & Sanders, W., (2004).Assessment of the anaerobic biodegradability of macropollutants. *Reviews in Environmental Science and Biotechnology*, *3*(2), 117-129.

[4] Elbeshbishy, E., Nakhla, G., & Hafez H.,(2012). Biochemical methane potential (BMP) of food waste and primary sludge: influence of inoculum pre-incubation and inoculum source. *Bioresource technology*, 110, 18-25.

[5] Neves, L., Oliveira, R., & Alves, M., (2004). Influence of inoculum activity on the bio-methanization of a kitchen waste under different waste/inoculum ratios. *Process Biochemistry*, *39*(12), 2019-2024.

[6]VDI, V.D.I., (2006). *4630: Fermentation of organic materials, characterisation of the substrate, sampling, collection of material data, fermentation tests.* Verein Deutscher Ingenieure (VDI), editor. VDI Handbuch Energietechnik. Berlin: Beuth Verlag GmbH, p. 44-59.

[7]Prabhudessai, V., Ganguly, A., & Mutnuri, S., (2013). Biochemical methane potential of agro wastes. *Journal of Energy*, 2013.

[8]Labatut, R.A., Angenent, L.T., & Scott, N.R., (2011). Biochemical methane potential and biodegradability of complex organic substrates. *Bioresource technology*, *102*(3), 2255-2264.

[9]Owen, W., et al., (1979). Bioassay for monitoring biochemical methane potential and anaerobic toxicity. *Water research*, *13*(6), 485-492.

[10] Lesteur, M., et al., (2010). Alternative methods for determining anaerobic biodegradability: a review. *Process Biochemistry*, *45(*4), 431-440.

[11] Chen, T.-H. &Hashimoto A.G.,(1996). Effects of pH and substrate: inoculum ratio on batch methane fermentation. *Bioresource Technology*, **56**(2-3), 179-186.

[12]Hashimoto, A.G.,(1989). Effect of inoculum/substrate ratio on methane yield and production rate from straw. *Biological wastes*, *28*(4), 247-255.

[13]Chynoweth, D., et al.,(1993). Biochemical methane potential of biomass and waste feedstocks. *Biomass and bioenergy*, *5(*1), 95-111.

[14]Raposo, F., et al.,(2006). Influence of inoculum to substrate ratio on the biochemical methane potential of maize in batch tests. *Process Biochemistry*, *41*(6), 1444-1450.

[15]Hansen, T.L., et al.,(2004). Method for determination of methane potentials of solid organic waste. *Waste Management*, *24*(4), 393-400.

[16]Telliard, W., (2001). Method 1684: Total, fixed, and volatile solids in water, solids, and biosolids. *US Environmental Protection Agency*. Washington.

[17]9045D, E.,(2004). Soil and Waste pH, part of Test Methods for Evaluating Solid Waste, Physical/Chemical Methods. Environmental Protection Agency.

[18]D3987-12, A.,(2012) Standard Practice for Shake Extraction of Solid Waste with Water, in *ASTM International*.

[19]D1067-16, A.,(2016) Standard Test Methods for Acidity or Alkalinity of Water, in *ASTM International*.

[20]Esposito, G., et al.,(2012). Bio-methane potential tests to measure the biogas production from the digestion and co-digestion of complex organic substrates. *The Open Environmental Engineering Journal*,. **5**(1).

[21]Angelidaki, I., et al.,(2009). Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. *Water science and technology*, *59*(5), 927-934.

[22]Zhang, C., et al.,(2014). Reviewing the anaerobic digestion of food waste for biogas production. *Renewable and Sustainable Energy Reviews*, 38, 383-392.

[23]Divya, D., Gopinath,L., & Christy,P.M., (2015). A review on current aspects and diverse prospects for enhancing biogas production in sustainable means. *Renewable and Sustainable Energy Reviews*, 42, 690-699.