Effects of Oil Palm Shell Biochar to Tuna Wastewater on Biogas Production and Methane Yield by Anaerobic Sequencing Batch Reactor (ASBR): Mesophilic Condition

K. Hussaro

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ABSTRACT

Tuna wastewater (TW), elephant manure (EM), chicken manure (CM), and chemical additive (oil palm shell biochar or OPS biochar (OB)) were co-digested on anaerobic sequencing batch reactor (ASBR) at control mesophilic temperature (35±2^{o}C) for 55 days. The purpose of this research was to study effects on biogas production by using different mixing ratio and comparing to with/without OB into mixing feedstock. Anaerobic co-digestion processes were performed in 250 L ASBR with 130-L fermentation area and 120-L of headspace within 5 different ratios of TW/EM/CM/OB: D1 (TECB of 5:1:1:1), D2 (TECB of 5:1:1:2), D3 (TECB of 5:1:1:3), D4 (TECB of 5:1:1:4), (TECB of 5:1:1:0). Adjusted feedstock for optimum C/N ratio by mixing OPS biochar resulted in significantly higher biogas production yield and methane content yield mixing without OPS biochar on feedstocks. At second stage (between day 21-44), the result has found that D2 with optimal C/N ratio of 25.73 generated the highest of cumulative biogas production, biogas yield, methane content, methane yield, which was 3,694.88 L, 0.87 L/kg feed.day, 75 %, and 0.58 % CH4/kg feed.day, respectively. D2 had biogas production of more than 5 %, 20 %, 25 %, and 30 % than D3, D1, D5, and D4, respectively. D4 was operated with higher OPS biochar than D1-D3, which was inhibition on methanogenesis phase to low biogas production. Therefore, this anaerobic co-digestion system D1-D3 can considered as the right choice system for tuna wastewater treatment which give a benefit on renewable energy (biogas) production.

1. INTRODUCTION

It was reported that Thailand exported 133,998 tons of processed canned tuna during January to March 2020 (The Bureau of Agricultural and Industrial Trade Promotion, Department of International Trade Promotion (DITP)). It is worth 532.47 million USD, which has increased about 4.56 % compared to the same period of last year [1]. Wastewater is by-products from tuna processing industry (TPI), which is collectively called WW and has become a serious environmental problem. It must be treated by wastewater treatment plants (WWTPs) prior to release into the environment. Waste from tuna processing generates 13.5-18.9 tons/day from 135 tons/day of raw materials (Chotiwat Manufacturing Co., Ltd., Songkhla Province), which consists of 35-35% wastewater (WW) and 25-35% solid waste (SW). Tuna processing is presented in Fig. 1. Production of WW and SW occurs within all stages of tuna processing, including defrosting, cleanup, steaming, cooling, white meat separation, canning, adding of oil or saltwater, cans cleaning, and sterilization. SW are estimated to be 12 tons from 35-40 tons/day of factory capacity, which is 25-35 % of feed stock tuna processing. Although, the coronavirus disease (COVID-19) has been endangering human health worldwide since before December 2019 [2,3], but the food processing industry is still important to keep on producing continuously. The strength of Thailand in tuna processing industry consists of readiness skills and labor skills. Developing of products was done via complete technology, and fish raw materials were easily sourced out from around the world. In addition, Thailand has a network covering raw material sourcing, production, as well as distribution channels in keys markets. Hence, enormous amounts of tuna wastes are produced in the tuna processing industry. Physico-chemical treatment methods and biological treatment methods are currently the most common wastewater treatments. Anaerobic digestion, aerobic treatment, and fungal treatment are presently the greatest common biological treatment methods [4,5].

Anaerobic digestion (AD) is considered as an advanced technology for wastewater treatment, due to its stage of art advantage, reduce pollution and serve as alternative fuel resources [6]. Biogas production occurs pending anaerobic digestion of organic wastes via a microbial community

[1]*Rajamangala University Technology Rattanakosin, Salaya, Phutthamonthon, Nakhon Pathom 73170, Thailand.*
**Corresponding author:* K. Hussaro; Phone: +66-88-940-8358; Fax: +66-2-441-6000 ext 2630; E-mail: kanokorn.hus@rmutr.ac.th.

with a multiple biochemical reaction, which can be used for heating and cooling, cooking, power generation, vehicle fuel, and gas-grid injection on transmission system of electricity. AD has more advantages than other biological treatment methods; aerobic treatment, physicochemical treatment, and others, in terms of facility of design, less capital investment requirement, and lower energy requirement [4,5]. AD produced biogas product through biological reaction. Main process involving four phases, which are hydrolysis, acidogenesis, acetogenesis, and methano-genesis phase. Overall AD process of organic materials digestion for biogas production is shown in Fig.2 [5,6,7]; Feedstock characteristics and process parameters are affecting on performance of biogas production [8].

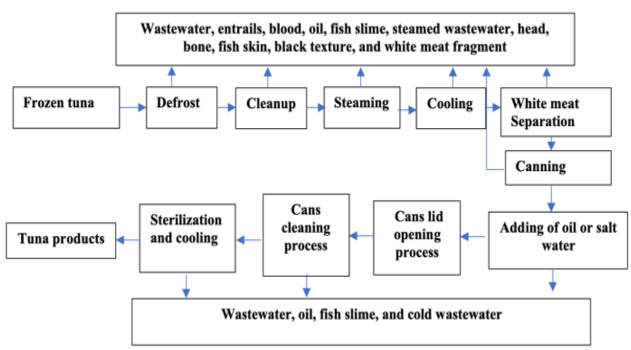

Fig.1. Production of tuna processing wastes.

For assuring process stability, aside from nutrient contents, others feedstock characteristics which very important for AD reaction are particle size and inhibitory compounds. In addition, parameters for AD process configuration design include organic loading rate (OLR), substate pH, moisture content, reaction temperature, hydrogen concentration, agitation rate, hydraulic retention time, and inoculum [6]. Moreover, process configuration design also needs to consider type of operations, which are batch types or continuous types, with single stage or multi-stage. Different digesters design for improving biogas production from different organic materials were studied, range from laboratory scale, pilot scale, demonstration scale, to industrial scale, popular type of industrial AD digester are Anaerobic Sequencing Batch Reactor (ASBR), Continuous Stirred Tank Reactor (CSTR), and Anaerobic Plug Flow Reactor (APFR). While, some conventional digesters are operated on inoculum retention, including Anaerobic Contact Reactor (ACR), Up-flow Anaerobic Sludge Bed Reactor (UASB), Up-flow Anaerobic Solid-State Reactor (UASS), Anaerobic Baffled Reactor (ABR), and Internal Circulation Reactor (ICR) [6,9]. However, UASB and ASBR can be applied on the industrial scale and pilot scale with wastewater treatment, due to efficiency in operating AD stable [10,11,12].

Previous research reported that digesting over swine wastewater in three reactors of ASBR, UASB, and UASS. The different reactor will be generating effects on performance, methanogenic genera, and volumetric methane production rate. Maximum methanogenic genera of Methanosaeta and Methaanospirillum showed for 81.37-90.83 % in the ASBR, while high volumetric methane production rate is at 1.679 L/L.d at the OLR of 8 g TS/L.d. The ASBR achieved better performance than USBR, due to terrible washout of extremely sludge wastewater in the USR [13].

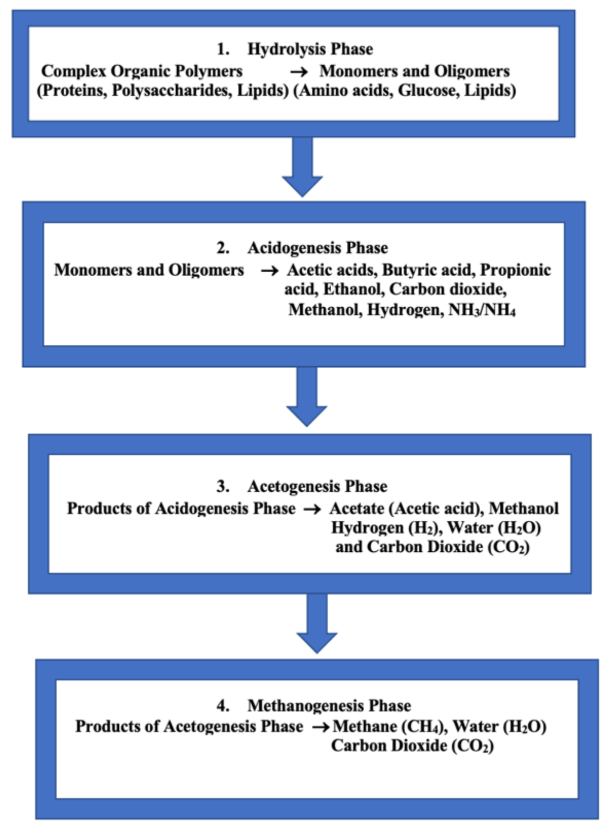

Fig. 2. Anaerobic Digestion Process.

6 L-ASBR have been experimented for study anaerobic fermentation of sucrose-rich systetic wastewater to produce biohydrogen by many researchers. Testing parameters are pH, hydraulic retention time (HRT), and organic loading rate. Result of studied showed that using pH 4.5, HRT 30 h, and OLR 11.0 kg/m^3.d, the maximum hydrogen production rate and yield were 3.04 L H_2/L reactor.d and 2.16 mol H_2/mol hexose, respectively [14]. Furthermore, digester ASBR can be applied on the treatment of flush diary manure using a cationic polyacrylamide (PAM) as it can provide relative high sludge retention of PAM efficiencies high-rate treatment of flush dairy manure, but there isn’t any approval to enhancing biogas yield [15]. On the other hand, a study of effects of additive antimicrobial chlortetracycline (CTC) with swine manure slurry in four AD 9.5 L lab-scale ASBR on 28-day cycles digestion, two without CTC and two with CTC-amended manure. It was found that there is significantly less volumetric

composition 13 % and 15% of methane for cycles 1 and cycles 2, respectively [16].

Wastes from tuna processing is rich in entrails, blood, oil, fish slime, head, bone, fish skin, black texture, and white meat fragment, which will be inhibited to the anaerobic digestion. To improve biogas production, therefore, some biological and chemical additives such as biochar are added. Enhancing biogas production, including process stability and biogas products (biogas quantity and quality), some others chemical additives such as pectin, activated carbon, silica gel, and etc. have been studied and reported [6]. The other interesting studied refer to effect of bamboo hydrochar (BHC) additives for fish waste processing in AD on methane yield, it was found that at dry mass ratio of of BHC to fish waste 1:2, it can obtained 292 L/kg-VS compared to the maximum biogas yield and 3410 kJ/kg-VS of the highest methane energy yield, via using hydrothermal carbonization (HTC) of BHC at 200 °C [17].

On the other hand, there are explanations on the effectiveness of biochar additives with wastewater sludge to biogas production on AD, which was operated with Gouglas for pylolysis. It was found that the cumulative highest methane production at 500 °C, which was 11% and 98% more than without biochar at 37 °C and 25 °C of AD temperature respectively. Therefore, biochar play importance role in enhancing methane production from wastewater sludge AD [18].

Some researchers selected some carbon-based nano-materials for improving biogas production. Organic wastes has high potential to transform to carbon-based nanomaterials biochar with three geometrical structures form; carbon nanotubes (CNTs), graphene, and fullerenes. CNTs have been investigated recently on a wide range of applications in biogas production which generally give benefit to enriching electroactive bacteria that includes Caloramator sp. (Geobacter sp.) and methanogenic archaea (Methano-sarcina, Methanosaeta, etc.). CNTs (Form of multi-wall carbon nanotube, MWCNTs) could enhance bacterial community and obtained 11.2 % of Bacteroridetes and 8.4 % of Firmicutes [19, 20]. Other studies on effects of CNTs (in form of multi-wall carbon nanotube, MWCNTs) to biogas production by AD from granular sludge wastes of beer industry under the mesophilic condition for 96 h. It was found that, cumulative methane content (CH_4) produced was higher than 1500 mg/L of MWCNTs, which was compared to the control one (151.8 versus 106 mL/g volatile-suspended granules) [20, 21].

This research has focused on effects of palm oil kernel biochar to organic materials on biogas production and methane yield via ASBR. Therefore, the aim of this study was to assess the influence of adding biochar (hydrothermal carbonization experiment) to raw materials (inoculum and tuna wastewater), which was operated in AB system at the mesophilic state. There were evaluated in form of biogas outputs (biogas composition and methane percentage).

2. MATERIALS AND METHODS

Substrates Preparation

Inoculum used in this research was elephant manure (EM) from Nongnuch Garden Pattaya, and chicken manure (EM) from a poultry farm in Chachoengsao province. Tuna wastewater (TW) was collected from a factory in Samut Sakhon province. The bone fragments and fish head were separated from the Tuna processing waste (consists of organic matter, bone fragments, fish head, fish skin, and flaked tuna scraps) prior to using. Collected substrates were stored at 25°C prior to characterization and used. Oil palm shell (OPS) biochar produced by hydrothermal carbonization process was used for this studied. The biochar production started with drying OPS in a hot air oven at 110 °C for 24 hours, then grinding and sieving to classify for 2-2.8 mm size, after that OPS will be introduced to a stainless-steel tube reactor operating in a muffle furnace at temperature 600 °C for 1 hour under the flow of N_2 at 150 cm^3/min. Heating rate was controlled at 10 °C/min, then OPS biochar (OB) was cool down to ambient temperature.

Experimental design and set-up

Full scale anaerobic co-digestion studies were operated in 250 L ASBR with 130-L working volume and with 120-L gas production headspace. Stainless steel ASBR digester of single digestion with two layers and operated continuously were operated at a constant mesophilic temperature (35±2 °C) through the hot water tank for 55 days. During this experiment, proper biogas production and varied feedstock ratio were used in the AD study. 250 L ASBR was set up in hot water tank, 250-L of stainless steel of AD, 200-L of water displacement system, table base, inlet feedstocks, overflow mash, water pump, power supply and control system, auxiliary heater, pressure gauges, temperature meter (TENMARS, model TM-747DU), and control valve, as shown in Fig.3. Feedstocks were tuna wastewater (TW)/elephant manure (EM)/chicken manure (CM)/ OPS biochar (OB)): TM:EM:CM:OB of 5:1:1:1, 5:1:1:2, 5:1:1:3, 5:1:1:4, 5:1:1:0 (by weight) for biogas production, hereafter referred to as D1, D2, D3, D4, and D5, respectively.

Five full scale co-anaerobic systems were fed of raw materials ratio for 55 days, which was performed daily for only one week (about 7 days). Initial study using TW and inoculum (animal manure) has been done to compare the effectiveness of OB synthesized at different ratio (1-4). Additional anaerobic co-digestion experiments were also operated using TW as feedstock to observe the effect of these OB in treatment of TW from tuna processing waste. For all digester of D1-D5, each digester was loaded with 439 g/L of TW, 88 g/L of EM, and 88 g/L of CM with 88 g/L, 176 g/L, 264 g/L and 352 g/L of OB for D1-D4, respectively.

Test and measurement

Total solids (TS) concentration, volatile solids (VS), total Kjeldahl nitrogen (TKN), potassium (K), and phosphorus (P) of animals' manure as an inoculum (CM and EM) were determined by Standard Methods of the American Public Health Association (APHA, 2005). The pH was measured with a SP-2100, Suntex pH meter. Proximate and ultimate analyses of each feedstock and OB were determined by ASTM standard test with CHNS analyzer (Automated C, H, N analysis). Concentration of methane (CH_4) and carbon dioxide (CO_2) were measured using a portable Geotech BIOGAS 5000 with accuracy of ±0.5% after calibration, which allowed 0-100% of CH_4 and CO_2, 0-25% of O_2, 0-10,000 ppm of H_2S, and 0-1,000 ppm of CO. First biogas composition was measured after 7 days of digestion, and then daily, for cumulative biogas yield, optimized biogas yield, CH_4 content (%), methane yield, and methane content (%). All digester performances were daily monitored through regular temperature and pH measurements.

3. RESULT AND DISCUSSION

Characterization of Substrates and OPS Biochar

The composition of tuna wastewater (TW), elephant manure (EM), chicken manure (CM), and OPS biochar (OB) used in the studies are shown in Table 1. It is widely known that the compositions of these substrates had affected on biogas production. In the ultimate analysis, TW and OB carbon contents were similar at 51.19 % and 63.52 % (by wt.), respectively. While, TW nitrogen content (4.89 % by wt.) was over six of that of the OB (0.81 % by wt.). However, EM and CM carbon contents were similar, at 28.06 % and 29.56 % (by wt.), respectively, which CM nitrogen content (3.24 % by wt.) was over twice of that of EM. The C/N ratios of CM, TW, EM and OB were 9.12, 10.47, 15.76, and 78.42, respectively. C/N ratios have been presented in Fig.4.

C/N ratios of TW and EM were out of acceptable range of optimal C/N ratio (15-30 of organic substrates in AD), which was also affected by the methanogen process. The higher 35 of C/N ratio occurred due to rapid consumption of nitrogen by methanogenesis, which it will no longer react to the leftover carbon content on substrates, and may cause a low biogas production even if the C/N ratios of feedstock had lower than 20 of C/N ratio due to ammonia accumulation in (NH_4) of ammonia. This substance (NH_4) will be increasing acidity (pH) on digester, which it is causing an effect on methanogenic bacteria and result in low biogas production [22,23]. The multiple feedstocks (in the optimum C/N ratio range) was obtained optimal biogas production. Anaerobic co-digestion was operated at a relatively hydraulic retention time of 55 days by using mixed TW, CM, and EM as a feedstock with/without OB. Resulting in a carbon to nitrogen ratio (C/N Ratio) were 19.14, 25.73, 31.25, 35.54, and 11.28 of D1, D2, D3, D4, and D5, respectively, as shown in Fig.5. D1 and D2 were nearly optimal C/N ratio range. It can be explained that the TW and OB had improving into optimal anaerobic co-digestion for biogas production.

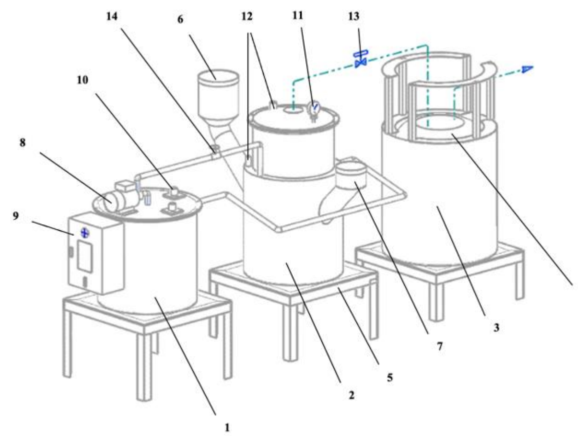

Fig.3. Full Scale ASBR-Anaerobic Digestion Equipment; (1) Hot Water Tank, (2) 250-L of Stainless Steel of Anaerobic Digester, (3) and (4) 200-L of Water Displacement System, (5) Table Base, (6) Inlet Feedstocks, (7) Overflow Mash, (8) Water Pump, (9) Power Supply and Control System, (10) Auxiliary Heater, (11) Pressure Gauges, (12) Temperature Meter (TENMARS, model TM-747DU), (13) and (14) Control Valve.

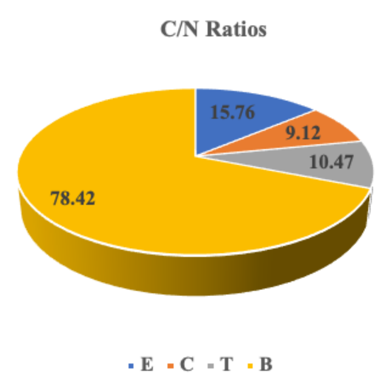

Fig. 4. Carbon/Nitrogen Ratio of TW, EM, CM, and OB.

In the proximate analysis, the VS of OB, TW, CM, and EM were 25.76 %, 39.11 %, 57.65 %, and 67.32 %, respectively, while that of the TS were 41.05 % of TW, 67.18 % of EM, and 75.25% of EM. Technically, all substrates are greater than 30 % of TS, there will be influent on AD to low biogas production, which agree well with the other hand [17]. In addition, the VS/TS ratio of CM, EM, and TW were 8.86, 0.89, and 0.95, respectively, indicating that the inoculum (EM and EM) contained insignificantly more organic substrate than the TW [24]. In the chemical analysis, TW showed that it is rich in fats

(37.05 %), which was the result of high carbon content and low nitrogen content in the molecules. TW had very less 10.47 of C/N ratio. Therefore, the experimental design should be cautious by using organic substrates twice for co-anaerobic digestion and operated on mesophilic condition in order to reduce inhibition effect on anaerobic digestion and adjust C/N ratio to optimal condition.

Biogas Production Characterization

The daily biogas volume (L/day) with different OB content for five OB was presented in Fig. 6, it was found that biogas production of all digesters (D1-D5) was obtained began rapidly on the seven days. The maximum daily biogas production at 21 days of D1-D5 was 90.84 L, 113.55 L, 107.87 L, 79.49 L, and 84.78 L, respectively. While the total cumulative biogas production in range 55 day were 2955.90 L, 3694.88 L, 3510.13 L, 2586.41 L, and 2758.84 L of D1-D5, respectively. From the digester of TW with/without OB showed that D2 had higher maximum daily biogas production and cumulative biogas than D3, D1, D5, and D4, respectively. As described above, the D2 had increased to 5 %, 20 %, 25 %, and 30 % of biogas production compared with a D3, D1, D5, and D4, respectively.

Table 1. Chemical Properties of Inoculum (EM and CM), TW, and OB

Analysis	Item	Tuna wastewater (TW)	Elephant manure (EM)	Chicken manure (CM)	OPS biochar (OB)
Ultimate Analysis	Carbon (% by wt.)	51.19	28.06	29.56	63.52
	Nitrogen (% by wt.)	4.89	1.78	3.24	0.81
	C/N ratio	10.47	15.76	9.12	78.42
Proximate Analysis	TS (%)	41.05	75.23	67.18	-
	VS (%)	39.11	67.32	57.65	25.76
	VS/TS	0.95	0.89	0.86	-
	Ash content (%)	1.48	17.89	30.17	0.79
	pH	6.8	7.86	7.59	-
Chemical Analysis	Proteins (%)	7.89	-	-	-
	Fats (%)	37.05	-	-	-

Similar studies on the highest daily biogas yield of 0.87 L/kg feed.day of D2 at 21 days had higher than another digester, which were 0.83 L/kg feed.day, 0.71 L/kg feed.day, 0.65 L/kg feed.day, and 0.61 L/kg feed.day of D3, D1, D5, and D4, respectively, as shown in Fig. 7. The mixture of OB was expected to improve biogas production efficiency. In comparison of with or without OB on D1-D3 and D5, It was found that D1-D3 has been maintained an optimal C/N ratio for methanogenic bacteria on anaerobic digestion. Thus, feedstocks with OB have significant buffering capacity to biogas production. Conversely, D4 was obtained lower biogas production than another digester (with/without OB), due to it might be related to the highest carbon content and highest C/N ratio and its activity. Therefore, rapid consumption of nitrogen was inhibition on methanogenesis phase to low biogas production. This result is in agreement with the other researchers [22,25].

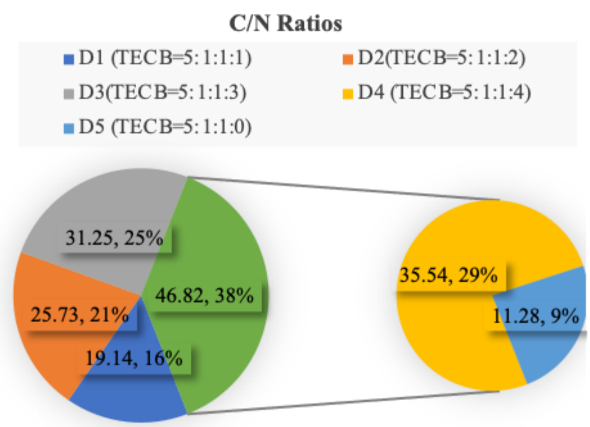

Fig. 5. Carbon/Nitrogen Ratio of D1, D2, D3, D4, and D5.

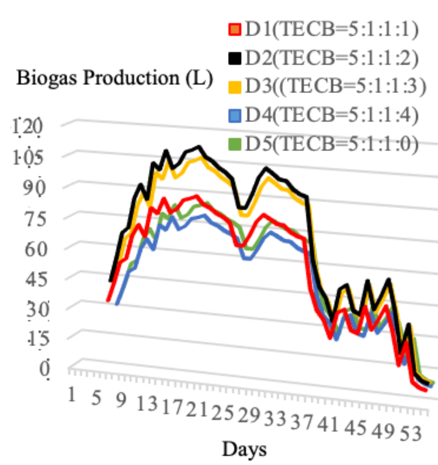

Fig. 6. Daily Biogas Production of TW and Inoculum (EM and CM) with/without OB and Five Differences: D1-D5.

Methane Production Characterization

Methane production during the ASBR-AD is shown in Fig. 8. It was found that methane content from adding OB to all co-digestion (D1-D3) was higher than that achieved from no OB adding (D5), which except D4 obtained lower methane content. Methane content of D1, D2, D3, and D4 were range of 32 % -70 %, 38 % - 75 %, 34 %- 70 %, and 31 % - 63%, while of without OB substrate (D5), methane content were range of 33 % - 64%. During the first stage (1-20th days), the average methane content and methane yield of all digesters were lower at 55 % and 0.55 % CH_4 /kg feed.day, respectively, as shown in Fig.9, but it remained closely stable at the end of the first stage. Going into the second stage (after 21th days)., it was increased to 15% over the highest peak of the first stage. The highest methane content and methane yield occurred on the 21st day were 75 %, 70 %, 64 %, and 63 %, and 0.58 % CH_4/kg feed.day, 0.54 % CH_4/kg feed.day, 0.51 % CH_4/kg feed.day, and 0.48 % CH_4/kg feed.day of D2, D1 and D3, D5, and D4, respectively. Meanwhile, the methane content and methane yield decreased slowly until day 44, after that there was sharp decrease of about 3.39 % of D1, 3.13 % of D1 and D3, 3.72 % of D4, and 3.57 % of D5 before finally at the end second stage, due to acetogenesis and methanogenesis stage was occurred more completely on the second stage [6].

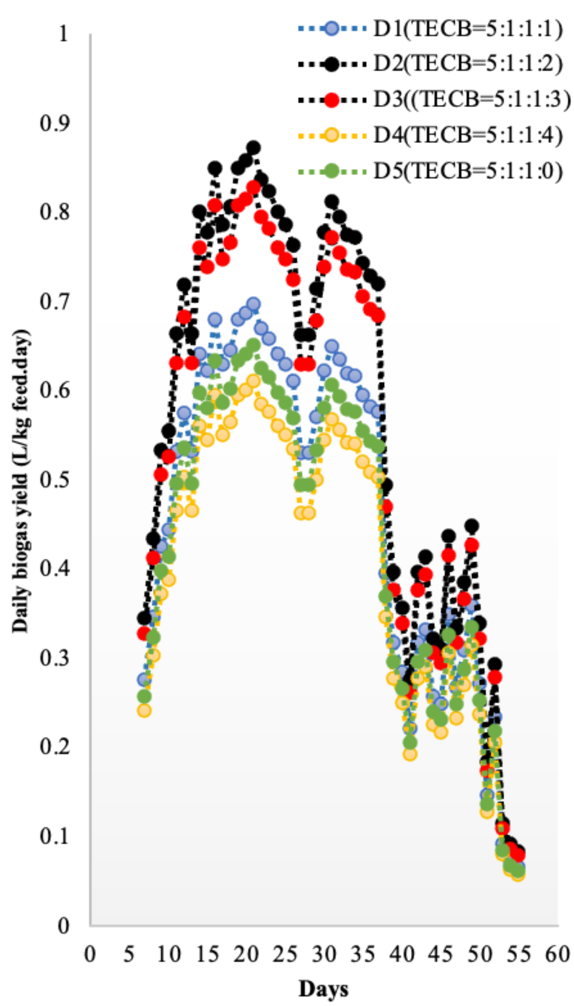

Fig.7. Daily Biogas Yield of TW and Inoculum (EM and CM) with/without OB and Five Differences: D1-D5.

It can be concluded that OB additive have some influence on methane content in biogas production. The methane yield and content in D2 was higher than that of others digester, the highest were 75 % and 0.58 % CH_4/kg feed.day, respectively, which led to 12% increase in highest methane content compared to the control proportion substrate and inoculum without OB (D5) as feedstock. These results clearly underline that co-anaerobic digestion amends biogas products, which were on the side of quantity and quality. This result agreed well with others studied [18], with highest methane content at 76.44 % in AD using fish processing waste and bamboo hydrochar (at ratio 1:3).

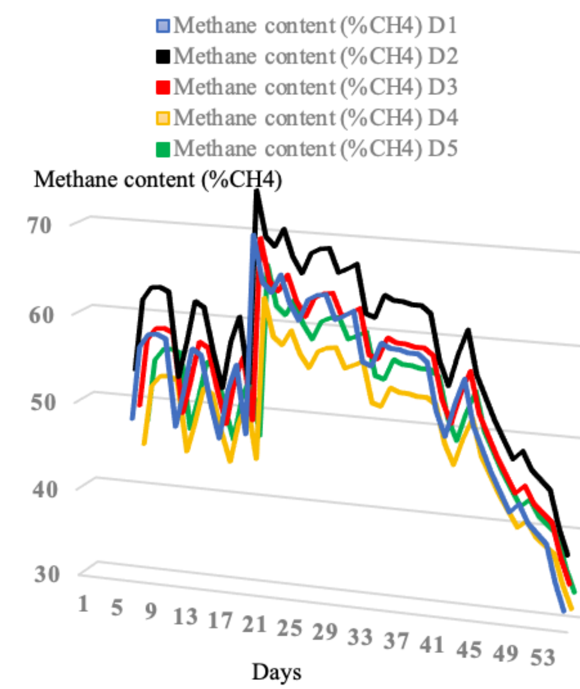

Fig. 8. Methane Content of TW and Inoculum (EM and CM) with/without OB and Five Differences: D1-D5.

Digester Temperature

For five ASBR digesters, average digester temperature was in the optimal mesophilic range, which there were 34.61 °C, 35.1 °C, 34.48 °C, 34.52 °C, and 34.92 °C of D1, D2, D3, D4, and D5, respectively, as shown in Fig. 10. It is well known that the temperature factor effects on the biodegradation of organic substrates into biogas production and stability methanogens. Especially, the mesophilic condition is the most parameter affecting the co-anaerobic digestion, which 25 °C – 45 °C refers to the optimal mesophilic range. Due to this, the co-anaerobic digestion was operated at a high temperature, in which there is no volatile fatty acid (VFA) accumulation compared to lower temperature [22]. Therefore, this system can provide more advantageous factors for methanogens which offer mesophilic co-anaerobic digestion. Most co-anaerobic digestions were operated under mesophilic condition; high mesophilic methanogenesis will be obtained more than hydrolysis/ acidogenesis using control temperature with ASBR digester.

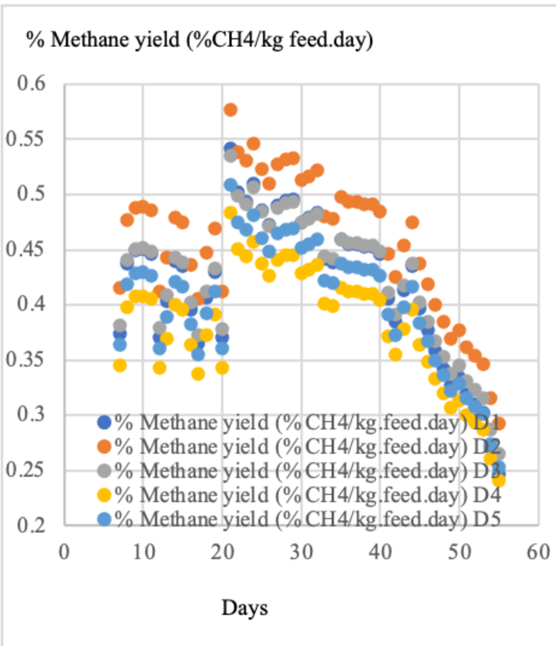

Fig. 9. Methane Yield of TW and Inoculum (EM and CM) with/without OB and Five Differences: D1-D5.

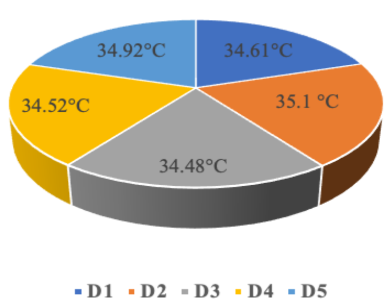

Fig. 10. Average Digester Temperature of TW and Inoculum (EM and CM) with/without OB and Five Differences: D1-D5

pH Characterization

The pH value was recorded throughout the whole digestion period by monitoring on anaerobic co-digestion of all five digesters with ASBR digester, the pH value of D1-D5 was shown in Fig. 11. The pH value found was in the range of 6.63-7.56, 6.50-7.40, 6.56-7.48, 6.89-7.63, and 6.18-7.41, of D1, D2, D3, D4, and D5 respectively. The pH average of D1-D5 were 7.09, 6.94, 7.01, 7.16, and 6.63, respectively, which were decreased from an initial 7.56 of D1, 7.40 of D2, 7.48 of D3, 7.63 of D4, and 7.41 of D5. This result clearly indicates that the use of biochar and tuna processing waste (D1-D3) were suitable for biogas co-anaerobic digestion due to the achievement of an optimum range to methanogens via maintaining neutral range of pH value, which was generally obtained optimum pH range at 6.5.7.5 [26]. Namely, a naturally obtained buffer system are carbonic-acid/bi-carbonate/carbonate and ammonia/ammonium equilibrium, that prevent too low (swing around 6.5 of pH value) and high pH value (swing around 10 pH value), respectively, as shown in Eq. (1) and Eq. (2) [6];

$$CO_2 + H_2O \Leftrightarrow H_2CO_3 \Leftrightarrow HCO_3^- + H^+ \Leftrightarrow CO_3^{2-} \Leftrightarrow 2H^{+.} \quad (1)$$

$$NH_3 + H^+ \Leftrightarrow NH_4^+$$
$$NH_4^+ + OH^- \Leftrightarrow NH_3 + H_2O. \quad (2)$$

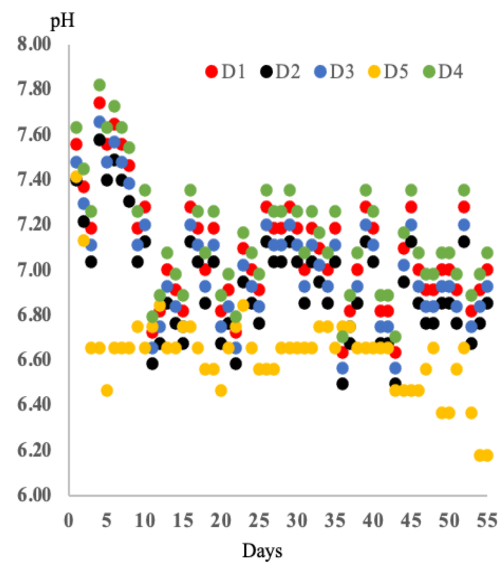

Fig. 11. pH Value of TW and Inoculum (EM and CM) with/without OB and Five Differences: D1-D5.

4. CONCLUSION

Anaerobic co-digestion of TW can be considered a promising alternative for improving the performance of anaerobic co-digesters and biogas efficiency of inoculum (EM and CM). It is clear that ASBR digester performs in converting organic substrates and OB additive by constant temperature (35±2 °C) through the hot water tank for 55 days. The investigated OB significantly enhanced biogas production. In this research, it has shown that significant optimization of cumulative biogas produced was 3,694.88 L with 75 % of highest methane content at 21st day, which was achieved by providing OB additive at D2 (TECB, 5:1:1:1:2). Comparing to with/without OB, it was found that the D2 ((TECB, 5:1:1:1:2) had increased more 25 % of biogas production than D5 (TECB, 5:1:1:0). So anaerobic co-digestion of TW, EM, CM with OB was proved to be good alternatives as renewable energy source in biogas production form. The advantage of the next research would be economic analysis and study of micro communities in order to have a better understanding of anaerobic co-digestion mechanisms.

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