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Anaerobic co-digestion of spent mushroom compost with striped fishpond sludge: The effects of C/N ratio on biogas production

Ngan, N. V. C.[1*], Viet, L. H.[2] and Thao, H. V.[3]

[1]Department of Water Resources, Can Tho University, Vietnam; [2]Department of Environmental Engineering, Can Tho University, Vietnam; [3]Department of Environmental Science, Can Tho University, Vietnam.

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Abstract The study evaluated co-digestion of spent mushroom compost (SMC) with striped fishpond sludge (FPS) in various C/N ratios in order to assess optimum proportion. Co-digestion of SMC and FPS performed better in methane potential than the sole digestion of FPS. As the C/N ratio increased, both biogas and methane potential increased and then declined. Higher C/N ratio set up, better co-digestion performance with stable pH and greater buffering capacity improved bio-methanation and greater biogas production. A C/N ratio of 40/1 showed optimum biogas yield and methane yield (283.25 L.kg VS_{added}^{-1} and 142.85 L.kg VS_{added}^{-1}) in a batch testing with hydraulic residence time of 60 days. In the batch experiment, methane concentration was recorded higher than 50% after two weeks of fermentation. For the semi-continuous experiment, the average biogas production rate was 102.69 L.kg VS_{added}^{-1} and the methane content greater was more than 50% after three weeks of testing. This result suggested that better performance of anaerobic co-digestion of SMC and FPS can be fulfilled by optimizing C/N ratio.

Keywords: Anaerobic co-digestion, C/N ratio, Methane production, Spent mushroom compost, Striped fishpond sludge

Introduction

The Mekong delta of Vietnam not only known as a rice-bowl area but also a region with high potential for aquacultural development of the country. In agriculture sector, rice straw produced in the Mekong delta was 26.86 million tons in 2016 (Ngan, Thuy and Phuong, 2018). There are several using of rice straw such as cattle feeding, onsite buried for composting, mushroom cultivation, selling to produce materials of light beton, paper, etc. (Nguyen and Tran, 2015). After mushroom cultivation process, there is leaft of spent mushroom compost (SMC) but no any treatment applied on this residue. In

[*] **Corresponding Author:** Ngan, N. V. C.; **Email:** nvcngan@ctu.edu.vn

aquaculture sector, the most growing product is small-pond culture of *Pangasianodon hypophthalmus* or striped catfish with 6,600 ha in 2019 (increased 22.2% to 2018) and produced 1.42 million tons. By applying on private segment, farmer do not care much on wastewater and sludge treatment but mostly direct discharge them into the effluent canal. Study of Anh *et al*. (2010) recorded the polluted parameters from striped catfish's sludge such as: COD of 1769 mg/L, BOD of 1061 mg/L, TSS of 6497 mg/L, total nitrogen of 45.6 mg/L, and total phosphorus of 22.7 mg/L that all far exceed the national standard of water discharge for aquatic wastewater.

During a recent decades, anaerobic digestion (AD) has been viewed as a proper technique for power recovery with enhancement manure and waste substrate. In particular, anaerobic co-digestion procedure showing good result on methane production because positive interaction of co-substrates (Agyeman and Tao, 2014). Fishpond sludge (FPS) - by richnes of organic and nutrient contents - was suggested as a co-digestion material for anaerobic process (Opurum *et al*., 2017; Opurum *et al*., 2015; Delaide *et al*., 2019). Study on co-digestion of SMC and pig manure in the Mekong delta recorded that SMC is possibly an additional material for energy recovery in the anaerobic fermentation process (Ngan and Fricke, 2012). This study aimed to investigate the effect of co-digestion SMC and FPS for biogas production in both of batch test and semi-continuous test.

Materials and methods

Feedstock preparation

Sample of FPS was collected from striped catfish farm in Thot Not district - Can Tho city - Vietnam. A pumping was used to pump sample from pond bottom to 300 L plastic containers, then transport them to laboratory for testing. The experiments were process in the same day of sludge sample collecting.

Sample of SMC was collected from mushroom farm in O Mon district - Can Tho city - Vietnam. The SMC were manually cut into approximate 1.0 - 2.0 cm long particles. The chopped SMC was air-dried at ambient temperature up to unchanged weight, and then they were manually mixed up until becoming a homogeneous form. The crude dried SMC were stored at room temperature in plastic bags.

Table 1. Analysis results of input materials

No.	Parameter	Unit	FPS	SMC
1	pH	-	6.84	-
2	Organic dried matter (ODM)	%	8.64	77.76
3	Carbon (C)	%	18.52	43.33
4	Total Kjeldahl nitrogen (TKN)	%	2.00	0.65
5	C/N ratio	-	9/1	67/1

Batch test

A total of six batch treatments were held in similar 20 L air-tight plastic reactor, each reactor has an outlet linked to a 15 L aluminum bag for biogas collection. To minimize the development of algae population that creates inner oxygen, all reactors were covered by black nylon bags throughout the testing period. There were two air-valves installed to the pipes in connection with the digester and the gas-bag so as to switch the gas off when the gas was being recorded. Similar reactors were introduced in previous study by Ngan & Fricke (2012).

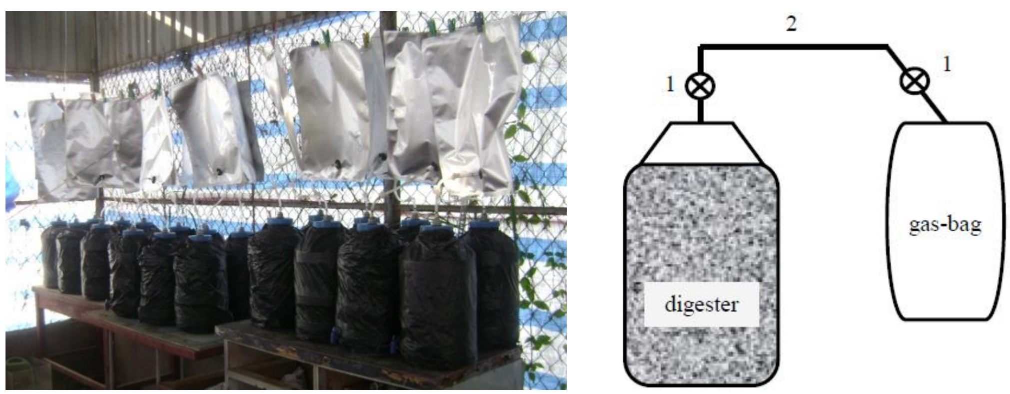

Figure 1. Treatment of batch test with (1) air-valve, and (2) gas pipe

The difference treatments of C/N for SMC and FPS with various proportions from 20/1 to 40/1 were shown in Table 2. In all the reactor (except the control treatment), FPS was input to adjust the volume of the working sample as 19 L. The remained volume of 1 L was left as buffer zone for gas stored so that the substrate will not accessed into the gas pipe. All treatments were mixed up by shaking the reactors manually once a day so as to increase the biogas production. Each of the treatments was in triplicates.

Table 2. The adding materials for testing treatments

	NT0 - control treatment	**Testing treatments with C/N ratios of**				
		20/1	***25/1***	***30/1***	***35/1***	***40/1***
SMC (g)	-	57.57	94.25	141.13	202.82	287.64
FPS (L)	19	18	18	18	18	18

Semi-continuous test

The semi-continuous anaerobic digestion with 20 L plastic reactor has been developed for this study. Succeed to the results from the batch test, this experiment applied co-digestion of SMC and FPS but only in the C/N ratio of 40/1. The testing conditions were similar to the batch test. There were three replicates for this treatment. The schematic outline of this reactor was shown in Figure 2.

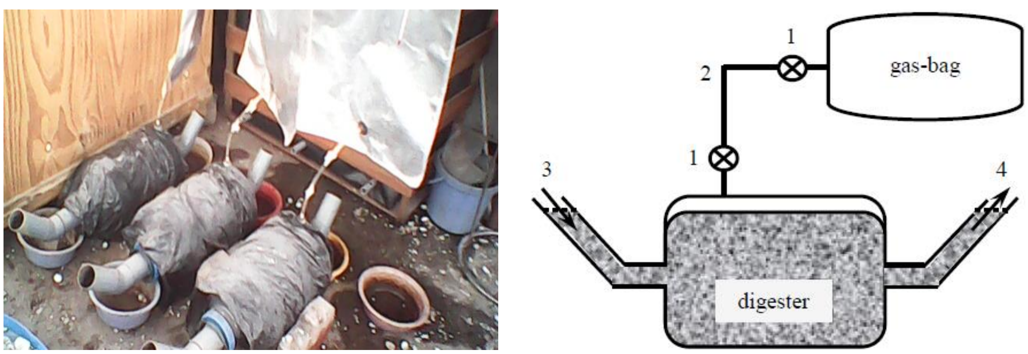

Figure 2. Semi-continuous treatment: (1) air-valve, (2) gas pipe, (3) inlet, (4) outlet

At the beginning, the material quantity of 16 days was fed once into the reactor, then fed daily from the day 17th onward. For the first 16 days, reactors were mixed up by manual shaking once a day. From the day 17th, only the action of feeding helped stir the substrate inside the reactors. This treatment was conducted for a period of 60 consecutive days.

Analysis methods

For both tests, substrates before and after the experiments were taken and analyzed for pH, carbon, total Kjeldahl nitrogen (TKN), and organic dry matter (ODM) according to the procedures in the Standard Methods for the Examination of Water and Wastewater (APHA, 1995). For the semi-continuous test, the substrates also collected and analyzed at every 20 days interval.

The daily biogas production was recorded by Ritter gas-meter (with the smallest scale of 20 mL), and the biogas components were monitored by a GA94 gas analyzer at every 4 days interval. The gas record was expressed at ambient temperature and at stable atmosphere pressure (1004 - 1006 mbar).

A specific biogas yield was calculated by dividing the total volume of biogas production after anaerobic fermentation with total organic dry matter (ODM) added initially. By using IBM SPSS 22.0 software, a one-way ANOVA was achieved.

Results

Batch anaerobic co-digestion between FPS and SMC

Biogas production

For co-digestion of FPS and SMC, the daily biogas production of all reactors rapidly increased on the day 10^{th} and reached a peak of 2.43 - 2.87 $mL.gVS^{-1}.day^{-1}$ within 16 - 20 days of digestion (Figure 3). Moreover, most of the co-digestion reactors displayed a very similar trend in the acceleration of biogas production excepting the treatment of C/N at 35/1. Co-digestion of C/N treatments, comprising ratio of 20/1, 25/1, 30/1 and 40/1 obtained the highest peak at the day 16^{th} while the control treatment and the co-digestion of C/N of 35/1 achieved the maximum of biogas volume on the day 20^{th}. No lag phase was detected during the anaerobic digestion of FPS and SMC. Thereafter, biogas production started to decrease rapidly and maintained low levels until the end of the experiment which could be due to the slow of biodegradable of substrates in reactors.

The large amount of methane content in reactors with the average value was 52.3 % (Table 3). The treatment with C/N ratio of 20/1 demonstrated the optimum values of CH_4 and CO_2 concentration compared with other treatments (Figure 5).

However, the cumulative biogas production from different treatments is shown in Figure 4. It is indicated that the accumulation of biogas production gave an opposite view when calculating based on the amount of VS added. It can be seen that the highest biogas production was observed from the only aquacultural sewage fermentation which recorded a maximum of 108.43 L.kg VS^{-1} added (1.81 L.kg $VS_{added}^{-1}.day^{-1}$) followed by the co-digestion reactors 20/1 (89.54 litters), 40/10 (83.59 litters), 25/1 (84.56 litters), 30/1 (76.54 litters), and 35/1 (75.08 litters). In the other hand, biogas yield of the mono-digestion was higher than 17.43%, 22.02%, 29.41%, 30.75%, and 22.09% that of co-digestion reactors of 20/1, 25/1, 30/1, 35/1, and 40/1 respectivelly.

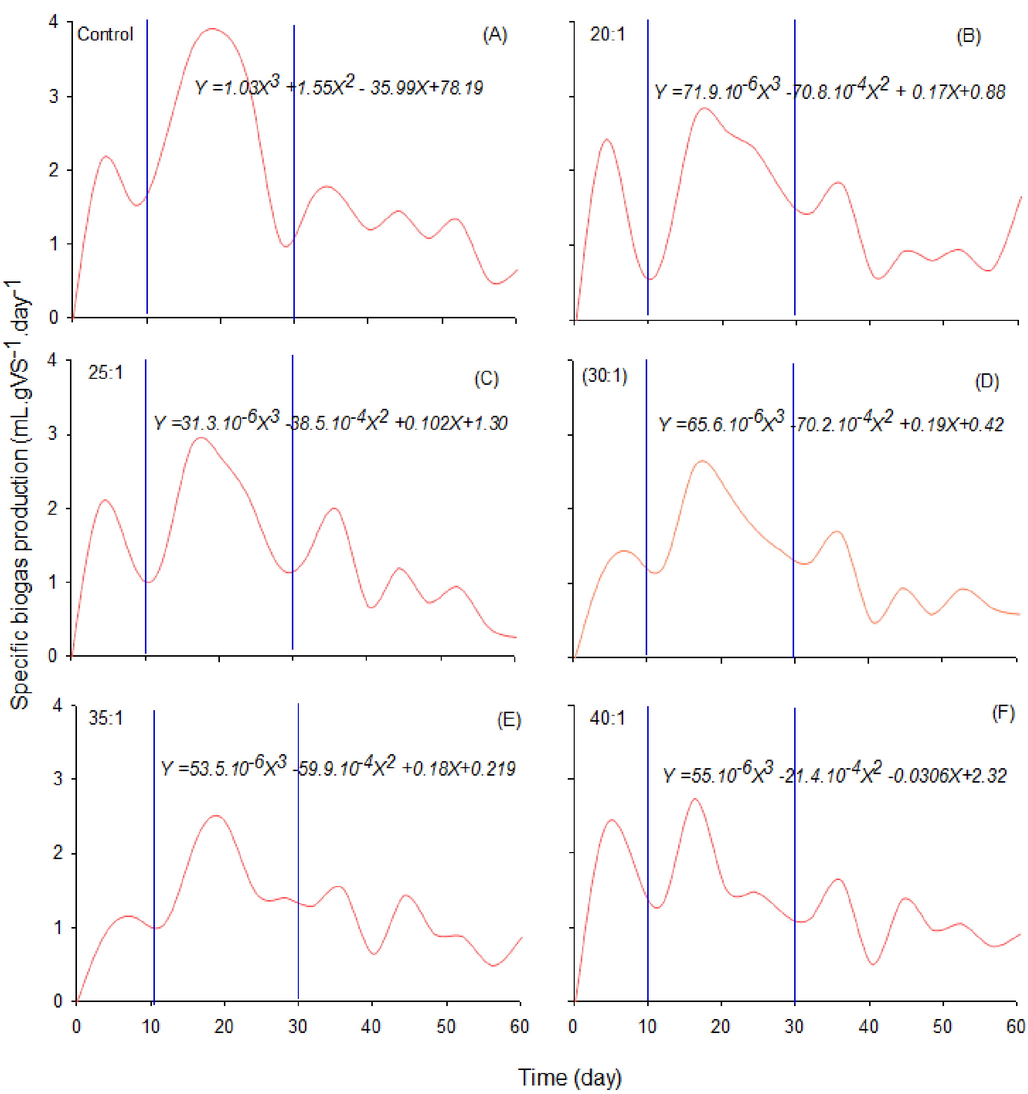

Figure 3. Daily biogas production from treatments

Table 3. Experimental results for different reactors

Parameters	**Reactors**					
	Control	***20/1***	***25/1***	***30/1***	***35/1***	***40/1***
Initial pH	6.84	6.73	6.69	6.69	6.65	6.72
Alkalinity (mg $CaCO_3.L^{-1}$)	1,766	1,833	1,900	2,067	2,165	2,267
Retention time (d)	60	60	60	60	60	60
Biogas yield (L.kg VS^{-1} degraded)	157.72	174.84	193.71	201.16	224.70	283.25
CH_4 yield (L.kg VS^{-1} degraded)	79.15	91.44	101.00	102.42	111.66	142.85
Average methane content (%)	50.19	52.30	52.14	50.91	49.69	50.43

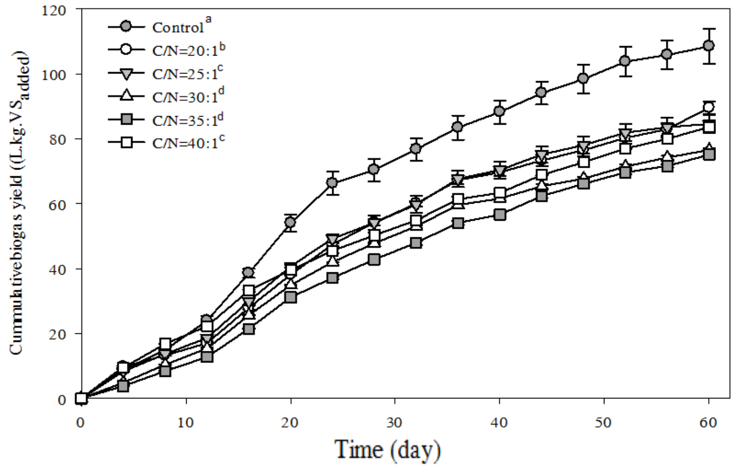

Figure 4. Cumulative biogas production in reactors

Biogas composition

The methane content of the biogas from all the treatments varied among the three periods, from 39.73 to 55.77% for the startup phase (the first 10 days), from 50.07 to 57.90% for the maximum biogas production from days 10th to 30th, and 37.65 to 58.73% for the suppression from days 30th to 60th which showed a relatively big deviation over the entire digestion (Figure 5).

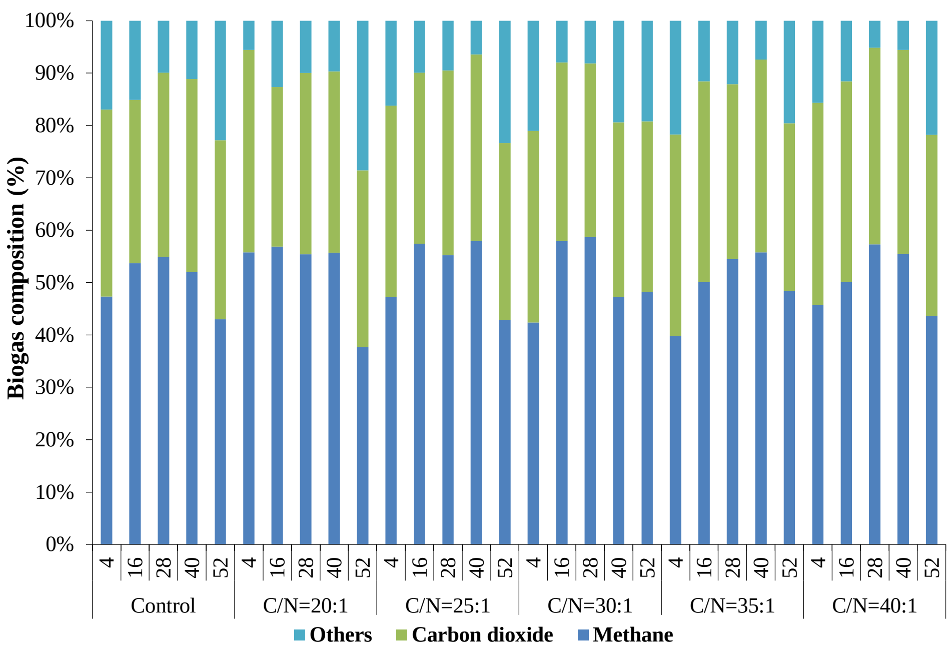

Figure 5. Biogas composition from various treatments

Volatile solids removal effectiveness for successful reactors

One of the most useful parameters to evaluate the efficiency of anaerobic digestion is the reduction in VS value. During the digestion process, VS are degraded to a certain extent and converted into biogas. It showed the reactor with sewage obtained the highest rate of VS destruction 69%, while reactor 40/1 had the lowest rate at 30% (Table 4).

Table 4. VS removal efficiency in reactors

Reactors (C/N)	Initial VS concentration	Final VS concentration	VS removal efficiency (%)
Control	73.87	23.09	69
20/1	113.20	55.23	51
25/1	143.27	80.73	44
30/1	179.73	111.34	38
35/1	227.70	151.62	33
40/1	293.65	206.99	30

The characteristic of biogas liquid

The data indicated a general trend that reactors containing SMC produced final digestates significantly lower concentrations of total Nitrogen (0.5 - 23.0%), and total Phosphorus (17.00 - 47.05%) than reactor digesting by mono-substrates excepting the reactor 40/1 (Table 5). However, fermented effluents in reactors FPS and SMC embraced the COD concentration was higher than FPS from 3.70 - 38.12%. Consideration of the feasibility of the fermented liquid before discharging which may put the pressure on local environmental problems, comprising the increase of greenhouse gas emission and eutrophication and water pollution.

Table 5. Characteristics of remaining digestate solutions in reactors

Reactors	Total Nitrogen (mg N/L)	Total Phosphorus (mg P/L)	COD (mg/L)
Control	204	17.83	1690.98
20/1	181	12.18	1756.10
25/1	169	14.17	1951.22
30/1	157	13.96	2341.46
35/1	204	9.98	2536.59
40/1	320	11.66	2731.71

Semi-continuous co-digestion between FPS and SMC

Biogas volume

Basing on the consequence from the batch digestion, co-digestion between FPS and SMC obviously demonstrated C/N ratio at 40/1 offering the highest methane yield with 283.25±2.12 $L.kgVS^{-1}$ degraded. Thus, experimentation on the co-digestion between FPS and SMC at C/N ratio 40/1 were implemented to evaluate the feasibility of biogas production in the context of semi-continuous digestion which widespread uses in reality.

The semi-continuous co-digestion of FPS and SMC could be divided into 3 periods: (i) initial feeding of 223.64 gVS.time^{-1} for a period of 16 days; (ii) daily feeding of 13.98 gVS.day^{-1} and (iii) without feeding (Figure 6). It is clear shown that daily biogas production was gradually increased during the stage (i) and (ii) from the day 8 to 72, and reached a peaking at 6.27±2.18 L.day^{-1}. This period could be attributed to the start-up and the steady phase where the feeding largely contributed to daily biogas production. Afterward, the daily biogas generation started to decrease rapidly for the remaining period which showed the suppression phase. Specifically, it was observed that after discontinuing the daily feeding for biogas reactors - biogas production continued the process of gas-forming until 12 days before witnessing the suppression period. It was suggested that recalcitrant polymers within SMC limited their degradation, and the lower amounts of soluble carbohydrates in the substrate resulted in slow hydrolysis and fermentation.

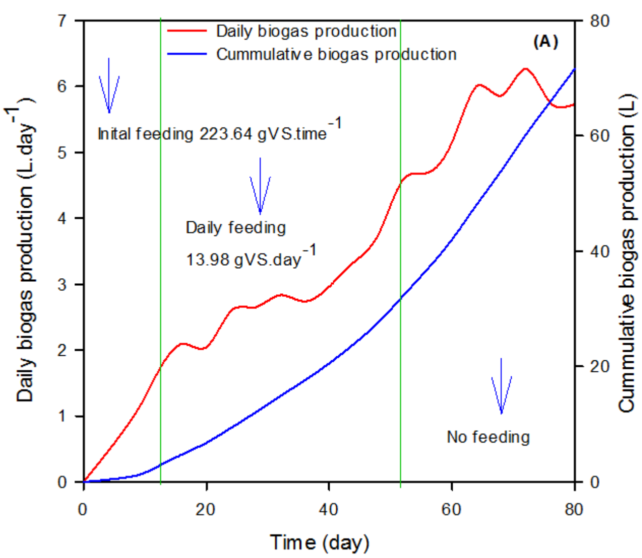

Figure 6. Biogas productions of semi-continuous reaction

Methane content

It indicated that methane content was speedily escalated in the startup and steady stage from 7.2 to 67% from the day of 8th to 60th (Figure 7). After 24 days of fermentation, methane concentration obtained around 50% in biogas composition which confirmed that the stabilization of methanogenesis stage.

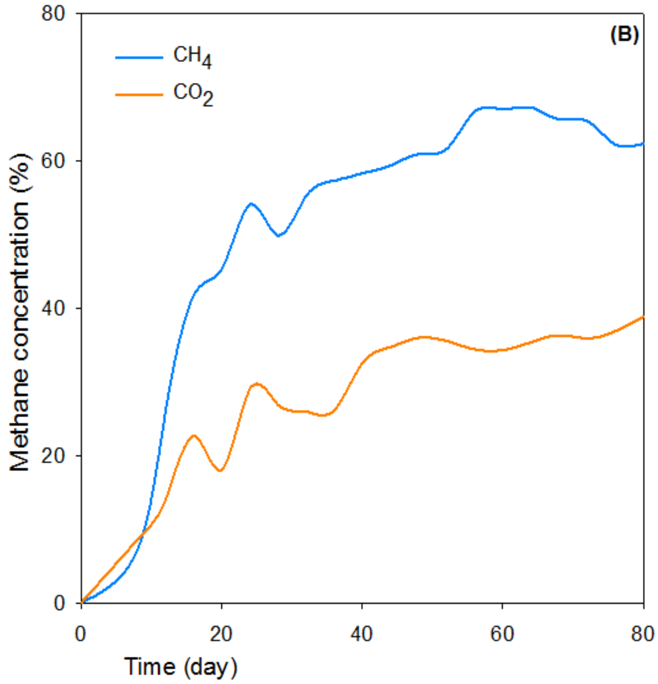

Figure 7. Methane concentration of semi-continuous reaction

Discussion

Batch anaerobic co-digestion between FPS and SMC

Biogas production

Results indicated that the co-digestion of FPS and SMC were significantly intensify the attainment of the biogas production in the early phase, the co-digestion is decelerated the accomplishment of the maximum of daily gas production at the first stage as compared with the control treatment. In the reactors, biogas was started to measure on the day 4th, containing a high CH_4 concentration from 47.33 to 58.73% as well as a slightly low CO_2 concentration from 35.7 to 38.63%. It indicated that fermentation form was due to methanogenesis process during this period (Kleyböcker *et al*., 2012).

The initial pH and alkalinity values were appropriated for biogas production as mentioned by previous studies on pH (Rajagopal *et al*., 2013; Abouelenien *et al*., 2010) and on alkalinity (Ren and Wang, 2004; Mahvi *et al*., 2004). It was found that the biogas yield of co-digestion reactors with C/N ratios at 20/1, 25/1, 30/1, 35/1 and 40/1 was 10.85%, 22.82%, 27.54%, 42.47% and 79.59% which higher than that of the control reactor, respectively. In parallel, methane yield of the co-digestion reactors 20/1, 25/1, 30/1, 35/1 and 40/1 were 15.52%, 27.61%, 29.40%, 41.07% and 80.48% respectively, which higher than the control reactor. The biogas yield and methane yield of reactor with C/N ratio of 40/1 were the highest among all the treatments. It is suggested that anaerobic co-digestion of FPS and SMC could considerably increase biogas yields if the appropriate proportion of straw is added.

It is noticeable that the accessibility of microorganisms in sludge could easily be digestible in comparison with the mixtures of SMC. It could be explained that the one underwent a composting process of substrate for a period of time in order to pasteurization softened materials and helped in the breaking down lignin (de-lignification). Over the progression, physio-chemical compositions of SMC changed substantially, especially the availability of carbon degradability and soluble organic matters for digesting had significantly diminished and remained stubbornness materials. It can be stated that the aforementioned process is unfavorable for increasing the amount of the produced biogas. Thus, the biogas production could be led to the lag phase or the effectiveness of digestion could be exhausted. However, co-digestion could accelerate methanogenesis as compared with mono-digestion phase when SMC being incorporated in reactors is clearly evident. Agyeman and Tao (2014) and El-Mashad and Zhang (2010) also state similar concern in their co-digestion studies.

Biogas composition

The recorded biogas composition was consistent with the result from daily biogas production in this study. In the first 4 days, the methane content had a faster increase among reactors and remained at a stable level until the end of digestion. This increasing could be observed in all the ratios indicating that the status reached the promptest methanogenesis stage. The average methane and carbon dioxide contents ranged between 49.69 - 52.30% and 33.93 - 37.93% which was similar to the findings in previous studies of El-Mashad and Zhang (2010), Ye *et al*. (2013), Nam *et al*. (2016). It is clearly shown that there was occurred uninhibition situation during the digestion. The availability of microorganisms from sludge-added solution which boosted the activity of microbiology in the startup phase (Liew *et al*., 2012). When the easily degraded matter was used up, co-digestion substrates produced a similarly methane content as the control treatment substrate.

Volatile solids removal effectiveness for successful reactors

It revealed that when the C/N ratio gradually increased from 20/1 to 40/1, the VS removal efficiency presented to decrease predisposition from 51% to 30%. This could be attributable to organic matter in the co-substrates being more easily degradable than the larger C/N ratio. It is stated inline to the findings in previous studies of Choi *et al.* (2020), Cerón-Vivas *et al.* (2019), Dioha *et al.* (2013).

The characteristic of biogas liquid

Biogas effluents contained sufficient concentration of essential elements that demonstrated the suitability of biogas effluent as N and P elements fertilizers for agriculture cultivation. The use of biogas effluents would become a promising technology to tackle environment related concerns. Thereby in all likelihood increasing their utilities revealed as cheap organic fertilizer sources (Nguyen *et al.*, 2015; Nguyen and Fricke, 2014).

Semi-continuous co-digestion between FPS and SMC

Biogas volume

The cumulative biogas production rate was estimated at around 86.12 $L.day^{-1}$ at the termination day. Consequently, the biogas yield obtained 102.69 $L.kgVS^{-1}$ added which equivalented roughly 1.11 $mL.gVS_{added}.day^{-1}$. Biogas production regarding semi-continuous co-digestion of FPS and SMC was lower approximately 39% than the batch co-digestion. It was found that process was not shown as effective as the fermentation of other sources. Anaerobic fermentation of SMC generates 122 $L.kgDM^{-1}$ of methane, while corn silage can produce 320 $L.kgDM^{-1}$ (Bisaria *et al.*, 1990). The biogas yield from other available substrates: manure (cow, pig, buffalo, poultry, horse) fluctuates from 270 to 800 $L.kgDM^{-1}$; agricultural residues (rice straw, wheat straw, maize straw, grass, mango leaves, foliage of parthenium, coffee pulp, corn stalk, casava peels) varies from 270 to 660 $L.kgDM^{-1}$; food wastes (household grease, whey, vegetable waste, fruit wastes, kitchen/restaurant wastes, left over's food, egg waste and cereals) altered from 200 to 980 $L.kgDM^{-1}$; aquatic plants or see weeds (algae, water hyacinth, giant kelp, cabana, salvinia) standardized in between 150 to 550 $L.kgDM^{-1}$ (Rajendran *et al*., 2012). Zak and Montusiewicz (2018) revealed that biogas production from SMC was not competitive enough.

Methane content

The trend displayed a slightly decreasing trend which fluctuated from 62.2 to 67.2% for the remaining periods. This range is similar to previous studies stated that the composition of methane from 63.7 to 74.1% (Agyeman and Tao, 2014) and from 54 to 69% (El-Mashad and Zhang, 2010) for the co-digestion of food waste and dairy manure. Other studies with at least one similar feeding material, there were 57.5 - 63.6% methane content recorded in the co-digestion of SMC and pig manure (Ngan & Fricke, 2012), while Opurum *et al.* (2017) reported 68% of methane content in the co-digestion of FPS and cow blood meal.

The average methane concentration was 56.7% which higher than batch experiments. Although the batch co-digestion experiments of FPS and SMC accomplished better the biogas yield, but the semi-continuous co-digestion proved the higher methane concentration (12%). Previous study on co-digestion of SMC and pig manure also recorded similar trend with 57.5 - 63.6% methane content from the semi-continuous treatment that higher than the 48.6 - 55.7% of the batch treatment (Ngan and Fricke, 2012). This finding demonstrated that the co-digestion between FPS and SMC is feasible for biogas production.

It is concluded that adjusting the proportions of mixture substrates in anaerobic co-digestion to obtain suitable feed characteristics including the C/N ratio is being an effective way to achieve desired digestion performance. Anaerobic co-digestion of SMC and FPS could increase the cellulase activity that is helpful in biodegradation of sludge, provide more nutrients in the reactor, and improve both biogas and methane production rate. For the batch anaerobic co-digestion of SMC and FPS, the optimum C/N ratio was recorded at 40/1, proving that more supplement of SMC could give better biogas production. This study offers the possibilities for improving methane yield from anaerobic co-digestion of SMC and FPS substrates that helps to reduce the environmental issues caused by these waste sources.

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