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Lab–scale Methane Fermentation Using Shrimp Pond Sludge and Some Available Biomass on Bến Tre in Vietnam

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The Mekong Delta of Vietnam is rich in unutilized biomass resources such as sludge discharged from shrimp, catfish culture, residue of sugar production (bagasse and molasses) and coconut candy factory (coconut pomace), which have the potential to satisfy the local energy demand. Although many projects are focused on biofuel production from these biomass resources, not much attention has been paid to developing an efficient utilization technology. Therefore, we started the present study investigated the optimal conditions for methane fermentation to achieve stable biogas production from the biomass feedstock of the Mekong Delta in Vietnam. This is the first report of using methanogenic bacteria in the shrimp pond sludge for the digestion of bagasse, molasses and coconut pomace, which were available biomass near the methane fermenter constructed beside shrimp pond at Bến Tre in Vietnam. As a result of a continuous input test of bagasse and molasses, the H_2S concentration in the collected gas was increased to 600 ppm and finally it had reached to 1,600 ppm by continuing to supply molasses. Therefore, an input test using coconut pomace instead of molasses was started. As a result, the generation of H_2S was reduced to several tens ppm. After a 118–day acclimatization test, biogas was generated smoothly. After then, a methane fermentation test using bagasse and coconut pomace was started. As a result, in the 2nd fermentation test, adding a mixture of bagasse and coconut pomace (2: 3) at 10% VS with HRT80 resulted in biogas (4.03 L, 894 mL g^{-1}VS)) equivalent to the effective volume of the fermenter (4 L).

Key words: bagasse, coconut pomace, H_2S, methane fermentation, molasses

INTRODUCTION

The Mekong Delta of Vietnam, which is located in the southeastern Indochina Peninsula, is one of the largest deltas in Asia with an area of around 40,519 km^2 (Cosslett and Cosslett, 2014) and a population of over 17 million as of 2011 (Nguyen and Ye, 2015). As the main agricultural zone in Vietnam, the Mekong Delta accounts for more than 50% of the country's agricultural production (50% of the rice, 70% of the fruit and 52% of the seafood products in 2011), and plays an important role in nationwide economic growth and food security (Garschagen *et al.*, 2012; Nguyen and Ye, 2015). This region is rich in unutilized biomass resources such as sludge discharged from shrimp, catfish culture, residue of sugar production (bagasse and molasses) and coconut candy factory (coconut pomace), which have the potential to satisfy the local energy demand. Although many projects are focused on biofuel production from these biomass resources, not much attention has been paid to developing an efficient utilization technology. Therefore, we started the project "Sustainable Development of Rural Area by Effective Utilization of Bio–wastes with Highly Efficient Fuel Cell Technology" within the framework of the Science and Technology Research Partnership for Sustainable Development (SATREPS) supported by the Japan Science and Technology Agency (JST) and the Japan International Cooperation Agency (JICA) since April 2015.

Biogas is a digester gas arising from the activity of methanogenic anaerobic bacteria which decompose organic matter. Its composition depends on the type of raw material subjected to the digestion process and on the method of conducting this process and is methane CH_4 (50–75%), carbon dioxide CO_2 (25–45%), hydrogen sulfide H_2S (0–1%), hydrogen H_2 (0–1%), carbon monoxide CO (0–2%), nitrogen N_2 (0–2%), ammonia NH_3 (0–1%), oxygen O_2 (0–2%) and water H_2O (2–7%) (Weiland, 2010).

General biogas produced from livestock manure, garbage, sewage sludge etc. contains 500–4,000 ppm H_2S (Alves *et al.*, 2013). When the biogas is utilized for power generation or hydrogen production, desulfurization process is indispensable (Abatzoglou and Boivin, 2009; De Arespacochaga *et al.*, 2014) because H_2S strongly deactivates catalyst materials for reforming and electrochemical reactions. The present study investigated the optimal conditions for methane fermentation

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to achieve stable biogas production from the biomass feedstock of the Mekong Delta in Vietnam. This is the first report of using methanogenic bacteria in the shrimp pond sludge for the digestion of bagasse, molasses and coconut pomace, which were available biomass near the methane fermenter constructed beside shrimp pond at Bến Tre in Vietnam.

MATERIALS AND METHODS

A: Biomass and seed inoculum for methane fermentation

For the biogas production, shrimp pond sludge discharged from shrimp culture was used as a source of mesophilic methanogenic bacteria for methane fermentation and acid production bacteria decomposed the organic material to low molecular compounds, which include organic acids etc., bagasse and molasses discharged by sugar factory and coconut pomace discharged during coconut milk production were used as fermentation substrates. These biomass were used to import the Kyushu University under the management of the Plant Protection Station, frieze–dried and ground by a Cyclotec 1093 sample mill (100–120 mesh, Tecator AB, Hoedanaes, Sweden), and further pulverized with a high–speed vibration mill (TI–100, CMT Co. Ltd., Fukushima) and used for next analysis. For the analysis of organic matter (VS) and ash content, the finely pulverized sample was heated at 600°C, and the VS content was obtained from the reduced weight and the ash content was obtained from the weight of the remaining residue. After then, these H, C and N contents were analyzed by CHN Corder (MT–5, Yanaco, Tokyo, Japan), and other elemental components were analyzed by an energy dispersive X–ray fluorescence analyzer (EDX7000, Shimazu, Kyoto, Japan).

For the lab–scale methane fermentation, the coconut pomace and molasses were used without any processing, but bagasse was ground by Cyclotec 1093 sample mill.

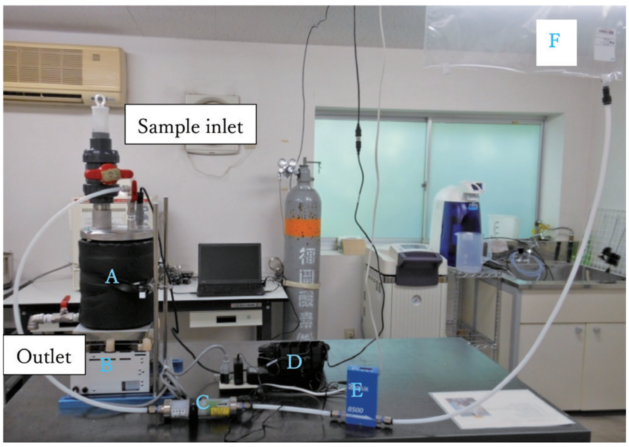

Fig. 1. Fermenter and other equipment for laboratory scale fermentation tests.
A: 4 L fermenter, B: magnetic stutterer, C: air drying tube, D: pH meter, E: digital flow meter, F: Tedlar gas sampling bag

B: Lab–scale acclimatization and fermentation using shrimp farm sluge

Lab–scale experiment was tried using 4 L stainless steel fermenter equipped a pH meter (Orion VERSA STAR equipped with an electrode: Orion 8107UWMMD, Thermo Fisher Scientific, MA, USA), 30 L Tedlar gas sampling bag (GL science, Tokyo, Japan), a digital mass flow meter (3810DS Ⅱ, Kofloc, Kyoto, Japan) and an air–drying tube with silica gel (GL science) (Fig. 1). The contents of fermenter were constantly stirred by VOLTEGA power hot stirrer with aluminum top plate (AS ONE, Oosaka, Japan). The monitoring of condition of methane fermentation was done by continuously pH measurement only. The biogas composition was analyzed by using Shimadzu Gas Chromatograph GC–8AT with TCD (Shimazu, Kyoto, Japan) using of 1 mL biogas from sampling bag. The SUS column used was SHINCARBON ST (50/80 mesh, 2.0 m × 3.0 mm I.D., Shimazu), the carrier gas used was Ar, and the flow rate was 50 mL min^{-1}, injection port temp. of 160°C, initial column temp. of 50°C, final column temp of 150°C with heating rate of 10°C min^{-1}, detector temp. of 150°C and current of 60 mA.

RESULTS

A: Elemental compositions of biomass

Elemental compositions of shrimp pond sludge and

Table 1. Mass and elemental content of the dry materials for methane fermentation at demo site and C/N ratio

Element	Sludge	Bagasse	Molasses	Coconut pomace
Mass content (DW%)				
VS	7.42	93.66	88.38	98.9
Ash	92.58	6.34	11.62	1.10
Moisture	57.18	21.19	32.19	69.71
Elemental content (DW%)				
H	0.55	5.84	4.29	7.93
C	2.20	47.70	44.01	55.11
N	0.16	0.28	2.20	0.94
P	0.03	0.85	0.04	1.88
K	5.57	12.03	21.34	17.94
Ca	4.49	3.69	8.28	2.01
Mg	1.77	1.02	1.16	0.77
S	2.39	2.27	5.54	2.02
Fe	11.29	2.82	0.47	0.75
Mn	0.22	0.38	0.04	0.17
Zn	–	0.21	0.02	0.19
Cu	0.03	0.18	0.02	0.19
Cl	2.19	3.26	10.45	3.09
Ni	0.02	0.05	–	0.07
Co	–	0.47	0.28	0.66
Si	52.35	12.78	–	0.30
Carbon to Nitrogen ratio				
C/N	14	170	20	59

these biomasses collected in Bến Tre, Vietnam, were listed in Table 1. The sludge had a low organic matter content and low utility value as a fermentation material, but had a high iron content. Coconut pomace had the highest carbon content, followed by bagasse and molasses. However, coconut pomace had a high moisture content and lacked preservability. Bagasse was the best for stable use as organic material in the demonstration site. Since molasses had a high carbon content and a large amount of low–molecular sugars (Hashizume *et al.*, 1966), it could be used as a material that promoted fast digestion and the growth of microorganisms but there was an issue for high sulphur content.

B–1: Acclimatization using shrimp farm sludge

This study was held at Kyushu University from August 17, 2015 to March 7, 2016 (80 days). Fig. 1 showed the setup for lab–scale methane fermentation (wet–type mesophilic fermentation) composed of a fermenter (4 L), pH meter and gas holder. On July 17, 2015, 1 kg of sludge (VS: 1.86%, Ash: 24.5%, Moisture: 73.6%), 3 L of tap water, 7.7 g VS of bagasse, and 2.4 g VS of molasses were added to a fermenter with effective volume of 4 L, and first acclimatization test was started, which was thermostatic at 35°C, and stirred at 300 rpm by VOLTEGA power hot stirrer. The pH at this point was 8.02, but the pH dropped as the culture continued. By the 32nd day, a total of 27 g VS of bagasse and 8.4 g VS of molasses were added. However, as the generation of biogas was very small, mineral salts (Table 2) were added on the 39th day to promote methane fermentation. When bagasse 30.8 g VS and molasses 9.6 g VS were added by the 43rd day, the pH dropped sharply to 5.49. The pH was increased to 6.5 on the 45th day, the biogas was generated about 0.36 L day^{-1}. Second acclimatization test was performed between March 8, 2016 and July 4, 2016 (118 days) using only bagasse as fermentation material. On March 8, 2016, the second acclimatization test was started by adding 1 kg of sludge, 3 L of tap water and 27.0 g of bagasse to the 4 L fermenter. The pH at the time of material input was 6.79, but gradually decreased after starting of the test. After that, there were total of 67.0 g VS of bagasse was added by the 7th day, biogas generation was confirmed within 7 days. And then, mineral salts (Table 1) and bagasse of were added sequentially. The fermentation material was added at intervals of 1 to 2 weeks, and gas generation was confirmed with each addition. By the 84th day after the start of this acclimatization, 107.0 g VS of bagasse was added. During the 98th to the 118th day, more than 0.1 L day^{-1} of biogas was generated. During this acclimatization period, the pH converged to about 6.25.

B–2: New acclimatization and fermentation test by continuous input of bagasse and molasses

In order to conduct a new continuous input test, a acclimatization test using the digested–liquid as an inoculum was planned. From the fermenter described above, all the digested liquid and the clay precipitated in the fermenter were taken out, all the instruments inside the fermenter were sterilized, the pH electrode was re–adjusted, and the fermenter was set again. After addition of 1 L of the above digested liquid with 3 L of tap water, an acclimatization again was started according to the schedule shown in Table 3, and add a mixture of bagasse and molasses at a rate of 20.0 g VS weekly for 58 days. After acclimatization, a continuous input test (March 7 to April 14, 2017) was started. Bagasse and molasses are mixed at a ratio of 9:1. Input of 12 g VS once every two days together with tap water and mineral salts to make 1/20 (200 mL) of the fermenter volume (4 L). After 200 mL of digested liquid was discharged, these mixtures were added through the inlet (hydraulic residence time of 40 days: HRT40). For the addition of mineral salts, 0.77 g of NH_4Cl or 0.43 g of urea was added as nitrogen compounds. In this case, the input material had a CN ratio of about 20. In this test, gas generation

Table 2. Minimum required amount of nutrient salts to maintain acetic acid consumption rate of 30 g L^{-1} d^{-1} (Takashima and Speece, 1989)

Nutrient source	Nutrient requirement	Salt requirement in fermenter	
	mg L^{-1} d^{-1}	Salt	mg 4 L^{-1}
Macro			
NH_4–N	100	NH_4Cl	1527.5
PO_4–P	4	KH_2PO_4	70.3
K	5		
S	10	Na_2SO_4	177.2
Ca	5	$CaCl_2 \cdot 2H_2O$	73.4
Mg	1	$MgCl_2 \cdot 6H_2O$	33.5
Micro			
Fe	1	$FeCl_3 \cdot 6H_2O$	19.4
Ni	0.2	$NiCl_2 \cdot 6H_2O$	3.2
Co	0.1	$CoCl_2 \cdot 6H_2O$	1.6

Table 3. Material input schedule in continuous dose test (HRT40)

Number of times	Date	Culture	Bagasse	Molasses	Mineral	NH_4Cl	Urea
		days	g VS		(times)	g	
①	7–Mar	0	10.8	1.2	0.5	0.77	–
②	9–Mar	2	10.8	1.2	0.5	0.77	–
⑤	15–Mar	8	10.8	1.2	0.5	0.77	–
⑥	17–Mar	10	10.8	1.2	1	0.77	–
⑧	21–Mar	14	10.8	1.2	1	0.77	–
⑨	23–Mar	16	10.8	1.2	2	0.77	–
⑪	27–Mar	20	10.8	1.2	2	0.77	–
⑫	29–Mar	22	10.8	1.2	2	–	0.43
⑮	4–Apr	28	10.8	1.2	2	–	0.43
⑯	6–Apr	30	10.8	1.2	2	–	1.10
⑳	14–Apr	38	10.8	1.2	2	–	1.10

Rows without counts have the same material added as the previous run, and the numbers in the inorganic columns indicate the ratios to the quantities in Table 1.

was delayed until the 4th day after the start of the test, but then biogas generation was steadily observed, and the gas composition was 45% CH_4 and 32% CO_2 on average from the start of the test. The biogas generation rate was 1.30 L day^{-1} and 280 mL of biogas was generated per g VS (Fig. 2). During the test period, the pH had changed between 6.5 and 6.7. The H_2S concentration in the gas collected in a Tedlar gas sampling bag in the last stage of the fermentation test was abnormally high and until reaching to 1600 ppm.

Table 4 showed the effect of added amount of mineral salts on biogas generation. Two times higher addition of mineral salts with urea as N source increased effectively biogas generation rate. If over application of urea was used, there were decreased the rate of biogas generation.

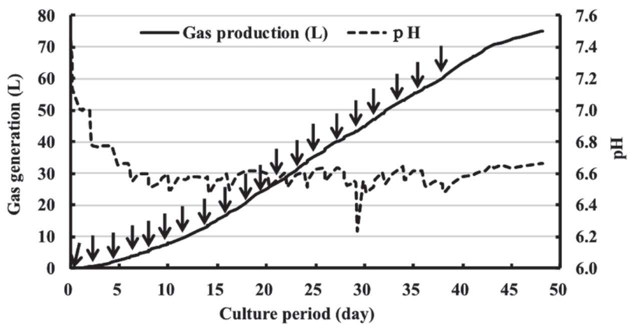

Fig. 2. Material input timing (displayed by arrow, input every 2 days) in continuous dose test (HRT40) and changes in gas generation and pH (March 7 to April 17, 2017)

B–3: Methane fermentation test by continuous input of bagasse and coconut pomace

In methane fermentation using molasses and bagasse, the generation of H_2S was increased, and the cause was thought to be the sulfur component in molasses (Table 1). Then, fermentation test using bagasse and coconut pomace was planned. For waiting to no longer observed the above production of biogas, we started acclimatization with bagasse and coconut pomace (July 25–November 8, 2017). During this period, the addition of 18 g VS and mineral salts (Table 2) was performed by HRT80 (Table 5 and 6), and biogas was generated for 1.25 L day^{-1} and the pH changed to 6.3 or more (Fig. 3 and 4).

The continuous addition test was conducted between November 16, 2018 and March 16, 2019, which was inputted once every four days at rate of 18 g VS equivalent (bagasse: coconut pomace = 2: 1) in the test from 1st to 88th days and at rate of 20 g VS equivalent (bagasse: coconut pomace = 2: 3) from 92th to 120th days (Table 7 and 8). Tap water and inorganic salts (twice the amount in Table 2) were suspended in 1/20 (200 mL) of the tank volume (4 L) and administered through the inlet. At that time, 200 mL of digestive fluid was discharged (HRT80). After that, gas generation was improved, and the final values during this fermentation test were 61% CH_4 and 34% CO_2. The fermentation rate was 4.03 L day^{-1} and 894 mL of biogas was generated per g VS (Fig. 5 and 6).

Table 4. Effect of added inorganic salt on biogas production

Incubation period	Bagasse	Molasses	Mineral	NH_4Cl	Urea	Biogas production	
	g VS		(times)	g		L day^{-1}	mL g^{-1}VS
7–Mar to15–Mar	10.8	1.2	0.5	0.77	–	0.46	77
17–Mar to 23–Mar	10.8	1.2	1	0.77	–	1.27	212
23–Mar to 29–Mar	10.8	1.2	2	0.77	–	1.34	223
29–Mar to 6–Apr	10.8	1.2	2	–	0.43	1.72	286
6–Apr to 14–Apr	10.8	1.2	2	–	1.10	1.57	262

Table 5. Input schedule for the 1st acclimatization using bagasse and coconut pomace

Number of times	Date	Culture	Bagasse	Molasses	Mineral	Urea
		days	g VS		(times)	g
①	25–Jul	0	12	4	1	0.43
②	8–Aug	14	12	4	1	0.43
③	24–Aug	30	12	4	1	0.43
④	5–Sep	42	12	4	1	0.43
⑤	12–Sep	49	12	4	1	0.43
⑥	19–Sep	56	12	4	1	0.43
⑦	26–Sep	63	12	4	1	0.43

Discharge: 200 mL, Input: 200 mL

Table 6. Input schedule for the 2nd acclimation using bagasse and coconut pomace

Number of times	Date	Culture	Bagasse	Molasses	Minerals	Urea
		days	g VS		(times)	g
①	11–Oct	0	20	10	–	–
②	15–Oct	4	20	10	–	–
③	19–Oct	8	20	10	–	–
④	23–Oct	12	20	10	–	–
⑤	27–Oct	16	12	6	1	0.43
⑥	31–Oct	20	12	6	1	0.43
⑦	4–Nov	24	12	6	1	0.43
⑧	8–Nov	28	12	6	1	0.43

Discharge: 200 mL, Input: 200 mL

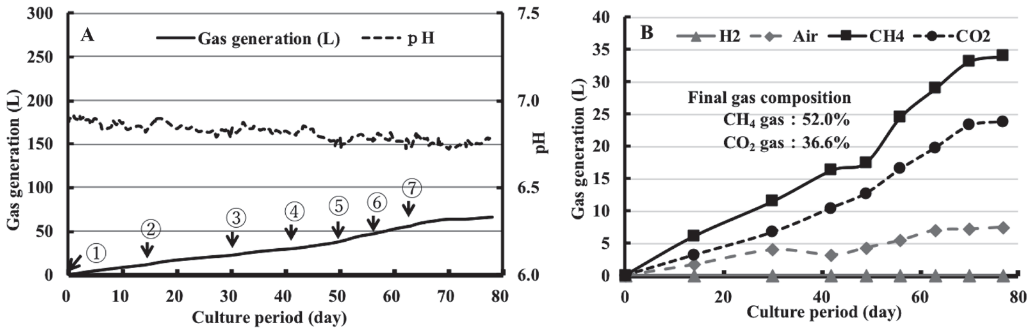

Fig. 3. A: Material input timing (①–⑦) and changes in gas generation and pH, B: Changes in biogas composition in 1st acclimatization (July 25 to September 26, 2018)

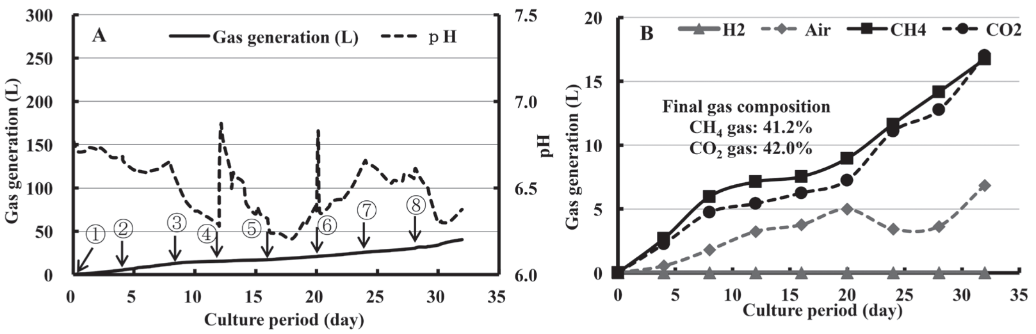

Fig. 4. A: Material input timing (①–⑧) and changes in gas generation and pH, B: Changes in biogas composition in 2nd acclimatization (October 11 to November 8, 2018)

Table 7. Input schedule for 1st methane fermentation using bagasse and coconut pomace (CP)

Number of times	Date	Culture	Bagasse	CP	Minerals (times)		Urea
		Days	g VS		Macro	Micro	g
①	16–Nov	4	12	6	1	2	0.43
③	24–Nov	12	12	6	1	2	0.43
⑤	2–Dec	20	12	6	1	2	0.43
⑦	10–Dec	28	12	6	1	2	0.43
⑨	18–Dec	36	12	6	1	2	0.43
⑪	26–Dec	44	12	6	1	2	0.43
⑬	3–Jan	52	12	6	1	2	0.43
⑮	11–Jan	60	12	6	1	2	0.43
⑯	15–Jan	64	12	6	1	2	0.43

Macro and micro minerals were added one time and two times of Table 2. Discharge is 200 mL and input are 200 mL on 4 days interval.

DISCUSSION

In an acclimatization test using shrimp pond sludge (B–1) and a subsequent methane fermentation test using bagasse and molasses (B–2), it was shown that methane fermentation was possible using sludge from a shrimp farm in Bến Tre, Vietnam collected as a seed inoculum and the waste biomass (bagasse and molasses) from the surrounding area. Next, we thought that it was necessary to add mineral salts to perform methane fermentation efficiently using these materials, and to confirm whether methane fermentation could be controlled only by monitoring the pH. Bagasse contained 42.2% of cellulose, 27.6% of hemicelluloses, 21.6% of lignin, 5.63% of extractives and 2.84% of ashes (de Moraes Rocha *et al*., 2015). These results are in good agreement with their previous results (Rocha *et al*., 1997; Gouveia *et al*., 2009), and with literature by other groups (Sanjuan *et al*., 2001). These values were also within the ranges stated by Triana *et al*. (1990), which are 40–50% for cellulose, 25–30% for hemicelluloses, and 20–25% of lignin. Chemical treatment had been shown

Table 8. Input schedule for 2nd methane fermentation using bagasse and coconut pomace (CP)

Number of times	Date	Culture	Bagasse	CP	Minerals (times)		Urea
		Days	g VS		Macro	Micro	g
①	19–Jan	0	12	6	1	2	0.43
③	27–Jan	8	12	6	1	2	0.43
⑤	4–Feb	16	12	6	1	2	0.43
⑦	12–Feb	24	12	6	1	2	0.43
⑧	16–Feb	28	8	12	2	2	0.86
⑨	20–Feb	32	8	12	2	2	0.86
⑪	28–Feb	40	8	12	2	2	0.86
⑬	8–Mar	48	8	12	2	2	0.86
⑮	16–Mar	56	8	12	2	2	0.86

Macro and micro minerals were added one time and two times of Table 2. Discharge is 200 mL and input are 200 mL on 4 days interval.

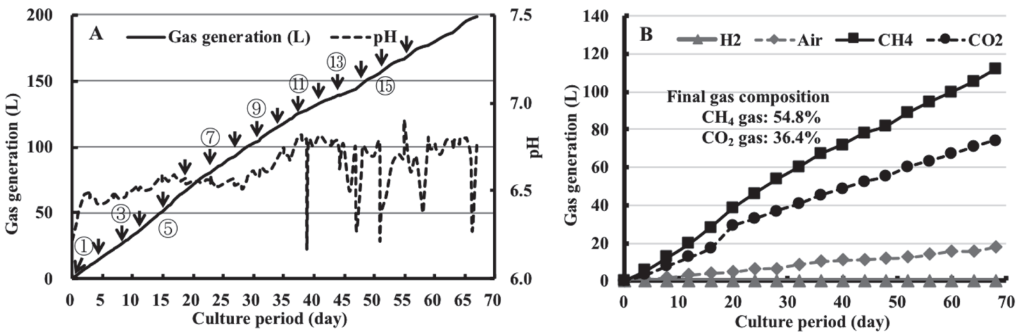

Fig. 5. A: Material input timing (①– ⑯ and changes in gas generation and pH, B: Changes in biogas composition in 1st methane fermentation (November 16, 2018 January to 15, 2019)

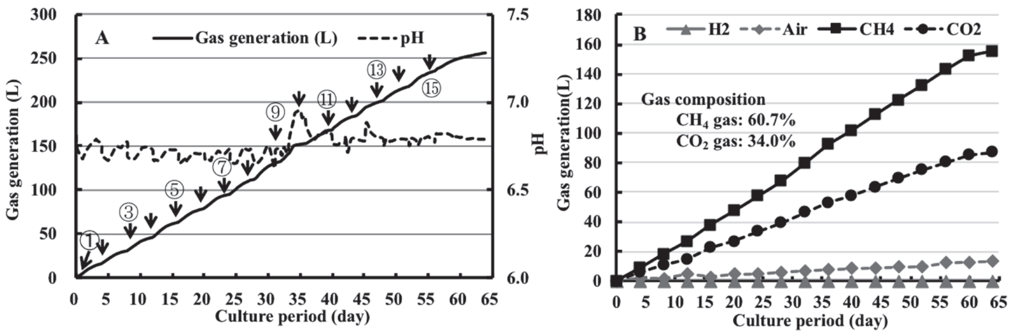

Fig. 6. A: Material input timing (①– ⑮ and changes in gas generation and pH, B: Changes in biogas composition in 2nd methane fermentation (January 19 to March 16, 2019)

to be effective for promoting methane fermentation using bagasse (Ishihara *et al*, 1988). Molasses was produced via the separation of sucrose crystals that follow the water evaporation from clarified juice (from sugarcane or beet) during the production of crystal sugar. The concentrated juice was facilitated sucrose crystallization. Sucrose crystals were removed via centrifugation, and the remaining viscous liquid was molasses. Molasses had 131–188 g L^{-1} of total sugar (mainly sucrose) and might be further recycled in this process in order to maximize sugar production (Senne de Oliveira Lino, 2018).

In the methane fermentation test with continuous input of a mixture of bagasse and molasses (9:1) at HRT40, the effect of adding inorganic salts was also investigated. As a result, the amount of biogas generated was maximized (1.72 L day^{-1}, 286 mL g^{-1}VS) by doubling the amount of addition of mineral salts other than the N source (Table 4). This yield was comparable to that of Ishihara *et al.* (1988) of methane fermentation in bagasse without chemical treatment. The reason why urea was selected as the N source at this time is that it could be obtained at a low price at the demonstration site. The CN ratio of 20 was set so that the CN ratio of the microorganism was about 10 and about half of the added carbon was bio–based. It was thought that it could be used for biogas generation. The biogas generation was decreased when the amount of N was added doubled. So, the amount of urea did not need to increase in proportion to other mineral salts.

In the methane fermentation at the demonstration site, we planned to separate the supernatant containing almost no inorganic substances such as clay from the sludge collected from the shrimp pond and use it as the seed inoculum and organic substances for methane fermentation. As the fermentation conditions in demonstration site differs from the initial acclimation test that started with sludge from the shrimp pond conducted at the lab scale, it was necessary to create fermentation conditions excluding the clay fraction in the sludge. Therefore, we started the methane fermentation test in secession B–2 using the digested liquid obtained in secession B–1 as next acclimatization test. During this acclimatization, the pH was reduced by the addition of molasses, and calcium hydroxide available at the demo site was added to prevent the pH from lowering. This suggests that a large amount of inorganic substances contained in the sludge suppressed the pH decrease in the previous a test using total sludge.

As a result of a continuous input test of bagasse and molasses, the H_2S concentration in the collected gas was increased to 600 ppm and finally it had reached to 1,600 ppm by continuing to supply molasses. This process was happened due to the addition of molasses. Therefore, an input test using coconut pomace instead of molasses was started. As a result, the generation of H_2S was reduced to several tens ppm. After a 118–day acclimatization test (Tables 5 and 6), biogas was generated smoothly (Figs. 3 and 4). Therefore, a methane fermentation test using bagasse and coconut pomace was started (Tables 7 and 8). As a result, in the 2nd fermentation test, adding a mixture of bagasse and coconut (2:3) at 10% VS with HRT80 resulted in biogas (4.03 L, 894 mL g^{-1}VS)) equivalent to the effective volume of the fermenter (4 L) as shown in Fig. 6.

AUTHOR CONTRIBUTIONS

Takeo YAMAKAWA designed the study, collected and analyzed the data, and wrote the first draft of the manuscript. Hajime MATSUBARA and Hiroyuki SGIMIZU designed and constructed the fermenter in this study. Mio SAKAMOTO engaged in this fermentation and collected the data about materials used in the study. Tran Trung GIANG collected and export to Japan the biomass and shrimp farm sludge at Bến Tre in Vietnam. Yusuke SHIRATORI managed and supported this study and edited the manuscript.

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