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Methane Production from Patchouli Oil Distillery Waste Using Wet Semi-Continuous and Dry Batch Anaerobic Digestion

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ABSTRACT
Patchouli oil distillery waste (PODW) was used as a substrate for methane production in wet semi-continuous and dry batch anaerobic digestion (AD). In wet semi-continuous AD, the system was operated with recirculation of effluent. Two reactors with 300 mL of working volume were operated for 45 days in three moderate stepwise-increased organic loading rates (OLR), which were 0.5, 1, and 2 g VS/(L.day). Wet semi-continuous AD with recirculation produced the highest methane production at OLR of 1 g VS/(L.day), which was 13.71 ± 2.28 (Nml/g VS). In dry batch AD, three different values of VS, which were 1.5 g VS; 3 g VS; and 6 g VS, were mixed with inoculum, after which distilled water was added to adjust the TS content in the reactor up to 17; 12; 20; 15; 26; and 21%. The experiments were conducted for 47 days. The highest methane yield of 13.87 ± 0.99(Nml/g VS) was obtained with TS 17% of 1.5 g VS.

Keywords: *Patchoulid Oil Distillery Waste, Methane Production, Anaerobic Digestion, Semi Continuous*

1. INTRODUCTION

Patchouli (Pogostemoncablin) is an industrially valued aromatic plant currently having a huge demand for its essential oil. The demand for this oil is very high in the international market. As a result, the high production of patchouli oil through its extraction process called steam distillation is also followed by a high amount of the residues. Steam distillation only yields 2.6% of patchouli oil, and 97.4% is ended up as residue [1]. This residue, later called patchouli oil distillery waste (PODW), is a lignocellulosic material that has a great potential as a resource of biomass for renewable energy through an aerobic digestion process.

Anaerobic digestion (AD) is gaining large attention nowadays both as a solution to environmental concern and also as an energy source. Anaerobic digestion breaks down organic matters into biogas dominantly containing methane and carbon dioxide. However, there are challenges for converting PODW into biogas. Anaerobic digestion has a shortcoming due to its environmental sensitivity as it can lead to an imbalance of methane-forming [2]. Thus, two types of anaerobic digestion processes are distinguished as a comparison, which are generally referred to as "wet" and "dry" anaerobic digestion processes.

For wet AD, the reactor usually used is a continuous stirred tank reactor (CSTR), where the active biomass is removed from the system, and a fresh substrate is fed at the same amount. Since there is a high probability of cell-washout during the removal of biomass, effluent recirculation was conducted in this work as it has been previously shown to have enriched methane yield and was able to overcome cell-washout without causing damage on CSTR [3]. To better understand the effects of recirculation in a semi-continuous reactor with high-solid content substrates, experiments were carried out with three different OLR levels and the same HRT for each OLR.

Typically, wet AD is used to treat sludge with TS content less than 15%. Meanwhile, PODW is a solid waste with a total solid of 15-50%. Thus, processing PODW using dry AD was of interest. Another challenge comes from the presence of patchouli oil in PODW since it has displayed various types of pharmacological activities such as anti-inflammatory, antiseptic, and antibacterial properties of its component(e.g., [1]). The major component of patchouli oil is patchouli alcohol that has been reported to have an antibacterial effect against several types of bacteria[4]. This effect thereafter makes patchouli oil act as an inhibitor against bacteria in anaerobic digestion. Therefore, the effect of this inhibitor in dry AD towards biogas production was also evaluated.

2. MATERIAL AND METHODS

2.1. Microorganism

Inoculum for wet AD was obtained from a digester that treats wastewater sludge (Vatten and MiljöiVäst AB, Varberg, Sweden). It was filtered through a 2-mm porosity sieve to remove sand, plastic, and other unwanted particles. It was then acclimatized for five days at 37o C before use. For dry AD, the inoculum was obtained from VästblekingeMiljö AB Biogas at Mörrum waste treatment plant in Mörrum, Karlshamn municipality. It was filtered through a 2-mm porosity sieve. After that, acclimatized for seven days at 37o C before use.

2.2. Synthetic Medium

For wet AD, the fresh substrate for the digestion was a synthetic basal medium (as nutrients supply) mixed with a certain amount of PODW. For dry AD, three kinds of substrate were used, i.e., microcrystalline cellulose (Avicel), PODW, and fresh patchouli plant. Avicel was obtained from Sigma-Aldrich and used as a synthetic substrate for patchouli oil inhibition study. PODW (both for wet and dry AD) and fresh patchouli plant were obtained from the local patchouli oil distillery industry in Bantul (Yogyakarta, Indonesia). Both were grounded and sieved to 40 mesh (0.42 mm) before use.

2.3. Bioreactor Setup

Wet semi-continuous AD was carried out for 45 days at 38oC using a 500 mL glass bottle as the reactor. The working volume was 300 mL. It contained free cells of inoculum from 100 g pelleted sludge and 200 mL of distilled water. Subsequently, the reactor was flushed with N2 gas for 3 minutes. The stirrer was set up at10 rpm speed. The reactor cap was made of plastic with two outlets for feeding and sampling. One outlet for sampling was connected to AMPTS (Automatic Methane Potential Test System) II Bioprocess Control version 3.0 and the other outlet was connected to the medium inside the reactor. The reactor was fed with PODW and recirculated supernatant with a total volume of 20 mL. At the same time, an equal amount of liquid was withdrawn from the reactor. The feeding composition is presented in Table 2.1.

Table 2.1 Feeding composition in recirculated reactor (RECIR) of wet semi-continuous anaerobic digestion

OLR ((gVS/L day)	20 mL Feeding Composition			PODW (g/mL)
	PODW (mL)	Basal Medium (mL)	Recirculated Effluent (mL)	
0.5	0.5	9.5	10	0.34
1	1	9	10	0.34
2	2	8	10	0.34

The dry AD was conducted in batch cultivation for 47 days. Two experiments were performed. In the first experiment, 3 g VS of inoculum and a certain amount of PODW (1.5g VS; 3 g VS; and 6 g VS) were mixed, giving an inoculum to substrate ratio (ISR)of 1:0.5; 1:1; 1:2 and TS of 17, 20, 26%, respectively. The influence of TS content in the reactor on methane production was evaluated by adjusting the TS contents of the substrate (PODW) into 12% (for 1.5 g VS); 15% (for 3 g VS); and 21% (for 6 g VS) by adding distilled water. Moreover, 1.5 g VS of fresh patchouli plant were also mixed with 3 g VS of inoculum (ISR of 1:0.5) without the addition of water, and the TS content was 17%. In the second experiment, 3 g VS of inoculum and 1.5 g VS of Avicel as the substrate were mixed. Thereby, the inoculum to substrate ratio was 1:0.5, and TS was 12% in the reactor. Different amount of patchouli oil, i.e.,0.05 g/L; 0.5 g/L; and 5 g/L, was added. The reactors used in this experiment were 120 mL of glass serum bottle. The bottles were sealed tightly with an aluminium crimp cap, and the headspace was filled with a gas mixture of 80% N2 and 20% CO2 to achieve anaerobic conditions. All the bottles were then incubated at 37ºC. The experimental design of dry batch is presented in Table 2.2.

Table 2.2 Experimental design of batch dry anaerobic digestion

Substrate	Inoculum to substrate ratio (ISR)(gVS basis)	Inoculum (g)	Substrate (g)	Water (mL)	TS Content of Reactor (%)
Avicel	1:0.5	33.71	1:50	2.29	12
Avicel	1:0.5	33.71	1:50	2.29	12
Avicel	1:0.5	33.71	1:50	2.29	12
Avicel	1:0.5	33.71	1:50	2.29	12
PODW	1:0.5	33.71	1:69	0.00	17
PODW	1:0.5	33.71	1:69	14.27	12
PODW	1:1	33.71	3.37	0.00	20
PODW	1:1	33.71	3.37	13.11	15
PODW	1:2	33.71	6.74	0.00	26
PODW	1:2	33.71	6.74	10.33	21
Fresh patchouli plant	1:0.5	33.71	1.81	0.00	17

3. RESULTS AND DISCUSSION

Patchouli oil distillery waste (PODW) is a lignocellulosic material that is present in a plentiful amount. PODW has the potential as a renewable energy source through anaerobic digestion to produce biogas. Still, there are challenges for converting PODW into biogas caused by the presence of patchouli oil residue left in PODW. Previously, Lukitawesa *et al.* [1]showed that fresh patchouli plant and its waste had the potential to be anaerobically digested through wet AD. Using 0.3 g VS of substrate and 0.3 g VS of inoculum with 50 ml addition of water, the corresponding methane yields were 86 and 179 NmL/g VS, respectively. It was further shown that the addition of 0.05, 0.5, and 5 g/L of patchouli oil reduced biogas production by 16.2, 27.2, and 100%, respectively.

In this work, wet AD of patchouli oil distillery waste was conducted for 45 days where the substrate was added daily, with the calculated OLR increased stepwise from 0.5 g VS/(L.day) for 16 days, 1 g VS/(L.day) for 13 days, and 2 g VS/(L.day) for 16 days. The method was based on Aslanzadeh*et al.* [2] with several modifications and different OLR inputs. respectively.

The use of PODW as a substrate and study of inhibition effect of patchouli oil towards biogas production in dry AD were never studied before. Therefore, this study used PODW as a substrate with different TS contents (17, 15, 20, 15, 26, dan 21) and investigated the effect of the same concentration of patchouli oil (0.05, 0.5, and 5 g/L) towards biogas production in dry AD. Table 3.1 and 3.2 present the important results of wet AD and dry AD of PODW.

Table 3.1 Average methane yield (Nml/g VS) of PODW in wet semi-continuous AD

HRT	OLR ((gVS/L day)	Average methane yield (Nml/g VS)	Patchouli alcohol concentration after 1 HRT *) (mg/mL reactor/day)
1	0.5	13.71 ± 2.28	0.19
2	1.0	12.50 ± 2.30	0.31
3	2.0	2.47 ± 0.58	0.77

Table 3.2 Average methane yield (Nml/g VS) of PODW in dry batch AD

Substrate	PODWTS 17% (1.5 g VS)	PODW TS 12% (1.5 g VS))	PODWTS 20% (3 g VS))	PODW TS 15% (3 g VS)	PODWTS 26% (6 g VS)	PODW TS 21% (6 g VS)	Fresh patchouli plant (TS 17%)
Average methane yield (Nml/g VS)	13.87 ± 0.99	11.45 ± 1.21	7.05 ± 0.96	5.95 ± 1.28	3.00 ± 1.00	2.46 ± 1.10	4.38 ± 1.71
Patchouli alcohol concentration inside the reactor *)(mg/ml)	0.14	0.10	0.25	0.19	0.40	0.34	0.85

*) Patchouli alcohol concentration was calculated based on the patchouli alcohol content in PODW and fresh patchouli stated in Lukitawesa *et al*. [1].

As shown in Table 3.1 and 3.2, wet semi-continuous AD at OLR 0.5 and 1.0 (gVS/L day) and dry batch AD with 1.5 g VS for TS 17and TS 12% had similar values of methane yield. However, when the concentration of patchouli alcohol is considered, wet semi-continuous was able to withstand the effect of inhibitor compared to dry batch AD. The reason for this could be that in semi-continuous, patchouli alcohol was fed into the reactor gradually, giving the digesting bacteria to adapt to the severe condition in a stepwise manner. This is not the case for dry batch AD, where the concentration of patchouli alcohol was high even from the beginning, which gave little time for the microbes to get adapted with the harmful environment. Furthermore, recirculated effluent helps in minimizing the risk of cell-washout and maintaining high cell concentration in the reactor[4]. This has substantiated the capability of semi-continuous in dealing with the inhibitor. Both systems exhibited a similar trend in which increasing in either OLR or VS or TS resulted in a reduction of methane yield possibly caused by the accumulated patchouli alcohol in the medium. The toxicity mechanism is usually through adsorption onto cell wall, which will affect its transport and protective functions and probably leads to damaged cells and reduced activities of microbial communities, which may have been stressed [4].In dry batch AD, a higher amount of TS could reduce the hydrolysis rate and could create higher physical limitations related to mass transfer. Liotta et al.[5] evaluated the effect of TS from 5 to 11.3% using carrot waste as a model complex of organic matter. It was found that the methane production rate decreased with higher solid content. Decreased mass transfer at high TS content could reduce the substrate accessibility to microbes, and could eventually lower the methane yield. Table 3.2further shows that the methane yield of PODW is significantly higher than that of fresh patchouli plants with the same TS content. The reason for the lower methane yield of fresh patchouli plants could be related to the higher patchouli oil content in the substrate.

Similar to the previous findings [1], the addition of patchouli oil into the medium with Avicel decreased the methane yields. The methane yield was reduced by 10.90% for the addition of patchouli oil of 0.05 g/L. An increase of patchouli oil concentration of 0.5 g/L reduced the methane yield by 14.98% compared to control. Meanwhile, the addition of patchouli oil of 5 g/L significantly decreases the methane yield by 48.38%.

4. CONCLUSION

Semi-continuous AD has a greater ability to deal with the effect of inhibitor compared to dry batch AD. More accumulation of inhibitor at higher OLR or VS/TS in both systems resulted in a decline in methane production. In dry batch AD, high TS content could affect the process performance negatively.

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