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Original Article

Effect of nitrogen and phosphorus on the performance of acidogenic and methanogenic reactors for treatment of biodiesel wastewater

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Abstract

A laboratory scale two-stage anaerobic process was studied in order to treat biodiesel wastewater. The first stage represented an acidogenic reactor while the second stage was a methanogenic reactor. The effect of nitrogen and phosphorus on the performance of both reactors was investigated. Biodiesel wastewater was adjusted so that its COD:N and COD:P within 100:0.1–1.1 and 100:0–1, respectively, and fed into the acidogenic reactor. After COD and pH adjustment, wastewater discharged from the acidogenic reactor was fed into the methanogenic reactor. The highest VFA concentration of 8.32 g/l was obtained from the acidogenic reactor at COD:N:P of 100:1.1:0.5. However, the highest CH_4 yield of 0.170 l CH_4/g $COD_{removed}$ was found at COD:N:P of 100:0.6:0.5 from the methanogenic reactor. Moreover, both nitrogen and phosphorus affected the acid composition produced in the acidogenic reactor and the long chain fatty acid consumption in the acidogenic and methanogenic reactors.

Keywords: biogas, acidogenic, methanogenic, fatty acids, biodiesel wastewater

1. Introduction

With a possible energy crisis, biodiesel is one of the most promising candidates as an alternative green energy with clean burning. However, wastewater from biodiesel processes is generated as a by-product with a high amount of lipid contents that must be treated. Many researchers focused on biodiesel wastewater treatment using physical and/or chemical processes (Suehara *et al*., 2005; Chavalparit and Ongwandee, 2009; Jaruwat *et al*., 2010; Rattanapan *et al*., 2011; De Gisi *et al*., 2013). Recently, an anaerobic digestion of wastewater was found as an attractive method because of its low energy requirement, low operating cost, and environmental friendly. However, biodiesel wastewater contains a high amount of oil and long chain fatty acids (LCFA) with low nitrogen and phosphorus contents, which limit the efficiency of any biological wastewater treatment system (Vidal *et al*., 2000; Siles *et al*., 2010). A two-stage anaerobic digestion approach has previously been reported to improve the conversion of organic substance to methane (Solera *et al*., 2002; Diamantis and Aivasidis, 2007; Göblös *et al*., 2008; De La Rubia *et al*., 2009; Luo *et al*., 2011). Two separated reactors are required for the selection and enrichment of different microorganisms. The acid forming and the methane forming bacteria are mainly responsible for an overall digestion. In the first stage, an organic matter is firstly hydrolyzed to sugars, fatty acids and amino acids by extracellular enzymes and then fermented by the acid-forming bacteria to short-chain fatty acids, alcohols, carbon dioxide and hydrogen (Khanal, 2008). Afterwards, they are subsequently converted to biogas (CH_4

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and CO_2) by methane forming bacteria in the second stage (De La Rubia *et al.*, 2009). Therefore, the first stage may act as a metabolic buffer and prevent pH shock to the methanogenic population (Solera *et al.*, 2002). Consequently, the two-stage systems can increase the stability of the process and prevent the inhibition effects from the overloading and toxic materials (Solera *et al.*, 2002).

In general, a theoretical minimum COD:N:P of 100:2: 0.3 and 100:0.7:0.1 is required for the highly loaded and lightly loaded anaerobic digestion, respectively (Khanal, 2008). However, the acidogenic and methanogenic bacteria have different nutrient requirements and growth kinetics (Rincón *et al.*, 2010). Consequently, this research aims to study the effect of nitrogen and phosphorus required in each stage of the anaerobic system. Also, the performance of acidogenic and methanogenic reactors during the anaerobic treatment of biodiesel wastewater was investigated.

2. Experimental Methods

2.1 Wastewater

Wastewater was obtained from the biodiesel production plant operated by the Specialized Research & Development Center for Alternative Energy from Palm Oil and Oil Crops, Prince of Songkla University, Thailand. This wastewater was a milky liquid containing high organic contents with low nitrogen and phosphorous content at a COD:N:P of 100:0.05:0.003 (Table 1). However, biodiesel wastewaters from various production plants were different according to different production methods.

2.2 Inoculum

The sludge (MLSS of 25 g/l) was collected from a full-scale up-flow anaerobic sludge blanket reactor treating wastewater from frozen seafood industry. In order to eliminate methane forming bacteria, the sludge was pretreated with heat–shock process (boil it under 100°C for 20 min) (Zhu and Béland, 2006) and inoculated into the first stage to work as acidogenic bacteria. Moreover, the sludge without heat–shock pretreatment was introduced into the second stage to work as methanogenic bacteria.

2.3 Reactors set-up and operation

Two up-flow closed anaerobic reactors (30 cm × 30 cm × 40 cm, a total volume of 36 l) were made of plastic tank with working volume of 25 l. The first reactors worked as acidogenic reactor and the second reactors worked as methanogenic reactor. Each reactor contained feeding ports at the bottom and effluent ports at the upper part of reactors with the up-flow mode. The top of both reactors were connected to a biogas collector. Between the two reactors, a balancing tank with a total volume of 1,000 l was used to supply the suitable nutrient for methanogenic reactor (Figure 1). All experiment was performed in parallel three sets of anaerobic system for triplication.

Biodiesel wastewater was supplemented with urea, NaH_2PO_4 and Na_2HPO_4 to obtain different ratios of COD:N:P. During the start-up period, the influent wastewater was diluted so that its COD was 0.9 g/l (OLR=1.8 g COD/(l·d)) and fed semi-continuously into the acidogenic reactor at HRT 0.5 d to reduce the substrate inhibition. Afterwards, the effluent of the acidification stage was collected in a balancing tank. Before the effluent from the acidogenic reactor was introduced to the sequential methanogenic reactor at HRT 1.0 d, the COD and pH adjustment was conducted. pH was adjusted to 7.0 using $Ca(OH)_2$ for the favorite growth of methanogen and COD concentration was adjusted by water dilution to obtain OLR of 6 g/l day in a balancing tank before feeding to the methanogenic reactor. All experiments were performed at

Table 1. Characteristics of biodiesel wastewater.

Parameters	Values
pH	9.23–9.38
Oil and grease (g/l)	28.8–36.5
Glycerol (g/l)	10.4–12.5
Methanol (g/l)	14.1–16.3
VFA (g/l)	1.8–9.45
LCFA (g/l)	19.3–19.9
Alkalinity (g $CaCO_3$/l)	0.497–1.02
COD (g/l)	216–242
BOD (g/l)	40.2–96.0
Nitrogen content (g/l)	0.106– 0.211
Phosphorus content (g/l)	0.007–0.038
Suspended solid (g/l)	5.51–19.7
Total solid content (g/l)	21.7–41.8

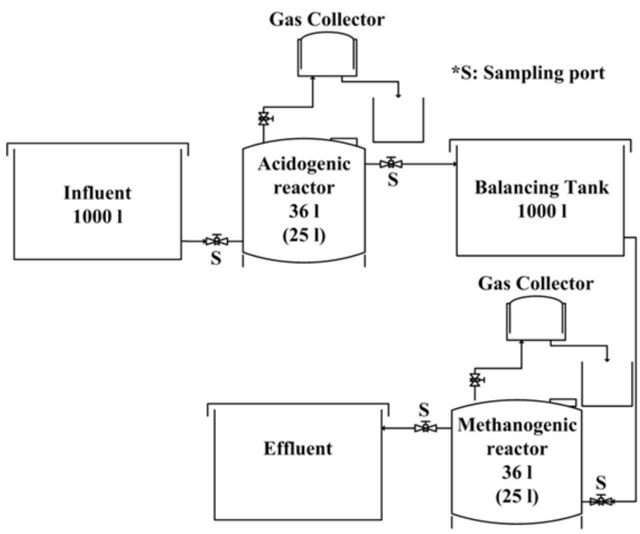

Figure 1. Schematic diagrams of two–stage anaerobic system (S = sampling port).

30±2°C until steady state (the constant COD removal and pH from effluent with the variation by less than 10% for five consecutive days). The influent and effluent of each reactor were sampled daily for pH measurements and every three days for COD analysis. At the initial and steady state, the influent and effluent of each reactor were sampled for VFA and LCFA analysis. Also, biogas production of each reactor was measured and analyzed daily.

2.4 Analytical methods

The characteristics of wastewater were analyzed according to the Standard Method of the APHA (APHA, AWWA, and WEF, 1998). VFA and LCFA were analyzed using a gas chromatograph HP6850 equipped with a flame ionization detector (FID) and a 30 m × 0.32 mm × 0.25 μm Stabilwax® –DA column. The liquid sample was centrifuged at 7,500×*g* for 15 min. For VFA analysis, the supernatant was acidified with 3 M phosphoric acid (1 ml of sample: 0.5 ml of acid) before injection to GC. For LCFA analysis, the liquid sample was mixed with *n*–heptanes (1 ml of sample: 0.5 ml of *n*–heptanes) before injection to GC. Biogas production was measured using a water replacement method. Moreover, the gas sample was taken from a gas collector using a precision analytical syringe. The biogas composition was analyzed by SHIMADZU GC-8A with the thermal conductivity detector and Porapak Q column with length of 1 m and 3.0 mm I.D.

3. Results and Discussion

3.1 Start-up of acidogenic and methanogenic reactors

COD and pH profiles during start-up period of acidogenic and methanogenic reactors are shown in Figure 2 and 3. Wastewater discharged from the biodiesel production plant was firstly diluted with a tap water so that its COD was 0.9 g/l (OLR=1.8 g COD/(l·d)) at HRT 0.5 d and fed into acid tank. It was found that it took a week to reach steady state and then the effluent from the acid tank was used as substrate for the methanogenic reactor. Afterwards, the COD influent in the

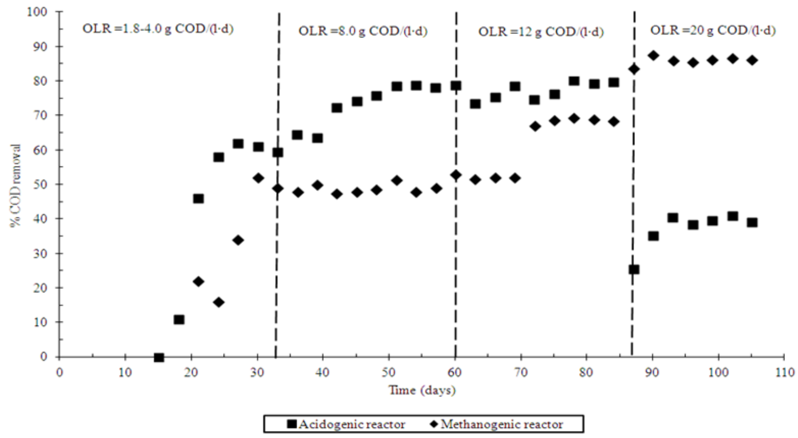

Figure 2. COD removal profiles during start-up period of acidogenic reactor (HRT 0.5 day) and methanogenic reactor (HRT 1.0 day).

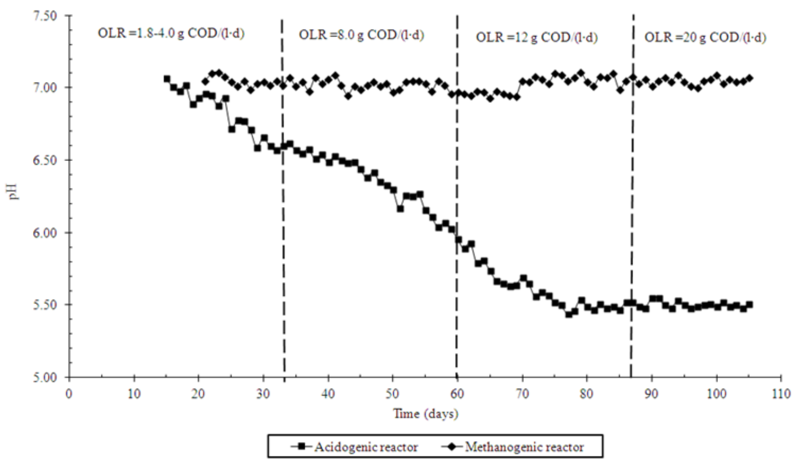

Figure 3. pH profiles during start-up period of acidogenic reactor (HRT 0.5 day) and methanogenic reactor (HRT 1.0 day).

acidogenic reactor was adjusted by stepwise increasing the COD of 10 g/l (OLR=20 g COD/(l·d)). It was found that the COD removal of acidogenic reactor was reached with 80% at OLR =12 g COD/(l·d) and decreased to 40% at OLR =20 g COD/(l·d) with pH 5.5. Total VFA and biogas productions at steady state were 1.64 g/l and 0.8 l/d, respectively from the acidogenic reactor.

In the methanogenic reactor, the COD removal was higher than 80-85% at OLR>8 g COD/(l·d). pH profiles were constant at 7.0–7.1. At steady state, total VFA concentration in the effluent and the biogas production from methanogenic reactor were 0 g/l and 3.0 l/d, respectively. The pH of the effluent from the acidogenic and methanogenic reactors were 5.5 and 7.0, respectively, which were suitable for acidogens (optimum pH of 5.5–6.5) and methanogens (optimum pH of 6.8–7.2) (Khanal, 2008). Therefore, both reactors were ready for further experiment to investigate the effect of nitrogen and phosphorus on the performance of two-stage anaerobic digestion of biodiesel wastewater.

3.2 Effect of nitrogen and phosphorus on acid production in acidogenic reactor

All experiments were carried out for 45 days. The result showed that the VFA production increased with an increase of nitrogen content from COD:N of 100:0.1 to 100:1.1 except at COD:P = 100:1.0 (Table 3). Moreover, the increase of phosphorus from COD:P of 100:0 to 100:0.5 gave a higher VFA production. High COD:P of 100:1 did not enhance VFA production. It was indicated that both nitrogen and phosphorus affected VFA production in the acidogenic reactor and it was necessary to improve the performance of two-stage anaerobic system treating biodiesel wastewater. Nitrogen and phosphorus is the essential components of a microbial cell (Khanal, 2008). Thus, the acid-forming bacteria in this stage required for their growth and synthesis of new cells and their metabolite products. The highest COD removal and VFA production of 45.0%, and 8.32 g/l, respectively, were found at COD:N:P of 100:1.1:0.5 (Table 3). This ratio was slightly different from theory for the anaerobic digestion (COD:N:P = 100:2:0.3) (Khanal, 2008). However, Argun *et al.* (2008) found that a COD:N:P ratio of 100:0.5:1 was an optimum for the acidogenic step for biohydrogen fermentation of wheat powder solution. Intanoo *et al.* (2012) reported that the COD:N:P of 100:6:0.5 was the optimum nutrient for a hydrogen production in the anaerobic digestion of alcohol wastewater. Therefore, the effect of N and P on an anaerobic digestion depends on the source of materials and organic loading rate.

The VFA concentration of 3.85-8.32 obtained from the effluent of acidogenic reactor in this experiment was acceptable for a methanogen activity in the subsequent step. The maximum limit of VFAs content for methanogen inhibition was 10.0 g/l (Khanal, 2008). With a low pH in the acidogenic reactor, the biogas production was just less than 6% of methane content. Others gas might be mainly hydrogen and

Table 2. Operating conditions of acidogenic and methanogenic reactor.

Operating parameters	Acidogenic reactor	Methanogenic reactor
HRT (day)	0.5	1.0
Flow rate (l/day)	50	25
OLR (g COD/(l·d))	20	6

Table 3. Performance of acidogenic reactor at HRT of 0.5 d and OLR of 20 g COD/(l·d) at steady state.

Exp.	COD:N:P	pH[a]	VFA concentration[a] (g/l)	LCFA concentration[a] (g/l)	COD removal (%)	Biogas production (l/d)	CH_4 content (%)
1	100:0.1:0	5.77	3.85	0.620	35.6	0.750	2.59
2	100:0.6:0	5.70	6.64	0.559	39.0	3.76	0.33
3	100:1.1:0	5.60	6.95	0.540	38.9	2.10	1.06
4	100:0.1:0.5	5.55	6.39	0.569	38.9	3.59	2.77
5	100:0.6:0.5	5.51	7.25	0.522	40.8	7.75	2.64
6	100:1.1:0.5	5.54	8.32	0.483	45.0	8.14	4.13
7	100:0.1:1	5.67	6.78	0.550	39.4	2.00	5.84
8	100:0.6:1	5.63	7.22	0.512	40.6	7.46	5.89
9	100:1.1:1	5.51	7.09	0.524	39.7	8.50	0.14

Note: [a]Values were determined from the effluent of acidogenic reactor; pH of influent was adjusted to 7.0.

carbon dioxide. The acetic acid (C2) was the main acid production in the experiment without phosphorus addition (Figure 4). With a higher amount of the nitrogen and phosphorus supplement, the amount of the higher long carbon chain production such as butyric acid (C4), valeric acid (C5), and caproic acid (C6) tended to increase. This result was similar to the work of Sreethawong *et al*. (2010) who reported that either deficient or excess nitrogen led to decrease in the hydrogen yield and an increase in the contents of valeric and propionic acids. The reason was that the produced hydrogen was used to form acids of longer carbon chain. However, heptanoic acid (C7) and caprilic acid (C8) were not found in both influent and effluent of the acidogenic reactor. It was also found that palmitic acid (C16) of 0.278 g/l and oleic acid (C18:1) of 0.436 g/l were the major acids in biodiesel wastewater. Therefore those acids were mostly consumed in the acidogenic reactor (Figure 5).

3.3 Effect of nitrogen and phosphorus on methane production in methanogenic reactor

The results showed that biogas production increased with an increase of nitrogen and phosphorus content except at COD:P > 100:0.5 (Table 4). However, the higher nitrogen ratio gave lower methane content. In contrast, the increase of phosphorus from COD:P of 100:0 to 100:0.5 can enhance the high methane content. However, the COD:P of 100:1 gave low methane content. The influent with COD:N:P of 100:1.1:0.5 gave the highest biogas production (15.5 l/d) and COD removal (91.5%). However, the highest CH_4 yield of 0.170 l CH_4/g $COD_{removed}$ was found at the COD:N:P of 100:0.6:0.5 with the biogas production of 14.1 l/d and COD removal of 91.3% (Table 4). This might be the influence of high VFA produced from acidogenic reactor on methane producing bacteria (Khanal, 2008). Sheng *et al.* (2013) reported that low total ammonia nitrogen concentration was beneficial to anaerobic digestion, while high total ammonia nitrogen concentration caused the inhibition of methane producing bacteria.

Although both nitrogen and phosphorus affected biogas and methane production in the methanogenic reactor, COD removal was not significantly different. Also, it can be observed visually that the lipid layer was formed at the top of both reactors. This phenomenon might cause the low methane yield found in this study (Table 4). Theoretical

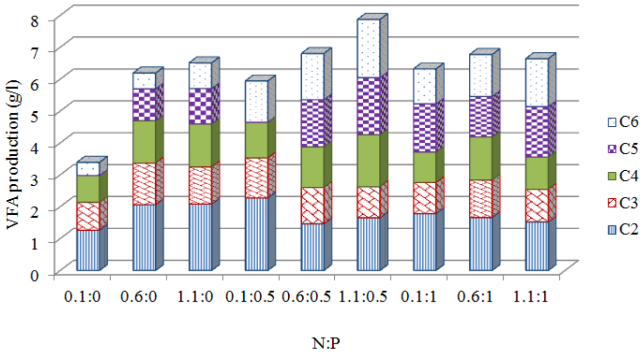

Figure 4. Effect of nitrogen and phosphorus on volatile fatty acid (VFA) production from acidogenic reactor at HRT of 0.5 d and OLR of 20 g COD/(l·d) (based on COD = 100).

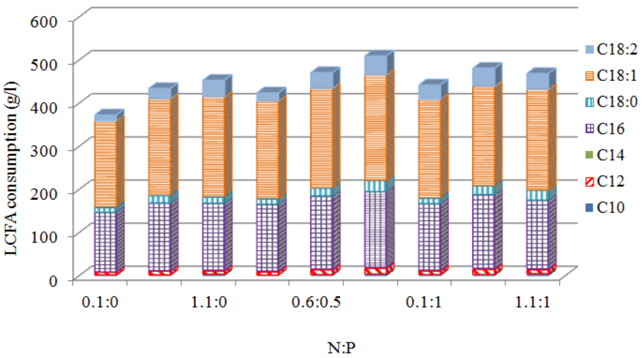

Figure 5. Effect of nitrogen and phosphorus on long chain fatty acid (LCFA) consumption from acidogenic reactor at HRT of 0.5 d and OLR of 20 g COD/(l·d) (based on COD = 100).

Table 4. Performance of methanogenic reactor at HRT of 1 d and OLR of 6 g COD/(l·d) at steady state.

Exp.	COD:N:P	pH[a]	COD removal (%)	Biogas production (l/d)	CH_4 content (%)	CH_4 yield (l CH_4/g $COD_{removed}$)
1	100:0.1:0	6.96	87.7	2.85	66.5	0.036
2	100:0.6:0	6.98	90.6	8.90	53.6	0.088
3	100:1.1:0	7.02	90.8	11.4	54.5	0.114
4	100:0.1:0.5	6.99	90.6	8.75	68.1	0.110
5	100:0.6:0.5	7.02	91.3	14.1	65.7	0.170
6	100:1.1:0.5	7.02	91.5	15.5	59.1	0.167
7	100:0.1:1	7.03	90.9	10.1	61.6	0.114
8	100:0.6:1	7.06	91.1	13.7	60.4	0.151
9	100:1.1:1	7.01	91.2	13.9	37.3	0.095

Note: [a]Values were determined from the effluent of methanogenic reactor; pH of influent was adjusted to 7.0.

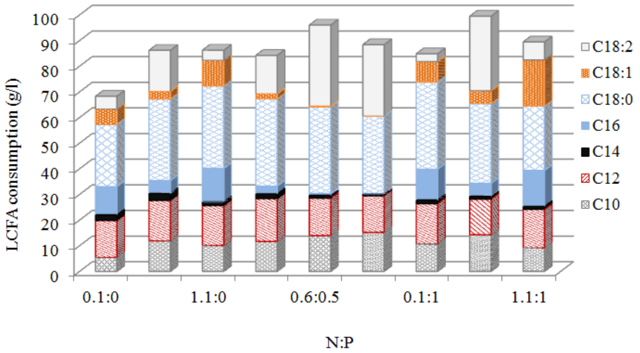

Figure 6. Effect of nitrogen and phosphorus on long chain fatty acid (LCFA) consumption from methanogenic reactor at HRT of 1 d and OLR of 6 g COD/(l·d) (based on COD = 100).

methane yield is 0.40 l CH_4/g $COD_{removed}$ at 35°C (Metcalf and Eddy, 2003). This problem might be solved by the application of a pretreatment step. Siles *et al.* (2011) stated that the application of acidification–electrocoagulation as a pretreatment step followed by the anaerobic digestion can improve the efficiency of COD removal, biogas production and methane yield.

Acetic acid and propionic acid from the effluent of acidogenic reactor was completely consumed in the methanogenic reactor. However, the VFA of 0.50 g/l with C4-C6 was still found in the effluent of methanogenic reactor (data not shown). Figure 6 shows the consumption of LCFA in the methanogenic reactor. Although stearic acid (C18:0) was not the major acid in the influent wastewater, it was mostly consumed in this stage. In theory, 72% of methane production comes from the decarboxylation of acetate (Khanal, 2008). From this study, it was found that not only an acetic acid was consumed in the methanogenic reactor but also either short or longer chain was consumed. Linoleic acid (C18:0) was consumed in the methanogenic reactor rather than in the acidogenic reactor. The different pH and hydrogen partial pressure between acidogenic and methanogenic reactors may affect a microbial community of acetogenic bacteria.

The overall efficiency of acidogenic and methanogenic reactors is presented in Table 5. It shows the high efficiency of COD, TS, VS, and SS removal. The overall mechanism for the treatment of biodiesel wastewater in this study did not occur only in the acidogenic and methanogenic reactors, but the treatment might also happen in the balancing tank. This tank was large and used for collecting the effluent of the acidogenic reactor before feeding to the methanogenic reactor. Moreover, the results showed that LCFA was slightly consumed in the methanogenic reactor. Although, the overall COD removal of 94.9% was obtained, the overall LCFA consumption was only achieved with about 53.4%. Moreover, the accumulation of lipid layers was found in both reactors. The high amount of LCFA required a longer time for the degradation in anaerobic system (Kuang, 2002).

4. Conclusion

The present study showed that nitrogen and phosphorus were significant factors for the two-stage anaerobic digestion treating biodiesel wastewater. The high methane production from biodiesel wastewater required the nitrogen and phosphorus supplements in the optimum content. The COD:N:P of biodiesel wastewater at a ratio of 100:0.6:0.5 was preferable to produce methane with the low nutrient requirement. Nitrogen and phosphorus also affected on the long chain fatty acid consumption. Palmitic acid (C16) and oleic acid (C18:1) were mostly consumed in the acidogenic reactor whereas stearic acid (C18:0) and linoleic acid (C18:0) was mostly consumed in the methanogenic reactor. This result indicated that the two-stage anaerobic system was a good approach to consume more availability substrate in biodiesel wastewater for methane production. However, pretreatment steps may be needed to improve biogas and methane production.

Table 5. Performance of acidogenic and methanogenic reactors at COD:N:P of biodiesel wastewater of 100:0.6:0.5.

Parameters	Acidogenic reactor	Methanogenic reactor	Overall
pH effluent	5.51	7.02	-
COD removal (%)	40.8	91.3	94.9
TS removal (%)	34.9	82.4	88.5
SS removal (%)	36.4	91.2	94.5
VS removal (%)	34.2	71.2	81.1
VFA (g/l) influent	0.449	7.25	-
VFA (g/l) effluent	7.25	0.5	-
LCFA (g/l) influent	0.994	0.522	-
LCFA (g/l) effluent	0.522	0.463	-
LCFA consumption (%)	47.5	11.3	53.4
Biogas production (l/d)	7.75	14.1	-
Methane content (%)	2.64	65.7	-

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