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Effect of pH, OLR, and HRT on performance of acidogenic and methanogenic reactors for treatment of biodiesel wastewater

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ABSTRACT

Two-stage anaerobic process was studied in order to treat biodiesel wastewater. The first stage represented acidogenic reactor while the second stage was methanogenic reactor. The effect of pH, hydraulic retention time (HRT), and organic loading rate (OLR) on performance of both reactors was investigated. The optimum condition was examined using response surface methodology with the Box–Behnken Design. In the acidogenic reactor, the optimum pH, HRT, and OLR were 6.48, 16 h, and 26 g COD/(l d), respectively. High VFA production of 9.35 g/l was achieved with the low methane production. In the methanogenic reactor, the optimum pH, HRT, and OLR were 6.95, 30 h, and 6 g COD/(l d), respectively. Biogas production of 19.1 l/d was obtained with the methane content of 66%. VFA was completely consumed. In comparison, the two-stage system showed higher efficiency (COD removal, biogas production, and methane yield) than the one-stage system.

Keywords: Biogas; Acidogenic; Methanogenic; Anaerobic treatment; Response surface methodology; Biodiesel wastewater

1. Introduction

With the increasing demand for energy, biodiesel represents an alternative green energy with clean burning. However, wastewater from biodiesel process is generated as byproduct with the high amount of lipid contents that must be treated. Some researches focused on biodiesel wastewater treatment using physical and/or chemical processes [1,2]. Recently, anaerobic digestion of wastewater was found as an attractive method because of its low energy requirement and environmentally friendly. Biodiesel wastewater contains high amount of long-chain fatty acids (LCFA), which limit the efficiency of the biological wastewater treatment system [3,4]. A two-stage anaerobic digestion approach has previously been reported to improve the conversion of organic substance to methane [5–12]. Two separated reactors are required for the selection and enrichment of different micro-organisms. The acid-forming and the methane-forming bacteria are mainly responsible for overall digestion. In the first stage, 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 [13,14]. Afterward, they

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are subsequently converted to biogas (CH_4 and CO_2) by methane-forming bacteria in the second stage [6]. Therefore, the first stage may act as a metabolic buffer and prevent pH shock to the methanogenic population [8]. In general, acidogenic and methanogenic bacteria have different nutrient requirements and growth kinetics [11]. In two-stage system, the separation of optimization of the hydraulic retention time (HRT) and organic loading rate (OLR) for acidification and methanogenesis can be employed in each stage [12]. Thus, short retention times are generally used in acidogenic reactors to wash out of methanogenic microorganisms [15]. Consequently, the two-stage systems can increase the stability of the process and prevent inhibition effects from overloading and toxic materials [8].

For the digestion of complex substrates, the performance of the hydrolysis and acidogenesis phase is very important. Due to rate-determining step during hydrolysis, the environmental and operational parameters for acid-phase and methane-phase digestion should be considered. Consequently, this research aims to study the optimum operating condition of pH, HRT, and OLR on a two-stage anaerobic system for biodiesel wastewater treatment plant. Although two-stage anaerobic systems have been reported for waste/wastewater treatment from oil, dairy, and fruit industries [6,7,9–11], the application of this system for biodiesel wastewater has never been reported. Also, the optimum condition for each reactor using response surface methodology (RSM) is an approach that has never been reported for two-stage anaerobic system. The RSM can determine the optimum condition with mathematical and statistical methods. Also, this method can analyze the interactions of experimental variables on desirable responses [16].

2. Methods

2.1. Wastewater

Wastewater was obtained from biodiesel production plant (Specialized Research & Development Center for Alternative Energy from Palm Oil and Oil Crops, Prince of Songkla University, Songkhla Province, Thailand). This wastewater was a milky liquid containing high chemical oxygen demand (COD), lipid as oil and grease content, glycerol and methanol with COD:N:P of 100:0.07:0.01 (Table 1). Therefore, the nitrogen and phosphorus adjustment was required for microbial activity. From our previous study [17], we found that the optimum COD:N:P for VFA and methane production for two-stage anaerobic digestion of biodiesel wastewater was 100:1.1:0.51 and 100:0.98:0.65, respectively. Therefore, biodiesel wastewater for each stage was supplemented with urea, NaH_2PO_4, and Na_2HPO_4 to obtain suitable COD:N:P. Also, the influent wastewater was diluted to COD of 10 g/l to reduce substrate inhibition.

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)	0.090–0.450
LCFA (g/l)	0.967–0.994
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

2.2. Inoculum

The sludge (MLSS of 25 g/l) was collected from a full-scale upflow anaerobic sludge blanket 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) [18] 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 setup and operation

The 36 l closed anaerobic reactors (30 cm × 30 cm × 40 cm) were made of plastic tank with working volume of 25 l. Two reactors were used as acidogenic reactor and methanogenic reactor for two-stage anaerobic system. The top of both reactors were connected to biogas collector. Between the two reactors, a 1,000 l balancing tank was used to supply the suitable nutrient and provide the selected flow rate for methanogenic reactor (Fig. 1). In comparison, the one-stage anaerobic system was also carried out using a 36 l closed anaerobic reactor.

In the two-stage anaerobic system, biodiesel wastewater with supplemented nutrient was fed semi-continuously to the acidogenic reactor. Afterward, the

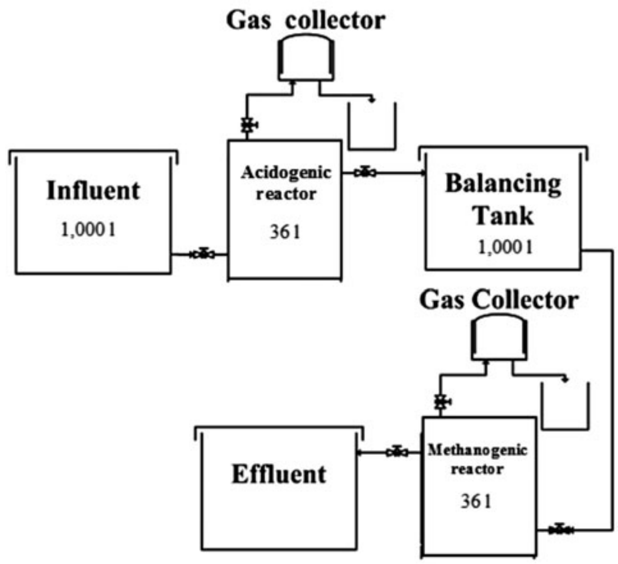

Fig. 1. Schematic diagrams of two-stage anaerobic system.

effluent of the acidification stage was collected in balancing tank. Before the acidified wastewater was introduced to the sequential methanogenic reactor, the suitable nutrient addition and pH adjustment was conducted. All experiment was performed at 30 ± 2˚C with triplication.

2.4. Experimental procedure

The Box–Behnken Design (BBD) was employed to design and analyze all data using Essential Regression and Experimental Design for Chemists and Engineers Version 5.0c running on Microsoft Excel 1998 [19]. A three-factor central composite design with three equidistant levels was performed to describe the nature of the response surface in the optimum region. The interactive effects of three variables; pH (x_1), OLR (x_2), and HRT (x_3) were investigated. The codes and real values for all variables are presented in Table 2. The 15 experimental runs for acidogenic and methanogenic reactors were required (Tables 3 and 4). Each experimental run was done in duplicate. A second-order polynomial equation was proposed to predict the optimal point as following equation:

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_1^2 + b_5x_2^2 + b_6x_3^2 + b_7x_1x_2 + b_8x_1x_3 + b_9x_2x_3 \quad (1)$$

where Y is the response, $b_0 - b_9$ are the regression coefficients variables. COD removal, VFAs content, biogas production, and methane content were considered as the response of the model.

Table 2
Variables and their levels for BBD

	Variables	Level −1	0	1
Acidogenic reactor	pH (x_1)	5	6	7
	OLR, g COD/(l d) (x_2)	10	20	30
	HRT, h (x_3)	6	12	18
Methanogenic reactor	pH (x_1)	6	7	8
	OLR, g COD/(l d) (x_2)	4	6	8
	HRT, h (x_3)	18	24	30

2.5. Analytical methods

The following parameters were analyzed according to the Standard Method of the APHA [20]: biochemical oxygen demand (BOD_5: Iodometric method), COD (Closed reflux method), total Kjeldahl nitrogen (TKN: Macro-Kjeldahl method), suspended solid (SS) and total solid (TS) (Gravimetric method), phosphorus (Ascorbic acid method), glycerol (titration method), and alkalinity (Titration method).

VFA and LCFA were analyzed using Gas Chromatography HP6850 equipped with a flame ionization detector 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. The temperatures of injector and detector were kept at 250˚C, while the column temperature was initially set at 50˚C for 1 min and then increased at a rate of 21.5˚C/min to 250˚C, and maintained at 250˚C for 1 min. 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. The temperatures of injector and detector were maintained at 290 and 300˚C, respectively. The column temperature was operated at 210˚C for 12 min and then ramped to 250˚C at a rate of 15˚C/min, and maintained at 250˚C for 8 min.

Biogas production was measured using water replacement method. Moreover, the gas sample was taken from gas collector at steady state using a precision analytical syringe (VICI precision sampling, Inc., Baton Rouge., LA, USA). The biogas composition was analyzed by SHIMADZU Gas Chromatography GC-8A with thermal conductivity detector and Porapak Q column with length of 1 m and 3.0 mm I.D. The inlet and detector temperatures were kept at 100˚C, while the column temperature was operated at 40˚C.

Table 3
Performance of acidogenic reactor

Exp. no.	pH	HRT (h)	OLR (g COD/(l d))	VFA (g/l)	LCFA consumption (g/l)	Biogas (l/d)	COD removal (%)
1	5	18	20	4.99	0.80	0.35	35.5
2	6	18	10	5.46	0.37	0.43	37.5
3	7	12	30	7.98	0.75	1.59	39.8
4	5	12	30	5.21	0.86	0.32	36.8
5	6	12	20	8.27	1.07	0.34	44.9
6	7	18	20	8.02	0.76	1.53	40.3
7	5	12	10	3.54	0.19	0.29	34.2
8	6	6	10	1.73	0.08	0.19	35.6
9	7	12	10	3.99	0.19	0.55	33.6
10	5	6	20	3.32	0.20	0.25	34.0
11	6	6	30	5.19	0.25	0.28	36.8
12	7	6	20	3.42	0.20	0.44	34.0
13	6	12	20	8.20	1.07	0.35	44.8
14	6	12	20	8.21	1.06	0.35	44.6
15	6	18	30	8.57	1.02	2.40	48.0

Table 4
Analysis of variance for the polynomial models

Terms	Responses for acidogenic reactor			Responses for methanogenic reactor	
	VFA production	LCFA consumption	COD removal	Biogas production	COD removal
b_0	−59.36*	−11.56*	−169*	−437.44*	−142.96*
b_1	18.24*	3.117*	68.59*	121.96*	56.67*
b_2	0.354*	0.142*	0.284$^{n.s.}$	9.200*	6.377*
b_3	0.623*	0.229*	0.297$^{n.s.}$	1.149$^{n.s.}$	1.715*
b_4	−1.673*	−0.256*	−6.062*	−8.929*	−4.096*
b_5	−0.01372*	−0.00315*	−0.0237*	−0.714*	−0.522*
b_6	−0.04483*	−0.00890*	−0.07223*	0.02491$^{n.s.}$	−0.03282*
b_7	0.05794*	−0.00255$^{n.s.}$	0.118*	0.05549*	−0.172$^{n.s.}$
b_8	0.122*	−0.00151$^{n.s.}$	0.224*	0.131$^{n.s.}$	0.02778$^{n.s.}$
b_9	0.00148$^{n.s.}$	0.00202*	0.04114*	0.08156$^{n.s.}$	0.00486$^{n.s.}$
R^2	0.997	0.989	0.981	0.952	0.976
R^2_{adj}	0.993	0.968	0.963	0.925	0.957
F-value	244.35	48.50	53.02	35.36	53.33
p-value	<0.0001	0.0002	<0.0001	<0.0001	<0.0001

Notes: Significant of estimated coefficient.
$^{n.s.}$not significant.
*$p < 0.05$.
R^2, correlation coefficient.
R^2_{adj}, correlation coefficient adjusted for degree of freedom.
F-value, ratio between mean squares of regression and residuals.

3. Results and discussion

3.1. Performance of acidogenic reactor in the two-stage anaerobic system

The effect of three variables: pH (x_1) = 5–7, OLR (x_2) = 10–30 g COD/(l d), and HRT (x_3) = 6–18 h was studied for acidogenic reactor. The experimental results are presented in Table 3. The predicted regression equations for VFA production (Y_1), LCFA consumption (Y_2), and COD removal (Y_3) as the responses of model are given in the following equations with only significant coefficients ($p < 0.05$);

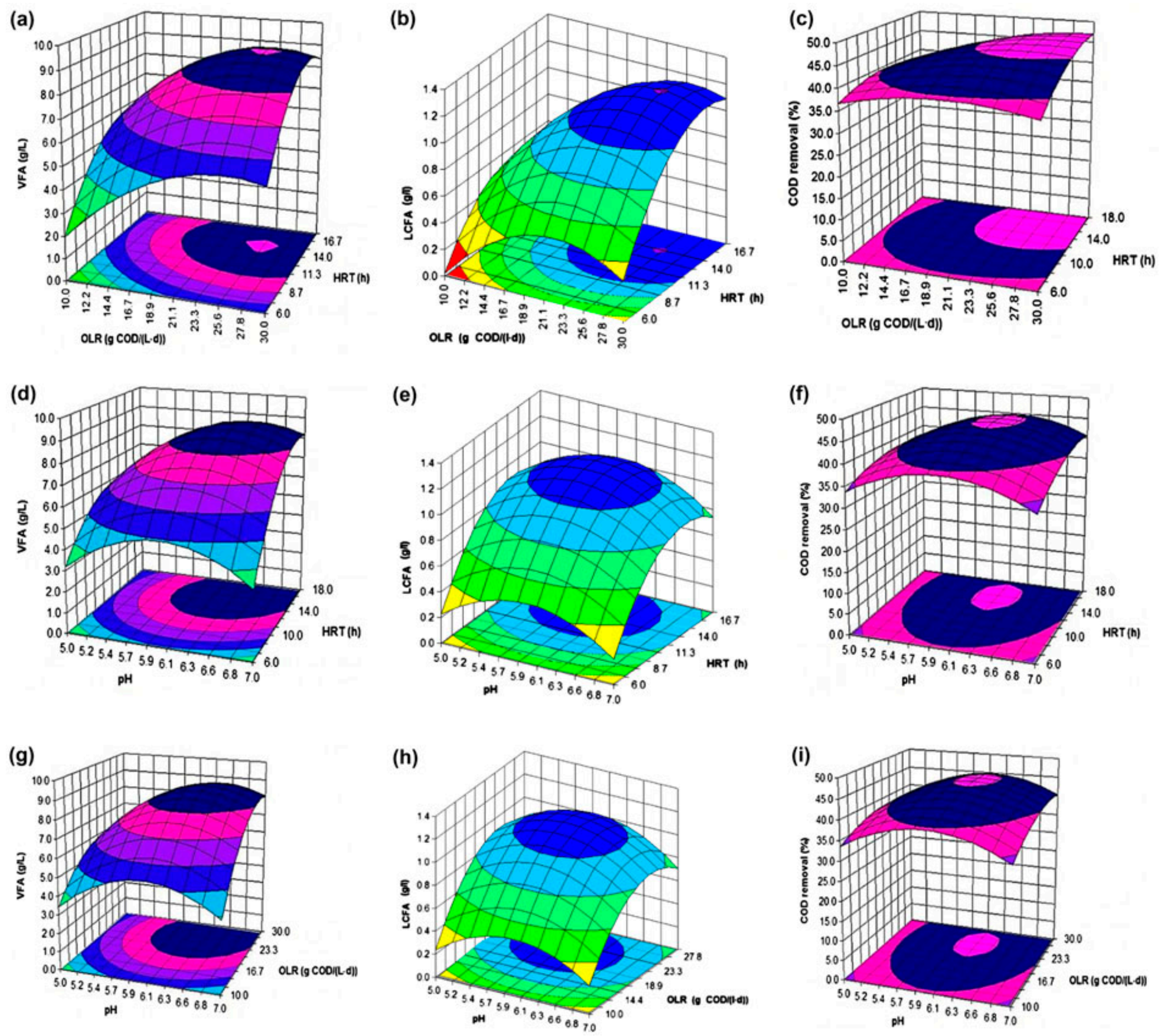

Fig. 2. The 3D response surface for the effect of pH, HRT, and OLR on VFA production ((a), (d), (g)), LCFA consumption ((b), (e), (h)) and COD removal ((c), (f), (i)) in acidogenic reactor.

$$Y_1 = 18.24x_1 + 0.354x_2 + 0.623x_3 - 1.673x_1^2 - 0.01372x_2^2 - 0.04483x_3^2 + 0.05794x_1x_2 + 0.122x_1x_3 - 59.36 \quad (2)$$

$$Y_2 = 3.117x_1 + 0.142x_2 + 0.229x_3 - 0.256x_1^2 - 0.00315x_2^2 - 0.0089x_3^2 + 0.00202x_2x_3 - 11.56 \quad (3)$$

$$Y_3 = 68.59x_1 - 6.062x_1^2 - 0.0237x_2^2 - 0.07223x_3^2 + 0.118x_1x_2 + 0.224x_1x_3 + 0.04114x_2x_3 - 169 \quad (4)$$

Table 4 shows analysis of variance for the polynomial models. The VFA production, LCFA consumption, and COD removal models were given with R^2 of 0.997, 0.989, and 0.981, respectively as showed in Eqs. (2)–(4), which explained 99.7, 98.9, and 98.1% of the variability data. These indicate that the values predicted from models are in a good agreement with the experimental data. The significance of the models was also confirmed by high F-values of regression with very low probability values (p-value < 0.0001). Moreover, the high precision index indicated an adequate signal and that the model can be used to navigate the design space [21,22]. From the high value of coefficient with very low p-value, pH (x_1) was major effect on VFA production, LCFA consumption, and COD removal. Also, the 3D response surface plots between various factors were analyzed as showed in Fig. 2 and the optimum

Table 5
Performance of methanogenic reactor

Exp. no.	pH	HRT (h)	OLR (g COD/(l d))	Biogas production (l/d)	Methane content (%)	COD removal (%)
1	6	18	6.00	7.20	56.9	89.3
2	7	24	6.00	18.0	66.0	94.8
3	8	18	6.00	3.67	60.3	88.0
4	6	24	4.00	7.96	59.9	89.3
5	7	18	4.00	9.47	64.2	90.0
6	8	24	8.00	3.89	60.0	87.3
7	6	24	8.00	9.37	58.5	89.6
8	7	18	8.00	15.6	64.0	91.3
9	8	24	4.00	3.65	60.2	88.3
10	6	30	6.00	11.3	56.1	90.7
11	7	30	8.00	17.2	65.0	92.9
12	8	30	6.00	10.9	62.2	90.0
13	7	24	6.00	18.2	65.3	95.0
14	7	24	6.00	18.2	66.1	94.5
15	7	30	4.00	15.0	63.4	91.8

condition was found. It clearly indicates that higher OLR and HRT gave higher VFA production. The highest VFA production of 9.42 g/l with LCFA consumption of 1.12 g/l and COD removal of 46.5%, respectively, was mathematically obtained at pH, HRT, and OLR of 6.48, 16 h, and 26 g COD/(l d), respectively. However, the higher values of HRT and OLR than their optimum values tended to produce lower VFA concentration. High HRT may allow methanogen to grow and convert VFA to methane [23]. Moreover, high OLR might accumulate the inhibiting substances such as propionic acid, butyric acid, and veleric acid [8,9], which affect microbial growth.

To verify the models, experiments were then performed at pH, HRT, and OLR of 6.48, 16 h, and 26 g COD/(l d), respectively. At steady state, the experimental VFA production of 9.35 g/l, LCFA consumption of 1.17 g/l, and COD removal of 41.5% were recorded. It can be visualized that the predicted and actual values were well in agreement with the error of 0.75, 4.46, and 10.8% for VFA production, LCFA consumption, and COD removal, respectively.

3.2. *Performance of methanogenic reactor in the two-stage anaerobic system*

Effluent from acidogenic reactor under optimum condition was used to load a methanogenic reactor. The effect of three variables; pH (x_1) = 6–8, OLR (x_2) = 4–8 g COD/(l d), and HRT (x_3) = 18–30 h was investigated. The experimental results are presented in Table 5. The predicted regression equations for biogas production (Y_4) and COD removal (Y_5) as the responses of the model are given in the following equations with only significant coefficients ($p < 0.05$);

$$Y_4 = 121.96x_1 + 9.200x_2 - 8.929x_1^2 - 0.714x_2^2 + 0.05549x_1x_3 - 437.44 \tag{5}$$

$$Y_5 = 56.67x_1 + 6.377x_2 + 1.715x_3 - 4.096x_1^2 - 0.522x_2^2 - 0.03282x_3^2 - 142.96 \tag{6}$$

The biogas production and COD models for methanogenic reactor were given with R^2 of 0.952 and 0.976, respectively. These explain the high level of correlation between the experimental and predicted values. Also, the high *F*-values with very low probability values (*p*-value < 0.0001) (Table 3) implied that most of the variations in the responses can be explained by the model equation. Similar to acidogenic reactor, pH (x_1) gave the high value of coefficient with very low *p*-value. Therefore, it indicated that pH was major effect on biogas production and COD removal. The response surface plots of biogas production and COD removal are showed in Fig. 3. It was found that with pH, HRT, and OLR of 6.95, 30 h, and 6.10 g COD/(l d), respectively, the highest biogas production of 19.6 l/d with COD removal of 94.3% was achieved. It clearly showed that pH is significant parameter that affects the biogas production. Most methanogens can grow in a very narrow pH range of 6.7–7.4 [24]. This explains why pH is more sensitive in the methanogenic reactor than in the acidogenic reactor. OLR and HRT also

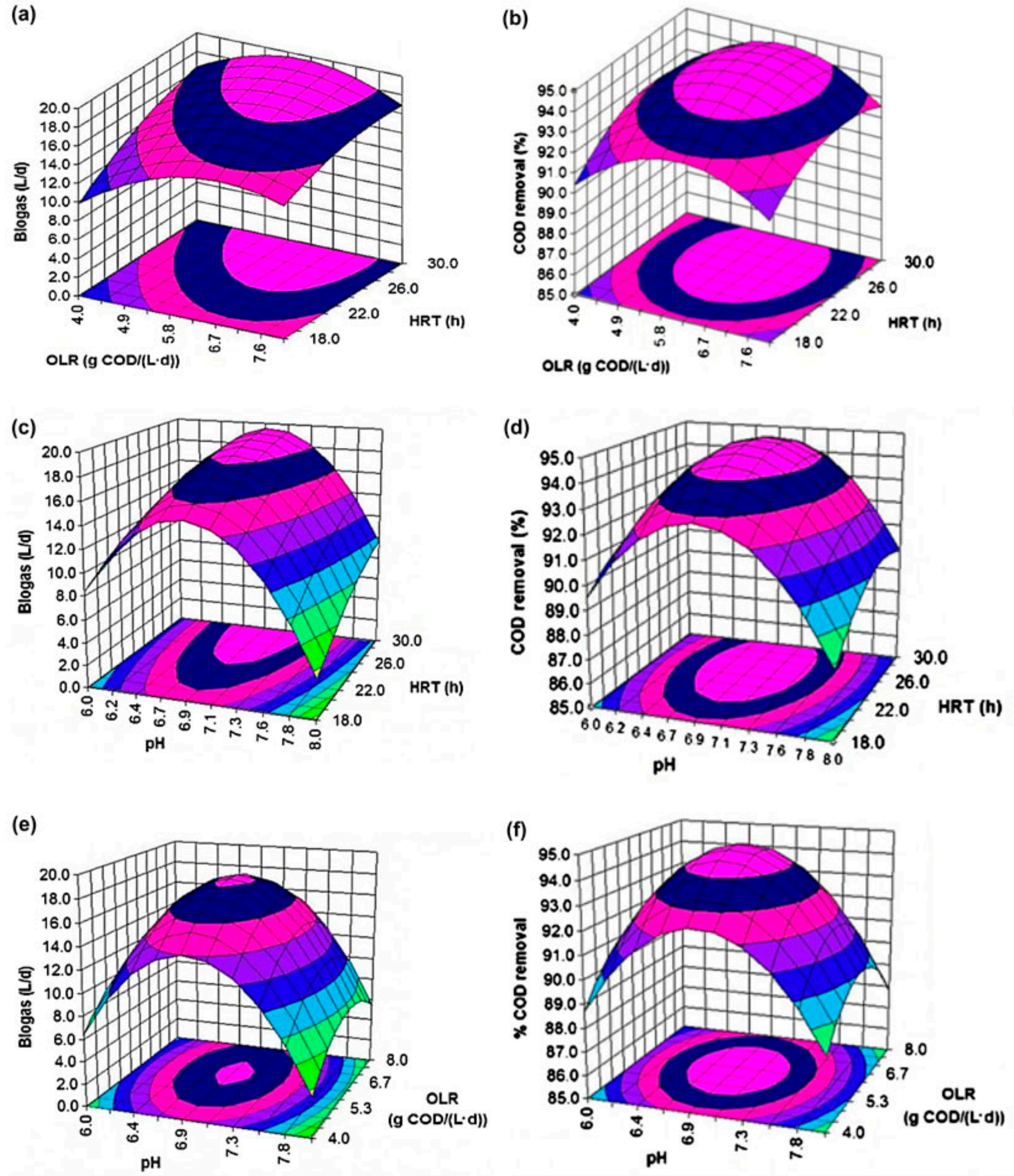

Fig. 3. The 3D response surface for the effect of pH, HRT, and OLR on biogas production ((a), (c), (e)) and COD removal ((b), (d), (f)) in methanogenic reactor.

affected the biogas production and COD removal. Yadvika et al. [25] reported that the optimum OLR produced maximum biogas and further increase in the amount of substrate cannot produce more biogas. HRT is the one important parameter. Shorter HRT is likely to decrease some active micro-organisms.

Confirmatory experiment was conducted under optimum condition at pH, HRT, and OLR of 6.95, 30 h, and 6.10 g COD/(l d), respectively. After the experiment was carried out until steady state, the experimental biogas production of 19.1 l/d was recorded with the error of 2.55% from predicted model (Eq. (5)). Also, COD removal of 93.5% was obtained from experiment with the error of 1.06% from predicted model (Eq. (6)). Moreover, the predicted methane yield was calculated from biogas model (Eq. (5)) with methane content of 66% and the value of 0.152 l CH_4/g $COD_{removed}$ was achieved. However, it was found to be slightly higher than the experimental methane yield (0.143 l CH_4/g $COD_{removed}$) with the error of 5.92%. It

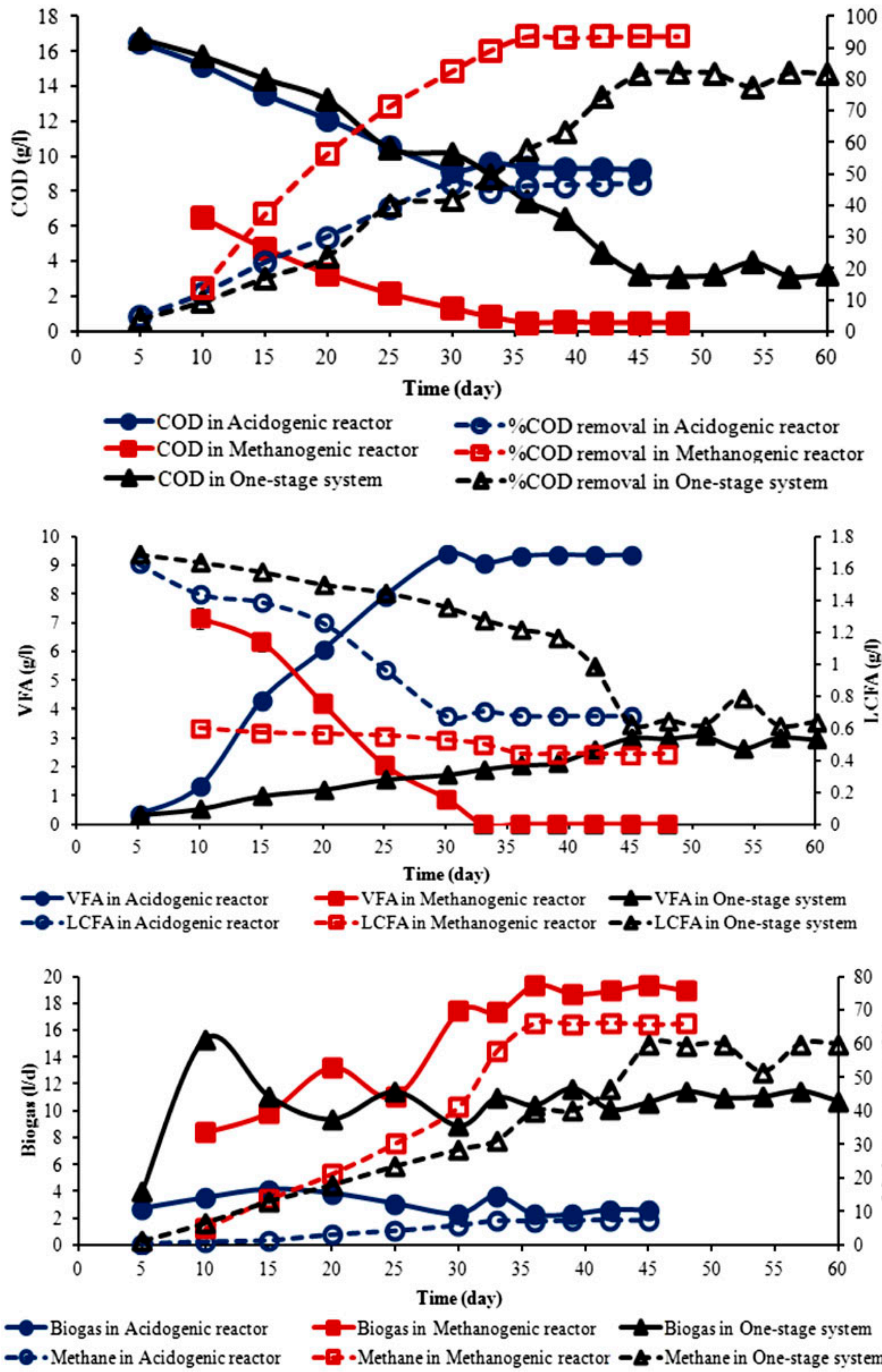

Fig. 4. The profiles of the COD, VFA, LCFA, biogas, and methane content during the anaerobic digestion of the two-stage system (circle symbol for acidogenic reactor and square symbol for methanogenic reactor) and the one-stage system (triangle symbol).

Table 6
Efficiency of two-stage and one-stage anaerobic system for biodiesel wastewater treatment

Parameters	Two-stage			One-stage
	Acidogenic reactor	Methanogenic reactor	Overall	
pH effluent	5.84 ± 0.03	7.13 ± 0.08	–	7.05 ± 0.16
COD effluent (g/l)	9.26 ± 0.84	0.488 ± 0.012	–	3.17 ± 0.05
COD removal (%)	46.5 ± 0.41	93.5 ± 0.26	97.2	81.7 ± 0.27
TS removal (%)	36.7 ± 0.11	85.3 ± 0.42	90.7	86.3 ± 0.39
SS removal (%)	30.4 ± 0.25	95.8 ± 0.14	97.1	90.1 ± 0.22
VS removal (%)	36.5 ± 1.08	75.6 ± 0.82	84.5	78.9 ± 0.97
LCFA effluent (g/l)	0.680 ± 0.00	0.441 ± 0.001	–	0.632 ± 0.01
VFA effluent (g/l)	9.35 ± 0.03	0	–	3.01 ± 0.04
Biogas production (l/d)	2.40 ± 0.17	19.1 ± 0.29	–	11.0 ± 0.35
Methane (%)	7.3 ± 0.15	66.0 ± 0.21	–	59.7 ± 0.20
Carbon dioxide (%)	35.4 ± 1.02	11.4 ± 0.16	–	6.45 ± 0.33
Methane yield (l CH_4/g $COD_{removed}$)	–	0.143	–	0.032

can be concluded that the predicted and experimental values were in close agreement. The mathematical models can be used to predict the efficiency of methanogenic reactor.

3.3. The comparison of one-stage and two-stage anaerobic digestion

The efficiency of two-stage anaerobic digestion under optimum pH, HRT, and OLR of 6.48, 16 h, and 26 g COD/(l d) (with COD influent of 17.3 g/l) and 6.95, 30 h, and 6 g COD/(l d) for acidogenic and methanogenic reactors, respectively, were presented in Fig. 4 and Table 6. In the acidogenic reactor, VFA production of 9.35 g/l was obtained with acetic acid, butyric acid, and propionic acid of 5.27 g/l (56.4%), 1.58 g/l (16.9%), and 0.96 g/l (10.3%), respectively. COD removal was 46.5 ± 0.41% and LCFA (mainly consisting of palmitic acid and oleic acid) was also consumed about 63.2% (Tables 6, and 7). The acidogenic bacteria in the first step consume the organic substrate and produce volatile fatty acids and CO_2 [11,26]. Wijekoon et al. [27] reported the VFA profile variation in the two-stage thermophilic anaerobic membrane bioreactor. They found that the effluent from the first stage mostly contained acetic and butyric acid with very low propionic acid concentration. The acetic acid can be converted to methane, whereas propionic acid is the inhibitory substance. Our results indicate that the effluent from the first stage contained the most favorable substrate for methane production in the second stage. Moreover, the accumulation of thick lipid layer at the top of acidogenic reactor was visible to naked eyes. After analysis, the composition of lipid layer was 50.7% glycerol and others component (with soap as main component). In methanogenic reactor, VFA was completely consumed whereas LCFA was consumed about 35.3% and COD of 93.5% was removed (Tables 6, and 7). No lipid layer was visibly found at the top of methanogenic reactor. Also, biogas of 19.1 l/d was produced with methane and carbon dioxide content of 66.0 and 11.4%, respectively (Fig. 4 and Table 6).

The efficiency of COD removal and biogas production for one-stage anaerobic system was compared with the two-stage anaerobic system. The pH, HRT, and COD of 6.95, 46 h, and 8.8 g COD/(l d) (with COD influent of 17.3 g/l), respectively, were used. It was found that 87.1% of COD was removed in the one-stage anaerobic reactor, while the two-stage anaerobic system respective removal reached 97.2%. Moreover, the VFA concentration of 3.01 g/l was still remained in the effluent with the lower biogas production of 11.0 l/d in the one-stage anaerobic system. Methane and carbon dioxide content of 59.7 and 6.45%, respectively, was obtained. It was found that the methane yield in the two-stage system was higher about five times than in the one-stage system (Table 6). Also, a huge thickening lipid layer was formed at the top of one-stage anaerobic reactor. It is showed that the two-stage anaerobic system gave a higher efficiency than one-stage system significantly. Similar observations for the efficiency of two-stage anaerobic digestion have been reported by Göblös et al. [9]. Moreover, Saddoud and Sayadi [28] and Luo et al. [5] found that the two-stage anaerobic digestion improved the performance of the anaerobic

Table 7
VFA and LCFA concentration at steady state of two-stage and one-stage anaerobic system for biodiesel wastewater treatment

Compositions	Influent concentration	Two-stage system		Effluent from one-stage system
		Effluent from acidogenic reactor	Effluent from methanogenic reactor	
VFA (g/l)				
Acetic acid (C2)	0.06	5.27	0	1.81
Propionic acid (C3)	0	0.96	0	0.42
Isobutyric acid	0	0.04	0	0
Butyric acid (C4)	0.04	1.58	0	0.31
Isovaleric acid	0	0.04	0	0.02
Valeric acid (C5)	0	0.44	0	0.04
Isocaproic acid	0	0	0	0
Caproic acid(C6)	0	0.68	0	0.27
Heptanoic acid (C7)	0	0.34	0	0.14
LCFA (g/l)				
Caprilic acid (C8)	0	0	0	0
Capric acid (C10)	0.056	0.010	0.002	0.015
Lauric acid (C12)	0.069	0.015	0.004	0.168
Myristic acid (C14)	0.011	0.004	0.001	0.003
Palmitic acid (C16)	0.598	0.220	0.165	0.151
Stearic acid (C18:0)	0.108	0.040	0.013	0.025
Oleic acid (C18:1)	0.727	0.267	0.172	0.181
Linoleic acid (C18:2)	0.282	0.124	0.083	0.087

digestion at high OLR without system failure compared to the one-stage anaerobic digestion.

It can be observed that the lipid layer and scum in the two-stage anaerobic system gave lower accumulation than those in the one-stage system. This phenomenon might cause the low methane yield found in this study (Table 6). Theoretical methane yield is 0.40 l CH_4/g $COD_{removed}$ at 35˚C [29]. This problem might be solved by the application of pretreatment step. Siles et al. [30] 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. There have been reported that methane yield from pretreated wastewater of biodiesel processing was 0.280–0.297 l CH_4/g $COD_{removed}$ [30,31].

4. Conclusion

The present study showed that pH, HRT, and OLR were the significant factors for the two-stage anaerobic digestion of biodiesel wastewater. Especially, pH was the major effect in both reactors; the acidogenic and methanogenic reactors. If the system is to be further optimized concerning the biogas production, the necessary volume should also be taken into account. Thus, specific focus should be given on the optimum (lower) HRT combined with the other two parameters. Also, RSM method was the successful tool for a construction of model equation with COD, VFA production, LCFA consumption, and biogas production as the responses. The predicted and actual values were well in agreement. The error was less than 11% and R^2 was higher than 95% for all models. In comparison, the two-stage system showed the higher efficiency than the one-stage system. Further study to increase the methane yield may be needed. The pretreatment steps might be the good approach for the improvement of biogas and methane production.

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