
ORIGINAL ARTICLE

Effect of organic loading rate on methane and volatile fatty acids productions from anaerobic treatment of palm oil mill effluent in UASB and UFAF reactors

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Abstract

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Effect of organic loading rate on methane and volatile fatty acids productions from anaerobic treatment of palm oil mill effluent in UASB and UFAF reactors
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Anaerobic treatment of palm oil mill effluent (POME) with the separation of the acidogenic and methanogenic phase was studied in an up-flow anaerobic sludge blanket (UASB) reactor and an up-flow anaerobic filter (UFAF) reactor. Furthermore, the effect of OLR on methane and volatile fatty acid productions in UASB and UFAF reactors was investigated. In this research, UASB as acidogenic reactor was used for volatile fatty acid production and UFAF as methanogenic reactor was used for methane production. Therefore, POME without pH adjustment was used as influent for the UASB reactor. Moreover, the synthetic wastewater with pH adjustment to 6.00 was fed into the UFAF reactor. The inoculum source for both reactors was the combination of POME sludge collected from the CSTR of a POME treatment plant and granule sludge collected from the UASB reactor of a frozen sea food industry treatment plant. During experimental operation, the organic loading rate (OLR) was gradually increased from 2.50 to 17.5 g COD/l/day in the UASB reactor and 1.10 to 10.0 g COD/l/day in the UFAF reactor. Consequently, hydraulic retention time

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(HRT) ranged from 20.0 to 2.90 days in the UASB reactor and from 13.5 to 1.50 days in the UFAF reactor. The result showed that the COD removal efficiency from both reactors was greater than 60.0%. In addition, the total volatile fatty acids increased with the increasing OLR. The total volatile fatty acids and acetic acid production in the UASB reactor reached 5.50 g/l and 4.90 g/l, respectively at OLR of 17.5 g COD/l/day and HRT of 2.90 days before washout was observed. In the UFAF reactor, the methane and biogas production increased with increasing OLR until an OLR of 7.50 g COD/l/day. However, the methane and biogas production significantly decreased when OLR increased up to 10.0 g COD/l/day. Therefore, the optimum OLR in the laboratory-scale UASB and UFAF reactors were concluded to be 15.5 and 7.50 g COD/l/day, respectively.

Key words : UASB, UFAF, OLR, methane production, Palm Oil Mill Effluent (POME)

บทคัดย่อ

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จากการศึกษาการบำบัดน้ำทิ้งโรงงานสกัดน้ำมันปาล์มแบบไวร์ออกาสแบบแยกเฟสของการสร้างกรดและการสร้างมีเทนในถังปั๊วิกรัฟ UASB (Up-flow anaerobic sludge blanket) และถังปั๊วิกรัฟ UFAF (Up-flow anaerobic filter) โดยทำการศึกษาผลของอัตราการป้อนสารอินทรีย์ต่อการผลิตกรดไขมันระเหยง่ายและมีเทนในถัง UASB และ UFAF ในงานวิจัยนี้จะใช้ถัง UASB เป็นถังผลิตกรดสำหรับสร้างกรดไขมันระเหยง่าย และจะใช้ถัง UFAF เป็นถังผลิตมีเทนสำหรับสร้างมีเทน ดังนั้นสารตั้งต้นที่ป้อนเข้าสู่ถัง UASB จะใช้น้ำทิ้งโรงงานสกัดน้ำมันปาล์มที่ไม่มีการปรับค่าพีเอช ในขณะที่สารตั้งต้นที่ถูกป้อนเข้าสู่ถัง UFAF จะใช้น้ำที่สีสันคร่าวะที่มีกรดระเหยง่ายเป็นองค์ประกอบและถูกปรับค่าพีเอชให้ได้เท่ากัน 6.00 สำหรับแหล่งของหัวเขี้ยวจุลินทรีย์สำหรับใช้กับถังทั้งสอง คือ จุลินทรีย์ผสมจากตะกอนจุลินทรีย์ในถัง CSTR ของระบบบำบัดน้ำทิ้งโรงงานสกัดน้ำมันปาล์มและกรดูลจุลินทรีย์ จากถัง UASB ของระบบบำบัดน้ำทิ้งโรงงานอาหารทะเลแห้งแข็ง ในระหว่างการดำเนินงานการทดลองอัตราการป้อนสารอินทรีย์จะเพิ่มขึ้นแบบเป็นขั้นตอนจาก 2.50 ถึง 17.5 กรัม ซีโอดี/ลิตร/วัน สำหรับถัง UASB และจาก 1.10 ถึง 10.0 กรัม ซีโอดี/ลิตร/วัน สำหรับถัง UFAF ซึ่งสอดคล้องกับระยะเวลาที่ใช้ในการเก็บกักน้ำเสียอยู่ในช่วงจาก 20.0 ถึง 2.86 วัน สำหรับถัง UASB และจาก 13.5 ถึง 1.50 วัน สำหรับถัง UFAF ตามลำดับ ผลการทดลองพบว่าประสิทธิภาพของ การกำจัดซีโอดีสำหรับถังปั๊วิกรัฟทั้งสองมากกว่า 80.0% กรดไขมันระเหยง่ายจะถูกผลิตเพิ่มขึ้นเมื่อเพิ่มอัตราการป้อนสารอินทรีย์ ปริมาณของกรดไขมันระเหยง่ายทั้งหมดและกรดอะซิติกในถัง UASB เท่ากัน 5.50 และ 4.90 กรัม/ลิตร ตามลำดับ ซึ่งได้รับที่อัตราการป้อนสารอินทรีย์เท่ากัน 17.5 กรัม ซีโอดี/ลิตร/วัน โดยระยะเวลาในการเก็บกักเท่ากัน 2.86 วัน ก่อนที่จะเกิดการระดับของจุลินทรีย์ออกจากระบบ ขณะที่ผลการทดลองในถังปั๊วิกรัฟ UFAF พบว่า การผลิตมีเทนและก๊าซชีวภาพจะเพิ่มขึ้นเมื่ออัตราการป้อนสารอินทรีย์เพิ่มขึ้นจนกระทั่งถึง 7.50 กรัม ซีโอดี/ลิตร/วัน โดยมีผลผลิตมีเทนสูงสุด เท่ากัน 0.342 ลิตรมีเทน/กรัม ซีโอดีที่ถูกใช้ไป และเมื่ออัตราการป้อนสารอินทรีย์เพิ่มขึ้นถึง 10.0 กรัม ซีโอดี/ลิตร/วัน การผลิตมีเทนและก๊าซชีวภาพจะลดลงอย่างมีนัยสำคัญ ดังนั้นอัตราการป้อนสารอินทรีย์ที่เหมาะสมสำหรับถัง UASB และถัง UFAF อยู่ที่ประมาณ 15.5 และ 7.5 กรัม ซีโอดี/ลิตร/วัน ตามลำดับ

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The production of palm oil, however, results in the generation of large quantities of polluted wastewater commonly referred as palm oil mill effluent (POME). Typically, 1.0 ton of crude palm oil production requires 5.0-7.5 ton of water; over 50.0% of which ends up as POME. Moreover, POME was high in organic content (COD 50.0 g/l, BOD 25.0 g/l) and contains appreciable amounts of plant nutrient (Borja *et al.*, 1996; Singh *et al.*, 1999; Ahmad *et al.*, 2005). If discharged, the untreated POME can cause considerable environmental problems.

With increasing demand for energy and cost effective environmental protection, anaerobic digestion biotechnology has become the focus of worldwide attention (Singh *et al.*, 1999). Moreover, it offers a positive environmental impact since it combines waste stabilization with net fuel production and allows the use of the effluent as fertilizer. POME consists of various suspended components. POME nutrient content is too low for aerobic treatment process, but sufficient for anaerobic treatment process (Chin *et al.*, 1996). One of the most notable developments in anaerobic treatment process technology is the up-flow anaerobic sludge blanket (UASB) reactor. The UASB reactor exhibits positive features, such as allows high organic loadings, short hydraulic retention time (HRT) and has a low energy demand (Paravira *et al.*, 2005). Granular sludge formation is the main distinguishing characteristic of UASB reactors as compared to other anaerobic technologies. In spite of the advantages of granular sludge, effective treatment of wastewater with flocculent sludge UASB reactors have been documented (Sayed and Lettinga, 1984; Gooden *et al.*, 2001). The high suspended solids content of POME can prevent the system from operating at high organic loading rate (OLR). Suspended and colloidal components of wastewaters in the form of fat, protein, and cellulose have adverse impact on UASB reactors' performance and can cause deterioration of microbial activities and wash out of active biomass (Tokian *et al.*, 2003). In addition, the up-flow anaerobic filter (UFAF) reactors also have potential to apply treatment of domestic sewage

and industrial wastewater containing relative low levels of organic materials. The reactor contains a microbial supporting material. Granulate micro-organisms exist not only in the spaces within the medium, but are also attached to its surface; a high-density microbial population is retained within the reactor, creating a hybridization of microbial floc and adhesion (Kazuhisa, 1997). The bioreactor for methane fermentation such as UASB and UFAF experience inherent problems when operated at high COD loads, due to the fact that the overall growth rate of acidogenic bacteria proceeds faster (10-fold) than that of methanogenic bacteria. When this occurs, inhibitory products such as volatile fatty acids and H₂ accumulate in the reactor, slowing down the entire process. In order to overcome this, two-phase processes consisting of acidogenic and methanogenic fermentation have been investigated (Kazuhisa, 1997).

In order to increase the stability of anaerobic digestion, two-phase anaerobic systems have been introduced. The physical separation of the acidogenic and methanogenic phase can increase stability because overloading of the methane reactor can be prevented by proper control of the acidification step (Dionopolou *et al.*, 1988). More phase separation also allows the maintenance of appropriated densities of the acid and methane formers in separate reactors and enables maximization of the acidification and methanogenesis reactors by applying optimal operation condition, determined by the metabolic and bio-kinetic properties of both groups (Stronach *et al.*, 1986). Other advantages of the two-phase configuration are that it increases significantly the specific activity of methanogens and enables the disposal of the fast growing acid formers. The acidification reactor can serve as a buffering system when the composition of the wastewater is variable. Moreover, it can help in the removal of the compounds, which are toxic to methane bacteria. Finally the acidification reactor provides a constant substrate for the methanogens, which are known to adapt slowly to varying substrate concentration and composition. The effluent of the acidogenic reactor contains mainly acetic acid, propionic acid and butyric acid. Further-

more, higher fatty acids are also found at lower concentrations. The type of fatty acids produced finally depends upon the type and composition of wastewater used as well as the physical parameters like temperature, pH, HRT and OLR (Stronach *et al.*, 1986; Kisaalita *et al.*, 1987; Dionopolou *et al.*, 1988; Alexiou *et al.*, 1994;).

Therefore, this research aims to study the effect of OLR on methane and volatile fatty acid productions from anaerobic digestion for POME in the UASB and UFAF reactors.

Materials and methods

1. Wastewater

POME was used as influent for the acidogenic UASB reactor obtained from the first pond wastewater system of Trang Palm Oil manufacturing process, Trang province, Thailand. After collection, POME was stored at -18°C and was thawed before using in experiments. The characteristic of the POME are summarized in Table 1. This wastewater are a viscous brown liquid containing fine suspended solid, COD of 95.0 g/l; BOD of 22.0 g/l; TS of 35.0 g/l; ash of 4.50 g/l; TKN of 1.08 g/l; SS of 12.0 g/l; oil&grease of 10.6 g/l; total phosphate of 0.47 g/l; PO₃³⁻-H-P of 0.15 g/l and pH of 4.35. The COD: N: P ratio in POME about 190:2.20:1.00, which shows that nutrients were just enough for anaerobic digester. It can be observed that COD, TS, SS and oil & grease contents of the wastewater were very high. The oil&grease inhibit microbial activity while the TS inhibit development of granule in UASB. Therefore, the POME pretreatment was required. The POME was pretreated by screening filtration two times to get rid of oil&grease and total solid. The characteristic of pretreatment wastewater are shown in Table 1. The data show values for each parameter containing COD of 72.0 g/l; TS of 12.5 g/l; SS of 7.00 g/l and oil&grease of 6.10 g/l. The COD, TS, SS and oil & grease removal after pretreatment were about 24.2%, 64.3%, 41.7%, 42.5%, respectively.

The composition of synthetic wastewater fed for the methanogenic UFAF reactor was (in

g/l): acetic acid 5.00, propionic acid 1.80, butyric acid 1.20 and diluted POME (Pind *et al.*, 2002).

2. Sludge seed

The inoculum for seeding was a mixture of sludge taken from the CSTR reactor of Asian Palm Oil manufacturing process (Krabi, Thailand) and granule was taken from the UASB reactor of Chotiwat Industry Co. Ltd. (Songkhla, Thailand). The granule from sea food industry was passed through a screening to remove debris before combined sludge from palm oil treatment process (1:1). In order to test the microbial activity of the sludge seed, 5.00 ml of the sludge mixture was added to 50.0 ml sucrose and acetate (as substrate with COD of 4.00 g/l) in a 120 ml serum bottle (Alper *et al.*, 2005). The produced gas was analyzed after 24 h. It was found that sludge seed had anaerobic activity and produced CH₄ (45.0%), H₂S (6.00 ppm) and N₂ (4.00 ppm).

3. Experimental reactors

The experimental reactor was performed using two different reactor designs (Figure 1). One was an UASB reactor with an active volume of 10.0 l with 9.00-12.8 cm internal diameter and a small funnel installed in the upper part acting as a gas separator. The lower part of the UASB had a smaller dimension than the upper part to avoid microbial washout from the reactor. The lower part had diameter and height about 9.00 and 80.0 cm, respectively, while the upper part had diameter and height about 10.5 and 60.0 cm, respectively. The other reactor was an UFAF reactor with an active volume of 5.00 l with 9.00 cm internal diameter, internal constrictor filled up to 4.50 l of its volume with poly vinyl chloride (PVC) tubular section, with a rough surface to retain the bacterial biomass (Paravira *et al.*, 2005). A tubular PVC microbial filter in the bioreactor was 2.00 cm in height, 1.80 cm internal diameter and 2.20 cm external diameter (Chavez *et al.*, 2005; Michaud *et al.*, 2005) (Figure 2). The UASB reactor and UFAF reactor were used for the acidogenic and methanogenic reactions of a two-stage process. The reactors were maintained at a temperature of

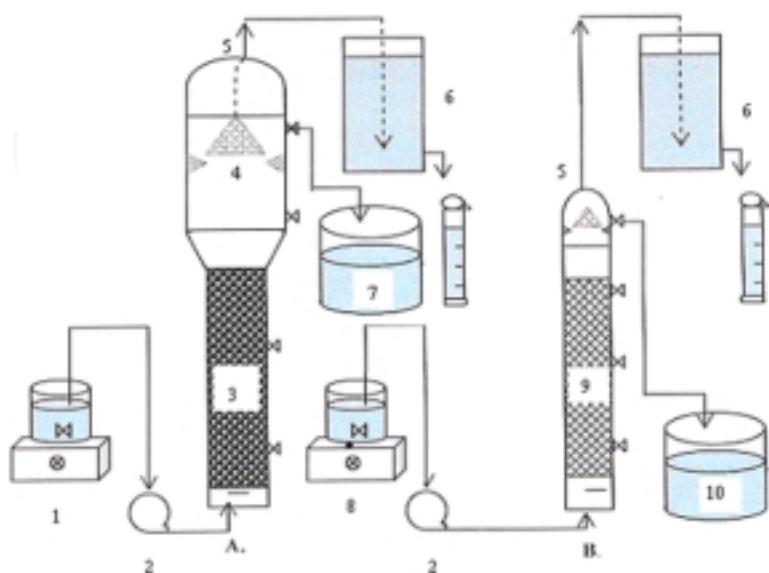


Figure 1. Schematic diagram of (A) the UASB and (B) the UFAF reactor.

1 = POME as influent for UASB	6 = Gas Collector
2 = Peristaltic pump	7 = Effluent from UASB
3 = Sludge blanket in UASB	8 = Synthetic wastewater as influent for UFAF
4 = Gas separator	9 = Microbial supporting materials in UFAF
5 = Gas Exit	10 = Effluent from UFAF



Figure 2. The microbial supporting materials in the UFAF reactor.

(A) The top view (B) The side view

(Color figure can be viewed in the electronic version)

$28.0 \pm 2.0^{\circ}\text{C}$. The UASB reactor was designed having a gas-biomass-liquid separator at the head of the column and an influent liquid distributor at the base. The top of reactors was connected to a gas collector bottle using a water displacement tank filled with an acidified brine solution to prevent CO_2 dissolution (Borja *et al.*, 1996).

4. Start-up period

The UASB and UFAF reactors used as

acidogenic and methanogenic reactors were inoculated with 3.00 and 1.50 l of sludge inoculums of VSS of 32.8 mg/l, respectively. In the start-up period both reactors were operated for 2 months at $28.0 \pm 2.0^{\circ}\text{C}$. Both reactors were initially fed with diluted POME (about 15.0-50.0 g COD/l). Calcium oxide (lime or CaO) was used for pH adjustment to 6.00 (Marchaim and Krause, 1991; Subbah *et al.*, 2004). Total alkalinity of reactors was controlled in the range of 2.50-4.00 g CaO/l. The influent

COD concentration was increased gradually by reducing the dilution factor with tap water. Organic loading rate (OLR) increased from 1.50 to 5.00 g COD/l/day and hydraulic retention time (HRT) was kept at 3.30 days for the both reactors.

5. Experimental operating condition

Experimental operation period began after steady-stage was observed during the start-up period. The influent for UASB reactor was diluted POME (COD of 50.0 g/l) without pH adjustment, while the influent for UFAF reactor was synthetic wastewater (COD of 15.0 g/l) with pH adjustment to 6.0. OLR increased stepwise from 2.50 to 17.5 g COD/l/day and 1.10 to 10.0 g COD/l/day, and HRT was kept from 20.0 to 2.86 days and 13.5 to 1.50 days for the UASB and UFAF reactor, respectively. The effluent for each reactor was sampled every 4 days for COD and VFA analysis. Also, gas samples for each reactor were analyzed daily for the CH₄ content and gas production.

6. Analytical methods

6.1 Total gas production and biogas composition

The total gas production was determined by monitoring volume of liquid displaced in a gas collector with inverted measuring cylinder. Moreover, the gas sample was taken from the top of each reactor using a precision analytical syringe (VICI precision sampling, Inc., Baton Rouge., LA, USA) to determine biogas composition by MULTIGAS analyzer Model MX2100 (OLDHAM; France).

6.2 Volatile fatty acid (VFA) content

The liquid samples for VFA determination were analyzed by gas chromatograph, HP 6850 series equipped with flame ionization detector (FID) (Agilent, USA). The capillary column (123-3232 DB-FFAP) was 30.0 m x 0.32 mm internal diameter, 0.25 µm film thickness of 5.00% phenyl and 95.0% dimethyl polysiloxane (Agilent, USA). The chromatographic optimum conditions of GC-FID system were a carrier gas flow rate of 13.3 ml/min, fuel gas (H₂) flow rate of 30.0 ml/min, oxidant gas flow rate (Air) of 300 ml/min, injector

temperature of 250°C and detector temperature of 240°C. A liquid sample was centrifuged at 7,500 x g for 15 min. After that 1.00 ml of supernatant was acidified with 1.00 ml of 3 M phosphoric acid. Then, the mixture was combined with 1.00 ml of 4-methyl valeric acid as internal standard. Before injecting into the GC column, the mixture was centrifuged at 7,500 x g for 15 min to remove suspended solid preventing any clogging in the GC column.

6.3 Chemical oxygen demand (COD)

The liquid samples were centrifuged at 7,500 x g for 15 min for CODs analysis. Potassium biphthalate (KHC₈H₄O₄, KHP) and distilled water were used as positive and negative control, respectively. The samples were digested by Spectroquant®, series TR 320 (MERCK, Germany), subsequently the samples were measured by Spectroquant®, series NOVA 60 (MERCK, Germany).

7. Calculation

- Organic loading rate (OLR)

The OLR can be varied by changing the influent COD concentration and by changing the flow rate (Eq. (1)). Changing the flow rate implies changing the HRT and the upflow velocity (Mahmoud *et al.*, 2003).

$$\frac{Q * COD_{infl}}{V} = \frac{COD}{HRT} \quad (1)$$

where Q is the influent flow rate (l/day), V is the volume of the reactor (l), COD_{infl} is the total influent COD (g/l).

- Percentages of total hydrolysis (H); acidogenic (A); and methanogenesis (M)

Hydrolysis, which indicated total degradation, acidogenesis, and methanogenesis were determined using the following equations (El-Mashad *et al.*, 2004; Halalsheh *et al.*, 2005):

$$\% \text{Hydrolysis} =$$

$$\frac{COD_{CH4} + (COD_{diseffl} - COD_{disinfl})}{COD_{infl} - COD_{disinfl}} \times 100 \quad (2)$$

%Acidification =

$$\frac{COD_{CH_4} + (COD_{VFAeffl} - COD_{VFAinfl})}{COD_{infl} - COD_{VFAinfl}} \times 100 \quad (3)$$

$$\% \text{Methanogenesis} = \frac{COD_{CH_4}}{COD_{infl}} \times 100 \quad (4)$$

where $COD_{diseffl}$ is the dissolved effluent COD, $COD_{disinfl}$ is the dissolved influent COD, COD_{infl} is the total influent COD, $COD_{VFAeffl}$ is the COD of effluent VFA, $COD_{VFAinfl}$ is the COD of influent VFA and COD_{CH_4} is the COD of methane production.

8. Statistical analysis

Data were analyzed using the data analysis toolbox in EXCEL software. The applied statistical analysis was ANOVA single factor with replication. To compare the mean values, Fisher's least significant difference (Fisher's LSD) at $t = 0.05$ was used.

Results and discussions

1. Performance of the UASB reactor

During the start-up period, the accumulation of VFA and COD removal in the UASB reactor tended to increase with increasing OLR from 1.50 to 5.00 g COD/l/day at HRT of 3.30 days. The maximum VFA of 4.00 g/l at OLR of 5.00 g COD/l/day was found at day 50. After that VFA decreased to about 1.60 g/l (Figure 3). However, COD removal remained constant at almost 40.0% after day 40 (Figure 4). The VFA decrease resulted from the biogas production. At initial start-up period, biogas production of 0.12 l/day was observed. However, at the end of this period the maximum biogas production of 5.98 l/day was found.

During the experimental operation period, the initial OLR was set at 2.50 g COD/l/day and HRT of 20 days. The OLR was then increased in steps to 5.00, 7.50, 10.0, 12.5, 15.0 and 17.5 g COD/l/day by reducing the HRT to 20.0, 10.0, 6.67, 5.00, 4.00, 3.33 and 2.86 days, correspondingly. The results show that the accumulation of VFA increased with increasing OLR. The maximum VFA was 5.50 g/l at OLR of 17.5 g COD/l/day.

After that the accumulation of VFA significantly decreased and washout was observed (Figure 5). In addition, total biogas production and methane yield increased with increasing OLR. The maximum total biogas production and methane yield were 25.5 and 7.01 l/day, respectively at OLR of 15.0 g COD/l/day (Figure 6). The maximum methane production rate of 0.695 l CH_4 /l/day was also found at OLR of 15.0 g COD/l/day (Table 2). However, %methanogenesis rapidly decreased when OLR was increased from 15.0 to 17.5 g COD/l/day. Conversely, %acidification showed no significant difference. This result indicated that the conversion of organic matter in wastewater to VFA was not different, but the conversion of VFA to CH_4 decreased with increasing OLR. The COD removal efficiency was more than 80.0% at OLR from 2.50 to 15.0 g COD/l/day (Figure 7). Furthermore, when OLR was increased from 15.0 to 17.5 g COD/l/day, COD removal rapidly decreased. However, more than 60.0% COD removal was achieved.

2. Performance of the UAF reactor

During the start-up period, the accumulation of VFA and COD removal in the UAF reactor tended to increase with the increasing OLR from 1.50 to 5.00 g COD/l/day at HRT of 3.30 days. The maximum VFA of 6.00 g/l at OLR of 5.00 g COD/l/day was found at day 50. After that VFA de-

Table 1. Characteristic of the palm oil mill effluent (POME) used in this study.

Parameter	Raw wastewater	Pretreatment wastewater
BOD (g/l)	22.0	18.0
Total COD (g/l)	95.0	74.0
Soluble COD (g/l)	72.0	68.0
TS (g/l)	35.0	14.0
SS (g/l)	12.0	7.50
Ash (g/l)	4.50	2.70
Oil and grease (g/l)	10.6	6.10
TKN (g/l)	1.08	0.980
Total phosphate (g/l)	0.473	0.433
PO ₄ ³⁻ -H-P (g/l)	0.147	0.125
pH	4.35	4.36

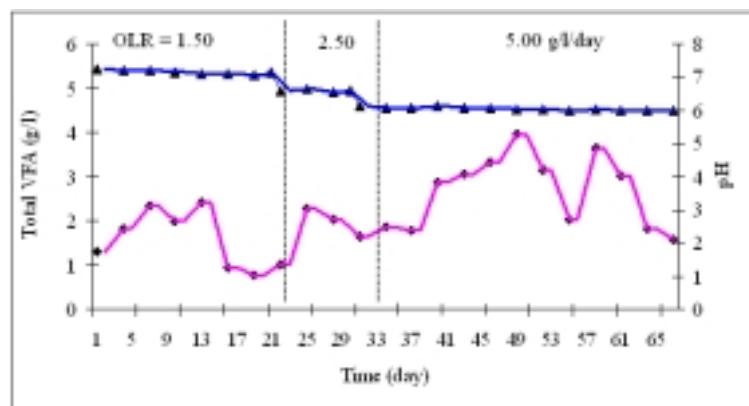


Figure 3. The pH and VFA production in UASB reactor during start-up period.
(▲) pH value (◆) Total VFA

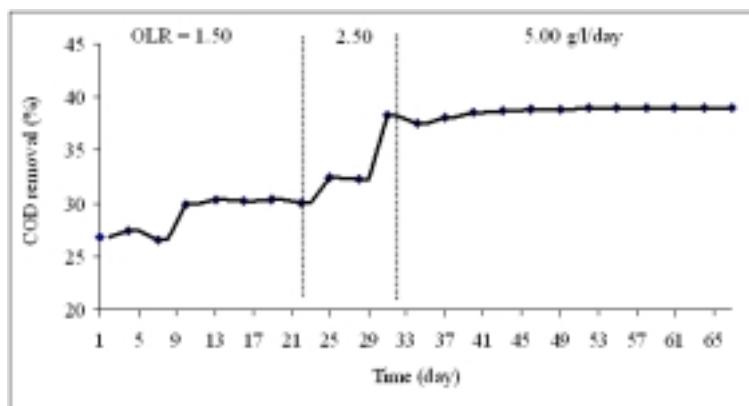


Figure 4. The COD removal with the increasing OLR in the UASB reactor during start-up period.

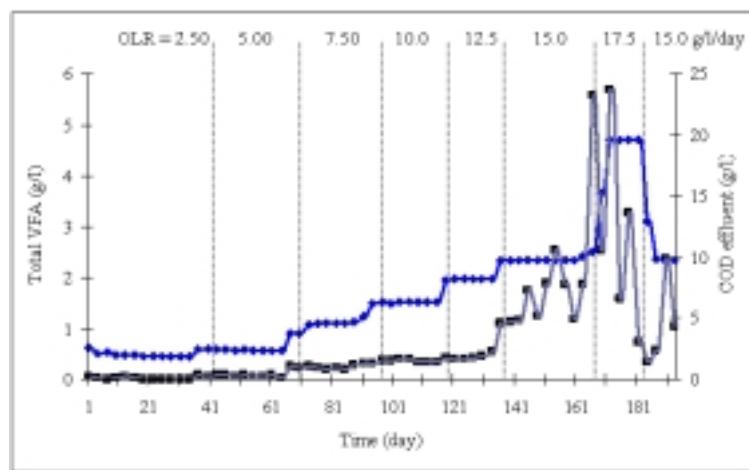


Figure 5. The VFA accumulation with the increasing OLR in the UASB reactor during experimental operation period. (◆) COD effluent (■) Total VFA

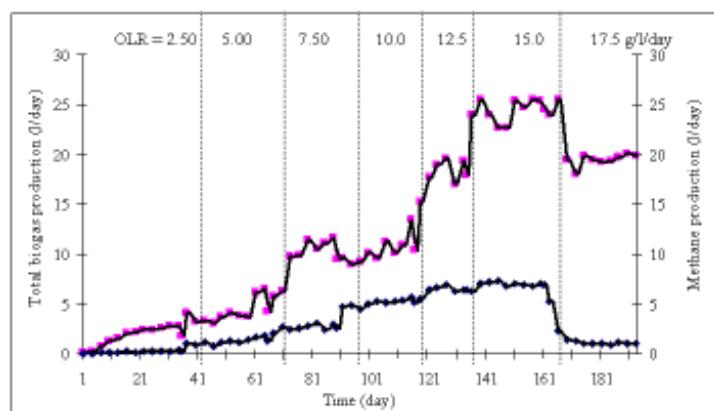


Figure 6. Biogas and methane production with the increasing OLR in the UASB reactor during experimental operation period. (■) Methane yield (◆) Total biogas

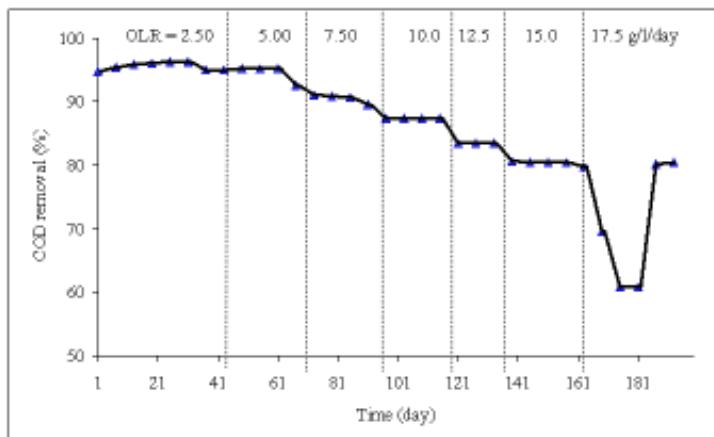


Figure 7. The COD removal in the UASB reactor during experimental operation.

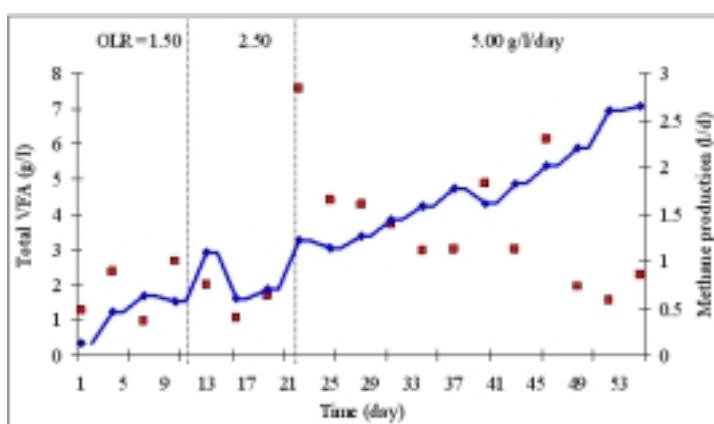


Figure 8. The VFA and methane production in UFAF reactor during start-up period.
(■) Total VFA (◆) Methane

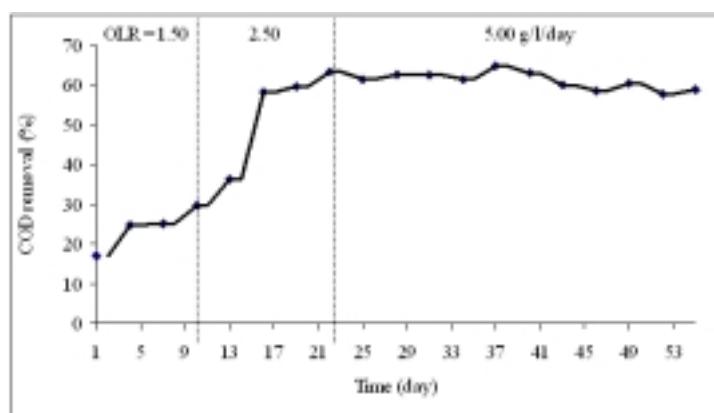


Figure 9. COD removal with the increasing OLR in the UASB reactor during start-up period.

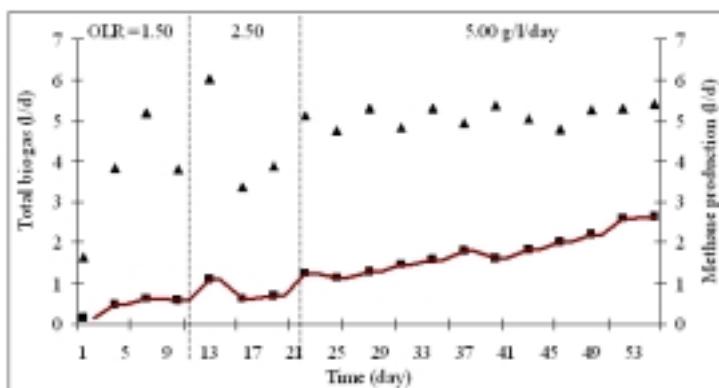


Figure 10. The methane and total biogas production in UFAF reactor during start-up period.
(▲) Total biogas (■) Methane

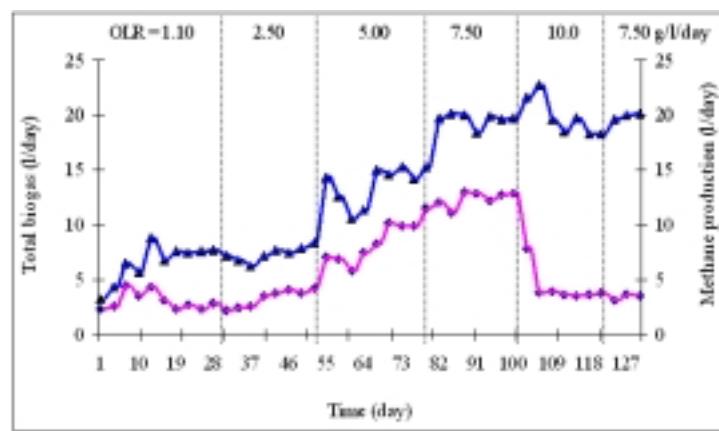


Figure 11. The methane and total biogas production in UFAF reactor during experimental operation. (▲) Total biogas (◆) Methane

Table 2. Experimental values a at steady-state condition in UASB reactor.

OLR (g COD/l/day)	2.50	5.00	7.50	10.0	12.5	15.0	17.5
HRT (day)	20.0	10.0	6.67	5.00	4.00	3.33	2.86
pH	6.71	6.63	6.32	6.52	6.47	6.43	6.03
Hydrolysis (%)	37.3	38.1	41.2	43.2	45.7	47.7	59.1
Acidogenesis (%)	33.7	33.5	32.6	31.6	30.4	33.1	32.3
Methanogenesis (%)	33.7	33.3	32.0	30.6	29.2	28.2	22.0
Qm^b (l CH ₄ /l/day)	0.028	0.146	0.277	0.534	0.654	0.695	0.141
Methane yield (l CH ₄ /g COD _{degraded})	0.012	0.031	0.040	0.061	0.063	0.058	0.013
VFA (g/l)	0.007	0.063	0.227	0.359	0.440	1.73	3.55
Soluble COD (g/l)	1.87	2.38	4.62	6.31	8.25	9.75	18.7
COD removal (%)	96.3	95.2	90.8	87.4	83.5	80.5	62.5

^a Values are averages during the steady-stage condition.

^b QM : methane production rate

Table 3. Experimental values a at steady-state condition in UAF reactor.

OLR (g COD/l/day)	1.10	2.50	5.00	7.50	10.0
HRT (day)	13.5	6.00	3.00	2.00	1.50
pH	7.03	7.01	7.02	7.01	7.02
Hydrolysis (%)	40.5	41.7	44.3	47.5	55.7
Acidogenesis (%)	32.2	31.4	31.5	30.0	24.3
Methanogenesis (%)	32.1	31.3	29.9	28.3	22.0
Qm^b (l CH ₄ /l/day)	0.486	0.774	1.99	2.54	0.706
Methane yield (l CH ₄ /g COD _{degraded})	0.482	0.346	0.465	0.420	0.107
VFA (g/l)	0.002	0.001	0.164	0.179	0.290
Soluble COD (g/l)	1.26	1.56	2.16	2.87	5.05
COD removal (%)	91.6	89.6	85.6	80.9	66.3

^a Values are averages during the steady-stage condition.

^b QM : methane production rate

creased to about 1.20 g/l (Figure 8). However, COD removal was found to be almost 60.0% after day 40 (Figure 9). Moreover, CO₂ was found to be the dominant gas at the initial period of start-up operation. Afterward methane yield became almost 50.0% of total biogas and achieved 2.65 l CH₄/day at 53 days (Figure 10).

During the experimental operation period, the initial OLR was set at 1.10 g COD/l/day and HRT of 13.5 days. The OLR was then increased in steps to 2.50, 5.00, 7.50 and 10.0 g COD/l/day by reducing the HRT to 13.5, 6.00, 3.00, 2.00 and 1.50 days, correspondingly. The results show that

the production of methane yield and total biogas production increased with increasing OLR. The maximum methane yield was 13.01 CH₄/day at OLR of 7.50 g COD/l/day. Then, the production of CH₄ significantly decreased (Figure 11) and wash-out was observed. Also, the maximum methane production rate of 2.54 l CH₄/l/day was found at OLR of 7.50 g COD/l/day (Table 3). However, %methanogenesis and %acidogenesis slightly decreased as OLR increased from 1.10 to 7.50 g COD/l/day and rapidly decreased when OLR increased from 7.50 to 10.0 g COD/l/day. This result showed that the conversion of organic matter

in wastewater to VFA and the conversion of VFA to CH_4 decreased with increasing OLR. Furthermore, the COD removal efficiency was more than 80.0% at OLR from 1.10 to 7.50 g COD/l/day and was decreased to about 60.0% at OLR from 7.50 to 10.0 g COD/l/day.

Conclusions

This study reveals that the OLR influenced methane and VFA production from anaerobic treatment in UASB and UFAF reactors. The VFA production in UASB and methane production in UFAF increased with the increasing OLR. However, the washout was found at OLR of 17.5 and 10.0 g COD/l/day in UASB and UFAF reactors, respectively. The maximum VFA accumulation of 5.50 g/l was achieved at OLR of 17.5 g COD/l/day and HRT of 2.86 days in UASB reactor. The maximum methane production was 7.00 l/day at OLR of 15.5 g COD/l/day in UASB reactor. Nevertheless, the maximum methane production was 12.5 l/day at OLR of 7.5 g COD/l/day in UFAF reactor. At the steady state, high soluble COD removal of 95.0% was achieved at OLR of 5.00 g COD/l/day and HRT of 20.0 day in UASB. In addition, COD removal was about 92.0% at OLR of 1.1 g COD/l/day and HRT of 13.5 days in UFAF. However, the COD removal from both reactors was greater than 60.0% during the experimental period.

Therefore, the optimum OLR for anaerobic treatment of POME in UASB and UFAF reactors was 15.5 and 7.50 g COD/l/day, respectively. Moreover, high VFA and methane production was achieved in UASB and UFAF reactors, respectively. Consequently, there is potential to use UASB and UFAF as acidogenic and methanogenic phase in two-phase anaerobic systems.

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