

CHAPTER 4

RESULTS AND DISCUSSION

Raw POME containing various concentrations of SS and O&G as shown in Table 3.3 were used to study the effect of SS and O&G on process performance and microbial characteristics of anaerobic hybrid reactor (AHR). Wastewater was upflow fed into AHR with 5 days of HRT. The influent feeding of POME was upflow through suspended microorganism in sludge zone and then up through attached biofilm and surrounding suspended microorganism in packed zone. At the steady state, performance efficiency, process stability and microbial characteristics in sludge and packed zones were evaluated. According to reactor operations found that the operation conditions can be dividing to four conditions (Figure 4.1) depending on SS and O&G concentrations and its performance, the study was divided into 4 phases as follows:

- Low-strength POME operation was operated at 5-7 g l⁻¹ of SS concentration
- High-strength POME operation was operated at 10-11 g l⁻¹ of SS concentration
- Shock load operation was operated at 12.5 g l⁻¹ of SS concentration
- Recovery and operational back to 10 g l⁻¹ of SS concentration

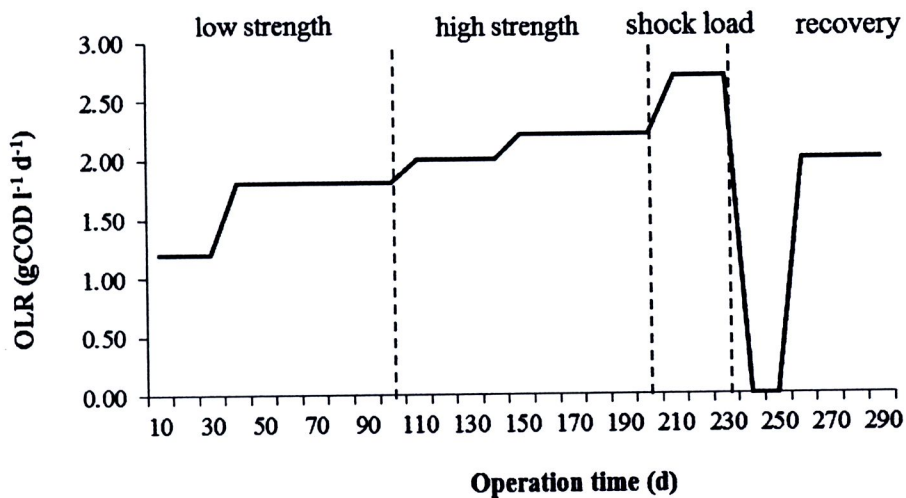


Figure 4.1 Organic loading rates of four phase operations

4.1 Low-strength POME operation

4.1.1 Process performance and stability at low-strength POME operation

Low-strength POME treatment system was operated with TCOD, SS and O&G in the range of 15-20, 5-7 and 0.9-1.9 g l⁻¹, respectively. At 5-7 g l⁻¹ of SS concentration with organic loading rate (OLR) 3.0-3.8 g COD l⁻¹ d⁻¹, once the system reached the steady state, it was continuously operated for more 4 of HRTs to ensure process stable. During the steady state, process performance and stability as well as microbial characteristics in sludge and packed zones of AHR were evaluated.

4.1.1.1 Sludge zone

At 5 g l⁻¹ of SS concentration with organic loading rate (OLR) 3.0 g COD l⁻¹ d⁻¹, the system reached to steady state within 4 cycles of HRT (20 d). The process was continuously operated for more 12 d to ensure process under steady state. During this period process performance and stability were evaluated (Table 4.1). The performance efficiency of sludge zone in AHR was shown in removal of TCOD, SCOD, SS and O&G at 82, 88, 70 and 81%, respectively, which was achieved under normal condition. POME containing concentration of SS 7 g l⁻¹ and O&G 1.4 g l⁻¹ corresponding to TCOD 20 g l⁻¹ was then upflow fed into AHR with OLR 1.9 g COD l⁻¹ d⁻¹ and 5 d of HRT. Performances in TCOD, SCOD, SS and O&G removal increased along with operation time and these values at 50, 92, 75 and 50% were achieved at steady state, respectively.

According to organic removal efficiency at low-strength POME, SCOD containing soluble organic compounds was easily biodegradable and most completely degraded (88-92%). TCOD which composed of suspended organic matters and O&G was more hardly biodegradable and its removal efficiency decreased to 50% while SS and O&G concentrations increased to 7 and 1.4 g l⁻¹, respectively. These some remaining undigested SS matters settled and accumulated in the bottom part of reactor. Even though increasing of SS removal efficiency from 70 to 75% was observed, but there were some part of SS that had accumulated in this zone. These suspended components might have been cell walls, organelles, short fibers, hemicelluloses, nitrogenous compound of proteins which requiring the longer retention time for satisfactory digestion (Ugoji, 1997). The phenomenon of O&G removal efficiency was difference from SS. O&G was the one of the hardly biodegradable organic matters in POME, however at low initial O&G concentration (0.9 g l⁻¹) feeding it could be removed to 81%.

Table 4.1 Process performance and stability at low-strength POME operation

Zone	Effluent concentration (mg l ⁻¹)										Biogas (ml d ⁻¹)	CH ₄ (ml d ⁻¹)	CH ₄ yield*
	TVA (mg l ⁻¹)	Alk (mg l ⁻¹)	TVA/Alk	pH	O&G	SS	TCOD	SCOD					
5 g SS l⁻¹													
Sludge	700 ± 0.2	2593 ± 0.6	0.27 ± 0.07	7.2 ± 0.5	171 ± 37	1500 ± 136	2700 ± 456	1080 ± 266					
Packed	320 ± 0.1	1702 ± 0.5	0.19 ± 0.06	7.4 ± 0.4	266 ± 18	1200 ± 67	2580 ± 331	810 ± 306					
Overall	570 ± 0.1	2818 ± 0.4	0.2 ± 0.06	7.3 ± 0.4	300 ± 36	1560 ± 122	3120 ± 520	820 ± 234			2281 ± 124	1362 ± 330	0.13 ± 0.04
7 g SS l⁻¹													
Sludge	1380 ± 0.1	2141 ± 0.2	0.64 ± 0.12	6.6 ± 0.4	634 ± 15	11800 ± 612	27500 ± 1026	1324 ± 323					
Packed	953 ± 0.1	1842 ± 0.4	0.52 ± 0.17	6.9 ± 0.2	285 ± 20	2450 ± 157	2406 ± 568	1004 ± 299					
Overall	1200 ± 0.1	2800 ± 0.3	0.43 ± 0.17	7.1 ± 0.3	700 ± 27	2800 ± 207	4600 ± 955	2100 ± 367			3200 ± 323	2200 ± 276	0.20 ± 0.12

Remark: *Methane yield unit: l CH₄ g⁻¹ COD_{removed}. Values are the mean of *n* values at steady state ± standard deviation, whereby *n* in the range of 20-30.

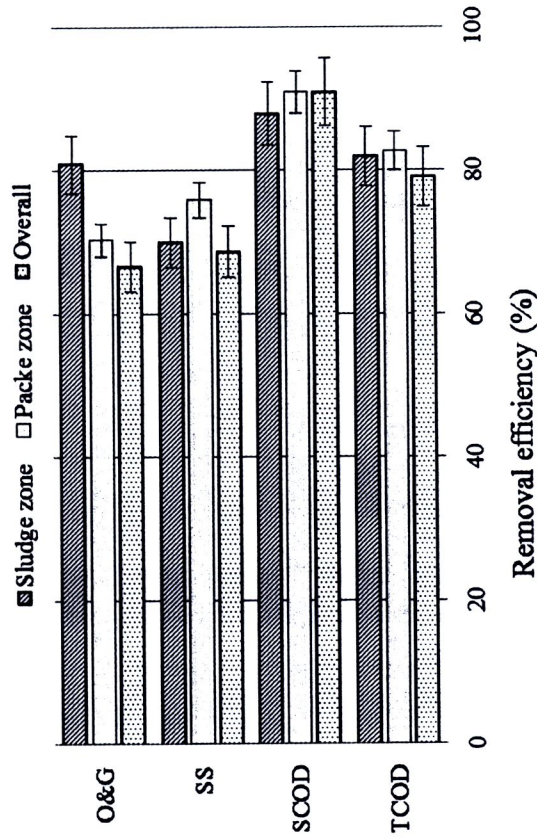


Figure 4.2 Performances AHR at low-strength POME

Increasing the OLR lead to O&G in influent POME increased. Performance of O&G removal decreased from 81 to 50% in POME with O&G at 1.4 g l^{-1} treatment. O&G of POME can be found in two phases that either suspend in the supernatant or float on the upper layer of the suspension. In this sludge zone, some of O&G might partially biodegraded from higher to lower molecular weight resulting in the lower in O&G biodegradation. Generally, O&G was hydrolyzed by bacterial enzymes under anaerobic condition and sensitive to pH and inhibitory substances. An optimum pH value for bacterial enzymes activities closes to neutral (Lee, 2006; Tchobanoglous et al., 2003). Therefore, O&G in this sludge zone might biodegrade to lower molecular weight under system condition of pH 7.2 and TVA/Alk ratio 0.27. Under operation at 0.9 g l^{-1} of O&G concentration, the environmental condition along sludge zone supported the removal efficiency. Additionally, during the operation pH value in this sludge zone was slightly acidic in the range of 6.2 - 6.6 and it might effected to O&G biodegradable. The profile of TVA in this zone was found in high concentration in the average of 1380 mg l^{-1} and lead ratio of TVA to Alk to 0.64.

The effect of pollution strength on the acidic-basic balance and process stability was represented by pH and TVA/Alk ratio. Generally, TVA/Alk ratio can be used as a measure of process stability (Fannin, 1997). The optimum of this value for anaerobic system is less than 0.3-0.4 which means that the process is considered to be satisfied operation without acidification risk (Grau et al., 1994). The process stability of this sludge zone closed neutral condition. The pH and TVA/Alk ratio values at 5 g l^{-1} of SS operation were 7.2 and 0.27, respectively including reactor performance was found in removal of organic matters in the system as shown in TCOD, SCOD, SS and O&G removal efficiency. The pH in sludge zone at 7 g SS l^{-1} was slightly acidic in the range of 6.2 - 6.6 with concentration of TVA at 1380 mg l^{-1} as reported in Table 4.1. Although pH slightly decreased from neutral but it did not reflect overall process stability because the buffer capacity of alkalinity was sufficient ($> 2000 \text{ mg CaCO}_3 \text{ l}^{-1}$).

4.1.1.2 Packed zone

In the packed zone at low-strength POME operation, TCOD, SCOD, SS and O&G removal efficiency were in the range of 83 ± 6 , 9 ± 2 , 65 ± 9 and $70\pm 5\%$, respectively. Most of the organic matter continued to biodegrade in the packed zone and the concentrations of these organic matters in the packed zone were lower than that in the sludge zone, as shown in Table 4.1. The partial biodegradation of TCOD, SCOD and SS which low molecular weight from the sludge zone were flown through packed zone made anaerobic microorganism easily biodegraded. Hence, it reflected in high removal of TCOD,

SCOD and SS in this packed zone. In addition, the biodegradation of O&G was lower in packed zone than that in sludge zone. The initial O&G concentration was low as 0.9-1.4 g l⁻¹ and after it was fed through sludge zone, the most easily biodegradable form of O&G was used up and some hardly biodegradable was partially digested in this zone and flown through packed zone. The remaining of O&G with low concentration was continually removed in packed zone not more than 70% and observed the thin layer of floating O&G on the surface of water like scum. With this form of O&G especially scum formation in packed zone might be effected to microbial contact for biodegradation and led to decrease the removal of O&G. The undigested O&G could be occurred in this phase. This case can be explained that colloidal components of POME in the form of O&G had adverse impact on reactor performance and caused deterioration of microbial activities (Borja and Banks, 1994; Torkian et al., 2003).

The process stability in this zone was maintained in pH 6.9 and 7.4, TVA/Alk ratio at 0.19 and 0.27, at 5 and 7 g l⁻¹ of SS, respectively. Less TVA accumulation was observed comparing of sludge zone. These conditions were flavor for anaerobic microorganisms to activate the higher performance of organic removal efficiency. The design of AHR by having the attached microorganism on nylon fiber as biofilm in the packed zone has the advantage to protect microorganism in the inner of biofilm. Therefore, biofilm in packed zone could help and maintain the balance of system.

4.1.1.3 Overall performance

The overall performance efficiency at low-strength POME was indicated by the removal efficiency of TCOD, SCOD, SS and O&G as well as biogas and methane production. These organic matters removal values were in the range of 77±9, 91±9, 64±5 and 58±9%, respectively, which shown in Table 4.1 and Figure 4.2. Cellulose was the main composition of SS. It was completely removed by showing 85-98%. Under this operation, normal conditions of process stability was observed with low VFA accumulation in the system as reported in Table 4.2. Biogas and methane production rate at 5 g l⁻¹ of SS concentration were achieved at 2.3 and 1.35 l d⁻¹, respectively. Methane yield was gained at 0.13 l CH₄ g⁻¹ COD_{removed} under this operation condition. Biogas and methane production increased to 3.2 and 2.2 l d⁻¹, respectively, and methane yield was obtained at 0.20 l CH₄ g⁻¹ COD_{removed} when the reactor operated at 7 g l⁻¹ of SS. The low methane yield obtained in this period probably was due to most of COD was consumed by microorganism to build up more cell than that to convert in biogas at the initial of startup period. This phenomenon was also found in the study of Chaiprasert et al. (2003).

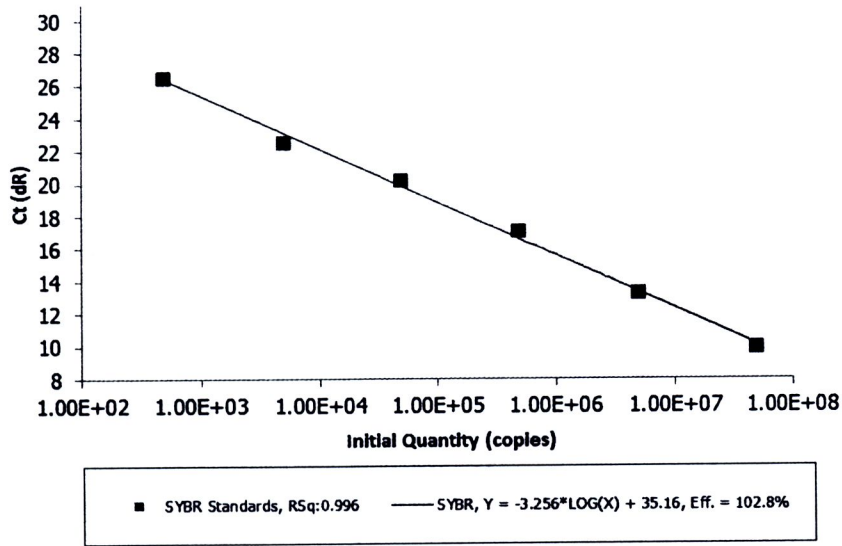
Table 4.2 VFA analysis of low-strength POME operation

VFA (C ₂ -C ₄) (g l ⁻¹)	SS concentration	
	5 g l ⁻¹	7 g l ⁻¹
Acetic acid	0.04	0.07
Propionic acid	0.13	0.16
Butyric acid	0.17	1.04

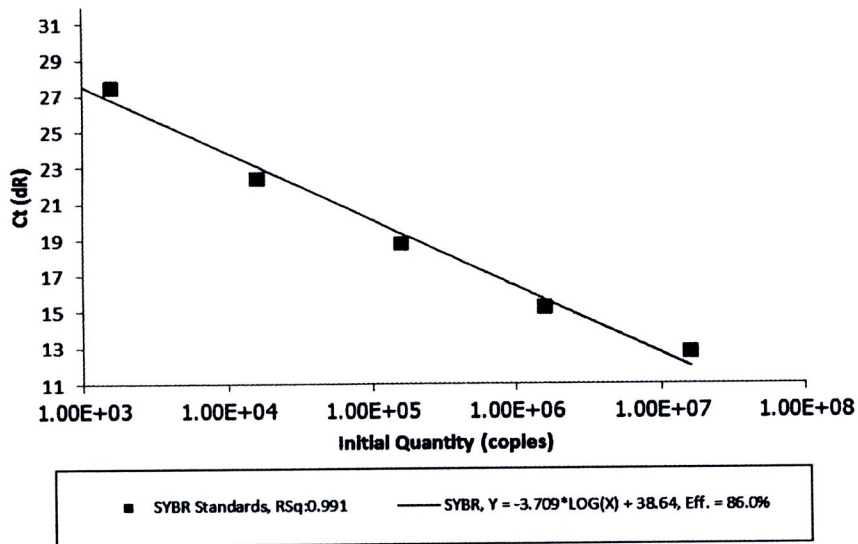
4.1.2 Microbial characteristics at low-strength POME operation

Microbial quantity was determined in terms of copy numbers of 16S rDNA of eubacteria and archaea by relative quantification real-time PCR (qPCR). Standard curves for eubacteria and archaea were generated using data derived from the serial dilution (10^2 - 10^7 copies rDNA) of plasmid DNA from standard eubacterial and archaeal strains. Figures 4.3a and 4.3b illustrate standard curves of eubacteria (EUB) and archaea (ARC), respectively. These standard curves had a high correlation coefficient in $R^2 = 0.996$ and 0.991 with slope = -3.286 and -3.709 for eubacteria and archaea, respectively. Based on the two sample replications the fluorescence detection system automatically calculated the population of eubacteria and archaea by comparing the C_t values, which cycles when the fluorescence from a sample crosses the threshold, from the unknown samples with the values on the standard curve.

The population of eubacteria and archaea was representative to non-methanogens and methanogens, respectively. In addition, microbial quality was monitored in term of microbial activity of non-methanogens and methanogens using glucose and acetic acid as specific substrate, respectively. The eubacterial and archaeal community was done by PCR-DGGE and DNA sequencing techniques.



(a) Standard curve for eubacterial population



(b) Standard curve for archaeal population

Figure 4.3 Standard curves of (a) eubacterial and (b) archaeal populations

4.1.2.1 Inoculum seed

The number of eubacteria (EUB) and archaea (ARC) in startup seed was 1.2×10^7 and 8.2×10^4 copies rDNA g^{-1} VSS, respectively (Table 4.3). The activity of eubacteria was found at $1.00 \text{ gCOD } g^{-1}$ VSS.d. Small number of archaea resulted in low methanogenic activity ($0.12 \text{ gCOD-CH}_4 \text{ g}^{-1}$ VSS.d) as shown in Table 4.4. Microbial communities of eubacteria and archaea are shown in Table 4.5 and 4.6. For the eubacterial community, 14 dominant bands were detected. Seven of them (S1, S3, S6, S7, S8, S9 and S10) were γ -Proteobacteria which acted as hydrolytic bacteria in anaerobic treatment system. γ -Proteobacteria (i.e., *Pseudomonas*) are common representatives of the microbial communities in anaerobic processes of solid substrates (Grotenhuis et al., 1991; McMahon et al., 2001; Rincon et al., 2006). *Pseudomonas* sp. is microorganism which has the ability for producing extracellular lipase enzymes that hydrolyze triglycerides to fatty acids and glycerol (Papasraskavas et al., 1992). Another 3 bands (S11, S12 and S13) were identified as members of acidogenic bacteria. All of them were *Bacteroidetes bacterium*, as shown in Table 4.5. Acetogenic bacteria were detected and represented by S2, S4 and S14 while S5 represented of other bacteria. These acetogenic bacteria were members of the *Firmicutes*, mostly represented by *Clostridiales bacterium* and *Acetobacter* sp. These results were in agreement with those observed by Rincon et al. (2006) at low organic loading rate. This genus showing an acetogenic metabolism, i.e, acetic acid is a final product of their metabolism for methanogens (Rincon et al., 2008). The dominants archaea communities were represented 5 bands (S1-S5). Two of them (S3 and S5) were members of acetoclastic methanogens. Other 3 bands (S1, S2 and S4) were hydrogenotrophic methanogens.

Table 4.3 Microbial quantity at low-strength POME operation

SS Concentration	EUB (Copies rDNA g^{-1} VSS)		ARC (Copies rDNA g^{-1} VSS)	
	Sludge zone	Packed zone	Sludge zone	Packed zone
Inoculum Seed	1.2×10^7		8.2×10^4	
5 g l^{-1}	4.4×10^8	8.9×10^7	1.4×10^5	2.8×10^6
7 g l^{-1}	3.2×10^7	2.7×10^8	1.3×10^4	8.0×10^4

Table 4.4 Microbial activity at low-strength POME operation

SS concentration	Non-methanogenic activity (g COD g ⁻¹ VSS d ⁻¹)		Methanogenic activity (g COD-CH ₄ g ⁻¹ VSS d ⁻¹)	
	Sludge zone	Packed zone	Sludge zone	Packed zone
Inoculum seed	1.00		0.12	
5 g l ⁻¹	1.08	0.11	0.11	0.18
7 g l ⁻¹	1.58	0.11	0.11	0.25

4.1.2.2 Low-strength POME operation

At steady state of low-strength operation, qualitative change in the 16S rDNA concentration of eubacteria in the sludge zone was slightly higher than that in packed zone for one order-level of microbial population. The eubacterial 16S rDNA concentration was 4.4×10^8 and 8.9×10^7 copies rDNA g⁻¹ VSS in sludge and packed zones, respectively which was observed in 5 g l⁻¹ of SS concentration. The archaeal gene concentration in packed zone was higher than sludge zone one-order level and in the concentration of 2.8×10^6 and 1.4×10^5 copies rDNA g⁻¹VSS, respectively. The growth of microbial populations was found in this operation related to the inoculum seed. The microbial activity of eubacteria and archaea in sludge zone closed to inoculum seed. The order-level of eubacterial and archaeal populations in sludge and packed zones was correlated and respected to their activities. The activity of eubacteria and archaea in sludge and packed zones was 1.08 and 0.43 g COD g⁻¹VSS d⁻¹ and 0.11 and 0.18 g COD-CH₄ g⁻¹VSS d⁻¹, respectively. At 7 g l⁻¹ of SS concentration operation found that microbial population has no change in eubacteria group while archaea decreased one order level in both sludge and packed zones. Eubacterial and archaeal populations (Table 4.3) in sludge and packed zones were 3.2×10^7 and 2.7×10^8 copies rDNA g⁻¹ VSS and 1.3×10^4 and 8.0×10^4 copies rDNA g⁻¹ VSS, respectively. However, increasing of microbial activity could detect under this operation. The activity of eubacteria and archaea in sludge and packed zones (Table 4.4) was 1.58 and 0.74 gCOD g⁻¹VSS d⁻¹ and 0.11 and 0.28 gCOD-CH₄ g⁻¹ VSS.d, respectively.

It seems that more acidogenesis occurred in the sludge zone where more methanogenesis was found in the packed zone. The results showed the high performance of organic removal efficiency and process stability. Overall system could maintain pH to neutral, less acid accumulation and the ratio of TVA/Alk < 0.4 It suggested that the rate of acid production by non-methanogens and the rate of acid consumption by methanogens in both zones were in harmony. Less acid accumulation was found in low concentration of acetic, propionic and butyric acids (Table 4.2). The results showed the order-level and activity of non-methanogens and methanogens with respect to the performance data.

DGGE and subsequent phylogenetic analysis were conducted to characterize the microbial community structures in the sludge and packed zones of AHR. Changes in microbial community structures were visualized the eubacterial and archaeal DGGE band patterns (Figure. 4.4). For 5 g l⁻¹ of SS concentration, total of 11 and 12 bands were sequenced and analyzed from eubacterial DGGEs in sludge and packed zones. The archaeal DGGEs were done for 8 and 7 bands in sludge and packed zones, respectively. The eubacterial and archaeal community illustrated in Table 4.5 and 4.6, respectively. Slightly change in profile of DGGE bands of eubacteria in sludge zone, packed zone and inoculum seed was found when SS concentration increased to 7 g l⁻¹ (Figure 4.4 lane B, A and S). Dominant eubacterial and archaeal bands founding in sludge and packed zone were 12 and 12 bands, and 11 and 9 bands, respectively.

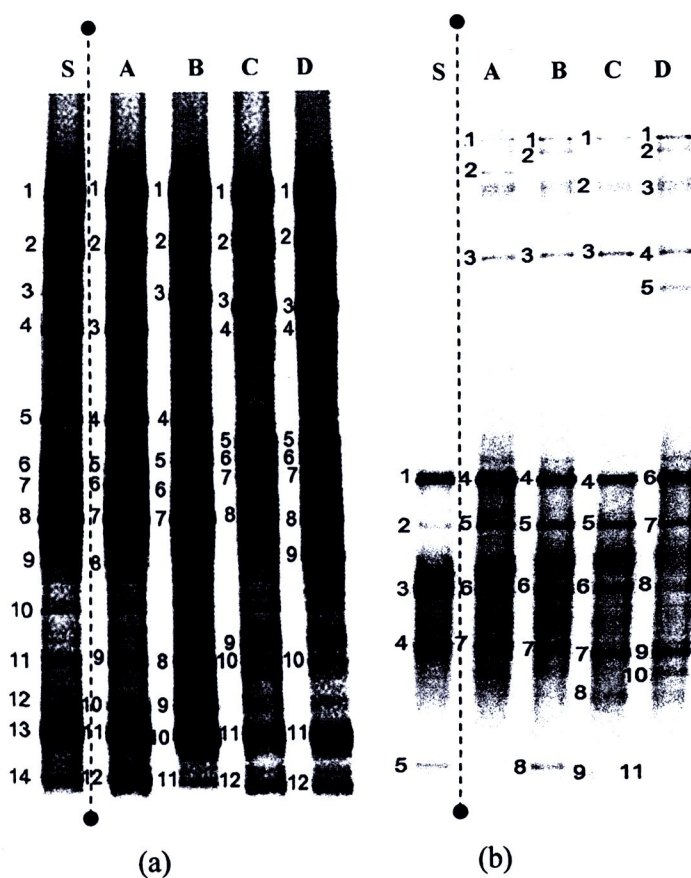


Figure 4.4 DGGE profiles of (a) EUB and (b) ARC of low-strength POME

Remark: Lane S: Inoculum seed, Lane A: Packed zone and Lane B: Sludge zone at 5 g SS l⁻¹;
Lane C: Packed zone and Lane D: Sludge zone at 7 g SS l⁻¹

Four DGGE bands of eubacteria from sludge zone (B1, B3, B5 and B7) and 5 bands that from packed zone (A1, A4, A5, A7 and A8) were belonged to hydrolytic bacteria (table 4.5). It can be seen that almost of the hydrolytic bacteria in these zones were represented by genus *Pseudomonas*, the same as in the inoculum seed (S1, S3, S5-6, S8-10). Moreover, it was still observed in the sludge zone of SS concentration at 7 g l^{-1} with total of 4 bands (D1, D3, D8-9) whereas 2 bands of inoculum (S5-6) with A4&B4 and A5&B5 faded out. Three couple bands (C1&D1, C3&D3 and C8&D8) were similar to the inoculum seed included D9, while C5&D5 and C9 were first observed. These first detecting bands were represented by *Firmicutes bacterium* and γ -*Proteobacterium*, respectively. *Bacteroidetes bacterium* appeared to be one of the most represented acidogenic bacteria in the sludge zone (B6, B8-10, D10-11) and the packed zone (A6, A9-11, C10-11). Six DGGE bands from sludge and packed zones were detected. The sequence from bands B6 & A6, B8, A9, D10&C10, B9 & A10 and B10, A11, D11&C11 clustered with inoculum seed S7, S11, S12 and S13, respectively. Four bands (C6-7, D6-7) were detected when SS increasing to 7 g l^{-1} and it was determined to be *Bacteroidetes bacterium*.

Table 4.5 The partial 16S rRNA gene sequences of EUB domain and organism with the best matching sequences determined by BLAST searches at low-strength POME operation

Affiliation	DGGE band	Similarity (%)	Accession no.	Bacteria
<i>Pseudomonas</i> sp. M130	S1 A1 B1 C1 D1	92	AB088750.2	Hydrolytic bacteria
<i>Pseudomonas entomophila</i> str. L48	S3 B3 C3 D3	92	CT573326.1	
<i>Pseudomonas</i> sp.	S5 A4 B4	97	AM886092.1	
<i>Pseudomonadaceae bacterium</i>	S6 A5 B5	94	AB545745.1	
<i>Pseudomonas</i> sp.	S8 A7 B7 C8 D8	97	AM886092.1	
<i>Pseudomonas pseudoalcaligenes</i>	S9 A8 D9	79	AF140011.1	
Uncultured γ - <i>Proteobacterium</i>	S10	82	EU167353.1	
Uncultured <i>Firmicutes bacterium</i>	C5 D5	94	FM896934.1	
Uncultured γ - <i>Proteobacterium</i>	C9	82	EU167353.1	
Uncultured <i>Bacteroidetes bacterium</i>	S7 A6 B6	94	CU926845.1	
Uncultured <i>Bacteroidetes bacterium</i>	S11 A9 B8 C10 D10	98	EU810898.1	
Uncultured <i>Bacteroidetes bacterium</i>	S12 A10 B9	76	GU955023.1	
Uncultured <i>Bacteroidetes bacterium</i>	S13 A11 B10 C11 D11	91	GU955023.1	
Uncultured <i>Bacteroidetes bacterium</i>	C6 D6	86	AB433139.1	
Uncultured <i>Bacteroidetes bacterium</i>	C7, D7	94	CU926845.1	
<i>Clostridiales bacterium</i>	S2 A2 B2 C2 D2	81	GU428556.1	Acetogenic bacteria
Uncultured <i>actinobacterium</i>	S4 A3 C4 D4	91	GU194237.1	
<i>Acetobacter</i> sp.	S14 A12 B11 C12 D12	98	GQ246703.1	



The last step for acid production in anaerobic digestion (acetogenesis), the eubacterial communities of acetogenic bacteria was sequenced from bands B2, A2, D2&C2, B3, D4&C4 and B11, A12, D12&C12 to type strain of *Clostridiales bacterium*, *Actinobacterium* sp. and *Acetobacter* sp., respectively. These three strains were also found in inoculum seed. Genus *Clostridium* was the predominant fermentation bacteria at low OLR of completely stirred tank anaerobic reactor treating two-phase olive mill solid residue (Rincon et al., 2008). In this study *Clostridiales bacterium* was classified in acetogenic bacteria regarding the study of Winter and Wolfe (1979).

The archaeal communities in sludge and packed zones was more diversified than inoculum seed (Table 4.6). The acetoclastic methanogens, 4 DGGE groups of bands (A6-B6-C6 and D8, B8, B2 and D2, and C9&D11) were sequenced to type strains of *Methanosaeta* sp., *Methanococcoides*, *Methanocaldococcus vulcanius* M7, and *Methanococcoides*, respectively. Five of them (A6-B6-C6&D8 and B8) as *Methanosaeta*, *Methanococcoides* were similar detecting bands as S3 and S5 in inoculum seed, respectively. Low acetate concentration (0.04 g l^{-1}) was found in the system at low - strength POME operation which contributed to suitable environment for these strains of acetoclastic methanogen growth. However, in this study *Methanosarcina* are not observed within AHR reactor at all. Both sludge and packed zones contained *Methanosaeta* sp. and *Methanococcoides* similar acetoclastic methanogen activity which was also confirmed by SMA test.

Hydrogenotrophic methanogens more diversified than acetoclastic methanogens and detected in the sludge zone more than in the packed zone. In sludge zone (Lane A and C), ten bands were detected and almost sequenced to strain of *Methanobacterium*, *Methanomicrobiaceae* and *Methanomicrobiales* and *Methanospirillum hungatei*. Hydrogenotrophic methanogens diversity in packed zone was quite same as sludge zone. There were only two bands (D5 and D10) when were first detected and identified to *Methanomicrobiaceae* and *Methanocelleus*, respectively. All of them were H_2/CO_2 -utilizer to CH_4 production under optimum range of pH at 6.6 - 7.8 (Garrity et al., 2004), which was in the same range of this study (7.2-7.4).

Table 4.6 The partial 16S rRNA gene sequences of ARC domain and organism with the best matching sequences determined by BLAST searches at low-strength POME operation

Affiliation	DGGE band	Similarity (%)	Accession no.	Bacteria
Uncultured <i>Methanosaeta</i>	S3 A6 B6 C6 D8	85	AY454766.1	
Uncultured <i>Methanococcoides</i>	S5 B8	89	AY454739.1	Acetoclastic methanogens
<i>Methanocaldococcus vulcanius</i> M7	B2 D2	90	CP001787.1	
Uncultured <i>Methanococcoides</i>	C9 D11	89	AY454739.1	
<i>Methanobacterium</i>	S1 A4 B4 C4 D6	93	GU936489.1	
<i>Methanobacterium</i>	S2 A5 B5 C5 D7	95	GU569395.1	
<i>Methanomicrobiaceae</i>	S4 A7 B7 C7 D9	87	GU129124.1	
Uncultured <i>Methanomicrobiales</i>	A1 B1 C1 D1	95	AY780566.1	Hydrogenotrophic methanogens
<i>Methanospirillum hungatei</i>	A2	99	AB517987.1	
<i>Methanobacterium</i>	A3 B3 C3 D4	97	GU936489.1	
<i>Methanobacteriaceae</i>	D5	89	GU129060.1	
<i>Methanoculleus</i>	D10	96	AB436897.1	
	C2, C8, D3			Unidentified

Remark: Lane S: Inoculum seed; lane A: Packed zone at 5 g SS l⁻¹; lane B: Sludge zone at 5 g SS l⁻¹; lane C: Packed zone at 7 g SS l⁻¹; lane D: Sludge zone at 7 g SS l⁻¹

4.2 High-strength POME operation

4.2.1 Process performance and stability at high-strength POME operation

High-strength POME operation was performed at OLR 4.0 and 4.8 g COD l⁻¹ d⁻¹ with containing SS at 10 and 11 g SS l⁻¹ and O&G at 1.9, 2.3 g l⁻¹, respectively. The process performance and stability in sludge and packed zones were investigated.

4.2.1.1 Sludge zone

The performance efficiency of AHR at 10 g SS l⁻¹ in removal efficiencies of TCOD, SCOD, SS and O&G were 50±4, 77±6, 40±5 and 41±2%, respectively (Table 4.7 and Figure 4.5). These values increased to 81±3, 85±5, 68±5 and 48±3, respectively, under AHR operation at 11 g SS l⁻¹. There was a slightly difference of organic removal in the sludge zone between low and high-strength POME operations. TCOD and SCOD removal were in the same range (~50-80%) with low-strength POME operation while SS removal decreased to 40-68%. This may be attributed to the increasing in the hardly biodegradable substrate gradient, which requires sufficient period for native microorganism to build cell up and degraded these organic matters to sample structure. However, almost of soluble organics matters as SCOD were degraded and converted to organic acid. It enhanced TVA value increasing to 2100±241 mg l⁻¹.

O&G removal decreased to 41-48% (Figure 4.5a) under this operation. It might be O&G forming to the phase of oil droplet and acidic environmental condition. Normally, the oil droplets of POME can either be found in two phases by suspension in supernatant or floating on the water surface. At the initial high concentration of O&G (1.9-2.3 g l⁻¹), it could be found oil droplet suspension and hardly attached by bacteria. As an interfacial phenomenon, the mechanism of lipid hydrolysis is a function of the “concentration” and quality of the interface (Rollo'n, 1999). Lipid degradation efficiency also depended on the environmental condition of the system. Under unsuitable environment affected to lipase activity, especially acidic pH (5.4-6.5) and TVA/Alk ratio (0.66-0.91) were in the range of acidic condition.

At high-strength POME operation, high organic matters were hydrolyzed and biodegraded to organic acids by hydrolytic and acidogenic bacteria indicating by high TVA values (1450-2100 mg l⁻¹).

Table 4.7 Process performance and stability at high-strength POME operation

Zone	Effluent concentration (mg l ⁻¹)										CH ₄ (ml d ⁻¹)	CH ₄ yield* (ml d ⁻¹)
	TVA (mg l ⁻¹)	Alk (mg l ⁻¹)	TVA/Alk	pH	O&G	SS	TCOD	SCOD	Biogas (ml d ⁻¹)	CH ₄ (ml d ⁻¹)		
10 g SS l⁻¹												
Sludge	2100 ± 0.2	3200 ± 0.3	0.66 ± 0.18	6.5 ± 0.2	1100 ± 0.2	6800 ± 0.2	10500 ± 0.2	2800 ± 0.1				
Packed	1600 ± 0.4	3100 ± 0.3	0.52 ± 0.14	7.0 ± 0.3	1470 ± 0.1	1630 ± 0.1	2300 ± 0.3	540 ± 0.1				
Overall	1980 ± 0.4	3060 ± 0.4	0.64 ± 0.15	6.2 ± 0.3	2300 ± 0.1	4300 ± 0.2	5300 ± 0.2	2070 ± 0.2	6300 ± 0.1	4200 ± 0.1	0.30 ± 0.08	
11 g SS l⁻¹												
Sludge	1450 ± 0.1	2000 ± 0.1	0.73 ± 0.07	5.4 ± 0.3	1200 ± 0.1	2780 ± 0.3	4550 ± 0.3	1900 ± 0.2				
Packed	1250 ± 0.1	2300 ± 0.1	0.54 ± 0.12	6.7 ± 0.3	340 ± 0.1	970 ± 0.3	1720 ± 0.3	240 ± 0.1				
Overall	1300 ± 0.2	2520 ± 0.3	0.52 ± 0.10	6.9 ± 0.3	1030 ± 0.1	2400 ± 0.3	3120 ± 0.2	1200 ± 0.2	4144 ± 0.3	3022 ± 0.2	0.15 ± 0.11	

Remark: *Methane yield unit: l CH₄ g⁻¹ COD_{removed}. Values are the mean of *n* values at steady state ± standard deviation (%); whereby *n* in the range of 20-30.

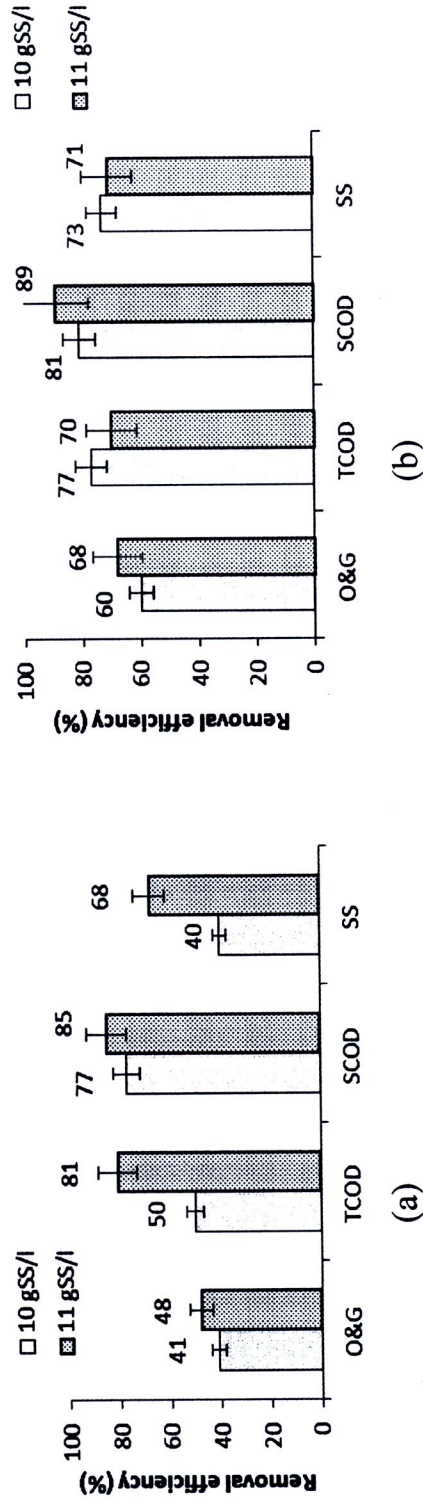


Figure 4.5 Process performances of (a) sludge and (b) packed zones at high-strength POME

Acidic condition might promote organic acid accumulation and meanwhile lipase deterioration was influenced, finally led to O&G removal reduction. Lipid-rich waste was indicated to problem of anaerobic digestion due to clogging, and may also cause mass transfer problems for soluble substrates since they become adsorbed to the microbial biomass surface (Pereira et al., 2004). The flotation of biomass due to adhesion of fat may also cause loss of active biomass because of washout (Cammarota et al., 2001). All these problems limit the operating efficiency of digesters, and a physico-chemical pre-treatment is usually applied in order to remove the lipid fraction before the anaerobic process. These results were similar to slaughterhouse wastewater treatment which the liquefaction of lipids was found to be rate-limiting when high amounts of suspended solids were present due to their low bioavailability (Sayed, 1988).

4.2.1.2 Packed zone

Increasing of SS and O&G concentration in the packed zone changed organic removal efficiency. At 10 g SS l⁻¹ operation, TCOD, SCOD, SS and O&G were 77±9, 81±4, 73±7 and 60±4%, respectively. These values changed to 70±6, 89±8, 71±10 and 68±9.0%, respectively, while SS load was increased to 11 g l⁻¹. These values were higher than that in sludge zone, especially O&G removal efficiency. It was due to the partially digested O&G from sludge zone was up flown to packed zone with high concentration and the system environmental condition was supported and favored to microbial degradation than that in sludge zone. Process stability of packed zone was maintained in normal condition by detecting pH 6.7-7.0 and TVA/Alk ratio 0.52-0.54 as shown in Figure 4.5b and Table 4.7. This condition promoted O&G biodegradation efficiency increasing. Most of it was digested in this packed zone not more than 70%. Similar results were found in packed zone at low-strength POME operation. The complete removal of O&G could not be occurred due to the phase of O&G in suspension. However, the observation of O&G accumulation was found at the top layer of suspension.

Although, anaerobic digestion in high concentration of SS and O&G influenced acidity condition in the sludge zone, but it was unaffected to environment of this packed zone. It meant that the rate of acid consumption was high by methanogens within deeper biofilm layer and less acid accumulation in this packed zone. In packed zone, biofilm has been formulated by growth of bacteria in supporting media. An acceleration of attached microbial growth is not only depended on nutrients but also attachment is a prerequisite step to the biofilm formation, the acceleration of biomass movement from sludge to packed zones would provide more cells to attach (Suraraksa, 1998). Substrate removal within biofilm composed of two processes: transportation of substrates by molecular diffusion and simultaneous substrates consumption by microorganisms. The magnitude of acid concentration gradient inside the deeper biofilm layer could not affect the methanogenic biofilm activity and biofilm in packed

zone could maintain the process stability. Therefore, under anaerobic digestion, volatile fatty acids were broken down into simpler molecule and utilized, especially, propionic acid and butyric acid were utilized by acetogenic bacteria to produce CO₂, hydrogen and mainly acetic acid. Then it was utilized and it produced methane gas by methanogens (Hosseini and Bitar, 2007), therefore TVA decrease appeared in this zone.

4.2.1.3 Overall performance

Overall results of high-strength POME operation indicated that high performance efficiency was achieved in this operation. Overall performance efficiency of TCOD, SCOD and SS removal under 10 g SS l⁻¹ operation were 73±6, 80±9 and 63±10%, respectively. These values changed to 87±3, 89±8 and 77±9%, respectively. Biogas and methane production rate in high-strength POME condition at 10 g l⁻¹ of SS were 6300 and 4200 ml d⁻¹, respectively. It was found that the rates of substrate uptake and methane production were correlated with the concentration of biodegradable TCOD.

Methane yield obtained at 0.3 l CH₄ g⁻¹COD_{removed} which the highest value of this study. This result closed to the methane production rate of POME treatment by AHR operating with OLR 1.75-7.50 g COD l⁻¹ d⁻¹ and the fresh feed was mix with recycle effluent (Najafpour et al., 2006). Therefore, this operation seems higher performance than that previous work because reactor operation of this study could produce high methane yield without recycle effluent. When SS and O&G concentrations increased to 11 and 2.3 g l⁻¹, respectively, although overall organic removal efficiencies still high but the trending of biogas and methane gas go down. It was signal of reactor performance in term of biogas production reduction. Duration of reactor operation at low-strength (5-7 g SS l⁻¹) and high-strength (10-11 g SS l⁻¹) POME, biogas and methane production rate increased along with increasing of SS and O&G concentrations until reactor operating under 11 g SS l⁻¹ that biogas and methane gas production decreased (Figure 4.6). It can be noted that operation condition (concentrations of SS and O&G) was the main factor to control the step of methane production. The concentrations of SS at 11 g l⁻¹ with 2.3 g l⁻¹ of O&G were noted to be the limiting concentrations of POME treatment with OLR 4.8 g COD l⁻¹ d⁻¹.

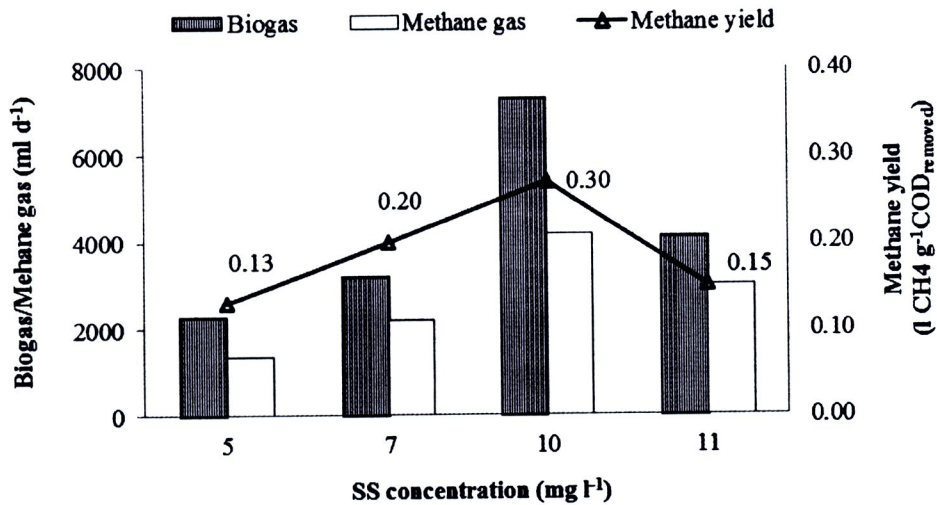


Figure 4.6 Biogas and methane production efficiencies at various SS concentrations

4.2.2 Microbial characteristics at high-strength POME operation

The numbers of microorganism in sludge and packed zones increased corresponding with SS and O&G concentrations. At high-strength POME, eubacterial and archaeal populations in both sludge and packed zones were in range of 10^8 - 10^9 and 10^5 - 10^6 copies rDNA g⁻¹VSS, respectively. At sludge zone of high-strength POME concentration (10-11 g SS l⁻¹), eubacteria and archaea were observed at 5.5×10^8 - 1.2×10^9 copies rDNA g⁻¹VSS and 3.2×10^5 - 1.5×10^6 copies rDNA g⁻¹VSS, respectively. Microbial quantity in the packed zone was close to that in the sludge zone, as shown in Table 4.8. In addition, the operation at 11 g SS l⁻¹ of SS, microbial population was higher than that packed zone of 10 g l⁻¹. At high-strength POME, microbial population and activity were parallel increased. Eubacterial activity of sludge and packed zones increased to 1.32-1.65 and 0.91-1.11 g COD g⁻¹VSS d⁻¹, respectively. Increasing of archaeal activity was detected in both sludge and packed zones (Table 4.9). These values were 0.14-0.16 and 0.26-0.34 g COD-CH₄ g⁻¹VSS, respectively. It confirmed that high OLR with SS and O&G stimulated the microbial activity and enhanced the growth of them by providing more organic substance as found in the study of Chan et al., (2010).

Table 4.8 Microbial quantity at high-strength POME operation

SS Concentration	EUB (Copies rDNA g ⁻¹ VSS)		ARC (Copies rDNA g ⁻¹ VSS)	
	Sludge zone	Packed zone	Sludge zone	Packed zone
10 g l ⁻¹	5.5 x 10 ⁸	8.6 x 10 ⁸	3.2 x 10 ⁵	9.2 x 10 ⁵
11 g l ⁻¹	1.2 x 10 ⁹	3.3 x 10 ⁹	1.5 x 10 ⁶	5.6 x 10 ⁶

Table 4.9 Microbial activity at high-strength POME operation

SS concentration	Non-methanogenic activity (gCOD g ⁻¹ VSS d ⁻¹)		Methanogenic activity (gCOD-CH ₄ g ⁻¹ VSS d ⁻¹)	
	Sludge zone	Packed zone	Sludge zone	Packed zone
10 g l ⁻¹	1.65	0.91	0.14	0.34
11 g l ⁻¹	1.32	1.11	0.16	0.26

According to microbial activity in the sludge and packed zone of AHR, it can be found that non-methanogenic activity (eubacteria) was enhanced by OLR and performed as hydrolysis and organic acid formation in sludge zone. High TVA production and accumulation was observed in this zone and it was not completely utilized due to the environmental condition not optimized to methanogenic activity. On the contrary, almost all of the organic acid was utilized in the packed zone by methanogens inside biofilm and therefore TVA was low in this zone. Results of VFA analysis were shown in Table 4.10. The acetic, propionic and butyric acid were accumulated in the range of 0.21-0.34, 0.03-0.16 and 0.05-1.04 g l⁻¹, respectively. The accumulation of VFA was found in higher concentration compared to low-strength POME operation. It can notice from acidic pH (5.4-6.5) and ratio of TVA/Alk > 0.5 in the sludge zone and these conditions could be affected to methanogenic performance. However, the design of AHR by having the attached microorganism on nylon fiber as biofilm in packed zone has the advantage to protect methanogens in the inner of biofilm. Therefore, biofilm in the packed zone could help and maintain the balance of the system to normal conditions of pH (6.7-7.0) and ratio of TVA/Alk (0.52-0.54) included high organic removal and methane production. It was noted that the packed zone of AHR acted as the methane producing zone.

Table 4.10 VFA analysis at high-strength POME operation

VFA (C ₂ -C ₄) (g l ⁻¹)	SS concentration	
	10 g l ⁻¹	11 g l ⁻¹
Acetic acid	0.21	0.34
Propionic acid	0.03	0.16
Butyric acid	0.05	1.04

Changes in eubacterial diversities in the sludge (Lane F and H) and packed zones (Lane E and G) were detected as illustrated in Figure 4.7. However, the microbial community did not change much from operation at low-strength POME. Most of hydrolytic bacteria in sludge and packed zones was similar to dominant hydrolytic bacteria in low-strength POME. There were 2 groups (E8, F7, G12, H11 and E10, G14, H13) of eubacterial bands first detected in this operation which closed to γ -*Proteobacterium* and α -*Proteobacterium* (Table 4.11). There are some researches isolated organisms from anaerobic digester of high solid and O&G contaminated wastewater and found to similar to bacteria found in this study. These organisms were *Clostridium* sp., *Peptococcus anaerobus*, γ -*Proteobacteria*, *Actinobacteria*, *Bacteroidetes* and *Deferribacteres* (Ugoji, 1997; Lee, 2006).

At the same time, there were some dominant bands faded out as *Pseudomonadaceae bacterium* (S6, A5 and B5), *Pseudomonadaceae bacterium* (S9, A8 and D9) and γ -*Proteobacterium* (S10). This occurrence might be related with SS and O&G removal efficiency decreasing due to high organic acid inhibition of lipase producing bacteria as genus *Pseudomonas*. According to Table 4.11 diversity of total acidogenic bacteria community increased from 16 to 20 bands, which was identified as *Bacteroidetes bacterium*. Phylum Bacteroidetes was the most dominant eubacteria that presented in the chemostat fed with long chain fatty acid at a dilution rate of 0.4 d^{-1} under steady-state condition. It was possible that increasing of *Bacteroidetes* bacteria at high-strength POME operation associated to increasing of O&G concentration in POME influent. Besides that, 12 bands of acetogenic bacteria were detected. Nine of them were still detected throughout reactor operation at low and high-strength POME included inoculum seed. Only 3 bands (E4 & F4 and G11) were first detected and identified as *Clostridiaceae bacterium* and *Acetobacter* sp., respectively. According to the results of acidogenic and acetogenic bacteria characteristics, it can be seen that high acidity conditions were generated from their activities, especially in the sludge zone.

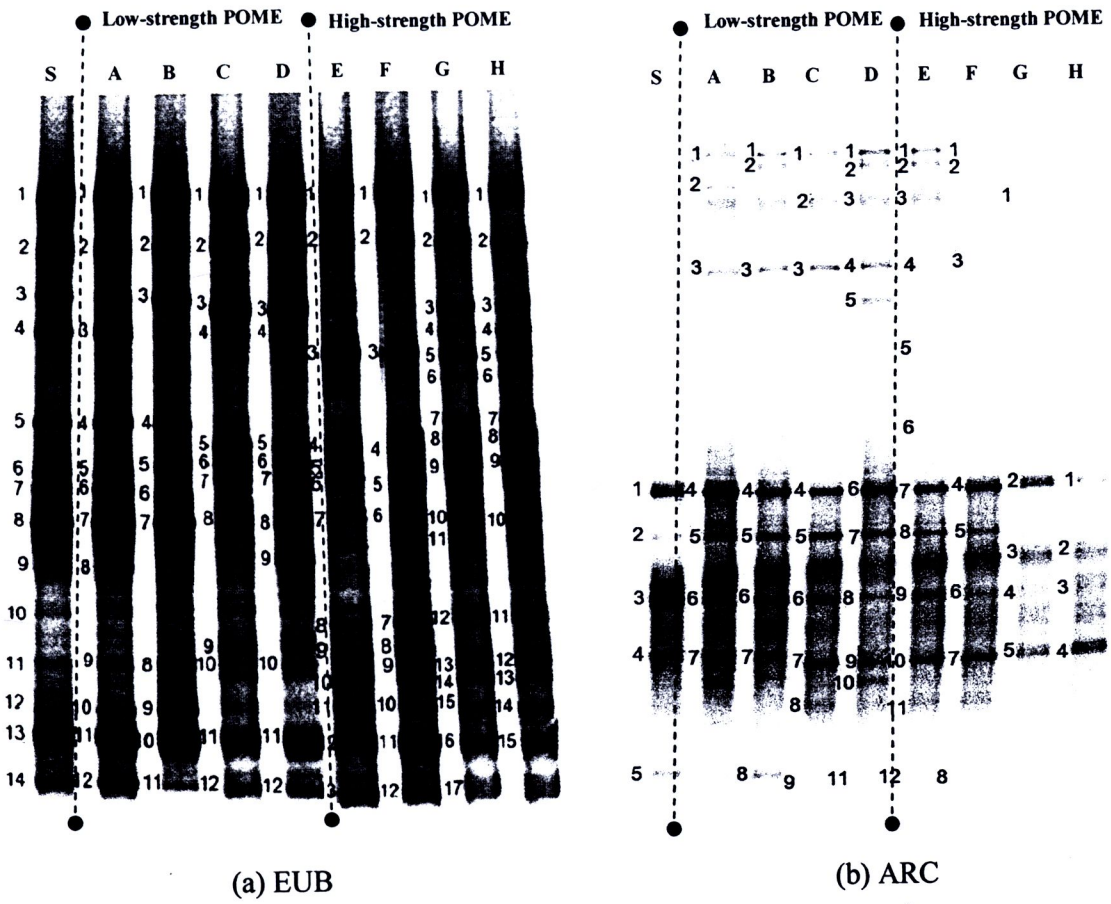


Figure 4.7 DGGE profiles of (a) EUB and (b) ARC at high-strength POME comparing to low-strength POME operations

Remark: Lane S: Inoculum seed, Lane A: Packed zone and B: Sludge zone at 5 g SS l⁻¹, Lane C: Packed zone and D: Sludge zone at 7 g SS l⁻¹, Lane E: Packed zone and F: Sludge zone at 10 g SS l⁻¹, Lane G: Packed zone and H: Sludge zone at 11 g SS l⁻¹

Table 4.11 The partial 16S rRNA gene sequences of EUB domain and organism with the best matching sequences determined by BLAST searches of high-strength POME operation

Affiliation	DGGE band										Similarity (%)	Accession no.	Bacteria
<i>Pseudomonas sp.</i> M130	S1	A1	B1	C1	D1	E1	F1	G1	H1		92	AB088750.2	
<i>Pseudomonas entomophila str.</i> L48	S3		B3	C3	D3			G3	H3		92	CT573326.1	
<i>Pseudomonas sp.</i>	S5	A4	B4					G7	H7		97	AM886092.1	
<i>Pseudomonadaceae bacterium</i>	S6	A5	B5								94	AB545745.1	
<i>Pseudomonas sp.</i>	S8	A7	B7	C8	D8	E7	F6	G10	H10		97	AM886092.1	Hydrolytic bacteria
<i>Pseudomonas pseudoalcaligenes</i>	S9	A8			D9						79	AF140011.1	
Uncultured γ - Proteobacterium	S10										82	EU167353.1	
Uncultured Firmicutes bacterium				C5	D5			G8	H8		94	FM896934.1	
Uncultured γ - Proteobacterium				C9		E9	F8				82	EU167353.1	
Uncultured γ - Proteobacterium						E8	F7	G12	H11		82	EU167353.1	
Uncultured delta Proteobacterium						E10		G14	H13		73	FN429803.1	
Uncultured <i>Bacteroidetes bacterium</i>	S7	A6	B6			E6	F5				94	CU926845.1	
Uncultured <i>Bacteroidetes bacterium</i>	S11	A9	B8	C10	D10		F9	G13	H12		98	EU810898.1	
Uncultured <i>Bacteroidetes bacterium</i>	S12	A10	B9			E11	F10	G15	H14		76	GU955023.1	Acidogenic bacteria
Uncultured <i>Bacteroidetes bacterium</i>	S13	A11	B10	C11	D11	E12	F11	G16	H15		91	GU955023.1	
Uncultured <i>Bacteroidetes bacterium</i>				C6	D6	E5		G9	H9		86	AB433139.1	
Uncultured <i>Bacteroidetes bacterium</i>				C7	D7						94	CU926845.1	
<i>Bacteroides</i>						E3	F3	G5	H5		87	EU136682.1	
<i>Clostridiales bacterium</i>	S2	A2	B2	C2	D2	E2	F2	G2	H2		81	GU428556.1	
Uncultured actinobacterium	S4	A3		C4	D4			G4	H4		91	GU194237.1	
<i>Acetobacter sp.</i>	S14	A12	B11	C12	D12	E13	F12	G17			98	GQ246703.1	Acetogenic bacteria
Uncultured <i>Clostridiaceae bacterium</i>						E4	F4				74	AB218300.1	
<i>Acetobacter sp.</i>								G11			98	GQ246703.1	
clone RWF5 16S ribosomal RNA								G6	H6		98	GQ921905.1	Other bacteria

Remark: Lane S: Inoculum seed, Lane A: Packed zone and B: Sludge zone at 5 g SS l⁻¹, Lane C: Packed zone and D: Sludge zone at 7 g SS l⁻¹, Lane E: Packed zone and F: Sludge zone at 10 g SS l⁻¹, Lane G: Packed zone and H: Sludge zone at 11 g SS l⁻¹

For the archaeal community, 8 dominant bands were detected in the sludge and packed zones at high-strength POME operation, as reported in Table 4.12. *Methanosaeta* was only one strain of acetoclastic methanogen which could be detected along the reactor operation at low and high POME included inoculum seed. *Methanocaldococcus vulcanius* M7 and *Methanococcoides* were also observed in this operation and they were identified from couple bands E2&F2 and E12&F8, respectively. *Methanomicrobiaceae* still was observed to be same as low-strength POME and inoculum seed. It can be seen that, hydrogenotrophic methanogenesis became the dominant pathway of methane formation in overall reactor operation and acidophilic methanogen as the genus *Methanobacterium* was detected (Demirel and Scherer, 2008). For acetoclastic methanogen, its activity was found and *Methanosaeta* sp., was detected in sludge zone by this acidic condition. This occurrence was also found by Xuefei and Nanqi (2007) that *Methanosaeta* sp., could digest acetate in an acidic environment ($\text{pH} < 5$).

Acetate was one of the most significant intermediates for methanogenesis in anaerobic digestion of POME. Acetoclastic methanogens was not observed in the packed zone at high POME strength operation. Surprisingly, syntrophic acetate oxidation and hydrogenotrophic methanogenesis was found in both sludge and packed zones of AHR. Syntrophic acetate oxidation was utilized by *Clostridia* within the phylum Firmicutes, whereas the H_2 -consuming methanogenesis was catalyzed by *Methanobacterium*. This mutual syntrophy theoretically yields energy is the same stoichiometry as acetoclastic methanogenesis ($\Delta G^\circ = -31.0 \text{ kJ mol}^{-1}$). Hattori (2008) mentioned that the oxidation of acetate could oxidize to H_2/CO_2 only when these products were subsequently utilized with syntrophic interaction between syntrophic acetate oxidizing bacteria and methogenic archaea, respectively.

Although, hydrogenotrophic methanogens seem diversified but almost all dominant bands such as *Methanoculleus* sp. (B8 and E11). *Methanomicrobiales* (E1 and F1) is a family of *Methanomicrobiales*, the same as *Methanomicrobiaceae*. Four bands (E2, F2, E12 and F8) also were classified in the same family of *Methanocaldococcaceae*. They were represented by *Methanocaldococcus vulcanius* M7 and *Methanococcoides* sp., respectively.

Table 4.12 The partial 16S rRNA gene sequences of ARC domain and organism with the best matching sequences determined by BLAST searches of high-strength POME operation.

Affiliation	DGGE band		Similarity (%)	Accession no.	Bacteria
Uncultured <i>Methanococcoides</i> sp.	S5	B9	89	AY454739.1	
Uncultured <i>Methanosaeta</i> sp.	S3	A6 B6 C6 D8 E9 F6 G4 H3	85	AY454766.1	Acetoclastic methanogens
<i>Methanocaldococcus vulcanius</i> M7		B2 D2 E2 F2	90	CP001787.1	
Uncultured <i>Methanococcoides</i> sp.		C9 D11 E12 F8	89	AY454739.1	
<i>Methanobacterium</i> sp.	S1	A4 B4 C4 D6 E7 F4 G2 H1	93	GU936489.1	
<i>Methanobacterium</i> sp.	S2	A5 B5 C5 D7 E8 F5	95	GU569395.1	
<i>Methanomicrobiaceae</i>	S4	A7 B7 C7 D9 E10 F7 G5 H4	87	GU129124.1	
Uncultured <i>Methanomicrobiales</i>		A1 B1 C1 D1 E1 F1	95	AY780566.1	
<i>Methanospirillum hungatei</i>		A2	99	AB517987.1	
<i>Methanobacterium</i> sp.		A3 B3 C3 D4 E4 F3	97	GU936489.1	Hydrogenotrophic methanogens
<i>Methanobacteriaceae</i>		D5	89	GU129060.1	
<i>Methanoculleus</i> sp.		D10	96	AB436897.1	
<i>Methanoculleus</i> sp.		B8 E11	96	AB436897.1	
<i>Methanobacterium palustre</i>		E5	91	EU293795.1	
<i>Methanobacterium</i> sp.		E6	95	GU569395.1	
<i>Methanobacterium palustre</i>		G3 H2	91	EU293795.1	
		C2 D3 E3 F1			Unidentified

Remark: Lane S: Inoculum seed, Lane A: Packed zone and B: Sludge zone at 5 g SS l⁻¹, Lane C:

Packed zone and D: Sludge zone at 7 g SS l⁻¹, Lane E: Packed zone and F: Sludge zone at 10 g

SS l⁻¹, Lane G: Packed zone and H: Sludge zone at 11 g SS l⁻¹

4.3 Shock load operation

4.3.1 Process performances and stability at shock load operation

After increasing OLR to $2.7 \text{ g COD l}^{-1} \text{ d}^{-1}$ with TCOD, SS and O&G at 30, 12.5 and 2.7 g l^{-1} , respectively. Process performance and stability as well as microbial characteristics were monitored same as previous study. During 27 d operation, the reactor shock load was occurred and results were shown below.

4.3.1.1 Sludge zone

High OLR with SS and O&G concentrations at 12.5 and 2.7 g l^{-1} impacted on the reactor performance in TCOD, SCOD, SS and O&G removal and stability. During 14 d reactor operation, overall organic removal efficiency decreased continuously and O&G removal was detected as the lowest value. It was lower than 40%. After reactor ran for 27 d, overall reactor performance in organic removal was unconscious while system pH became lower than 4.0. These results indicated the shock load condition. TCOD, SCOD, SS and O&G removal performance decreased to 54 ± 4 , 72 ± 3 , 49 ± 9 and $34 \pm 9\%$ respectively. SCOD was higher biodegradable due to the soluble organic matters were in the dissolved form of smaller molecular weight and easier for biodegraded by microorganism. Less removal of SS and O&G was observed which less than 50% and led to TCOD removal reduction. High accumulation of SS was observed in the sludge zone due to its hardly degradable and need more time to degrade by anaerobic microorganisms. For O&G, it was hydrolyzed by bacterial enzymes which sensitive to pH and inhibitory substances under anaerobic condition. A dramatic drop in 30% O&G removal was found under this overload feeding POME condition. Meanwhile, O&G could be removed over 50% depending on the initial concentration of SS and O&G feeding under normal condition operation.

Low performance and stability founding in this operation was signal of failure conditions as illustrated in Figure 4.8. Under this operation condition, system pH and ratio of TVA/Alk were strong acidic (4.42) and lower buffer capacity (>0.8) that it could affect inhibiting the lipase producer bacterial activity. Low performance and stability founding in this operation responded under shock load condition which was subjected to organic shock load. It indicated that OLR at $2.7 \text{ g COD l}^{-1} \text{ d}^{-1}$ with SS concentration at 12.5 g l^{-1} was over feeding POME to AHR. Shock load resulted in full organic acid substrate penetration expose organisms to very high concentrations of substrate which they may not be able to metabolize (Woolard, 1997), finally led it to be toxic in its activity. It seems that the sludge zone had a more violent effect or the shock load than the packed zone as indicated by low organic removal performance. It might be course microorganisms in form suspended sludge directly attack with organic substances in the system and got inhibiting by high acidic environment. This occurrence was also found

by Mendoza- Espinosa and Stephenson (2001) that fixed-film systems perform better than suspended growth reactors during shock loadings of primary settled sewage. Other research suggested that high rate organic waste treatment anaerobic reactors are more likely to fail due to the present of lipids and long chain fatty acid (LCFA) inhibiting the growth of acidogens, acetogens and methanogens as well as the washout of the sludge. Although lipids were shown to enhance biogas production significantly but the high potency of this substrate could easily lead to an overload of the reactor (Angelidaki et al., 1990).

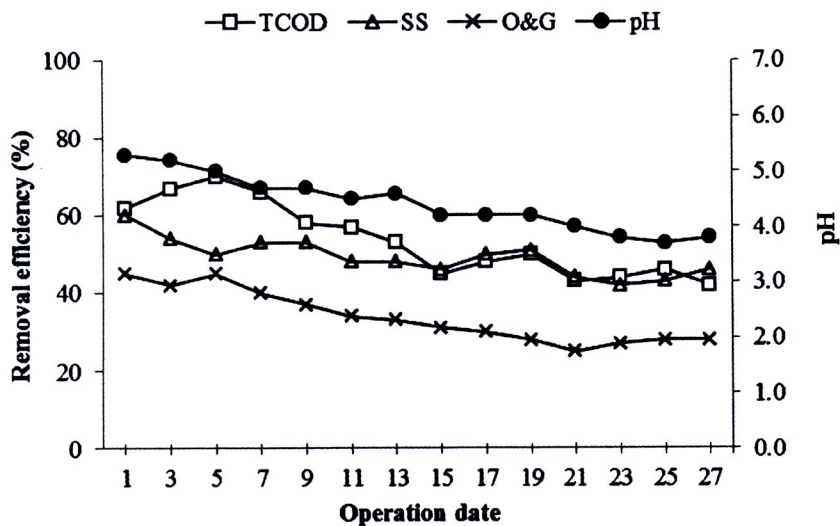


Figure 4.8 Organic removals and pH of sludge zone at shock load condition

4.3.1.2 Packed zone

AHR configuration provided different characteristics of organic matter structure in sludge and packed zones. Most of the organic matters were primaries digested in sludge zone and continued to biodegrade in packed zone. The concentrations and complex molecules of this zone were lower than that in sludge zone. This was reflected in high removal of TCOD, SCOD and SS in this packed zone under shock load condition. TCOD, SCOD and SS and O&G removal were maintained at 71 ± 6 , 73 ± 7 and $69\pm 7\%$, respectively, during 23 d reactor operation. Moreover, less organic acid accumulation was observed in this packed zone which indicated by TVA value at 1284 mg l^{-1} and pH of 6.41 as given by Figure 4.9. Only O&G removal was different from other organic matters. O&G removal was deteriorated when reactor was ran for 10 d by detecting of its removal decreased to $43\pm 6\%$. Although, the TCOD removal efficiency decreased wherewith increasing of OLR, the performance of packed zone was capable of removing more substrate at high loading rates.

Literature survey shows that the concentration of attached biomass in the hybrid reactor increases with increasing OLR. Higher the OLR, the larger will be the ratio of attached biomass to the total biomass in the hybrid reactor. In the case when the greater part of the biomass was fixed on a support material, the greater part of substrate will be consumed by the biofilm and lesser part remains for the suspended biomass. This indicates that attached biomass would have made greater contribution to the TCOD removal than the suspended biomass which would be the reason for the higher TCOD removal rates at higher OLR (Jianlong et al., 2000). It can be noted that packed zone of AHR was higher resistance to very high OLR than that sludge zone. However, very high organic acid concentration especially acetate (0.92 g l^{-1}) in this zone could affect methanogens activity inside biofilm reflecting to inhibit methane generation.

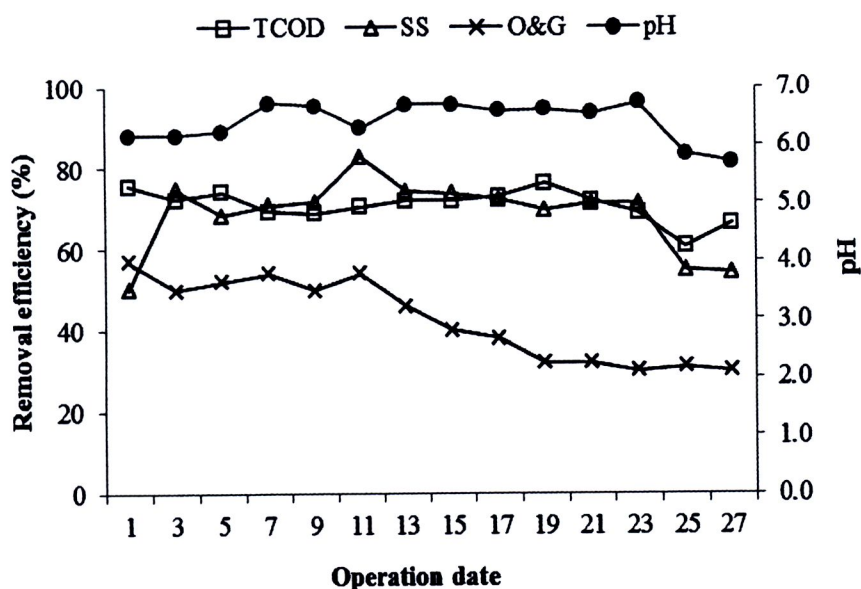


Figure 4.9 Organic removals and pH of packed zone at shock load condition

4.3.1.3 Overall performance

Low performance at shock load operation indicated by TCOD, SCOD, SS and O&G removal efficiency decreasing. Hardly biodegradable organic matters as SS and O&G were removed less than 50% whereas TCOD and SCOD were 57 ± 2 and $68 \pm 7\%$, respectively. Maximum biogas and methane gas production were detected at initial operation time not less than 4000 ml d^{-1} and 2000 ml d^{-1} , respectively, but it came to zero while operation time at 27th day as shown in Figure 4.10. It indicated

that the system approached to failure condition. Failure condition was influenced form high accumulation of undigested organic as TCOD ($10,660 \text{ mg l}^{-1}$) and SS ($2,460 \text{ mg l}^{-1}$) included organic acid (TVA = $1,850 \text{ mg l}^{-1}$) in the system. Very low pH and VFA analysis revealed high concentration of acetic acid, propionic acid and butyric acid (Table 4.13). Acetic acid and propionic acid were the main components; their concentrations were 0.90 and 0.20 g l^{-1} , respectively. Therefore, controlling the metabolism of microflora towards acetate formation is a key factor to achieve a high methane yield.

It can be noted that reactor operation at 12.5 g l^{-1} of SS concentration with 2.7 g l^{-1} of O&G was performed under shock load condition. Sludge zone was influenced to shock load as first part of AHR because it was prior encountered with organic matters and suspended sludge characteristic has high sensitivity to high acidic condition more than packed zone. Biofilm was claimed that it apparently inherits the advantages of load attenuation in completely mixed systems as well as strength of attached growth systems against shock loads in activated sludge biofilm system (Seetha et al., 2010).

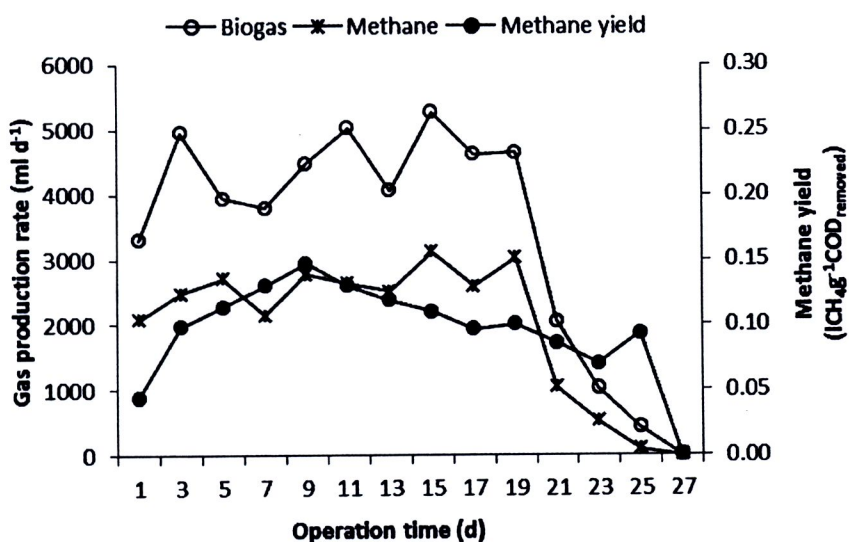


Figure 4.10 Gas production and methane yield at shock load condition

Table 4.13 VFA concentrations at shock load condition

VFA (C ₂ -C ₄)	Concentration at (g l ⁻¹)
Acetic acid	0.92
Propionic acid	0.20
Butyric acid	0.09

4.3.2 Microbial characteristics at shock load operation

Under shock load conditions, non-methanogenic activity in both sludge and packed zones has been high (1.52 - 1.70 g COD g⁻¹VSS d⁻¹). It indicated that the native bacteria could grow and resist high concentrations of organic substances, especially acidogen which could perform under optimum range pH at 5.5–5.9 (McCarty and Mosey, 1991) same as the system pH of shock load. High accumulative acetate (0.92 g l⁻¹) was observed in the sludge zone. This range of acetate concentration is higher than acetogenic methanogen could existence (≤ 0.02 g l⁻¹ of acetate) (Garand et al., 2005). Detecting low organic acid concentration (1,284 mg l⁻¹) in the packed zone reflected the activity of organic acid utilizer as methanogens. Its activity was 0.12 g COD-CH₄ g⁻¹VSS d⁻¹. Microbial population and community changing coordinated with microbial activity. Eubacterial population in sludge and packed zones decreased form normal condition. Eubacterial populations were 3.3 x 10⁸ and 1.1 x 10⁹ copies rDNA g⁻¹VSS, respectively. Changing of archaeal population was obviously observed in the sludge zone by decreasing to 9.4 x 10³ copies rDNA g⁻¹VSS while packed zones was 1.8 x 10⁴ copies rDNA g⁻¹VSS. Data shown in Table 4.14.

Table 4.14 Microbial quantity and activity at shock load condition

Zone	Microbial quantity		Microbial activity	
	EUB (Copies rDNA g ⁻¹ VSS)	ARC (Copies rDNA g ⁻¹ VSS)	EUB (gCOD g ⁻¹ VSS d ⁻¹)	ARC (gCOD-CH ₄ g ⁻¹ VSS d ⁻¹)
Sludge zone	3.3 x 10 ⁸	9.4 x 10 ³	1.70	0.09
Packed zone	1.1 x 10 ⁹	1.8 x 10 ⁴	1.52	0.12

Microbial communities were changed in both eubacteria and archaea as shown in Table 4.15 and 4.16. It was found that there were many dominant bands of eubacteria that had faded out (Figure 4.11a). Five bands (I1&J1, I5&J6 and I4) of hydrolytic bacteria remained in this operation and they were identified to be *Pseudomonas* sp. M130, γ -*Proteobacterium* and γ -*Proteobacterium*, respectively. These strains are normally abundant in high O&G residual wastewater. Acidogenic and acetogenic bacteria were detected in sludge zone higher than packed zone by detecting of 6 bands and 4 bands, respectively. Moreover, *Bacteroidetes bacterium* was the one of dominant acidogenic bacteria same as inoculum seed and normal condition operations.

Table 4.15 The partial 16S rRNA gene sequences of EUB domain and organism with the best matching sequences determined by BLAST searches at shock load condition

Affiliation	DGGE band	Similarity (%)	Accession no.	Bacteria
<i>Pseudomonas</i> sp. M130	I1 J1	92	AB088750.2	Hydrolytic bacteria
Uncultured γ - <i>Proteobacterium</i>	I5 J6	82	EU167353.1	
Uncultured γ - <i>Proteobacterium</i>	I4	82	EU167353.1	
Uncultured <i>Bacteroidetes bacterium</i>	J5	94	CU926845.1	Acidogenic bacteria
Uncultured <i>Bacteroidetes bacterium</i>	I6	98	EU810898.1	
Uncultured <i>Bacteroidetes bacterium</i>	J7	76	GU955023.1	
Uncultured <i>Bacteroidetes bacterium</i>	J4	86	AB433139.1	
<i>Clostridiales bacterium</i>	I2 J2	81	GU428556.1	Acetogenic bacteria
<i>Acetobacter</i> sp.	I9 J9	98	GQ246703.1	
Uncultured <i>Clostridiaceae bacterium</i>	I3 J3	74	AB218300.1	
clone RWF5 16S ribosomal RNA	I7	98	GQ921905.1	Other bacteria

Remark: Lane I: Packed zone and J: Sludge zone at shock load operation (12.5 g SS l⁻¹)

All of the archaeal communities were faded out under this shock load condition, especially, acetoclastic methanogens which might be inhibited by high concentration of acetate at 0.92 g l⁻¹ (Table 4.14). Total five bands were detected: two bands in sludge zone and 3 bands in packed zone (Table 4.16 and Figure 4.11b). They were represented by *Methanobacterium* sp., *Methanoculleus* sp. and *Methanospirillum hungatei*. All of them were members of hydrogenotrophic methanogen and also classified to be acid-tolerant methanogens (Demirel and Scherer, 2008). The elevated reactor pH and high internal acetate concentration in anaerobic treatment system could indicate the activation of syntrophic acetate oxidation.

It is mechanism by which acetate is oxidized to carbon dioxide followed by reduction of carbon dioxide by the hydrogen to methane production which was carried out by hydrogenotrophic methanogen (Schnurer et al., 1999). Factors relating to the occurrence of syntrophic acetate oxidation included a high salt concentration and high concentration of VFAs resulting in inhibition of acetate methanogenesis (Schnurer et al., 1996). The final factor supporting the relationship between hydrogenotrophic methanogenesis and syntrophic acetate oxidation is the lack of acetoclastic methanogen of sludge and packed zones. The increasing of hydrogenotrophic methanogen activity at this condition would help to maintain the reduced hydrogen partial pressure that is favorable for syntrophic acetate-oxidizing bacteria as *Clostridia*. It is now clear that under shock load conditions, hydrogenotrophic methanogenesis took the place of acetoclastic methanogenesis.

Table 4.16 The partial 16S rRNA gene sequences of ARC domain and organism with the best matching sequences determined by BLAST searches at shock load condition

Affiliation	DGGE band	Similarity (%)	Accession no.	Bacteria
<i>Methanobacterium</i> sp.	I1 J1	93	GU936489.1	Hydrogenotrophic methanogens
<i>Methanoculleus</i> sp.	I2	96	AB436897.1	
<i>Methanospirillum hungatei</i>	I3 J2	93	AB517987.1	

Remark: Lane I: Packed zone and J: Sludge zone at shock load operation (12.5 g SS l^{-1})

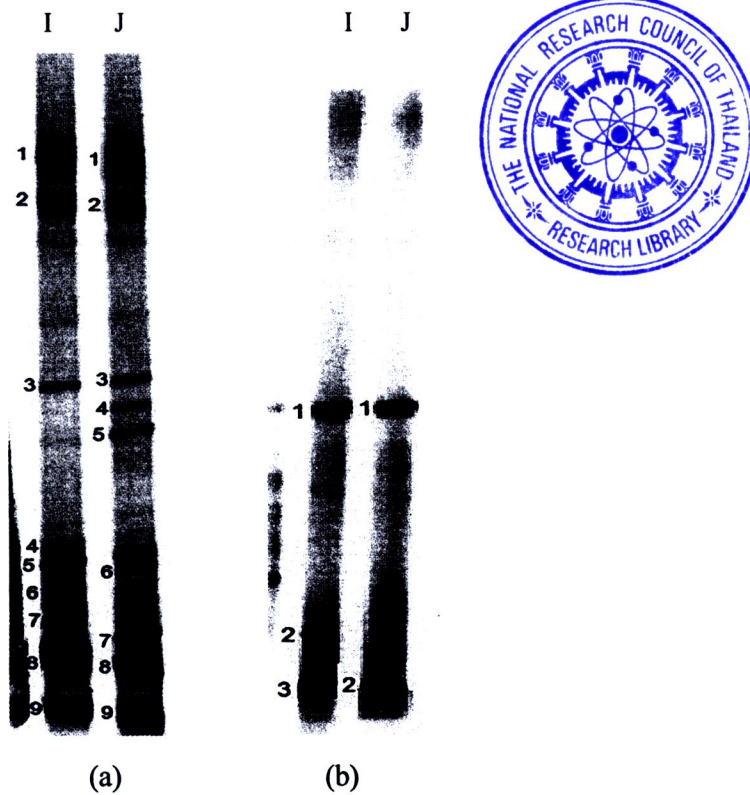


Figure 4.11 DGGE profiles of (a) EUB and (b) AR at shock load condition

Remark: Lane I: Packed zone and J: Sludge zone

4.4 Recovery and operational return to 10 g SS l⁻¹

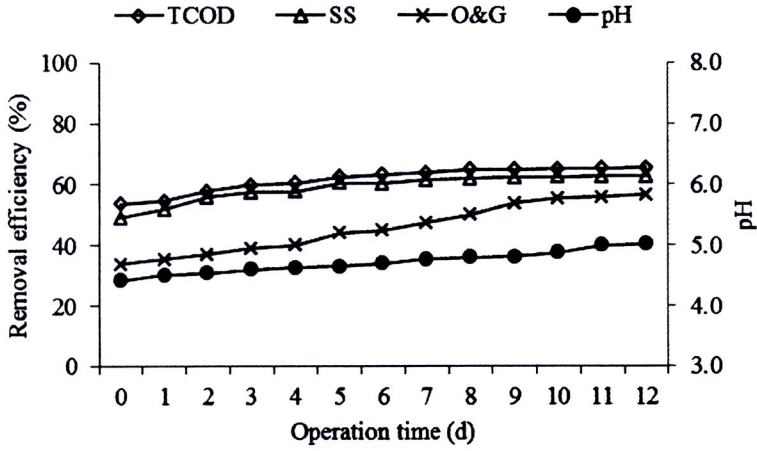
4.4.1 Recovery

Shock loads are common in all kinds of industries and they may have an important harmful effect on anaerobic biological processes, causing destabilization of the microbial populations. VFA accumulation can acidify the reactor and therefore inhibit methanogenic microorganisms (Pozo et al., 2000). In this case the reactor entered an accumulation and inhibition cycle which implies its total collapse. Shock loads of 30 g COD l⁻¹ d⁻¹ with 12.5 and 2.7 g l⁻¹ of SS and O&G concentrations, respectively.

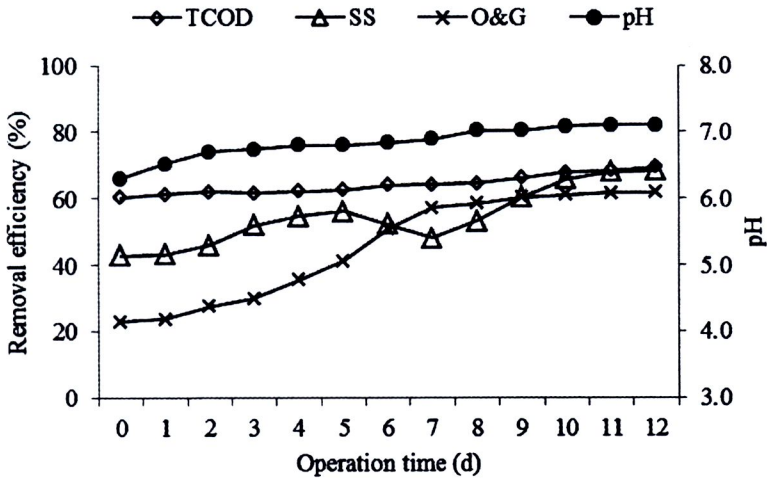
After anaerobic system failed, it stopped feeding of raw POME for 12 days without buffer chemical addition. The system recovery was carried by using effluent recirculation at 50 ml sec⁻¹ of velocity. The recirculation pipes were inserted into influent injection point under AHR and other end-pipe was connected to effluent release point. The effluent was re-fed constantly into the reactor in upflow direction as same as influent. Effluent recirculation could provide suspended organic matters in the sludge zone to be more attacked with microorganisms in the system before flowed up to the packed zone. Effluent recirculation has been applied to enhance the treatment efficiency of livestock wastewater resulted in obviously increasing of BOD and SS removal to 81.3 and 77.1%, respectively, in comparison with the values of 50.2 and 49.3% without effluent recirculation (Lian et al., 2006). During 12 d effluent recirculating, the normal condition of process performance and stability of the sludge and packed zones were resumed.

However, the sludge zone still showed slightly acidic conditions more than the packed zone. The pH varied in the range of 4.8-5.0 while organic removal performances moved up and stable at 65±6, 63±4 and 56±7% of TCOD, SS and O&G, respectively, as given by Figure 4.12a. Packed zone showed higher performance of organic removal efficiency than that sludge zone due to biofilm activity. Under steady state with neutral pH, high organic removal was obtained at 68±4, 53±7 and 52±9% of TCOD, SS and O&G, respectively. These values were shown in Figure 4.12b. Complete shock load recovery was indicated by the stability of overall process performance and stability. It can be noted that shock load was recovered in short time (12 d) because of biofilm in AHR has high performance to resist and overcome the stress condition. Other research found the benefit of biofilm that it provides satisfactory organic matter removal efficiencies, even when using high organic loading rates, under stress operating conditions, such as shock loads, very low hydraulic retention time or low temperature, the reactor shows a quite stable performance (Pozo et al., 2000). Other parameters as biogas and methane production rate were also recovered by detecting of biogas and methane production rate step-increased and stable at 2200 and 1300 ml d⁻¹, as illustrated by Figure 4.12c.

(a) Sludge zone



(b) Packed zone



(c) Gas production rate

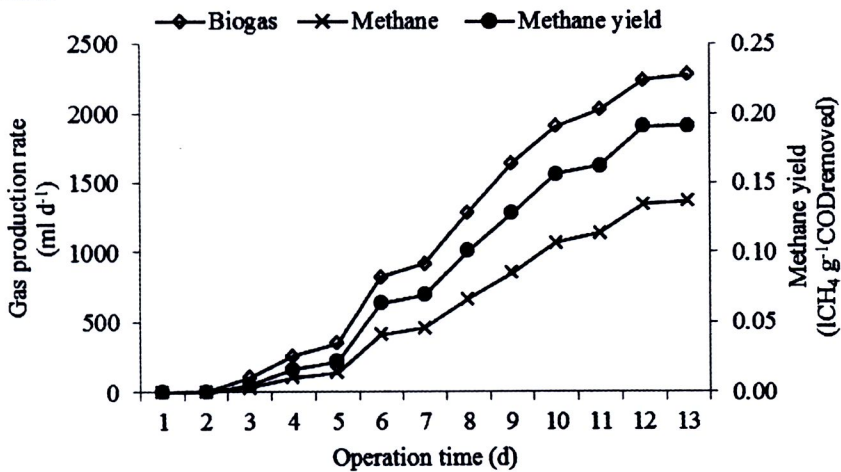


Figure 4.12 Overall organic removals and stability of (a) sludge and (b) packed zones, and (c) gas production rates of recovery condition

4.4.2 Reactor operational return to 10 g SS l⁻¹

Overall process stability once closed to neutral range and process performance was stable, reactor was operated back to lower strength of POME. POME with TCOD, SS and O&G at 20, 10 and 1.8 g l⁻¹, respectively. POME was then started fed to the reactor and operated at HRT of 5 d. After 42 d of operation time, the reactor could maintain overall performance and stability was under normal conditions and passed into steady state. At this steady state, process performance, stability and microbial characteristics in sludge and packed zones were evaluated.

4.4.2.1 Sludge zone

Increasing organic removal efficiency in sludge zone confirmed that shock load recovery was favorable. Organic removal in TCOD, SCOD, SS and O&G obtained at 48, 61, 53 and 39%, respectively. Undigested TCOD, SS and O&G accumulating in the system were observed that they not only composted in new influent but also residues from previous operation. These pollutants composting in effluent were 10320, 4672 and 1170 mg l⁻¹, respectively, as shown in Table 4.17. O&G removal efficiency was obtained below 40% might be caused by acidic conditions in this sludge zone. Slightly acidic environment with pH 6.3 and TVA/Alk at 0.72 accounted the low performance activity of lipid degradable bacteria included a long adaptation period for native microbial communities in the reactor would be required.

Comparison on process performance between this operation and the previous operation with the same SS concentration at 10 g l⁻¹ found that process performance of both operations were not much different, as demonstrated by Figure 4.13. In previous operations, TCOD, SCOD, SS and O&G were removed at 50±4, 77±6, 40±5 and 41±2% respectively, whereas these values of this operation after shock load recovery were obtained at 48±5, 61±6, 53±5 and 39±8%, respectively. The only surprising point that was observed was SS removal of this operation was higher than that previous operation. It indicated that the changing in non-methanogens activity or communities might have occurred and could enhance the SS removal efficiency. Not only overall process performances of both operations were similar, but also process stability which represented by pH (6.3) and TVA/Alk ratio (0.66-0.72). These results indicated that process performance and stability of sludge zone operating at 10 g SS l⁻¹ after recovery was maintained in the same range with the previous operation which operated under normal conditions.

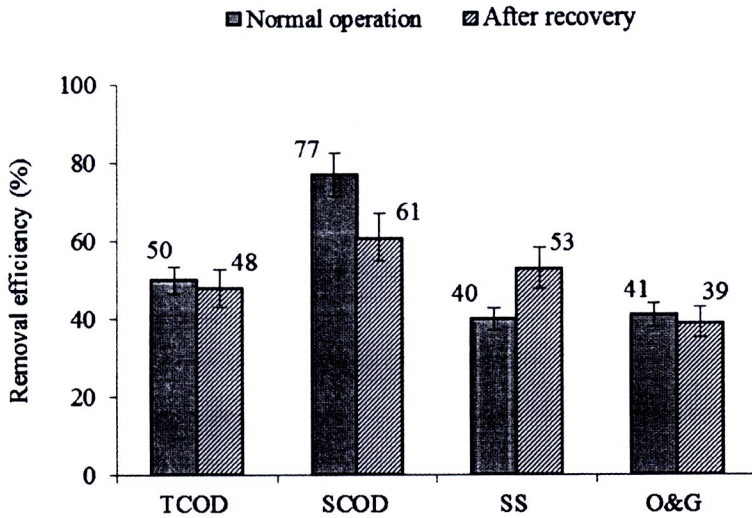


Figure 4.13 Organic removals of sludge zones at normal and after recovery operations at 10 g SS l^{-1}

4.4.2.2 Packed zone

AHR configuration supported biofilm generation in packed zone. Nylon fiber was supporting media for bacteria immobilization and protection of microbial washout. This foundation caused better process performance and stability than sludge zone by microbial biofilm activity. Performances of organic removal efficiencies were higher than that sludge zone by it moved up to 70 ± 5 , 84 ± 10 , 57 ± 6 and $60 \pm 4\%$ of TCOD, SCOD, SS and O&G, respectively. Increasing of O&G removal absolutely coursed from normal environmental system with low accumulated TVA (1200 mg l^{-1}) and system pH at 7.1 which optimum to lipase activity. Process performance and stability of this packed zone were compared with the packed zone of reactor operation at normal condition with the same concentration of SS at 10 g l^{-1} . The trend of both performance and stability were corresponding. These phenomena are illustrated in Figure 4.14. These results confirmed that biofilm has high advantage and performance to maintain overall microbial activity and led to high process performance and stability. The anaerobic reactor composting supporting media for biofilm formation has already been reported for its benefits. It is very appropriate system for pre-treatment of wastewater with high organic load and high solids concentration (Pozo et al., 2000).

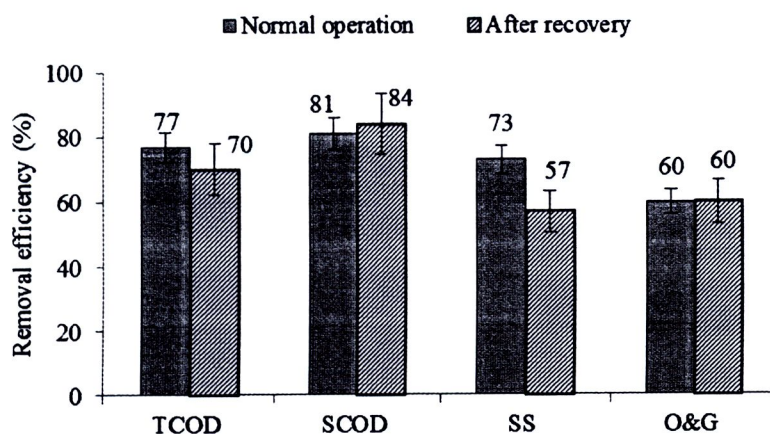


Figure 4.14 Organic removals of packed zones at normal and after recovery operations at 10 g SS l^{-1}

4.4.2.3 Overall performance and stability

Overall process performances in TCOD, SCOD, SS and O&G obtained at 68 ± 4 , 80 ± 8 , 53 ± 7 and $52 \pm 9\%$, respectively. After the system passes on shock load and was recovered, step-increasing of biogas was detected and stable at 5470 ml d^{-1} at steady state. Methane composition was approximately 60% or 3290 ml d^{-1} of methane production rate and methane production yield obtained at $0.20 \text{ l CH}_4 \text{ g}^{-1} \text{COD}_{\text{removed}}$. According to overall performance and stability of this operation were high, the same as the normal operation but the methane yield was not observed. Methane yield was different by obtained at 0.19 and $0.30 \text{ l CH}_4 \text{ g}^{-1} \text{COD}_{\text{removed}}$ of this operation and previous and operation, respectively. This case might be causes of methanogen activity deteriorated and inhibited from shock load. It needs to recover their cell and adapt their activity with new environment under normal condition in this period, therefore led to low performance of methane production.

According to observing high SS removal efficiency and SS accumulation in the system of this operation leading to composition of SS was interested to analyze. SS was analyzed its composition to identify the specific organic matters as cellulose, hemicellulose and lignin which normally contained in POME. The result showed that lignin was the highest quantity of $849 \pm 120 \text{ mg l}^{-1}$ (31%) follow by cellulose at $255 \pm 63 \text{ mg l}^{-1}$ (9.3%) and hemicellulose at $79 \pm 30 \text{ mg l}^{-1}$ (2.9%). These characteristics might be a cause of high SS accumulation in the system even high non-methanogenic activity was observed. Many microorganisms are capable of degrading and utilizing these matters as carbon and energy sources which depend on their ability to produce the specific enzyme (Tuomela et al., 2000).

Table 4.17 Process performance and stability of reactor operation at SS concentration of 10 g l⁻¹ under normal and after recovery conditions

Zone	TVA (mg l ⁻¹)	Alk (mg l ⁻¹)	Effluent concentration (mg l ⁻¹)						Biogas (ml d ⁻¹)	CH ₄ (ml d ⁻¹)	CH ₄ yield*
			TVA/Alk	pH	O&G	SS	TCOD	SCOD			
Normal operation											
Sludge	2100 ± 0.2	3200 ± 0.3	0.66 ± 0.18	6.5 ± 0.2	1100 ± 0.1	6800 ± 0.6	10500 ± 0.4	2800 ± 0.1			
Packed	1600 ± 0.4	3100 ± 0.3	0.52 ± 0.14	7.0 ± 0.3	1470 ± 0.1	1630 ± 0.4	2300 ± 0.2	540 ± 0.1			
Overall	1980 ± 0.4	3060 ± 0.4	0.64 ± 0.15	6.2 ± 0.3	2300 ± 0.1	4300 ± 0.4	5300 ± 0.4	2070 ± 0.4	6300 ± 0.2	4200 ± 0.2	0.30 ± 0.08
After recovery											
Sludge	2000 ± 0.2	2750 ± 0.2	0.72 ± 0.14	6.3 ± 0.5	1170 ± 0.1	4672 ± 0.5	10320 ± 0.4	3130 ± 0.3			
Packed	1200 ± 0.1	2425 ± 0.2	0.5 ± 0.12	7.1 ± 0.3	452 ± 0.1	1582 ± 0.2	3154 ± 0.2	1280 ± 0.1			
Overall	1450 ± 0.2	2550 ± 0.1	0.57 ± 0.16	6.8 ± 0.3	670 ± 0.1	2740 ± 0.3	4860 ± 0.6	1281 ± 0.1	5470 ± 0.1	3290 ± 0.3	0.20 ± 0.02

Remark: *Methane yield unit: l CH₄ g⁻¹ COD_{removed}. Values are the mean of *n* values at steady state ± standard deviation (%); whereby *n* is in the range of 20-30 d.

4.4.3 Microbial characteristics at operational back to 10 g SS l⁻¹

At the end of shock load until normal condition of process stability of reactor operation back to 10 g l⁻¹ of SS concentration was resumed, microbial activities studies were carried out. High performance of non-methanogen was found in packed zone of 1.14 g COD g⁻¹ VSS d⁻¹ while this value at 1.47 g COD g⁻¹ VSS d⁻¹ was detected in sludge zone. It can be seen that non-methanogens has high resistance to undesirable environmental effecting from high organic load, which showed by high TCOD, SS and O&G removal. Non-methanogen was change in its population comparable with activity and the number of microbial population in packed zone higher than sludge zone around two order-level as reported in Table 4.18. Non-methanogens population was detected at 5.6 x 10⁷ and 3.5 x 10⁹ copies rDNA g⁻¹ VSS in sludge and packed zone, respectively. High pH and less organic acid accumulation in packed zone enhanced both methanogen activity and population.

Methanogen activity moved up to 0.18 g COD-CH₄ g⁻¹VSS d⁻¹, in comparison with the values of 0.12 g COD-CH₄ g⁻¹VSS d⁻¹ of shock load, whereas microbial population also increased to 6.4 x 10⁵ copies rDNA g⁻¹VSS. Methanogen activity and population in sludge zone were less than that packed zone due to the high organic acid accumulating with low pH (6.3) of system obstructed VFA utilization by methanogen and also effected to the slow growth rate of methanogen. Its activity was found at 0.12 gCOD-CH₄ g⁻¹VSS d⁻¹, in the meantime its population was detected 5.1 x 10³ copies rDNA g⁻¹VSS.

Table 4.18 Microbial quantity and activity at reactor operational return to 10 g SS l⁻¹

Zone	Microbial quantity		Microbial activity	
	EUB (Copies rDNA g ⁻¹ VSS)	ARC (Copies rDNA g ⁻¹ VSS)	EUB (g COD g ⁻¹ VSS d ⁻¹)	ARC (g COD-CH ₄ g ⁻¹ VSS d ⁻¹)
Sludge zone	5.6 x 10 ⁷	5.1 x 10 ³	1.47	0.12
Packed zone	3.5 x 10 ⁹	6.4 x 10 ⁵	1.14	0.18

Eubacterial community was more diversified than shock load and new hydrolytic bacteria were detected. The eubacterial profile and communities were illustrated by Figure 4.15a and Table 4.19, respectively. Twelve dominants bands (K1-6, L1-4 and L6-7) were detected and most of them was first detected in both sludge (Lane K) and packed (Lane L) zones. *Pseudomonas* sp. which belong to class Gammaproteobacteria was only one strain detected throughout reactor operation at normal operation, shock load and recovery (K1 and L1) conditions. Other bands were detected in different positions in DGGE profile and they were identified to uncultured γ - *Proteobacterium* (K2 and L2), *Bacillus pumilus* (K3 and L3), γ -*Proteobacterium* (K4-6 and L6-7), *Bacillus* sp. (L4).

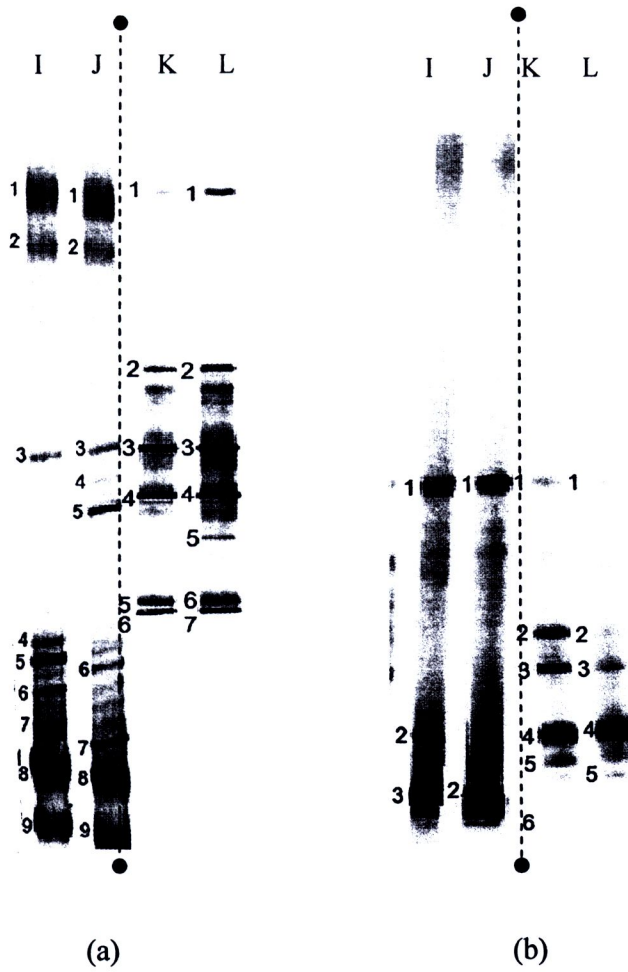


Fig 4.15 DGGE bands of (a) EUB and (b) ARC of shock load and recovery and operational return to 10 g SS l^{-1} .

Remark: Shock load; Lane I: Packed zone, lane J: Sludge zone,

Recovery and operational back to 10 g SS l^{-1} : Lane K: Packed zone,

Lane L: Sludge zone

It was surprising that most of the hydrolytic bacteria was Gram-negative rod shaped bacteria as *γ-Proteobacterium*. This result showed that Gram-negative rod shaped bacteria was the dominating hydrolytic bacteria in shock load recovery condition. It might be because of that a Gram-negative bacterium has high resistance more than Gram-positive bacteria. Cell membrane of Gram-negative bacteria composed of an inner membranes followed by a much thinner peptidoglycan layer and an outer membrane while Gram-positive has only is one membrane surrounding each bacterial cell (<http://www.protocol-online.org/biology-forums/posts/11093.html>). Gram-negative oval shaped bacteria were found to dominate the fixed as well as suspended biomass of organic shock load condition.

Shock loads changed the bacterial types of the reactor from Gram-positive rods to Gram-negative oval shaped bacteria. It was found that certain Gram-negative oval shaped bacteria could withstand organic shock load conditions (Seetha et al., 2010). Nevertheless, *Bacillus* sp. which Gram-positive bacteria producing extracellular hydrolytic enzymes such as alpha-amylase, mannanase and chitinase and have long been used for the production of secretory enzymes for industrial purposes (Schallmeyer et al., 2004) were first detected in this operation. *Bacillus* and *Pseudomonas* were classified to Zymogenous bacteria which was one group of four soil microorganism. This population increased gradually when specific substrate was added to the soil and both of them belonged to the cellulose decomposers, nitrogen utilizing bacteria and ammonifiers (Winogradsky, 1925). Founding of *Bacillus* and *γ-Proteobacterium* in the system of recovery and back to operation at 10 g SS l⁻¹ relevant to cellulose and hemicellulose accumulation in the system included and high non-methanogenic activity might be the cause of their activities.

POME is well known as high composting suspended solid wastewater. The group of non-methanogenic microorganisms responsible for the fermentation process consists of facultative and obligate anaerobic bacteria (Metcalf & Eddy, 2003). Extra cellular enzymes excreted by the fermentative bacteria catalyze the hydrolysis reactions. Although most biopolymers are readily degradable, the cellulose of highly lignified plant material (straw, wood, etc.) has been shown to be resistant to hydrolysis (Lynd et al., 2002). High accumulative SS in the system under shock load run on shock load recovery might elevate the diversity of hydrolytic bacteria. Moreover, *Bacillus* sp. was identified to hydrogen-producing bacteria as same as *Clostridium* sp. which found in high-strength SS and O&G wastewater and raw POME treatment using an anaerobic sequencing batch reactor included that it able to decolorize the POME from dark brown to very light yellow (O-Thong et al., 2007; Liu et al., 2009; Lee et al., 2010). It was possible that these bacteria acted as hydrogen producer and supplied to hydrogen-consumer methanogen under hydrogenotrophic methanogenesis.

Acetogenic bacteria oxidized the final products in the acidogenic phase in an appropriate substrate for methanogenic bacteria, which were hydrogen, CO₂, and acetate. From the products that were produced by acidogenic bacteria, only hydrogen and acetate can be directly used by the methanogens. Under shock load recovery, an acidogenic bacterium was not detected while an acetogenic bacterium was detected only uncultured *Clostridium* sp. (L5). These results can be explained that acetogenic bacteria performed as hydrogen-producing bacteria or acetate oxidizing bacteria because of interdependent relationship with hydrogenotrophic methanogens. These results were also confirmed by low concentrations of acetate, propionate and butyrate in effluent.

Dominant acetoclastic methanogens became more diversified and it seems acetoclastic methanogenesis was resumed as illustrated by Figure 4.15b and Table 4.20 Increasing of methanogenic community respecting to increasing of methanogenic activity which indicated the activity of acetoclastic methanogens detecting in this condition. Total 5 bands (K2, K5-6, L2 and L5) were detected and identified to the uncultured strain of *Methanococcoides* sp., *Methanosaeta* sp., *Methanosarcinales archaeon*, and *Methanosarcina* sp. This was a clear indication that acetoclastic methanogens were recovering from the inhibition caused by the acetate accumulation. Two genera of methanogens, *Methanosaeta* sp. and *Methanosarcina* sp. were known to dominant this acetoclastic methanogens. Particularly, *Methanosaeta* sp. was observed to be most abundant in the seed sludge, but their numbers decreased fast as the acetate concentration increased.

Increasing in acetate levels was accompanied by an increase in *Methanosarcina* sp. (Demerel and Scherer, 2008). Low acetate (0.02 g l⁻¹) of this system was the main occasion to presenting of *Methanosaeta* sp., *Methanosarcina* sp., however it was also detected which might because of optimum concentration of acetate during shock load recovery before system reached to steady state and it remained through reactor operation. Hydrogenotrophic methanogens were kept in this system by six dominant bands (K1, K3-4, L1, L3-4) were namely sequenced to *Methanobacterium*, *Methanomicrobiaceae*, and *Methanoculleus* sp. This group could help to utilized hydrogen pressure by utilized hydrogen using as an electron acceptor to methane formation meanwhile acted as hydrogen scavenger of acetate-oxidizing bacteria balancing hydrogen pressure in the system.

Table 4.19 The partial 16S rRNA gene sequences of EUB domain and organism with the best matching sequences determined by BLAST searches at shock load comparing to shock load recovery conditions

Affiliation	DGGE band				Similarity (%)	Accession no.	Bacteria
<i>Pseudomonas sp.</i> M130	I1	J1	K1	L1	92	AB088750.2	
Uncultured γ - <i>Proteobacterium</i>	15	J6			82	EU167353.1	
Uncultured γ - <i>Proteobacterium</i>	14				82	EU167353.1	
Uncultured γ - <i>Proteobacterium</i>			K2	L2	99	EF629561	Hydrolytic bacteria
<i>Bacillus pumilus</i>			K3	L3	99	FJ976612	
γ - <i>Proteobacterium</i>			K4		96	EF629561	
<i>Bacillus sp.</i>				L4	98	AY818029	
γ - <i>Proteobacterium</i>			K5	L6	96	EF629561	
γ - <i>Proteobacterium</i>			K6	L7	96	EF629561	
Uncultured <i>Bacteroidetes bacterium</i>		J5			94	CU926845.1	Acidogenic bacteria
Uncultured <i>Bacteroidetes bacterium</i>	16				98	EU810898.1	
Uncultured <i>Bacteroidetes bacterium</i>		J7			76	GU955023.1	
Uncultured <i>Bacteroidetes bacterium</i>	18	J8			91	GU955023.1	
Uncultured <i>Bacteroidetes bacterium</i>		J4			86	AB433139.1	
Uncultured <i>Bacteroidetes bacterium</i>					94	CU926845.1	
<i>Clostridiales bacterium</i>	12	J2			81	GU428556.1	Acetogenic bacteria
Uncultured <i>actinobacterium</i>					91	GU194237.1	
<i>Acetobacter sp.</i>	19	J9			98	GQ246703.1	
Uncultured <i>Clostridiaceae bacterium</i>	13	J3			74	AB218300.1	
uncultured <i>Clostridium sp.</i>				L5	79	FJ609997	
clone RWF5 16S ribosomal RNA	I7				98	GQ921905.1	Other bacteria

Table 4.20 The partial 16S rRNA gene sequences of ARC domain and organism with the best matching sequences determined by BLAST searches at shock load comparing to shock load recovery condition

Affiliation	DGGE band				Similarity (%)	Accession no.	Bacteria
Uncultured <i>Methanococcoides sp.</i>			K6		89	AY454739.1	
Uncultured <i>Methanosaeta sp.</i>			K2	L2	91	GU475192	Acetoclastic methanogens
Uncultured <i>Methanosarcinales archaeon</i>			K5		93	AB077212	
Uncultured <i>Methanosarcina sp.</i>				L5	82	AY454773	
<i>Methanobacterium sp.</i>	I1	J1	K1	L1	93	GU936489.1	Hydrogenotrophic methanogens
<i>Methanomicrobiaceae</i>			K3	L3	87	GU129124.1	
<i>Methanoculleus sp.</i>	I2		K4	L4	96	AB436897.1	
<i>Methanospirillum hungatei</i>	I3	J2			93	AB517987.1	

Remark: Shock load at 12.5 g SS l⁻¹: Lane I: Packed zone; lane J: Sludge zone,

Recovery and back operation at 10 g SS l⁻¹: Lane K: Packed zone;

Lane L: Sludge zone.

4.5 Comparison of reactor performance and microbial characteristics among operating conditions

4.5.1 Performance and stability

Overall reactor operation was illustrated in Figure 4.16. Organic removal performance in TCOD, SCOD, SS and O&G varied according to step-increasing of organic load. Over 60% of overall TCOD and SCOD removal were obtained at the range of OLR 3.0-4.8 g COD l⁻¹ d⁻¹ with SS 5-11 g l⁻¹ which classified in low (5-7 g SS l⁻¹) and high-strength POME (10-11 g SS l⁻¹). The range of OLR was realistic to be applied in POME treatment. Puempaiboon and Chowattanasak, 2001 have been studied POME treatment in anaerobic digester with pre-treated POME at OLR 4.53 kg COD l⁻¹ d⁻¹ and HRT of 7 d. The results of TCOD and BOD removals were 64 and 92%, respectively.

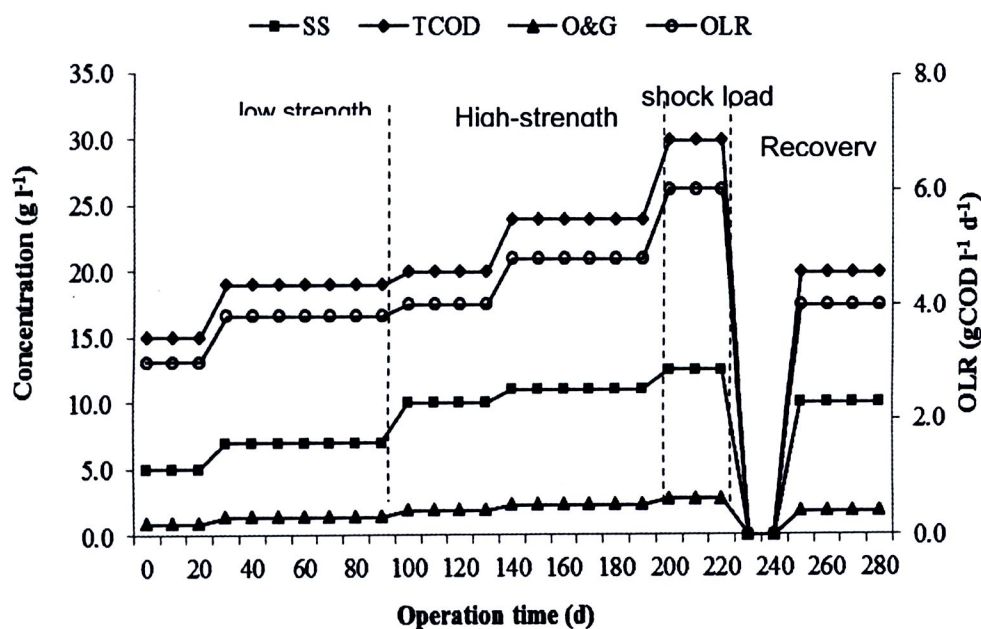


Figure 4.16 Overall reactor operations through 290 d of operation time

Deteriorations of removal efficiency of SS and O&G were detected along with its initial concentrations. When influent SS and O&G increased to 11 and 2.3 g l⁻¹, respectively, biogas and methane production rate decreased even organic removal was maintain higher than 50%. TCOD, SCOD, SS, O&G and cellulose removal are reported in Table 4.21.

Table 4.21 Process performance and stability through 290 d of reactor operation

Parameters	SS influent concentration (g l ⁻¹)					
	5	7	10	11	12.5	10
pH _{eff}						
- Sludge	7.2 ± 0.5	6.6 ± 0.4	6.5 ± 0.2	5.4 ± 0.3	4.42 ± 0.2	6.3 ± 0.5
- Packed	7.4 ± 0.4	6.9 ± 0.2	7.0 ± 0.3	6.7 ± 0.3	6.41 ± 0.2	7.1 ± 0.3
- Overall	7.3 ± 0.4	7.1 ± 0.3	6.2 ± 0.3	6.9 ± 0.3	5.02 ± 0.3	6.8 ± 0.3
TVA _{eff} (mg l ⁻¹)						
- Sludge	700 ± 0.2	1380 ± 0.1	2100 ± 0.2	1450 ± 0.1	2337 ± 0.3	2000 ± 0.2
- Packed	320 ± 0.1	953 ± 0.1	1600 ± 0.4	1250 ± 0.1	1284 ± 0.4	1200 ± 0.1
- Overall	570 ± 0.1	1200 ± 0.1	1980 ± 0.4	1300 ± 0.2	1850 ± 0.3	1450 ± 0.2
Alk _{eff} (mg l ⁻¹)						
- Sludge	2593 ± 0.6	2141 ± 0.2	3200 ± 0.3	2000 ± 0.1	1990 ± 0.3	2750 ± 0.2
- Packed	1702 ± 0.5	1842 ± 0.4	3100 ± 0.3	2300 ± 0.1	2040 ± 0.4	2425 ± 0.2
- Overall	2818 ± 0.4	2800 ± 0.3	3060 ± 0.4	2520 ± 0.3	2100 ± 0.3	2550 ± 0.1
TVA/Alk						
- Sludge	0.27 ± 0.07	0.64 ± 0.12	0.66 ± 0.18	0.73 ± 0.07	1.17 ± 0.22	0.72 ± 0.14
- Packed	0.19 ± 0.06	0.52 ± 0.17	0.52 ± 0.14	0.54 ± 0.12	0.63 ± 0.30	0.5 ± 0.12
- Overall	0.2 ± 0.06	0.43 ± 0.17	0.64 ± 0.15	0.52 ± 0.10	0.88 ± 0.28	0.57 ± 0.16
TCOD removal (%)						
- Sludge	82 ± 11	50 ± 5	50 ± 4	81 ± 3	54 ± 4	48 ± 5
- Packed	83 ± 3	89 ± 6	77 ± 9	70 ± 6	71 ± 6	70 ± 5
- Overall	79 ± 5	77 ± 9	73 ± 6	87 ± 3	57 ± 2	68 ± 4
SCOD removal (%)						
- Sludge	88 ± 6	92 ± 4	77 ± 6	85 ± 5	72 ± 3	61 ± 6
- Packed	91 ± 2	90 ± 2	81 ± 4	89 ± 8	73 ± 7	84 ± 10
- Overall	91 ± 9	88 ± 9	80 ± 9	89 ± 8	68 ± 7	80 ± 8
SS removal (%)						
- Sludge	70 ± 6	75 ± 5	40 ± 5	68 ± 5	49 ± 9	53 ± 5
- Packed	76 ± 4	65 ± 9	73 ± 7	71 ± 10	69 ± 7	57 ± 6
- Overall	69 ± 5	64 ± 5	63 ± 10	77 ± 8.8	49 ± 4	53 ± 7
O&G removal (%)						
- Sludge	81 ± 5	50 ± 6	41 ± 2	48 ± 3	34 ± 9	39 ± 8
- Packed	70 ± 5	70 ± 4	60 ± 4	68 ± 9	43 ± 6	60 ± 4
- Overall	67 ± 7	58 ± 9	56 ± 8	55 ± 9	30 ± 10	52 ± 9
Cellulose removal (%)	81 ± 11	79 ± 18	80 ± 17	82 ± 8	77 ± 12	80 ± 8
Methane production (l d ⁻¹)	1362 ± 0.3	2200 ± 0.2	4200 ± 0.2	3022 ± 0.4	0	3290 ± 0.4
Methane yield (l CH ₄ g ⁻¹ CODremoved)	0.13 ± 0.04	0.20 ± 0.12	0.30 ± 0.08	0.15 ± 0.11	0.13 ± 0.06	0.20 ± 0.02

Values are the mean of n values ± standard deviation (%); whereby n in the range of 20-30 values.

Under low and high-strength operations, sludge zone was found in TVA accumulation higher than packed zone and it reflected to lower pH and acidic environment in the sludge zone. These conditions resulted in different roles of microorganism in the AHR. Organic concentration had marked effect on methanogenic activity. Methanogenic activity was found to decrease steadily with the increase in accumulated TVA and acetate concentration in the system. In sludge zone, TVA was high at 2337 mg l⁻¹ (Table 4.21) has an intense effect on methanogenic activity to decrease at 0.09 g COD-CH₄ g⁻¹ VSS d⁻¹ due to the suspended microorganism character compared to biofilm in packed zone. Sludge zone was the first part of AHR which organic matters were attracted and decomposed by suspended sludge in this zone. There are a lot of organic acid were produced and accumulated in this zone which flavor condition of facultative bacteria. Therefore the sludge zone was acted as hydrolysis zone of AHR. Packed zone was detected as methanogenesis zone of AHR because the process stability with pH and TVA/Alk was normal condition and suitable for methanogenic activity more than that sludge zone.

Other than organic removal efficiency, biogas and methane production could indicate the performance of reactor. Biogas and methane production rate increased from 2000 to 6000 and 1300 to 4200 ml d⁻¹, respective at reactor operating at 5 to 10 g l⁻¹ of SS concentrations (Figure 4.16). These values became lower while initial SS concentration moved up to 11 g l⁻¹ and O&G 2.3 g l⁻¹. It was noted that this operation condition was the breakpoint of reactor performance and stability of POME treatment in AHR. The shock load occurred when reactor was fed by influent containing 11 g SS⁻¹ and 2.7 g O&G l⁻¹. Under shock load condition, overall organic removal decreased lower than 60% in sludge zone, while packed zone could still maintain process performance better than that sludge zone. This occurrence of packed zone caused of microorganism in biofilm resisting acidic environment and balancing of microbial activity in organic acid production and utilization. However, methanogenesis step in both sludge and packed zones was inhibited by high TVA accumulation and finally led to stop of biogas and methane production as demonstrated in Figure 4.17.

the range of 1.08-1.70 g COD g⁻¹VSS d⁻¹. The trending of non-methanogen changed after shock load recovery by detecting of high activity in packed zone at 2.09 g COD g⁻¹ VSS d⁻¹ while sludge zone was detected at 1.77 g COD g⁻¹ VSS d⁻¹. Tendency of microbial population was same as community. Under normal condition of sludge zone, 16S rDNA representing of microbial population was detected in the order-level 10⁷-10⁹ copies rDNA g⁻¹ VSS. High organic acid detecting in shock load condition inhibited suspended non-methanogenic activity but it not impacted to non-methanogenic biofilm in packed zone and led to microbial population in this zone was higher than sludge zone. At recovery and operation back to 10 g SS l⁻¹, microbial population was not much changed from shock load by detecting in order-level at 10⁹ copies rDNA g⁻¹VSS. These results indicated that microbial activity and community could clarify the relationship of process performance and stability with microbial dynamic better than microbial population.

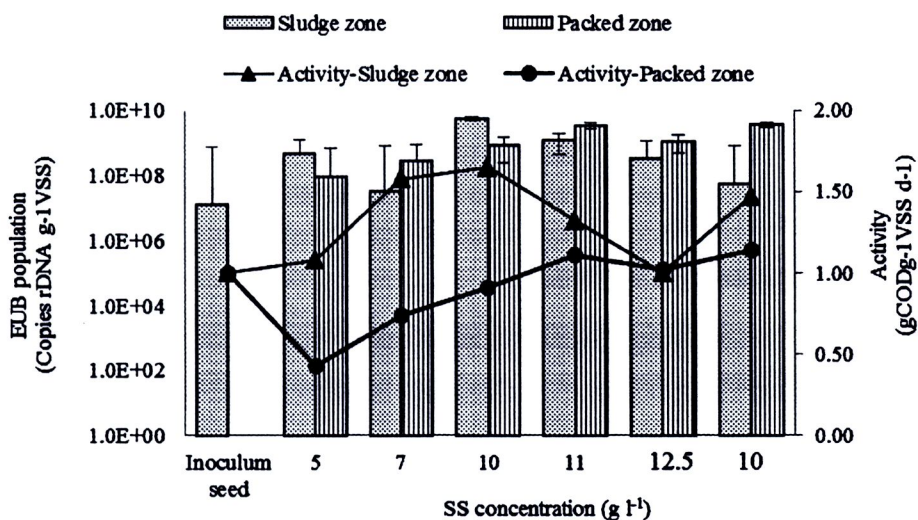


Figure 4.18 Non-methanogenic activities and populations

In anaerobic digestion, organic matters are degraded to methane and carbon dioxide in discrete steps by the concerted action of several different metabolite groups of microorganisms. The first step for most digestion process is hydrolysis during which, particulate matters are converted to soluble compounds that can be hydrolyzed further to simple monomers to be subsequently utilized by fermentative bacteria. The group of non-methanogenic microorganisms responsible for the fermentation process consists of facultative and obligate anaerobic bacteria (Metcalf & Eddy, 2003). In this study, hydrolytic bacteria community were abundant by *Pseudomonas*, γ -*Proteobacterium* and *Bacillus* which specific performed as O&G and suspended solid included cellulose digester. Diversity of these bacteria was influenced by concentration of SS and O&G and system environment. Non-methanogenic community is illustrated by Figure 4.19 and Table 4.22.

Under normal process stability at low and high-strength POME found that *Pseudomonas*, γ -*Proteobacterium* were dominant bacterium and at high-strength it more diversified than lower strength due to the high concentration of nutrient for growing and promoting activity of them. However, almost of them fated out when the system entranced to shock load. High acid accumulative in system influenced to specific group of hydrolytic bacteria insight the system. After shock load was recovered and operation at lower SS concentration at 10 g l^{-1} for 42 d of operation the system entranced to steady state. When normal condition was resumed γ -*Proteobacterium* was detected included new bands representing of *Bacillus* meanwhile partial SS and O&G accumulations in the system were observed. Change in hydrolytic bacteria community indicated that microbial community was affected by concentrations of SS and O&G which hardly biodegraded and need specific group of bacterium to remove.

Acidogenesis is step of the hydrolysis products are absorbed by the cells of other non-methanogen as fermentative bacteria to be fermented or anaerobically converted into compounds such as alcohols, short-chain fatty acids, formic acid, carbon dioxide, hydrogen, ammonia and sulfide. Only one acidogenic bacteria was detected in this study. It was sequenced to strain of *Bacteroides*, while dominant acetogenic acteria were *Clostridium* and *Acetobacter* sp. Both of them were detected through reactor operations. *Clostridium* could be detected under shock load due to relationship among this bacterium and hydrogenotrophic methanogen. The increasing of hydrogenotrophic methanogenic activity helped to maintain the reduced hydrogen partial pressure that is favorable for syntrophic acetate-oxidizing bacteria as *Clostridia*.

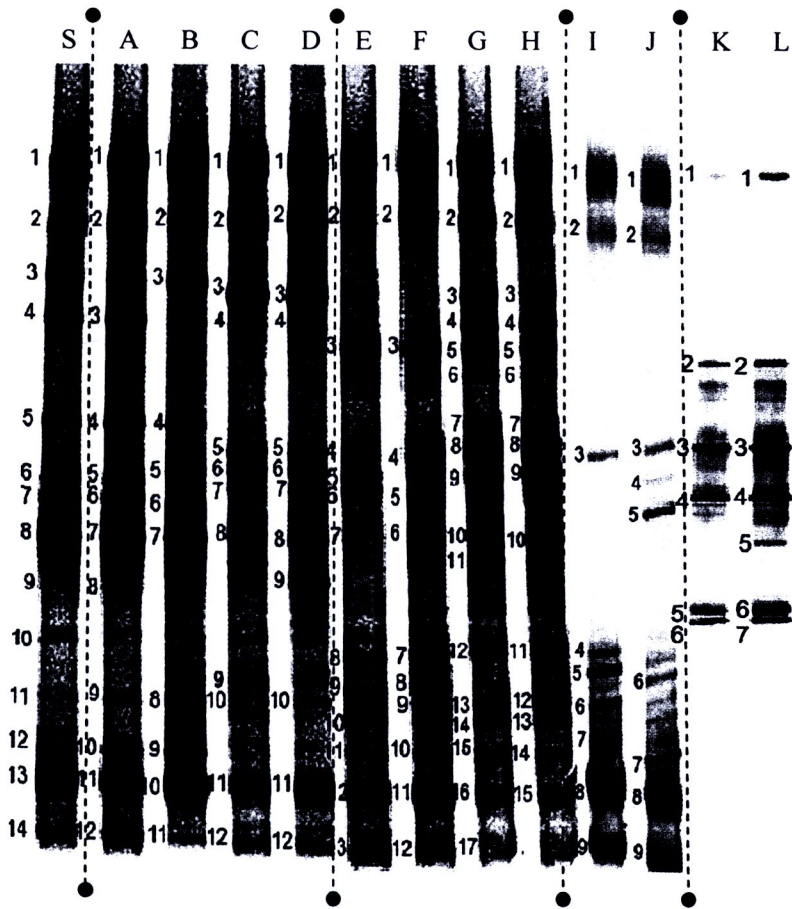


Fig 4.19 DGGE profiles of EUB of all reactor operations

Remark: Seed: Lane S,

Low-strength: Lane A: Packed zone at 5 g SS l⁻¹; Lane B: Sludge zone at 5 g SS l⁻¹;
Lane C: Packed zone at 7 g SS l⁻¹; Lane D: Sludge zone at 7 g SS l⁻¹.

High-strength: Lane E: Packed zone at 10 g SS l⁻¹; Lane F: Sludge zone at 10 g SS l⁻¹;
Lane G: Packed zone at 11 g SS l⁻¹; Lane H: Sludge zone at 11 g SS l⁻¹

Shock load at 12.5 g SS l⁻¹: Lane I: Packed zone; Lane J: Sludge zone,
Recovery and operational back to 10 g SS l⁻¹: Lane K: Packed zone;
Lane L: Sludge zone

Table 4.22 The partial 16S rRNA gene sequences of EUB domain and organism with the best matching sequences determined by BLAST searches of all reactor operations

Affiliation	♀		Low-strength			High-strength					Shock load			After recovery		Similarity (%)	Accession no.	Bacteria
	S	♀	A	B	C	D	E	F	G	H	I	J	K	L				
<i>Pseudomonas</i> sp. M130	+		+	+	+	+	+	+	+	+	+	+	+	+	+	92	AB088750.2	Hydrolytic bacteria
<i>Pseudomonas entomophila</i> str. L48	+		-	+	+	-	-	-	+	-	-	-	-	-	-	92	CT573326.1	
<i>Pseudomonas</i> sp.	+		+	+	-	-	-	-	+	-	-	-	-	-	-	97	AM886092.1	
<i>Pseudomonadaceae bacterium</i>	+		+	+	-	-	-	-	-	-	-	-	-	-	-	94	AB545745.1	
<i>Pseudomonas</i> sp.	+		+	+	+	+	+	+	+	-	-	-	-	-	-	97	AM886092.1	
<i>Pseudomonas pseudoalcaligenes</i>	+		+	-	-	+	-	-	-	-	-	-	-	-	-	79	AF140011.1	
Uncultured γ -proteobacterium	+		-	-	-	-	-	-	-	-	-	-	+	+	+	82	EU167353.1	
Uncultured <i>Firmicutes bacterium</i>	-		-	-	+	+	-	-	+	-	-	-	-	-	-	94	FM896934.1	
Uncultured γ -proteobacterium	-		-	-	+	-	+	+	+	-	+	+	+	+	+	82	EU167353.1	
Uncultured γ -proteobacterium	-		-	-	-	-	-	+	+	+	+	+	+	+	+	82	EU167353.1	
Uncultured <i>delta proteobacterium</i>	-		-	-	-	-	+	+	+	+	+	+	-	-	-	73	FN429803.1	
<i>Bacillus pumilus</i>	-		-	-	-	-	-	-	-	-	-	-	+	+	+	99	FJ976612	
<i>Bacillus</i> sp.	-		-	-	-	-	-	-	-	-	-	-	-	-	+	98	AY818029	

Table 4.22 The partial 16S rRNA gene sequences of EUB domain and organism with the best matching sequences determined by

BLAST searches of all reactor operations (Cont').

Affiliation	SS ₁		Low-strength			High-strength			Shock load		After recovery		Similarity (%)	Accession no.	Bacteria		
	S	S	A	B	C	D	E	F	G	H	I	J				K	L
Uncultured <i>Bacteroidetes bacterium</i>	+	+	+	+	-	-	+	+	-	-	-	+	-	-	94	CU926845.1	Acidogenic bacteria
Uncultured <i>Bacteroidetes bacterium</i>	+	+	+	+	+	+	-	+	+	+	-	-	-	-	98	EU810898.1	
Uncultured <i>Bacteroidetes bacterium</i>	+	+	+	+	-	-	+	+	+	+	-	+	-	-	76	GU955023.1	
Uncultured <i>Bacteroidetes bacterium</i>	+	+	+	+	+	+	+	+	+	+	+	+	-	-	91	GU955023.1	
Uncultured <i>Bacteroidetes bacterium</i>	-	-	-	-	+	+	+	-	-	+	-	+	-	-	86	AB433139.1	
Uncultured <i>Bacteroidetes bacterium</i>	-	-	-	-	+	+	+	-	-	-	-	-	-	-	94	CU926845.1	
<i>Bacteroides</i>	-	-	-	-	-	-	+	+	+	+	-	-	-	-	87	EU136682.1	Atetogenic bacteria
<i>Clostridiales bacterium</i>	+	+	+	+	+	+	+	+	+	+	+	+	-	-	81	GU428556.1	
Uncultured <i>actinobacterium</i>	+	+	+	+	+	+	+	-	-	+	-	-	-	-	91	GU194237.1	
<i>Acetobacter</i> sp.	+	+	+	+	+	+	+	+	+	+	+	+	-	-	98	GQ246703.1	
Uncultured <i>Clostridiaceae bacterium</i>	-	-	-	-	-	-	+	+	-	+	+	+	-	+	74	AB218300.1	
<i>Acetobacter</i> sp.	-	-	-	-	-	-	-	-	+	+	-	-	-	-	98	GQ246703.1	
clone RWF5 16S ribosomal RNA										+	+				98	GQ921905.1	Other bacteria

Remark: Lane S: inoculum seed, Low-strength: Lane A: Packed zone at 5 g SS l⁻¹; lane B: Sludge zone at 5 g SS l⁻¹, Lane C: Packed zone at 7 g SS l⁻¹; lane D: Sludge zone at 7 g SS l⁻¹.

High-strength: Lane E: Packed zone at 10 g SS l⁻¹; lane F: Sludge zone at 10 g SS l⁻¹;

Lane G: Packed zone at 11 g SS l⁻¹; lane H: Sludge zone at 11 g SS l⁻¹

Shock load at 12.5 g SS l⁻¹: Lane I: Packed zone; lane J: Sludge zone

Recovery and operation back to 10 g SS l⁻¹: Lane K: Packed zone; lane L: Sludge zone



4.5.2.2 Methanogens

Change in methanogenic community was detected follow step increasing of organic load. Under normal condition of low and high-strength operation, dominant acetoclastic methanogens were dominant by *Methanosaeta* and *Metahnococcoides*. It resulted in high activity and methane production performance detecting which indicated by methanogenic activity (SMA) and methane production rate. Methanogenic activity and population increased according with its community as showed in Figure 4.20. Acetoclastic methanogen was completely inhibited by shock load at ORL 6.0 g COD l⁻¹ d⁻¹ with SS and O&G concentrations of 12.5 and 2.7 g l⁻¹, respectively, due to very high organic acid accumulation in the system. After shock load recovery by effluent recirculating and re-fed with low organic load of SS and O&G at 10 and 1.8 g l⁻¹, respectively. The normal process performance was resumed and resulted in acetoclastic methanogen became to be dominant group. This phenomena directly impacted to methanogenic population in the system. *Methanosaeta* sp. and *Methanosarcina* sp. were known to dominant acetoclastic methanogens. Increasing in acetate level was accompanied by an increase in *Methanosarcina* sp., whereas, *Methanosaeta* sp. disappeared due to its less resistance to high concentration of acetate. Therefore, acetoclastic methanogenesis of methane formation was performed by *Methanosarcina* sp.

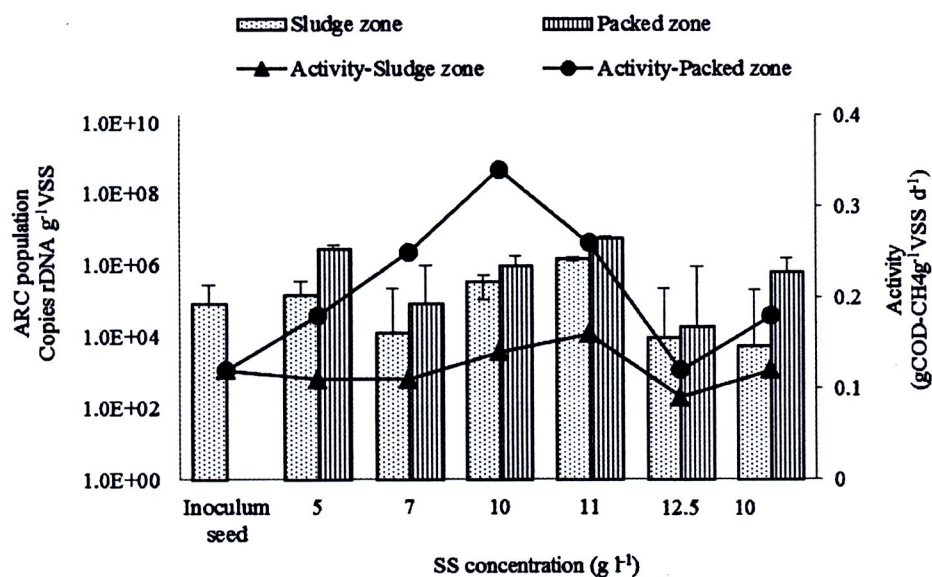


Figure 4.20 Methanogenic activities and populations

Figure 4.21 and Table 4.23 demonstrate methanogenic community. High organic acid accumulation induced the hydrogenotrophic methanogenesis instead of methane formation by acetoclastic methanogenesis. At high-strength POME (10-11 g SS l⁻¹) operation, maximum methane yield at 0.30 l CH₄ g⁻¹COD_{removed} was obtained at 10 g l⁻¹ of SS concentration but this value decreased to 0.15 l CH₄ g⁻¹COD_{removed} when operating at 11 g SS l⁻¹ while high methanogenic activity and organic removal efficiency were observed. This occurrence was suspicious about the mechanism of methane formation and microorganism activity in the system. The results showed that syntrophic acetate oxidation and hydrogenotrophic methanogenesis was dominant group in both sludge and packed zones of AHR while acetoclastic methanogens was not observed in the packed zone at high POME strength operation. The oxidation of acetate could oxidize to H₂/CO₂ only when these products were subsequently utilized with syntrophic interaction between syntrophic acetate oxidizing bacteria and methanogenic archaea, respectively. Syntrophic acetate oxidizing bacteria was identified to *Clostridia* within the phylum Firmicutes, which dominant acetogen at high POME strength operation, whereas the H₂-consuming methanogenesis was catalyzed by *Methanobacterium*. Other hydrogenotrophic methanogens were *Methanomicrobiales*, *Methanocaldococcaceae* and *Methanoculleus* sp. For acetoclastic methanogen, its activity was found and *Methanosaeta* sp. was detected in sludge zone by this acidic condition due to it was protected by granule formation and prevent high organic acid affection to inside *Methanosaeta* sp.

High organic acid in shock load inhibited all acetoclastic methanogen but some hydrogenotrophic methanogen was observed. *Methanobacterium*, hydrogenotrophic methanogen and acid-tolerant methanogens, was observed in this condition. However, it might be non-active under shock load because biogas and methane production were zero. Once reactor was recovered, acetoclastic methanogenesis became dominant partway of methane formation. Acetoclastic methanogen more diversified than low and high-strength POME and dominant strain were *Methanosaeta* sp. and *Methanosarcina* sp.

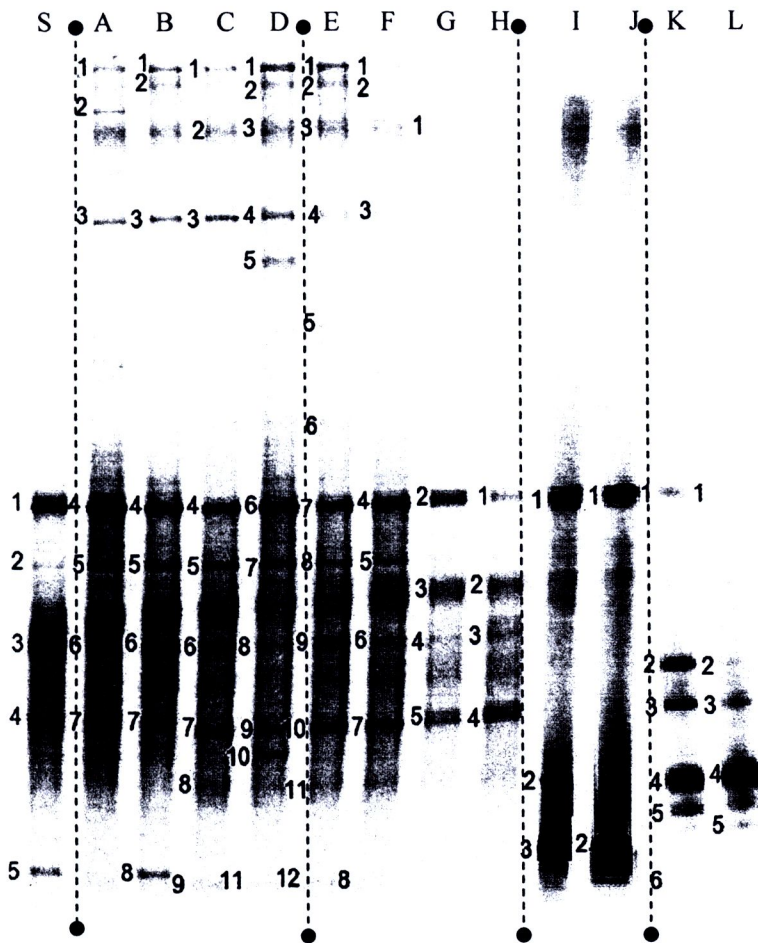


Fig 4.21 DGGE profiles of ARC of all reactor operations

Remark: Seed: Lane S,

Low-strength: Lane A: Packed zone at 5 g SS l⁻¹, Lane B: Sludge zone at 5 g SS l⁻¹;

Lane C: Packed zone at 7 g SS l⁻¹; Lane D: Sludge zone at 7 g SS l⁻¹.

High-strength: Lane E: Packed zone at 10 g SS l⁻¹; Lane F: Sludge zone at 10 g SS l⁻¹;

Lane G: Packed zone at 11 g SS l⁻¹; Lane H: Sludge zone at 11 g SS l⁻¹

Shock load at 12.5 g SS l⁻¹: Lane I: Packed zone; Lane J: Sludge zone,

Recovery and operational back to 10 g SS l⁻¹: Lane K: Packed zone; Lane L: Sludge zone.

Table 4.23 The partial 16S rRNA gene sequences of ARC domain and organism with the best matching sequences determined by BLAST searches of all reactor operations

Affiliation	SS	Low-strength				High-strength				Shock load		After recovery	Similarity (%)	Accession no.	Bacteria	
		A	B	C	D	E	F	G	H	I	J					K
Uncultured <i>Methanococcoides</i> sp.	+	-	+	-	-	-	-	-	-	-	-	-	-	89	AY454739.1	
Uncultured <i>Methanoseta</i> sp.	+	+	+	+	+	+	+	+	+	+	+	+	+	85	AY454766.1	
<i>Methanocaldococcus vulcanius</i> M7	-	-	+	+	+	+	+	+	+	+	-	-	-	90	CP001787.1	
Uncultured <i>Methanococcoides</i> sp.	-	-	+	+	+	+	+	+	+	+	-	-	-	89	AY454739.1	Acetoclastic methanogens
uncultured <i>Methanosarcinales</i> archaeon	-	-	-	-	-	-	-	-	-	-	+	+	-	93	AB077212	
uncultured <i>Methanosarcina</i> sp.	-	-	-	-	-	-	-	-	-	-	-	-	+	82	AY454773	
<i>Methanobacterium</i> sp.	+	+	+	+	+	+	+	+	+	+	+	+	+	93	GU936489.1	
<i>Methanobacterium</i> sp.	+	+	+	+	+	+	+	+	+	+	-	-	-	95	GU569395.1	
<i>Methanomicrobiaceae</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	87	GU129124.1	
Uncultured <i>Methanomicrobiales</i>	-	+	+	+	+	+	+	+	+	+	-	-	-	95	AY780566.1	
<i>Methanospirillum hungatei</i>	-	+	-	-	-	-	-	-	-	-	-	-	-	99	AB517987.1	
<i>Methanobacterium</i> sp.	-	+	+	+	+	+	+	+	+	+	-	-	-	97	GU936489.1	Hydrogenotrophic methanogens
<i>Methanobacteriaceae</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	89	GU129060.1	
<i>Methanoculleus</i> sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	96	AB436897.1	
<i>Methanoculleus</i> sp.	-	+	-	-	-	-	-	-	-	+	+	+	+	96	AB436897.1	
<i>Methanobacterium palustre</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	91	EU293795.1	
<i>Methanobacterium</i> sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	95	GU569395.1	
<i>Methanobacterium palustre</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	91	EU293795.1	
<i>Methanospirillum hungatei</i>	-	-	-	-	-	-	-	-	-	+	+	+	+	93	AB517987.1	Unidentified

Remark: Lane S: inoculum seed, Low-strength: Lane A: Packed zone at 5 g SS l⁻¹; lane B: Sludge zone at 5 g SS l⁻¹; Lane C: Packed zone at 7 g SS l⁻¹; lane D: Sludge zone at 7 g SS l⁻¹. High-strength: Lane E: Packed zone at 10 g SS l⁻¹; lane F: Sludge zone at 10 g SS l⁻¹; Lane G: Packed zone at 11 g SS l⁻¹; lane H: Sludge zone at 11 g SS l⁻¹, Shock load at 12.5 g SS l⁻¹; Lane I: Packed zone; lane J: Sludge zone, Recovery and operation back to 10 g SS l⁻¹; Lane K: Packed zone; lane L: Sludge zone.