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Original Article

Enhancement of sludge granulation in anaerobic treatment of concentrated latex wastewater

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Abstract

Recently, the upflow anaerobic sludge blanket (UASB) reactor has become attractive for wastewater treatment with low energy requirement and biogas production. However, the start-up of an UASB reactor depends on the formation of granules. Therefore, this research aims to study the effect of $AlCl_3$, $CaCl_2$ and temperature on the granule formation process using real concentrated latex wastewater. The result shows that the optimum chemicals concentration of $AlCl_3$ at 300 mg/l enhanced the biomass accumulation and sludge formation process. Approximately 50% of large granular size (0.5 mm < d < 0.8 mm) was obtained with a specific methanogen activity (SMA) of 0.14 gCOD/gVSS/d after 28 days. The COD removal efficiencies were gradually improved until reaching 50% at the end fermentation. Furthermore, the result shows that increasing temperature did not promote granular size. In addition, the granular sludge (R1) (positive control), crushed sludge (R2) and crushed sludge with 300 mg/l of $AlCl_3$ (R3) was examined in 2-l UASB system. It was also found that reactor with $AlCl_3$ supplement (R3) could provide a large granule size (d > 0.8 mm) within 35 days, whereas the large granular sizes in reactor without $AlCl_3$ supplement (R2) became visible within 63 days. Moreover, this experiment found that R1, R2 and R3 could reach steady state within 40, 55 and 45 days, respectively.

Keywords: granulation, concentrated latex wastewater, UASB, $AlCl_3$, $CaCl_2$

1. Introduction

Anaerobic wastewater treatment has become more attractive as a low energy requirement process. One of the most notable developments in anaerobic wastewater treatment is an upflow anaerobic sludge blanket (UASB) reactor. The UASB system can retain high biomass concentration in the presence of upflow wastewater velocity and biogas production. Sludge granulation is the main distinguishing characteristics of UASB reactors as compared to other anaerobic technologies (Liu and Tay, 2004). This granulation process allows UASB reactors to obtain loading rates higher than the conventional sludge processes. The resulting reduction in reactor size and required area for the treatment leads to lower investment costs. In addition, the operating costs are low due to the absence of aeration (Hulshoff Pol *et al*., 2004).

However, the start-up of a UASB reactor is dependent on the formation of granules. Granular sludge involves different bacterial groups, and physico-chemical and microbiological interactions. Granulation may be initiated by bacterial adsorption and adhesion to inert matter, inorganic precipitates and/or to each other through physico-chemical interactions and syntrophic relationships (Schmidt and Ahring, 1993; Yu *et al*., 2001a). It has been shown that some metal ions, such as Ca^{2+}, Fe^{2+}, Al^{3+} and Mg^{2+}, enhance the granulation and play an important role in microbial aggregation

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(Schmidt and Ahring, 1993; Shen *et al*., 1993; Yu *et al*., 2001a; 2001b). Besides, the abiotic environment, such as ionic strength, hydrogen-ion concentration, temperature and mixing, can influence on the granulation process (Schmidt and Ahring, 1996; Hulshoff Pol *et al*., 2004).

When the UASB system is seeded with non-granular anaerobic sludge, it can take several months. Yu *et al*. (2001a and b) studied the effect of aluminium chloride ($AlCl_3$) and calcium chloride ($CaCl_2$) on the sludge granulation process. The 4,000 mg-COD/l of soluble synthetic wastewater was used as feed to the UASB reactors at the 2.0 g-COD/l/d of organic loading rate. The results showed the introduction of $AlCl_3$ at a concentration of 300 mg/l reduced the sludge granulation time by approximate one month (Yu *et al*., 2001a). In addition, the $CaCl_2$ addition from 150 to 300 mg/l enhanced the biomass accumulation and granulation process (Yu *et al*., 2001b).

Most researchers studied sludge granulation using synthetic wastewater (Yu *et al*., 2001a and b; Britz *et al*., 2002; Show *et al*., 2004). However, the effect of fats, long chain fatty acids and suspended solids in the real wastewater can cause the granulation process to be difficult. Therefore, this research aimed to enhance granule formation in the real wastewater. Concentrated latex wastewater was used in this experiment. The effect of $AlCl_3$, $CaCl_2$ and temperature on the sludge granulation was investigated. Moreover, the granular sludge is sometimes limited and unavailable nearby. Therefore, the non-granular sludge could be an alternative inoculum source for UASB reactors. This research also studied the performance of UASB system seeded with non-granular sludge.

2. Materials and Methods

2.1 Wastewater and inoculum

Concentrated Latex Wastewater used in this research was obtained from equalizing pond (EQ) located at Chalong Concentrated Latex Industry Co., Ltd., (Songkhla, Thailand). The physical and chemical characteristics of wastewater are given in Table 1. The rubber residue in wastewater was removed with 0.5 mm sieve screening and the pH of the wastewater was adjusted to 6.8-7.2 by calcium oxide.

2.2 Inoculum

The inoculum source was granular sludge taken from UASB reactor of Hongyen Chotiwat Industry Co., Ltd. (Songkhla, Thailand). The granules were passed through a screening to remove debris. For different wastewater adjustment, inoculum preparation was required. The 50% of the sludge collected from Hongyen Chotiwat industry was anaerobically cultured for 2 weeks in mixture wastewater containing of the 2:1 (v/v) of Hongyen Chotiwat and Chalong Concentrated Latex wastewater. The 150 ml of supernatant was removed every 3 days and then 150 ml of new mixed wastewater was replaced. The ratio of mixed wastewater was changed to 1:1 and 0:1 every 2 weeks. The pH was maintained in a range of 6.8-7.2 by calcium oxide every wastewater transfer. Moreover, biogas production was released everyday to decrease pressure in the bottle. After 6 weeks of cultivation, the granular sludge was stirred 15-20 minutes to obtain non-granular inoculum.

2.3 Experimental reactors

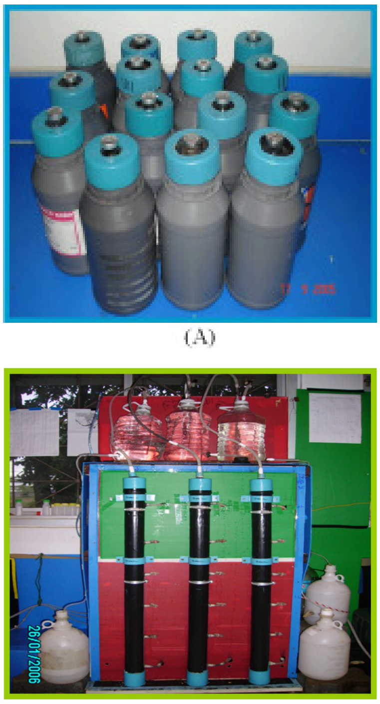
The 1-l bottles with an internal diameter of 10 cm and a height of 22 cm (Figure 1A) were used in a semi-continuous experiment. The working volume of bottles was 800 ml. In addition, UASB reactors with an internal diameter of 2.75 cm and height of 100 cm (Figure 1B) were used in a continuous experiment. The working volume of the UASB reactor was 2 l. Six sampling ports were installed at the height of 8, 23, 38, 53, 68 and 83 cm, respectively.

2.4 Experimental procedure

The effect of $AlCl_3$ (150 and 300 mg/l), $CaCl_2$ (150 and 300 mg/l) and temperature (30, 37 and 45°C) on the sludge granulation was studied in 1-l bottles. The 10% (v/v) of non-granular inoculum was used. The 150 ml of supernatant was removed and then the freshly concentrated latex

Table 1. The physical and chemical characteristics of concentrated latex wastewater.

Parameters	Wastewater for experiments with 1-l bottles	Wastewater for experiments with 2-l UASB reactor
BOD (mg/l)	1991	1855
COD (mg/l)	3973	3350
SS (mg/l)	367	340
TKN (mg/l)	407	390
NH_3- N (mg/l)	274	271
Org-N (mg/l)	133	133
VFA (mg/l)	1610	1628
Temp. (°C)	25.5	27.5
pH	1.75	1.95



(A)

(B)

Figure 1. The 1-l bottles (A) and UASB reactors (B) used in this work.

wastewater was replaced semi-continuously every 3 days. The sludge from each experiment was taken once a week to determine sludge volume index (SVI) and granular size. At the end of experiment, COD, suspended solids (SS), volatile suspended solids (VSS) and specific methanogen activity (SMA) was analyzed. Also, the volume of gas samples from each reactor were measured daily for gas production.

In addition, the performance of UASB was investigated for sludge granulation in the start-up period. The granular sludge (R1) (positive control), non-granular sludge (R2) and non-granular sludge with 300 mg/l of $AlCl_3$ (R3) were examined in 2-l UASB reactors. The 30% (v/v) of inoculum was used. The concentrated latex wastewater (average COD of 4,000 mg/l) with pH adjustment in a range of 6.8-7.2 was fed continuously into the UASB reactor. Organic loading rate (OLR) was operated at 1 gCOD/l/day (0.50 l/d), and hydraulic retention time (HRT) was kept at 4 days. The effluent from each reactor was sampled for COD analyses every 5 days. The sludge for each reactor was taken every week to analyze SVI, SMA and granular size. Moreover, biogas production was determined daily.

2.5 Analytical methods

The liquid samples were taken CODs analysis. The 2.2 ml Solution A (MERCK, Germany) and 1.8 ml Solution B (Merck, Germany) was used. Then 1 ml sample was added to the mixture of Solution A and B that already were mixed well for 2-3 min. The samples were centrifuged at 8,000 rpm for 15 min. Potassium biphthalate ($KHC_8H_4O_4$, KHP) and distilled water were used as positive and negative controls, respectively. The samples were digested by Spectroquant®, series TR 320 (MERCK, Germany), subsequently the samples were measured by Spectroquant®, series NOVA 60 (MERCK, Germany).

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

Sludge taken from the bottom sampling port was measured for granular size, SS, VSS, SVI, and SMA. The granular size was measured by stage and ocular micrometers with magnitude of 1,500 times. The sludge pictures were digitalized and granular size was analyzed on Photoshop version 10. Moreover, the sludge samples were dried to determine SS then was ashed at 550°C to obtain VSS. To evaluate SVI value, the sludge samples were allowed to settle in 100 ml cylinder for 30 minutes (APHA *et al*., 1998).

The SMA test was conducted using 50 ml sludge sample cultured on 50 ml synthetic wastewater in a 120 ml serum bottle (Smolder *et al*., 1995). The synthetic wastewater contained 10 ml/l acetic acid in the following solution: 132 mg/l $(NH_4)_2SO_4$, 75.5 mg/l $NaH_2PO_4·H_2O$, 50 mg/l $CaCl_2·2H_2O$, 90 mg/l $MgSO_4·7H_2O$, 10 mg/l yeast extract, 0.3 ml/l nutrient solution. The nutrient solution consisted of 1.5 g/l $FeCl_3·6H_2O$, 0.15 g/l H_3BO_3, 0.13 g/l $CuSO_4·5H_2O$, 0.18 g/l KI, 0.12 g/l $MnCl_2·4H_2O$, 0.06 g/l $Na_2MO_4·2H_2O$, 0.12 g/l $ZnSO_4·7H_2O$, 0.15 g/l $CoCl_2·6H_2O$ and 10 g/l EDTA. The biogas production was passed through a 2 M NaOH solution to adsorb CO_2 then, the remaining CH_4 was measured over a 10-h period. The SMA was calculated from the slope of the methane production curve, divided by the content of sludge solid (g), and expressed as gCOD/gVSS per day (theoretical 350 ml of CH_4 produced from 1 gCOD).

All data were analyzed by using the data analysis toolbox in SPSS for window 11.0 software. Statistical significance (p = 0.05) of the experimental data was tested using one way ANOVA statistical program.

3. Results and Discussion

3.1 Effect of $AlCl_3$ and $CaCl_2$ on sludge granulation

Five treatments; 150 mg/l of $AlCl_3$, 300 mg/l of $AlCl_3$, 150 mg/l of $CaCl_2$, 300 mg/l of $CaCl_2$, and no chemical (control) addition were conducted at room temperature (30$\pm$2°C). The results show that the size of granules increased from initial pinpoint size to reach 0.8 mm within 28 days. The granules with diameters of 0.5 mm < d < 0.8 mm was found visibly in 150 and 300 mg/l of $AlCl_3$, 150 and 300 mg/l of $CaCl_2$ and no chemical addition at 21, 14, 21, 28 and 21 days, respectively (Figure 2). Moreover, the sludge volume index (SVI) in 300 mg/l of $AlCl_3$ addition had significantly a

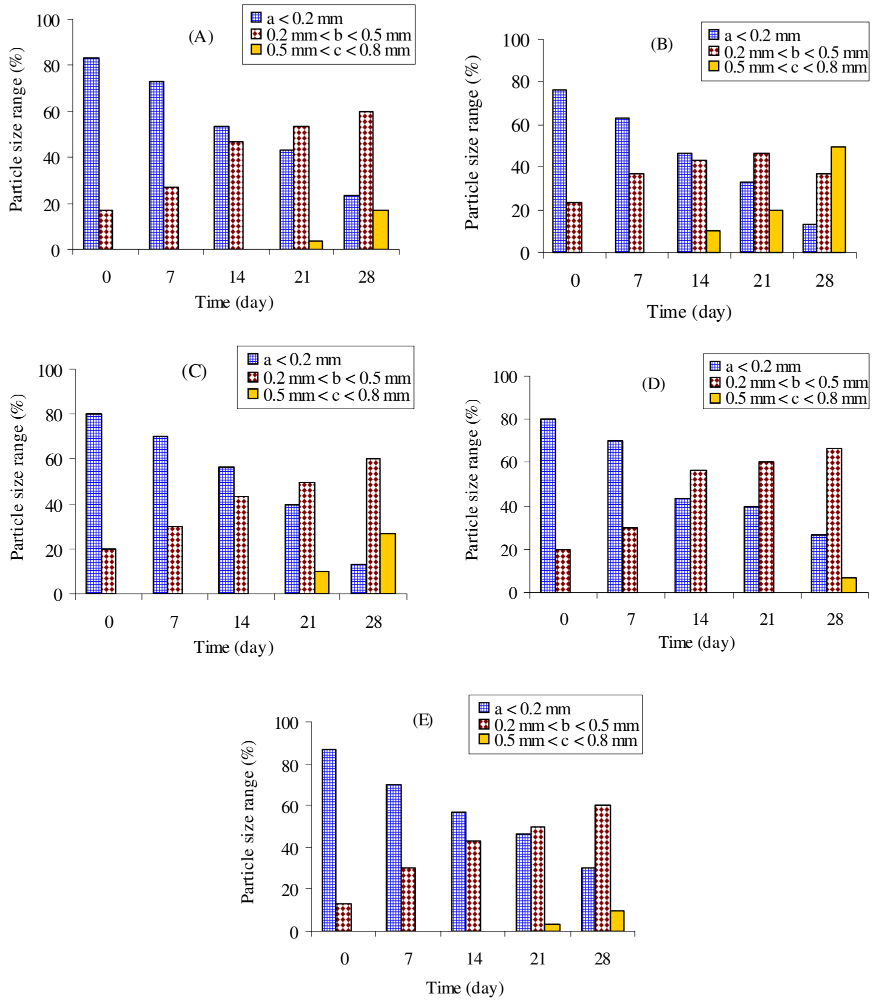


Figure 2. Effect of $AlCl_3$ and $CaCl_2$ on size distributions of granules. (A) $AlCl_3$ 150 mg/l; (B) $AlCl_3$ 300 mg/l; (C) $CaCl_2$ 150 mg/l; (D) $CaCl_2$ 300 mg/l and (E) the control (no chemical addition).

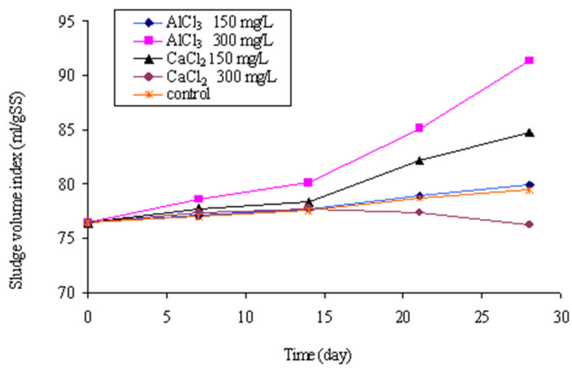


Figure 3. Effect of $AlCl_3$ and $CaCl_2$ on SVI.

higher SVI value (91 ml/gMLSS) than others after 14 days (Figure 3). In addition, $CaCl_2$ 150 mg/l supplement can promote to increase density and precipitation property. Nevertheless, the addition of $CaCl_2$ 300 mg/l had slower granulation and lower SVI comparing to all experiments (Figure 3). However, the $AlCl_3$ and $CaCl_2$ addition were significantly no influence on pH and gas production. As shown in Figure 4, the biogas production decreased during day 1 to 7 and then increased after being refilled with fresh wastewater.

In addition, the SMA values ranged from 0.07-0.14 gCOD/(gVSS-d) at the end of experiment. The highest SMA was found with the presence of $AlCl_3$ 300 mg/l (0.14 gCOD/(gVSS-d)) (Table 2). Furthermore, the biomass concentration in terms of MLVSS increased in parallel with the addition of $AlCl_3$ and $CaCl_2$, except $CaCl_2$ of 300 mg/l. The high ratio of VSS/SS was found in the treatments with $AlCl_3$ of 300 mg/l (VSS/SS = 0.82) and $CaCl_2$ of 150 mg/l (VSS/SS = 0.78). This result indicated that more microorganisms accumulated in the system with suitable concentration of trace element. Besides, the COD removal in the treatments with $AlCl_3$ and $CaCl_2$ addition had more COD removal efficiency than treatment with no chemical addition. The highest COD removal (61%) was found in the treatment with $AlCl_3$ of 300 mg/l supplement (Table 2).

Correspondingly, this study found that $AlCl_3$ of 300

Table 2. Effect of $AlCl_3$ and $CaCl_2$ addition on % COD removal, VSS, SS and SMA at 30 days.

Experiment	COD removal(%)	VSS(g/l)	SS(g/l)	SMA(gCOD/(gVSS-d))
$AlCl_3$ 150 mg/l	59 ± 0.33^{b}	0.85 ± 0.04^{b}	1.15 ± 0.04^{b}	0.09 ± 0.00^{bc}
$AlCl_3$ 300 mg/l	61 ± 0.19^{a}	1.08 ± 0.04^{a}	1.32 ± 0.08^{a}	0.14 ± 0.01^{a}
$CaCl_2$150 mg/l	$61\pm0.28b^{a}$	1.06 ± 0.02^{a}	1.36 ± 0.03^{a}	0.10 ± 0.01^{b}
$CaCl_2$ 300 mg/l	57 ± 0.39^{c}	0.49 ± 0.03^{c}	0.72 ± 0.03^{c}	0.07 ± 0.01^{d}
control	56 ± 0.61^{c}	0.41 ± 0.03^{c}	0.72 ± 0.03^{c}	0.08 ± 0.00^{c}

Note: 1. Average initial COD = 4.0 g/l and Average initial SS = 0.37 g/l
2. Means with the same letter are not significantly different at $p<0.05$ according to ANOVA statistical analysis

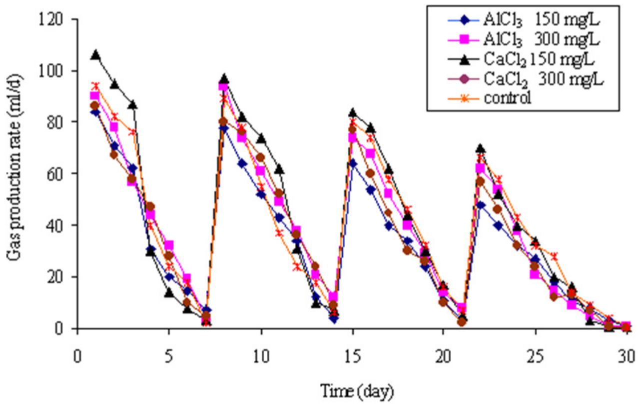


Figure 4. Effect of $AlCl_3$ and $CaCl_2$ on biogas production.

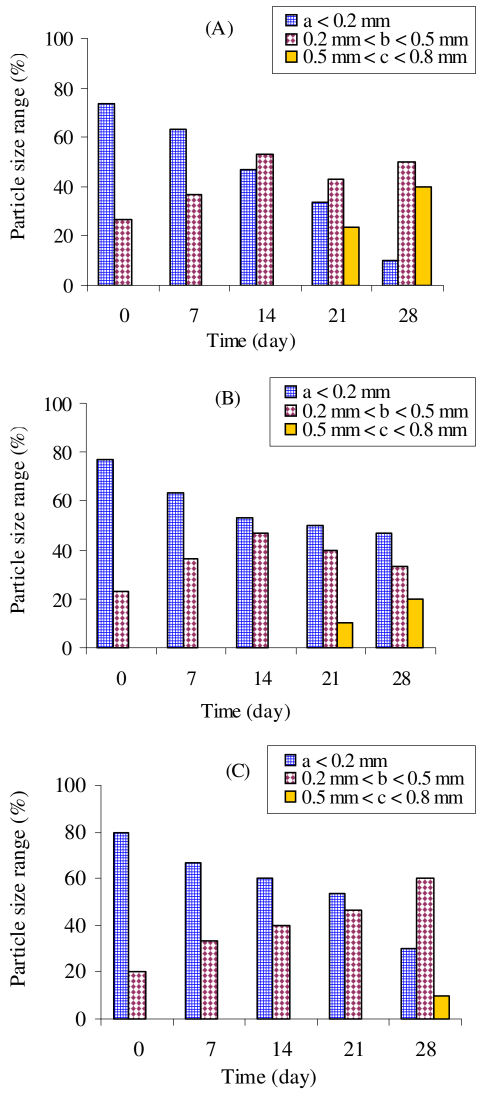


Figure 5. Effect of temperatures on size distributions of granules. (A) 30°C (B) 37°C and (C) 45°C.

mg/l had a positive influence on sludge granulation process by reduction time to achieve a larger size and increasing COD removal, concentration of biomass and methanogenic activity. This result was agreement with previous study by Yu *et al*. (2001a). They found that $AlCl_3$ of 300 mg/l in the synthetic wastewater (4,000 mg/l) enhanced the sludge granule process in the UASB by allowing aggregates to form faster and to achieve a larger size, resulted in a shortened start-up period for UASB reactors (Yu *et al.,* 2001a).

We also found that the addition of $CaCl_2$ of 150 mg/l gave a benefit on sludge granulation process. Conversely, $CaCl_2$ of 300 mg/l slightly induce granulation. This may cause from high concentration of Ca^{2+} obtained from CaO (the concentration of 500-600 mg/l) for pH adjustment. This result was contradictory from Yu *et al.* (2001b). They reported that the optimum calcium concentration for the granulation was from 150 to 300 mg/L using synthetic wastewater (4,000 mg/l). However, some researchers found that the presence of calcium concentrations at 100-200 mg/l had a positive impact on granulation (Cail and Barford, 1985; Mahoney *et al*., 1987). In contrast, some researchers reported that calcium did not promote granulation and may be detrimental to granule formation at high calcium concentration of over 500 mg/l (Alibhai and Forster, 1986; Thiele *et al*., 1990) or 600 mg/l (Yu *et al*., 2001b). Therefore, the effect of $CaCl_2$ may depend on characteristics of wastewater.

The result indicated that $AlCl_3$ seem more effective for granulation process than $CaCl_2$. Liu *et al*. (2003) reported that bacteria have negatively charged surfaces under usual pH conditions. The introduction of multi-valence positive ion leads to reduce electrostatic repulsion between negatively charged bacteria and consequently promote anaerobic granulation. The valence of $AlCl_3$ (Al^{3+}) was more than $CaCl_2$ (Ca^{2+}). This may be the explanation for the efficiency of $AlCl_3$.

3.2 Effect of temperature on sludge granulation

The treatments with 300 mg/l $AlCl_3$ addition were conducted at room temperature (30±2°C), 37°C and 45°C. The granule size distributions are shown in Figure 5. At the end of experiment, the granules with diameters of 0.5 mm < d < 0.8 mm was found more than 40% at room temperature but less than 20% at 45°C. Moreover, SVI in room temperature condition (87 ml/gSS) was significantly higher than others (Figure 6). Veiga *et al*. (1997) found that the production of extracellular polymers (ECPs) in methanogenic granules at 30°C was 1.5 times higher than at 42°C. Liu *et al.* (2003) reported that ECPs could change the surface negative charges of the bacteria, and thereby bridge neighbor cells physically to each other as well as with other inert particulate matters. This may be the explanation why the sludge at room temperature was found larger size and provided a better settling property than at 45°C.

However, the biogas production at 45°C was higher than at room temperature and 37°C (Figure 7). At 30 days, the COD removal efficiency increased with higher temperature. The COD removal efficiencies at room temperature, 37°C and 45°C were 59, 62 and 68%, respectively (Table 3). The results from this work corresponded to other works. Generally, the reaction rates in the thermophilic condition proceed faster than under mesophilic conditions (Yu *et al.*, 2002; Ahn and Forster, 2002a). Ahn and Forster (2002b) found that the COD removal efficiency and biogas production at 45°C was higher than at 35°C. Moreover, biomass concentration (in terms of VSS) at 45°C was significantly lower than at room temperature and 37°C. At higher temperature, the conversion of substrate to products was notably more favorable than biomass accumulation. SMA at 45°C was slightly lower than at room temperature and 37°C (Table 3). Ahn and Forster (2002b) reported that methane yield increased with increasing temperature from 35 to 45°C but decreased with increasing temperature from 45 to 55°C. Similarly, Yu *et al*. (2002) showed that the percentage of methane at 55°C was lower than at 37°C with organic loading rate of 8-24 gCOD/(l-d). From the larger sizes of granule, higher SVI and SMA obtained at room temperature, the operation at room temperature was indicated to be the optimum temperature for sludge granulation although the higher temperature gave more COD removal.

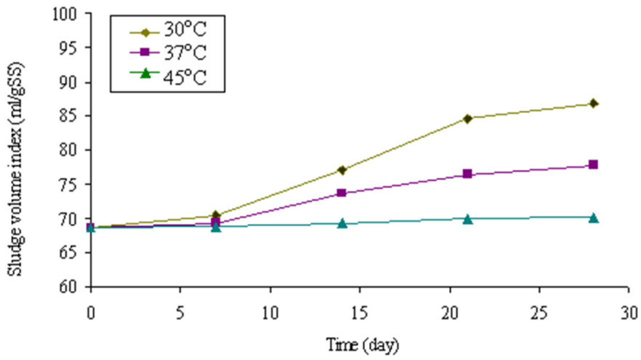


Figure 6. Effect of temperatures on SVI.

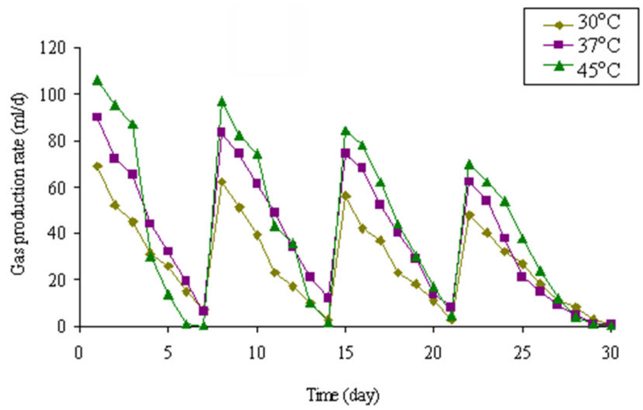


Figure 7. Effect of temperatures on biogas production.

3.3 Performance of UASB system

Three UASB reactors seeded with different inocula; granular sludge (R1), non-granular sludge (R2) and non-granular sludge with 300 mg/l of $AlCl_3$ addition (R3) were operated concurrently for 70 days at start-up period. The results show that the granules with diameter of d > 0.8 mm were found visibly in R2 and R3 at day 63 and 35, respectively. At the end of the experiment, the granules with diameter of d > 0.8 mm from R1, R2, and R3 were 70, 20, and 47% (Figure 8). In addition, SVI of sludge from R3 with $AlCl_3$ supplement increased rapidly during 45 to 70 day and was significantly higher than SVI of sludge from R2 without

Table 3. Effect of temperatures on % COD removal, VSS, SS and SMA at 30 days.

Experiment	COD removal(%)	VSS(g/l)	SS(g/l)	SMA(gCOD/(gVSS-d))
room temperature	59±0.21^c	0.80±0.04^a	1.03+0.03^a	0.12±0.00^a
37°C	62±0.24^b	0.68±0.03^a	0.94±0.04^a	0.12±0.00^a
45°C	68±0.24^a	0.38±0.03^b	0.58±0.03^b	0.10±0.00^b

Note: 1. Average initial COD = 4.0 g/l and Average initial SS = 0.37 g/l
2. Means with the same letter are not significantly different at $p<0.05$ according to ANOVA statistical analysis

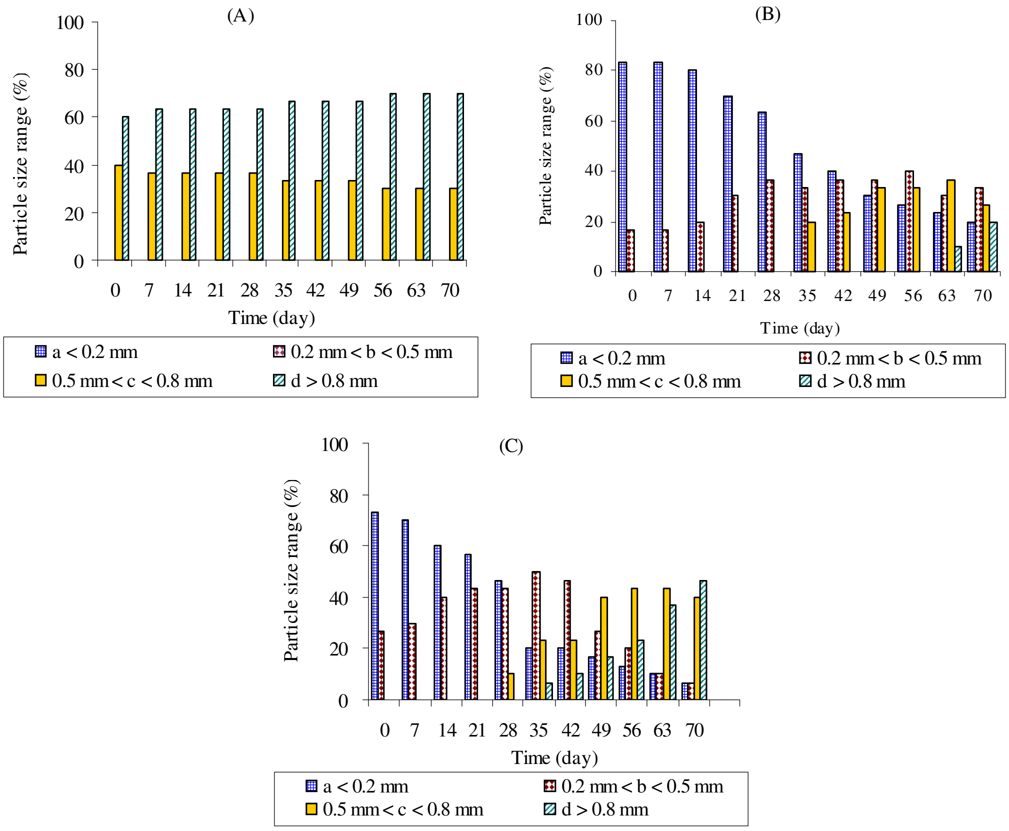


Figure 8. Size distributions of granules in the UASB reactors seeded with different inocula. (A) granular inoculum, R1 (B) non-granular inoculum, R2 and (C) non-granular inoculum with 300 mg/l $AlCl_3$ supplement, R3.

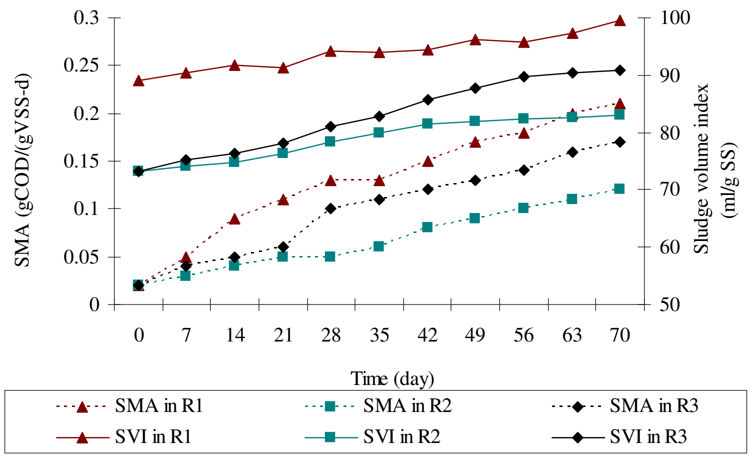


Figure 9. SMA and SVI values of sludges in the UASB reactors seeded with difference inoculum; granular inoculum (R1), non-granular inoculum (R2) and non-granular inoculum with 300 mg/l $AlCl_3$ supplement (R3).

chemical addition (Figure 9). As well as SMA, the sludge from R3 gave the methane activity higher than the sludge from R2 (Figure 9). However, the SMA obtained from this experiment was lower than the SMA from the experiment reported by Yu *et al*. (2001a and b). The different of initial SMA and characteristics of wastewater may influence on SMA at steady state. Yu *et al*. (2001a and b) investigated the granulation in synthetic wastewater with the initial SMA of 0.26. The initial SMA in this experiment was 0.02 gCOD/(gVSS-d). As well as, the concentrated latex wastewater (sulfate-rich wastewater) may result in the competitive between sulfate reducing bacteria and methanogen.

The COD removal efficiencies of the three reactors are illustrated in Figure 10. The result shows that the COD removal in experiment with granule inoculum (R1) could reach steady state at 40 days while the COD removal in

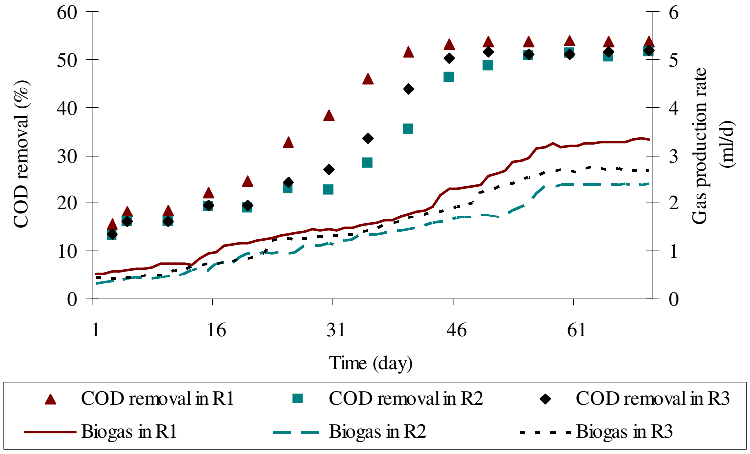


Figure 10. COD removal and biogas production in the UASB reactors seeded with difference inoculum; granular inoculum (R1), non-granular inoculum (R2) and non-granular inoculum with 300 mg/l $AlCl_3$ supplement (R3).

experiment with non-granular inoculum (R2 and R3) could reach steady state at 60 and 45 days, respectively. Addition, the pH was 7.15-7.50 and alkalinity was about 3,000-3,500 mg $CaCO_3$/l during the steady state (data not shown). This indicated that the stability was found at the steady state. Moreover, it was found that the maximum biogas productions were obtained at 3.34, 2.40 and 2.65 l/day for R1, R2 and R3, respectively at the end of operation (Figure 10). These results indicated that the presence of $AlCl_3$ 300 mg/l had enhanced granulation with good settleability and shorten time to obtain large granular size. Moreover, $AlCl_3$ addition can promote to reach steady state earlier.

The better performance on SMA, SVI, COD removal and biogas production was found in the experiment seeded with granular sludge (R1). However, the non-granular anaerobic sludge with $AlCl_3$ addition have potential to used as inoculum during start-up of UASB system when difficult to find granular sludge to be the inoculum source.

4. Conclusions

The $AlCl_3$ supplement to concentrated latex at concentrations of 150 and 300 mg/l enhanced the granule formation and biomass accumulation. Also, $CaCl_2$ of 150 mg/l gave a benefit on sludge granulation process. However, $CaCl_2$ of 300 mg/l did not stimulate granulation. The $AlCl_3$ at concentration of 300 mg/l was found to be the optimum chemicals addition to develop granule. Furthermore, increasing temperature did not promote granular size. The sludge at room temperature was larger size and better settling property than at 45°C. However, the biogas production, COD removal efficiency at 45°C was higher than at room temperature and 37°C.

In the UASB reactors inoculated with non-granular sludge and 300 mg/l of $AlCl_3$ addition gave larger granular size and higher SVI than experiment with non-granular sludge and no chemical addition, even though the experiment with seeded granular sludge gave a better performance than using non-granular sludge. In the case of granular sludge limitation, the non-granular anaerobic sludge with $AlCl_3$ addition may be the alternative inoculum for UASB start-up.

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