

Adaptation and Nucleation of Anaerobic Granules under Synthetic and Natural Polymers

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Abstract. Nucleation or granule nuclei formation is an important step in the formation of anaerobic granules. This research was aimed to investigate the formation and time for granule nuclei under the addition of synthetic and natural cationic polymers. In addition, the increase activity of syntroph microorganism in granule nuclei by adaptation with volatile fatty acid (VFA) was also studied. Three UASB reactors were used namely R1, R2 and R3 as control, addition of synthetic and natural polymers, respectively. Three reactors were operated under identical conditions. VFA was used as main substrate to adapt seed sludge and built syntroph microbial nuclei. Based on size distribution, nucleation was achieved at day 69 in R2 with average diameter size about 126 μm , size distribution 50.7% of $>100 \mu\text{m}$ -aggregates and SVI 42 $\text{ml.g}^{-1}\text{SS}$. The activities of specific non-methanogens and methanogen in reactors added polymers (R2 and R3) were lower than control reactor. However, adaptation of specific microorganism succeed due to their activities from methanogens, propionate- and butyrate- degrading microorganism were higher than initial activities. R2 showed as better cationic polymer compared to chitosan for shortening nucleation phase.

Keywords: Cationic polymer, syntroph, nucleation, aggregates, anaerobic granules

1. Introduction

The formation of granule is multistep processes involving physicochemical and biological forces resulting on the formation of multi layered granules (Hulshoff Pol et al. 2004; Liu and Tay 2004). Multi layered anaerobic granule consisted several layers in which each layer is occupied by specific microorganism (Batstone et al. 2004; Satoh et al. 2007). Fewer researches consider on the investigation of the formation of multi layered anaerobic granules since researches mostly investigated the characteristics of naturally formed multi layered anaerobic granules which were naturally formed 1 – 2 years. Some researchers divided granulation into two steps namely nuclei formation (nucleation) and nuclei maturation (Liu et al. 2003; Zheng et al. 2006). They concluded that nucleation is considered as the main essential step and starting point for granulation.

Mass culturing of anaerobic granules is needed to provide good anaerobic granules which leads to reduce the start-up phase of industrial scale anaerobic reactor and cost of anaerobic granules seeds. However, start-up period which is related to the formation of anaerobic granules can be approximately 2-8 months (Zhou et al. 2006). Mature granules from other reactors are normally used to shorten start-up period. However, transportation and availability of this mature granule can also be drawback factors. Therefore, non-granular

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anaerobic was frequently used as seeding sludge for reactor since it is affordable and largely available (Bhunja and Ghangrekar 2008; Show et al. 2004).

Consider to the time for constructing multi layered anaerobic granules, this research was focused on the main step of nuclei formation in granulation process. Nuclei formation can be shortened by the addition of synthetic and natural polymers. Adaptation of seed sludge with mixed volatile fatty acid (VFA) during nucleation was conducted since syntroph microbial nuclei was preferred for its superior characteristics of substrate degradation and granules size development (El-Mamouni et al. 1997). The effect of synthetic and natural polymers on nucleation process was elucidated. Microbial characteristics such as sludge morphology, size distribution of aggregates, sludge volume index (SVI), suspended solid (SS) and volatile suspended solid (VSS) and specific microbial activity were also investigated.

2. Material and Methods

2.1. Microorganism Seed and Wastewater

Initial seed sludge was obtained from open pond of cassava wastewater treatment plant in Chonburi Province, Thailand. Seed sludge was then sieved by 100- μm sieving instrument resulting average initial seed sludge was approximately 46 μm . Wastewater used in this research was synthetic wastewater adopted from (Smolders et al. 1994). The composition of synthetic wastewater were $(\text{NH}_4)_2\text{SO}_4$ 132 mg.l^{-1} , Na_2HPO_4 75.5 mg.l^{-1} , CaCl_2 50 mg.l^{-1} , MgSO_4 90 mg.l^{-1} , yeast extract 10 mg.l^{-1} and nutrient solution 0.3 ml. Mixed VFA was used as main carbon source consisted of acetate, propionate and butyrate (ratio 2:1:1) based on COD.

2.2. Cationic Polymer and Reactors

Medium molecular weight polyacrylamide 8265 (MEM 8265) and chitosan were used as synthetic and natural polymers, respectively. Optimum addition of polymers were based on Jar test (El-Mamouni et al. 1998; Resmanto 2010). The addition of polymers were approximately 2 $\text{mg.g}^{-1}\text{SS}$ and 13 $\text{mg.g}^{-1}\text{SS}$ for MEM 8265 and chitosan, respectively. Polymer additions were arbitrarily conducted every increase of OLR resulting four times addition of polymers until the end of nucleation phase.

Upflow anaerobic sludge blanket reactors were used in this research with diameter 86 mm, height 860 mm and working volume 4.5 l. Sampling ports were provided along height of reactors. Recirculation line were built from up to bottom of each reactor providing high velocity circulation about 1 m.h^{-1} . UASB 1 (R1), UASB 2 (R2) and UASB 3 (R3) were reactors as control, MEM 8625 and chitosan addition, respectively.

2.3. Operation Condition for Nucleation

Nucleation phase was initiated by inoculation of seed sludge (11.44 g VSS.l^{-1} and 13.17 g SS.l^{-1}) in all reactors. The first addition of MEM 8265 and chitosan were added concomitantly with seed sludge inoculation. Thereafter, polymers were routinely added at every increase organic loading rate (OLR). Reactors were operated under intermittent condition to ease adaptation of microorganism to substrate. OLR of 0.5 $\text{kg COD.m}^{-3}.\text{d}^{-1}$ was firstly applied for all reactors. OLR in all reactors were stepwisely increased until 1.5 $\text{kg COD.m}^{-3}.\text{d}^{-1}$. Increase of OLR was aimed to adapt microorganism to specific substrate used in this research. Circulation of wastewater was applied from top to bottom reactor with velocity of 1 m.h^{-1} to provide proper shear force for granulation. Nucleation phase under different polymer was investigated for their average diameter of aggregates, nuclei ratio and nucleation time. Initial nuclei can be classified based on its size which is range between 100 – 600 μm (Zheng et al. 2006). Time for nuclei formation was investigated as its relationship with the increase of nuclei size. The nuclei phase was terminated until nuclei ratio achieve $\pm 50\%$ of total sludge (Wu et al. 2009).

2.4. Analysis

pH, alkalinity, total volatile acid (TVA) and COD of influent and effluent were analyzed daily. While, SS, VSS, SVI and specific microbial activities were analyzed every the end of OLR. pH, alkalinity, TVA, COD, SS, SVI and VSS were analyzed based on Standard Methods (APHA 2005). ECP was analyzed based on cold extraction (Zhou et al. 2007). Specific methanogenic and non-methanogenic activities were analyzed based on Soto et al. 1993. Microbial activity included the activities of glucose degrading microorganism – GDM, propionate – PAM and butyrate – BAM degrading microorganism and methnogen – MTN. Glucose

was analyzed using Glucose Analyzer YSI 7100 MBS. Gas chromatography (GC) Shimadzu 14B with flame ionization and thermal conductivity detectors were used to analyze propionate/butyrate and methane concentration, respectively. Size distribution was analyzed based on image processing of MATLAB 2009. Samples of aggregate were viewed and captured by stereomicroscope Olympus SZX12. The diameter size and distribution were then analyzed by image processing of MATLAB 2009. The morphology of microbial nuclei was analyzed by scanning electron microscopy (SEM) in order to know the morphology of dominant microorganism in nuclei (Batstone et al. 2004).

3. Results and Discussion

3.1. Reactor Performance

COD removal in R1 and R2 sharply declined to 40-50% after OLR was increased to $1.0 \text{ kg COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$. However, COD removal of R3 tended to be stable at OLR $1.0 \text{ kg COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$. Low COD removals of R1 and R2 were caused by the accumulation of acids in reactors (data not shown). The decrease of COD removal (approximately 50-60% of COD removal) was also happened in R3 at day 40 when OLR was increased to be $1.5 \text{ kg COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$. However, COD removal of R3 increased again about 85% at day 55. R1 and R2 showed constant COD removal of 80-90% during operation of OLR $1.5 \text{ kg COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$.

TVA/Alkalinity ratio was higher in R1 and R2, approximately more than 0.5, at day 10 (increased OLR $1.0 \text{ kg COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$). While, TVA/alkalinity ratio in R3 was approximately lower than 0.4. TVA/alkalinity ratio less than 0.4 indicated that anaerobic reactor could accommodate minor fluctuations of pH and more than 0.4 indicated that the instability of anaerobic reactor could be expected (Behling et al. 1997). The decrease of COD removal can also be explained by VFA compositions inside reactor. From the data (not shown), VFA compositions of R1 and R2 at day 10 - 16 were approximately $1200\text{-}1700 \text{ mg}\cdot\text{l}^{-1}$ of acetic acid and $100\text{-}250 \text{ mg}\cdot\text{l}^{-1}$ of propionic acid. The TVA/Alkalinity ratio during reactor operation showed stable ratio between 0.2 – 0.5. Therefore, it can be concluded that there was no critical instability in all reactors until OLR $1.5 \text{ kg COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$.

3.2. Microbial Characteristics in UASB Reactors

VSS concentration represented biomass concentration inside reactor. VSS concentration decreased from initial condition until OLR $1.5 \text{ kg COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$, i.e., 64.2%, 63.1% and 49.2% for R1, R2 and R3 respectively. The utilization of mixed VFA as substrate seemed also affected on decreasing of biomass inside reactor due to slow generation time of VFA adapted microorganism such as butyrate-, propionate- degrading microorganism and acetoclastic methanogen compared with acidogenic microorganism (Gujer and Zehnder 1983). Chitosan showed possibility for retaining more biomass inside reactor although the tendencies of all reactor is similar.

Nucleation is initial and important step of anaerobic granulation. Granule nuclei was defined as aggregates with a diameter of $100 - 600 \mu\text{m}$ (Zheng et al. 2006). Therefore, size distribution of seed sludge is essential factor to be observed in this research. Average diameter size of initial sludge was approximately $46 \mu\text{m}$. At the end of nucleation phase (Table 1), R2 showed the highest size distribution of $>100 \mu\text{m}$ -aggregates approximately 50.7 % followed by R2 and R1 which showed approximately 40.0 and 24.1 %, respectively. It indicated that nucleation phase was achieved in R2 at day 69 or at the end of OLR $1.5 \text{ kg COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$. Mean aggregate size in R2 also higher compared to R3 and R1 as 126.0, 96.6 and $71.7 \mu\text{m}$, respectively. The effect of MEM 8625 and chitosan can be clearly seen after OLR $1.0 \text{ kg COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ since it showed not significant size distribution and mean aggregates size from initial until end of OLR $1.0 \text{ kg COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$. The addition of MEM 8625 could affect on nucleation by its faster nuclei formation and higher mean aggregate size. Wu, et al., (2009), investigated nucleation time of dispersed sludge without polymer addition for 146 days resulting average sludge diameter about $127.1 \mu\text{m}$. Synthetic polymers were used by several researchers to enhance granulation process which lead to shorten start-up period of reactor operation (El-Mamouni et al. 1998; Show et al. 2004; Uyanik et al. 2002).

Table 1: Size distribution and mean aggregate size at day 69 in all reactors

Reactor	Size distribution (%)		Mean aggregate size (μm)
	< 100 μm	> 100 μm	
R1	75.94	24.06	71.73
R2	49.03	50.69	126.02
R3	60.04	39.96	96.64

SVI represents the volume of sludge in ml to settle under current time (normally 30 minutes) with unit of ml settled sludge per mg suspended solids ($\text{ml.g}^{-1}\text{SS}$). Low SVI value shows better settling characteristics of granule. SVI value of initial seed sludge used in this research was very low as 75.93 $\text{ml.g}^{-1}\text{SS}$. All reactors showed decrease of SVI at day 69. The best SVI was found at R2 which showed as the lowest SVI of 42 $\text{ml.g}^{-1}\text{SS}$ among R1 and R3 as 68 and 60 $\text{ml.g}^{-1}\text{SS}$. It seemed that MEM 8625 showed the better performance to coagulate seed sludge than chitosan resulting on good settling of microbial aggregates. Chitosan seemed not to good as cationic polymer for granulation using mixed VFA as substrate since there were more dispersed microorganism in R3 which was showed by high turbidity of supernatant part during SVI analysis compared to other reactor.

3.3. Specific Microbial Activity

Microbial activity analyses were conducted in order to investigate the adaptation phase of seed sludge to specific substrate (mixed VFA) in order to built syntroph microbial nuclei. The activities of glucose-, propionate- and butyrate-degrading microorganism as well as methanogens were investigated at every end of OLR as shown in Table 2. Table 2: Specific microorganism activity in all reactors

Reactor	GDM		PAM		BAM		MTN	
	Initial	Day 69						
R1	2.93	1.96	0.24	1.14	0.20	1.25	0.12	0.25
R2	2.93	1.09	0.24	0.86	0.20	1.03	0.12	0.17
R3	2.93	1.35	0.24	0.62	0.20	0.93	0.12	0.15

- Activity of microorganism in unit of $\text{g COD.g}^{-1}\text{VSS.d}^{-1}$. GDM: glucose degrading microorganism, PAM: propionate degrading microorganism, BAM: butyrate degrading microorganism, MTN: methanogens

GDM represents acidogenic microorganism activity to degrade glucose and produce acids. From Table 2 above, it can be seen that GDM activity decreased from initial until day 69. GDM in R1, R2 and R3 decreased 33.1%, 62.8% and 53.9% from their initial activities, respectively. Adaptation of sludge to mixed VFA probably caused the decrease of GDM.

PAM activity increased from initial until day 69. PAM and BAM represent syntroph microorganism activity which degrade acids to acetic acid prior to be utilized by methanogen to produce methane and carbon dioxide. PAM activities in R1, R2 and R3 were 375%, 258% and 158% higher from their initial activities. BAM activities of all reactors also increased as 525%, 415% and 365% from initial activities in R1, R2 and R3, respectively.

MTN activity of all reactors slightly increased as 108%, 42% and 25% from initial activities. Slight increased of MTN activities compared to PAM and BAM activities were probably caused by the accumulation of acetate resulted from propionate and butyrate degradation which possibly inhibited or decreased methanogen activity. From the results of activity test, only GDM activity decreased from its initial activity because of the utilization of mixed VFA as main substrate. While other activities increased from their initial activity. However, the highest increase of PAM, BAM and MTN activities was observed in R1 as control reactor followed by R2 and R3. One of advantage of anaerobic granules is their effusion substrate degradation due to its complex microorganism consortia inside granules (Batstone et al. 2004; Satoh et al. 2007). Since nucleation is the beginning of granulation, microorganism consortia may not well established. It needs to be confirmed about the negative effects of accumulation of MEM 8625 and chitosan for microorganism (Wang et al. 2005).

SEM was used to analyze initial and nuclei morphology in order to know dominant microorganism in nuclei. Initial sludge consisted of filament, rod and cocci-shaped microorganism. Microorganism in R1 was

not different with initial microorganism which was dominated by cocci microorganism. However, different dominant microorganism was observed in R2 and R3 which long rod and filament shaped microorganism were dominant microorganism. Different dominant microorganism in R1, R2 and R3 were probably caused the dominant VFA inside reactors. The shape of nuclei in R2 and R3 were bigger than control reactor. However, the utilization of mixed VFA as main substrate caused irregular and rough nuclei due to lack extracellular polymer production by microorganism.

4. Conclusion

It was possible for the addition of cationic polymer to enhance nucleation phase as a part of granulation process. Nucleation with synthetic cationic polymer MEM 8625 showed shorter nucleation time, bigger nuclei size and high aggregates distribution $> 100 \mu\text{m}$ than chitosan. However, it is needed to be deeply elucidated about the optimum of addition of polymers since the activities of specific microorganism in reactors with polymer addition (R2 and R3) were lower compared to control reactor (R1). Mixed VFA as substrate can be used for adapt seed sludge and built syntroph microbial nuclei. However, based on SEM results, the shape of microbial nuclei may not too smooth and irregular due to lack of ECP production by microorganism

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