EFFECT OF SUBSTRATE FEEDING CONCENTRATION ON INITIAL BIOFILM DEVELOPMENT IN ANAEROBIC HYBRID REACTOR

B. Suraraksa*

Department of Environmental Technology, The Joint Graduate School
of Energy and Environment, King Mongkut's University of Technology
Thonburi (KMUTT), Tungkru, Bangkok 10140, Thailand

A. Nopharatana

Pilot Plant Development and Training Institute, King Mongkut's University
of Technology Thonburi (KMUTT), Tungkru, Bangkok 10140, Thailand

P. Chaiprasert, S. Bhumiratana

Department of Biotechnology, School of Bioresources and Technology,
King Mongkut's University of Technology Thonburi (KMUTT),
Tungkru, Bangkok 10140, Thailand

M. Tanticharoen

National Center of Genetic Engineering and Biotechnology,
National Science and Technology Development Agency (NSTDA),
Phaholyothin Rd., Klongluang, Pathumthani 12120, Thailand

Received 21 May 2003, Accepted 10 September 2003

ABSTRACT

To elucidate the effect of substrate concentration on biofilm development, glucose concentrations of 500 and 1,000 mg/L were used. At an early stage, biofilm development at both concentrations was not significantly different ($P=0.621$). After removing suspended biomass at 24 operational hours, the biofilm development at high substrate concentration was higher than at lower concentration. At 72 operational hours, the amounts of attached biomass at low and high glucose feeding were 9.04 ± 1.17 and 28.58 ± 2.72 g VSS/m², respectively. The activities of acidogens, acetogens, and methanogens at the low glucose concentration were 0.334, 0.016 and 0.003 g COD/g VSS/h, and those at the high glucose concentration were 0.145, 0.003 and 0.001 g COD/g VSS/h, respectively. Moreover, the ratio of methanogenic activity at low glucose concentration was higher than at high glucose concentration. The glucose utilization at low and high feeding concentrations was 33% and 27%, respectively. These results indicated that rapid

* Corresponding author-email address: benjaphon@pdti.kmutt.ac.th, benjaphon@biotec.or.th

biofilm development by using high substrate concentration would be less beneficial if unbalance of methanogenic ratio was found in biofilm.

Keywords : *Anaerobic hybrid reactor; biofilm; microbial activity; start-up; substrate concentration.*

1. INTRODUCTION

Anaerobic hybrid (AH) reactors are a high rate anaerobic reactor (HRAR) type which has the sludge bed at the bottom part and the supporting media at the upper part of the reactor. This type of reactor combines the advantages of the anaerobic filter (AF) and upflow sludge bed reactor (USB) and yields a better treatment efficiency compared to an UASB¹ reactor. Moreover, the supporting media in the AH reactor can help to increase the solids retention time by diminishing short circuiting, improving gas/solids/liquid separation, providing a surface for the attachment of biomass, and therefore resulting in a performance comparable with an AF reactor^{2,3,4}. Many researchers indicate that the higher amounts of media in the reactor positively affect the performance of the AH reactor^{2,3,5,6,7}. The results further suggest that the amount of attached biomass in the packed bed zone plays a vital role in stabilizing the entire system. Biofilm systems have several advantages above the conventional activated sludge systems due to their ability to support a variety of microbial populations.

In Thailand, this type of reactor has been developed for treating wastewater from rice starch factories, which contain high amount of organic substances and suspended solids. However, the slow growth of anaerobic bacteria on the supporting media is still one of the limiting factors resulting in a long start-up period. Therefore, the acceleration of the attached biomass growth on the supporting media might be the key to shortening the start-up period of this type of reactor. Nevertheless, there is a remaining problem for this reactor. Most nutrients were often found to be completely removed before entering the packed zone, resulting in limited nutrient availability for attached microbial growth^{7,8,9,10,11,12}. As such, a long start up period is observed.

Moreover, the microbial population in the biofilm is an important factor in anaerobic digestion because the performance of this system is dependent on a symbiosis of two microbial groups: non-methanogens and methanogens. Each microbial group is different with respect to its nutrition, physiology, and pH requirements; therefore, a balance of each microbial group leads to high biogas production. Several researchers have studied the microbial characteristics and populations in the sludge zone of AH reactors^{3,10,13}. Imai *et al.*¹³ studied the characteristics of sludge granules in the sludge zone of an AH reactor. They found that the AH reactor possessed the features of a two-phase anaerobic process, in which the two groups of bacteria were separated in different parts of the reactor. The upper part of the sludge zone contained a methane forming species, *Methanothrix*-like bacteria. The lower part of the zone contained acid forming species, *Bacilli*- and *Cocci*-like bacteria. There is, however, less research concerning the microbial population and characteristics of the biofilm on supporting media, especially anaerobic biofilms.

Therefore, this research aims to study the effects of substrate concentration on initial biofilm development, in terms of the amount of attached biomass and the ratios of microbial population.

2. MATERIALS AND METHODS

Reactors and supporting media:

Two acrylic cylindrical AH reactors (9.4 cm × 86.5 cm) with a total volume of 6 liters and working volume of 5.55 liters were used in the study. The AH reactor consisted of 2 zones: packed and suspended zones. The upper part of the reactor was packed with supporting materials from top to middle height, with the rest of the reactor acting as a sludge zone. Nylon fiber, 40 cm in length, was used as supporting material and was held inside the AH reactor with stainless steel wire. The specific area of the supporting material was 10,130 m²/m³. A schematic diagram of the AH reactor is shown in Figure 1.

Operation of the AH reactors:

The start-up of the AH reactors were carried out by using 5.5 g VSS/L anaerobic digestion sludge obtained from a tapioca starch factory. The synthetic wastewater, modified following

Figure 1 : Schematic flow chart of anaerobic hybrid reactor and nylon fiber contained in AH reactor.

Smolders *et al.*¹⁴ (Table 1), was continuously fed into the reactor inlet by a peristaltic pump at 1.0 m/h upflow feeding velocity, which can be calculated to transport microbial sludge of 0.1 mm diameter size from the sludge zone to the supporting materials¹⁵. To supplement the carbon source, glucose was added at concentrations of 500 and 1000 mg/L. All experiments were performed at ambient temperature (35±2°C). After being operated for 24 hours, the suspended

biomass in both reactors was removed and the attached microbial growth in the reactor was used to study the effect of substrate feeding concentration on biofilm development. Samples of wastewater and biomass in the reactors were taken every 12 hours at 20, 40, 60 and 80 cm reactor heights.

Table 1. The composition of synthetic wastewater

Composition of synthetic wastewater					
Glucose*			Nutrient Solution		
(NH ₄) ₂ SO ₄	132	mg/L	FeCl ₃ .6H ₂ O	1.500	mg/L
NaH ₂ PO ₄ .H ₂ O	75.5	mg/L	H ₃ BO ₃	0.150	mg/L
CaCl ₂ .2H ₂ O	50.0	mg/L	CuSO ₄ .5H ₂ O	0.130	mg/L
MgSO ₄ .7H ₂ O	90.0	mg/L	KI	0.180	mg/L
Yeast Extract	10.0	mg/L	MnCl ₂ .4H ₂ O	0.120	mg/L
Nutrient Solution	0.30	mL	Na ₂ MO ₄ .2H ₂ O	0.060	mg/L
			ZnSO ₄ .7H ₂ O	0.120	mg/L
			CoCl ₂ .6H ₂ O	0.150	mg/L
			EDTA	10.0	mg/L

*Remark : glucose concentrations of 500 and 1000 mg/L

Microbial activities in the AH reactors:

Samples of suspended and attached biomass taken from the reactor were left overnight to eliminate any remaining substrates. The microbial activities were tested in 75 ml glass bottles sealed with rubber septa retained with screw caps. Each bottle contained 50 ml of specific medium (pH 7.0±0.2) for each microbial group: 0.1% (w/v) glucose for acidogenic activity, 0.1% (v/v) propionic acid for acetogenic activity, 0.1% (v/v) acetic acid for acetoclastic methanogenic activity. When inoculating the sample into the specific media, the gas phase of the bottles was flushed with nitrogen gas. The bottles were then incubated at 37°C for 12 hours for acidogens, and 24 hours for both acetogens and acetoclastic methanogens. All activity tests were carried out in triplicate.

The specific maximum activities (g COD/g VSS/d) of acidogens, acetogens and acetoclastic methanogens were estimated as the rate of glucose degradation and as the rate of acid (propionate and acetate) degradation, respectively¹⁶.

Analytical method:

To analyze substrate utilization, the glucose concentration was measured by using a lactate-glucose analyzer (model YSI 2300 STAT PLUS, QUASAR). Volatile fatty acids were analyzed by using gas chromatography (model GC 14B, SHIMADZU) fitted with a hydrogen flame ionization detector. The amounts of suspended and attached biomass in the reactors were determined by measuring volatile suspended solids (VSS). Before quantification, the biomass attached to the supporting media was sloughed off by sonication for 10 minutes¹⁷. After that, the samples were dried overnight at 105°C and then placed in a furnace at 550°C for 3 hours. The VSS was estimated as the difference between the dried and burned masses¹⁸.

Statistical analysis:

The mean rates of biomass quantification data were compared using a single factor analysis of variance (ANOVA). Significance was determined at the 95% level of the confidence limit ($P=0.05$). To assess any significant difference of biofilm development at the low and the high substrate concentration in feeding, the analysis of variance was followed by a t -test (95% confidence limit).

3. RESULTS AND DISCUSSION

Effect of substrate concentration on the quantity of biofilm in AH reactor

Two AH reactors were continuously fed with glucose concentrations of 500 and 1000 mg/L at an upflow feeding velocity of 1.0 m/h. Figure 2 shows that attached biomass on supporting media at both concentrations increased continuously with time. During the first 24 hours, the quantity of attached biomass in both reactors was not significantly different ($P=0.621$). After suspended biomass was removed for study of the attached microbial development, the attached biomass concentration was obviously different between the low (500 mg/L) and high (1000 mg/L) glucose concentrations ($P=0.0013$). A rapid colonization was observed at the high glucose concentration. This result was due to high substrate availability for attached biomass growth. At 72 hours, the quantity of attached biomass at the low and high substrate concentrations was 9.04 ± 1.17 and 28.58 ± 2.72 g VSS/m², respectively.

Figure 2 : Comparison of biofilm development on supporting media when using glucose concentration of 500 and 1000 mg/L upflow feeding velocity.

Figure 3 shows a profile of suspended biomass through the reactor height at low and high substrate supplement during the first 24 operational hours. The result of both reactors was not

Figure 3 : Profile of suspended biomass concentration throughout the reactor height: (a) glucose concentration of 500 and (b) glucose concentration of 1,000 mg/L.

significantly different ($P=0.8324$). The suspended biomass in the packed zone increased with time, as did the attached biomass. The correlation between suspended biomass in the packed zone and attached biomass on the supporting media in both reactors was determined to be linear (Figure 4). This indicated that biofilm development in this period was mainly governed by microbial attachment. After taking off the suspended biomass at 24 hours, glucose transported to the packed zone was increased, resulting in high available substrate for attached microbial growth. In addition, glucose utilization in the packed zone at both reactors increased with time. A glucose concentration of 240 mg/L was utilized in the packed zone at the high glucose concentration while only 90 mg/L glucose was used at the low concentration. The microbial growth yield of attached biomass at the low and the high glucose concentrations was 0.02 kg VSS/kg COD_{re}. As a result, a high quantity of attached biomass was found at the high glucose concentration because of high available substrate for microbial growth. This indicates clearly that the substrate concentration in the packed zone is an essential factor for biofilm development in term of attached microbial growth.

Figure 4 : Correlation between attached biomass on supporting media and suspended biomass in bulk liquid.

Effect of substrate concentration on the quality of biofilm in AH reactor

Apart from the biomass concentration, the ratio of each microbial group is an important factor in anaerobic digestion leading to biogas production. The three main microbial groups involved in biogas production are: acidogens, acetogens, and acetoclastic methanogens. Figure 5 shows the specific microbial activity of attached biomass at the low and the high glucose concentration. When biofilm development in both reactors was governed by growth (after 24 operational hours), the activity of acetogens and methanogens was dramatically decreased, whereas the acidogenic activity was almost constant. Moreover, the specific microbial activity at the low glucose concentration was found to be more active than at the higher concentration. After 72 operational hours, the activity of acidogens, acetogens, and methanogens at the low glucose feeding concentration was 0.334, 0.016 and 0.003 g COD/g VSS/h, respectively, whereas at the high concentration the activity was 0.145, 0.003 and 0.001 g COD/g VSS/h, respectively.

Wanner and Gujer¹⁹ and Lens *et al.*²⁰ reported that the acidogenic bacteria (fast growing microorganisms) were mainly located in the outer layer, while the acetogens and methanogens (slow growers) were deeper inside the biofilm, scavenging substrate from the fast growers. Also the contact time between substrate and microbial cells at 1.0 m/h upflow velocity (0.03 day of HRT) was short, resulting in low growth of microorganisms inside the biofilm. As a result, a rapid decrease in acetogenic and methanogenic activities was the effect of substrate diffusion. Furthermore, biomass was determined as volatile suspended solid, which included microbial cells and extracellular polysaccharides – it is a characteristic of biofilm growth to assist attachment²¹. Higher slime production resulted in a smaller the fraction of microbial cells in the biofilm²². Moreover, this could be a barrier between the substrate and the microbial cells

Figure 5 : Comparison of the specific activity of trophic microbial groups of attached biomass on supporting media using glucose concentrations of 500 and 1000 mg/L: (a) acidogenic activity, (b) acetogenic activity, and (c) methanogenic activity.

on the supporting media, resulting in substrate diffusion. Jia *et al.*²³ noted that acidogenesis produced more exopolymer than acetogenesis and methanogenesis. Since a high glucose concentration stimulates acidogenesis and exopolymer production, the specific microbial activity at the high glucose concentration was lower than at the low glucose concentration.

The ratio of specific microbial activity of each group in biofilm was further determined to compare the effect of the substrate concentration. It was found in Figures 6 and 7 that the ratio of acidogenic activity in both reactors increased with time, while the ratio of acetogens and methanogens decreased. This result was due to substrate diffusion and the short contact time between substrate and microorganisms (0.03 day of HRT) affecting growth of acetogenic and methanogenic bacteria, which were located inside the biofilm. Zhang and Noike²⁴ reported that the acetic-utilizing methanogens were unable to exist at short HRT, because the doubling time of this microbial group was about 12 hours. Besides the acidogenic bacteria, rapid growing bacteria, was located in the outer biofilm. Therefore, an increase of acidogenic activity in biofilm was found. Furthermore, the high glucose concentration in the feed supported acid-forming bacteria growth. As a result, the ratio of the acidogenic population at the high substrate concentration was higher than at the lower concentration. This result indicates that a low glucose feeding concentration could maintain the methanogenic activity better than a high concentration

Figure 6 : Ratio of specific activity of trophic microbial groups in the biofilm at 24, 36, 48, 60 and 72 operational hours at low glucose concentrations.

Performance of biofilm on substrate reduction

Figure 8 shows that the efficiency of glucose utilization at both reactors decreased with time. This result relates to the ratio of methanogenic activity in the biofilm, which is shown in Figures 6 and 7. Low methanogenic activity resulted in low glucose reduction. Although a high glucose

Figure 7 : Ratio of specific activity of trophic microbial groups in the biofilm at 24, 36, 48, 60 and 72 operational hours at high glucose concentration.

Figure 8: Glucose utilization in the reactor when feeding with glucose concentrations of 500 and 1000 mg/L.

concentration in feeding promoted a high quantity of biofilm (Figure 2), the activity of methanogenic bacteria was low (Figure 5c). Therefore, glucose utilization at the low feeding concentration was higher than at the high glucose concentration in spite of a lower quantity of biofilm. After 72 hours, the glucose utilization at the feeding glucose concentration of 500 and 1000 mg/l was 33% and 27%, respectively. This result showed that the relative number and the balance ratio of methanogenic activity in the biofilm would be more beneficial than rapid biofilm development.

4. CONCLUSIONS

The overall results show that substrate concentration in feeding affects the biofilm growth on supporting media. Though attachment is a prerequisite, the subsequent growth of attached cells is a dominant process for rapid biofilm development. A higher substrate concentration results in lower microbial activities, even with higher biomass production. It might be that the biomass composition at higher concentrations of substrate contains higher exopolymer on the supporting media. Furthermore, the microbial activity in the biofilm is another factor for controlling the performance of the reactor. High ratios of acetogenic and methanogenic activities in the biofilm at low substrate feeding, result in high glucose utilization in spite of the low amount of attached biomass. These results show that both the quantity and the quality of the attached biomass are important factors for controlling the stability of the reactor. As a result, the substrate concentration in influent should be optimized to suit the attached microbial growth and the microbial activity.

REFERENCES

1. Elmitwalli, T.A., Zandvoort, M.H., Zeeman, G., Bruning, H. and Lettinga, G., (1999), *Water Science and Technology*, vol. 39, no. 5, pp. 177-185.
2. Oleszkiewicz, J.A. and Thadani, V.J., (1988), *Environmental Technology Letters*, vol. 9, pp. 89-100.
3. Chung, Y.C. and Choi, Y.S. (1993), *Water Science and Technology*, vol. 27, no. 1, pp. 187-194.
4. Jahren, S.J., Rintala, J.A. and Odegaard, H. (1999), *Water Science and Technology*, vol. 40, no. 11-12, pp. 67-75.
5. Oleszkiewicz, J.A., Hall, E.R. and Oziemblo, J.Z. (1986), *Environmental Technology Letters*, vol. 7, pp. 445-452.
6. Kennedy, K.J., Gorur, S.S., Elliott, C.A. and Andras, E. (1989), *Water Research*, vol. 23, no. 11, pp. 1397-1405.
7. Suraraksa, B., Wangnai, C., Chaiprasert, P., Tanticharoen, M., and Bhumiratana, S. (1998), "Performance of an Anaerobic Hybrid Reactor Combining a Filter and a Sludge Bed. In: *Biotechnology for a Self Sufficient Economic*," In: *Biotechnology for a Self Sufficient Economic*, The 10th Annual Meeting of the Thai Society for Biotechnology and Meeting of the National Center for Genetic Engineering and Biotechnology, Bangkok, Thailand, pp. 96.
8. Guiot, S.R. and van den Berg, L. (1984), *Biotechnology Letters*, vol. 6, no. 3, pp. 161-164.

9. Guiot, S.R. and van den Berg, L. (1985), *Biotechnology and Bioengineering*, vol. 27, no. 6, pp. 800-806.
10. Fang, H.H.P. and Kwong, T.S. (1995), *Environmental Technology*, vol. 16, pp. 13-23.
11. Borja, R., Alba, J. and Banks, C.J. (1996), *Process Biochemistry*, vol. 31, no. 3, pp. 219-224.
12. Wu, M., Wilson, F., and Tay, J. H. (2000), *Bioresource Technology*, vol. 71, pp. 151-157.
13. Imai, T., Ukita, M., Sekine, M., Nakanishi, H. and Fukagawa, M. (1998), *Water Science and Technology*, vol. 38, no. 8-9, pp. 377-384.
14. Smolders, G.J.F., Van der Meij, J., Van Loosdrecht, M.C.M. and Heijnen (1994), *Biotechnology and Bioengineering*, vol. 43, no. 6, pp. 461-470.
15. Noiklueb, C., Chaiprasert, P., Tanticharoen, M., and Bhumiratana, S., 1998, "Effect of Flow Rate and Mode of Recirculation on Biofilm Development during Start-up Process in an Anaerobic Fixed-Film Reactor," In: *Biotechnology for a Self Sufficient Economic, The 10th Annual Meeting of the Thai Society for Biotechnology and Meeting of the National Center for Genetic Engineering and Biotechnology*, Bangkok, Thailand, p. 97.
16. Araki, N. and Harada, H. (1994), *Water Science and Technology*, vol. 29, no. 10-11, pp. 361-368.
17. Poulsen L.K. (1995), "Protocol for the use of Fluorescent Labeled Oligonucleotide Probes", DTU, Denmark, pp 18.
18. APHA.(1995), "Standard Method for the Examination of Water and Wastewater," 19th ed., Washington, D.C., APHA/AWWA/WPCF, pp. 2.23-5.16.g.
19. Wanner, O. and Gujer, W. (1984), *Water Science and Technology*, vol. 17, pp. 24-44.
20. Lens, P., de Beer, D., Cronenberg, C.C.H., Houwen, F.P., Ottengraf, S.P.P., Verstracte, W. (1993), *Applied Environmental and Microbiology*, vol. 59, pp. 3803-3810.
21. Allison, D.E. (1998), *Biofilm Journal*, vol. 3, no. 2, 20.
22. Lopes, F.A., Vicira, M.J., and Melo, L.F. (2000), *Water Science and Technology*, vol. 41, pp. 105-111.
23. Jia, X.S., Furumai, H. and Fang, H.H.P. (1996), *Environmental Technology*, vol. 17, pp. 283-291.
24. Zhang, T.C. and Noike, T. (1994), *Water Research*, vol. 28, no. 1, pp. 27-36.