사)한국지하수토양환경학회
춘계학술대회 2001. 4.13-14
한양 대학교 신소재공학관

PHYSICOCHEMICAL CHARACTERIZATION OF UASB GRANULAR SLUDGE WITH DIFFERENT SIZE DISTRIBUTIONS

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ABSTRACT

Upflow anaerobic sludge blanket (UASB) system employs granular sludge to treat various wastewaters including landfill leachate. CH_4 production of the granules determines overall performance of a UASB reactor. Sludge granules are developed by self-granulation of microorganisms and dynamic balance between granule growth and decay results in coexistence of granules with different sizes in the reactor. In this study, granules taken from a laboratory-scale UASB reactor were classified into 4 groups based on their diameters and their physicochemical characteristics were investigated. Each group was analyzed for settling ability, specific methanogenic activity (SMA), and elemental content. Settling ability was proportional to granule diameter, suggesting effective detainment of larger granules in the reactor. When acetate or glucose was used as a substrate, all groups showed relatively slight difference in SMA. However SMA with a volatile fatty acid mixture showed significant increase with granule diameter, suggesting better establishment of syntrophic relationship in larger granules. Larger granules showed higher value of SMA upon environmental changes (i.e., pH, temperature, or toxicant concentration). Comparative analysis of elemental contents showed that content (dry weight %) of most tested elements (iron, calcium, phosphorus, zinc, nickel, and manganese) deceased with granule diameter, suggesting importance of these elements for initial granulation. Taken together, this study verified experimentally that physicochemical properties of granules are related to granule size distributions. Overall results of physicochemical characterization supports that larger granules are better applicable to UASB system.

Keywords: Mesophilic sludge, methane, size, sludge granule, UASB reactor.

1. INTRODUCTION

UASB reactor is a biological wastewater treatment system that uses granular sludge. The granules convert organic compounds to CH_4 under anaerobic conditions. Spontaneous association of microorganisms produces granular sludge of methanogenic microbial consortia. The ability of

microorganisms to form granular sludge is important for settling ability and metabolic versatility of granular sludge. However granulation mechanism is not yet well understood.

UASB granules contain several different metabolic groups of bacteria that participate in sequential metabolic processes to produce CH_4; four major steps are hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Macromolecules of organic compounds are converted to soluble monomers by hydrolytic bacteria. Acidogens produce acetate, other volatile fatty acids (VFAs), and H_2/CO_2 from the monomers. Acetogenic bacteria oxidize VFAs (e.g., propionate and butyrate) other than acetate to acetate and H_2/CO_2. Then methanogens use hydrogen or acetate to produce CH_4. Approximately two-thirds of the produced CH_4 is derived from acetate [1].

Because oxidation of propionate or butyrate is energetically unfavorable under methanogenic conditions, its oxidation is possible only when H_2 is removed fast. Low hydrogen pressure can be achieved by interspecies H_2 transfer from hydrogen-producing acetogens to hydrogen-utilizing methanogens [1, 2]. Therefore unfavorable thermodynamics can be overcome by syntrophic association between propionate- or butyrate-degrading bacteria and hydrogenotrophic methanogens.

The granule architecture is based on spatial organization of methanogenic microbial consortia. Integrated studies of fluorescent *in situ* hybridization and confocal laser scanning microscopy (CLSM) revealed that the outer layer of a sludge granule consisted of fermentative bacteria while the inner layer or core mostly contained methanogens (archaebacteria) [3, 4]. Besides, the inner layer or core showed microcolonies of syntrophic association between hydrogenotrophic methanogens and propionate-oxidizers [3, 5]. Such physical proximity between two metabolic-populations can allow the energetically unfavorable oxidation to occur.

Sludge granules with high CH_4-producing activity and good settling ability are necessary to achieve successful operation of UASB reactors. CH_4 production is generally analyzed to monitor the performance of UASB reactors. The SMA assay is the direct method of choice to test the methanogenic activity of granular sludge. SMA is determined by measuring CH_4 production rate of a sludge sample with a substrate [6].

Various sizes of granules coexist in a UASB reactor. Size distribution of granules in the reactor can be influenced by granule growth resulted from bacterial aggregation and growth as well as by granule decay due to bacterial death and granule disintegration. Washout of granules from the reactor also affects the size distribution. Average size or size distribution of granules depends on operating conditions of UASB reactors [1, 7].

Performance of a UASB reactor is based on integrated activity of granules with various sizes that coexist in the reactor. However few reports are available to describe performance of granules with different sizes [7, 8]. In this study, granules were sampled from a laboratory-scale UASB reactor and classified based on their sizes. The classified granules were used to investigate their physicochemical properties to understand characteristics of granular sludges with various sizes. Elemental content of each size group was also examined and compared each other. The

knowledge obtained in this study will provide insight into applicability of different-sized granular sludges to UASB system.

2. MATERIALS AND METHODS

Operation of UASB reactor

This study employed a laboratory-scale UASB reactor (5 l; inner diam., 9 cm) fed with a synthetic medium containing glucose [6] and operated at 35°C. The inoculum was anaerobically digested sludge obtained from a waste treatment plant located in Pusan, Korea. Initial operation conditions were 24 h of hydraulic retention time (HRT) and 1 g/l of influent COD. As COD removal efficiency increased to greater than 90%, HRT or influent COD was changed to increase COD load stepwise from 1 to 4 g COD/l·day. The COD load of 4 g COD/l·day was achieved from day 135 of operation by setting HRT at 12 h while keeping the influent COD of 2 g/l. COD removal efficiency was maintained greater than 90% from day 40 regardless of HRT and influent COD employed in this study. The reactor showed stable CH_4-production of 6.0 - 6.5 l/day from day 160. SMA reached a plateau from day 150. Granule samples were taken from a port at a height of 18.5 cm from the bottom of the reactor on days 220 and 227 and used for this study.

Classification of sludge granules

Microbial granules taken from the UASB reactor were classified into 4 groups using sieves in a waterbath containing the synthetic medium (35°C) used for feeding the reactor. The classified granules were used for the SMA assay immediately.

Analytical methods

Sizes of granules were determined by measuring their diameters as described previously [9]. One hundred granules were randomly selected from each group and used to measure their diameters.

SMA was measured in duplicate with 40-ml serum bottles sealed with butyl rubber stoppers and aluminum crimps. The reaction volume (10 ml) in each bottle was composed of granular sludge (approximately 20 mg VSS) and basal medium (pH 7.0) supplemented with a substrate (2 g COD/l) [6]. Bottles were flushed with N_2/CO_2 (70:30, v/v) gas for 5 min and incubated at 35°C in a waterbath shaker (100 rpm). Additional substrate (1.5 g COD/l) was supplied after 38 h and the pH was re-adjusted to 7.0. Then the bottles were re-flushed with N_2/CO_2. After 1 h of incubation, 50 μl of gas in the bottle headspace was analyzed periodically for its methane content. Unless otherwise specified, substrate and incubation temperature were sodium acetate and 35°C, respectively. Other substrates employed for the SMA assay were glucose and a VFA mixture (acetate:propionate:butyrate = 4:1:1, based on COD). CH_4 content and soluble COD were measured as described [10]. The amount of biomass (VSS) in each bottle was measured at the end of each assay as described previously [10]. SMA was calculated

according to Yukselen [11]. To examine the effect of environmental toxicants on the metabolic activity of granular sludge, the SMA assay was conducted at the presence of $CuCl_2$ (0.5 mg/l) or phenol (26 mg/l) in each serum vial.

Elemental analysis

Each group of sludge granules was rinsed gently with sterile distilled water, dried at 80°C and then used for elemental analysis. Elemental analysis was performed using inductively coupled plasma atomic emission spectroscopy (ICP-IRIS; Thermo Jarrell Ash, Franklin, MA, USA).

3. RESULTS AND DISCUSSION

Size classification of sludge granules

Granules were classified into 4 groups based on their diameters (Fig. 1). Diameter distribution of each group was as follows (number average diameter of in the parenthesis): group 1, 0.02 - 0.04 mm (0.03 mm); group 2, 0.05 - 0.09 mm (0.07 mm); group 3, 0.10 - 0.14 mm (0.12 mm); and group 4, 0.15 - 0.23 mm (0.19 mm). Total (unclassified) granules showed number average diameter of 0.13 ± 0.07 mm (data not shown). Overall granule sizes were relatively small probably due to low concentration of substrate employed in the reactor [1, 7].

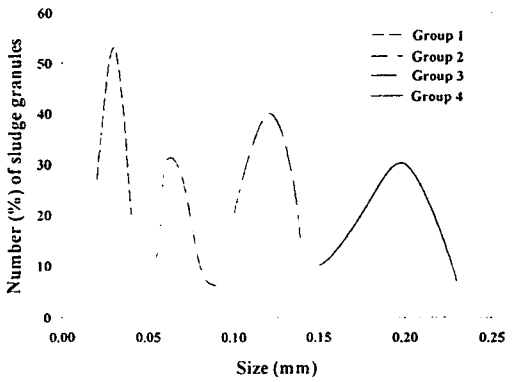

Fig. 1.Size distribution of granular sludge after sieving. Size was determined by measuring diameters of granules as described in Materials and Methods.

Settling ability of granules

Since UASB system does not employ any supporting materials to form or maintain granules, constituents of granules exclusively determine settling ability of granules. Granules with good settling ability are necessary to retain high biomass concentration to treat wastewaters. Settling ability of granules was proportional to their diameters as determined by sludge volume

index (Fig. 2). This suggested that large granules could be retained more effectively in the reactor at short HRTs.

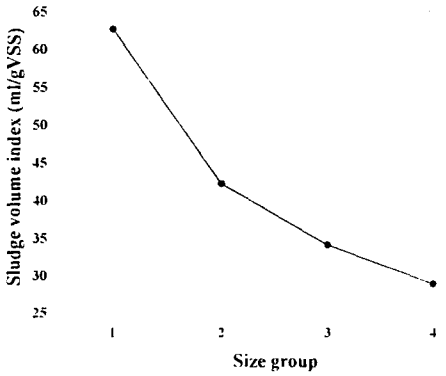

Fig. 2. Settling ability of each group of granules as determined by sludge volume index.

Effect of substrates on specific methanogenic activities

Successful operation of UASB system requires microbial granules not only with good settling abilities but also with high activities. Anaerobic sludge granules consist of microorganisms spatially organized to perform metabolic cooperation, which eventually results in CH_4 production [1, 3]. Each group of granules was examined for its methanogenic activity with different substrates. Methanogenic activity of granules was measured using the SMA assay.

When glucose was used as a substrate, all granules showed similar values (0.40 - 0.46 gCH_4-COD/gVSS·day) of SMA, suggesting that all granules in the reactor played similar roles to convert glucose to methane regardless of their sizes (Fig. 3). Since fermentative bacteria in the outer layer degrade glucose, glucose degradation does not seem to be affected by mass transfer limitation under the substrate concentration used in this study. When acetate was used a substrate, all groups showed relatively slight difference in SMA. Group 2 showed a maximum methanogenic activity of 0.49 ± 0.08 gCH_4-COD/gVSS·day while group 4 showed a minimum methanogenic activity of 0.37 ± 0.01 gCH_4-COD/gVSS·day

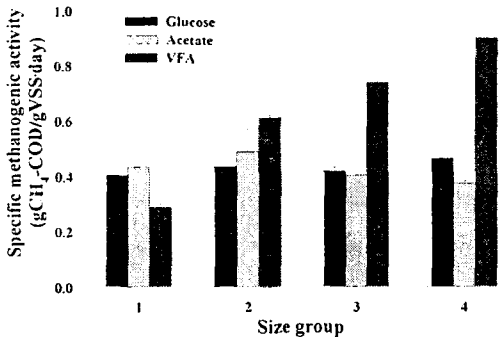

Fig. 3. SMA of each granule group with different substrates. Error bars represent standard deviations of the mean of 4 measurements; the SMA assay was performed 2 times and duplicate samples were used for each time.

This result partially supported the previous report by Alphenaar et al [8] that acetoclastic methanogenic activity of granules decreased with granule diameter although the size distribution of granules shown in this study was different from that of granules in the study [8]. When glucose is used as a substrate, fermentative bacteria in the outer layer of granules degrade glucose before it defuses into the granules. The products of glucose diffuse into the granules where methanogens convert them to CH_4. When acetate is used as a substrate, the outer layer comprised of fermentative bacteria could limit transfer of acetate from the bulk to the inner layer or core of granules where acetate consumers are located; acetoclastic methanogens and syntrophic association of acetate oxidizers and hydrogenotrophic methanogens [3, 4]. Kitsos et al. [12] found the diffusion rate of acetate in an anaerobic biofilm was only 7% of that in water. Groups 3 and 4 showed lower SMA values with acetate than glucose. The difference between SMA values with acetate and glucose could partially explain the mass transfer limitation. On the other hand, groups 1 and 2 showed higher SMA values with acetate than glucose, suggesting that granules in the size distributions would be affected by kinetic (glucose conversion to acetate) limitation rather than mass transfer limitation.

Compared to values of SMA with glucose or acetate, SMA with a VFA mixture showed significant difference depending on granule diameters (Fig. 3). SMA with a VFA mixture increased as granule diameter increased, suggesting better establishment of syntrophic relationship in larger granules. Degradation of VFA from a wastewater is based on syntrophic association of H_2-producing acid-oxidizers with H_2-utilizing methanogens [1]. Groups 2, 3, and 4 showed higher values of SMA with the VFA mixture than acetate only. This could be explained by that the groups contained dominant *Methanosaeta* spp. over *Methanosarcina* spp and/or well-developed syntrophic relationship between acid oxidizers and hydrogenotrophic methanogens. Gradual dominance of *Methanosaeta* spp. and/or gradual development of the syntrophic association could increase difference between values of SMA with acetate and VFA as

granulation proceeded. Only two genera, *Methanosaeta* and *Methanosarcina* are known to grow on acetate. *Methanosaeta* spp. dominate over *Methanosarcina* spp under low concentration of acetate [1]. Granule density increased with size of granules employed in this study as determined by observations of granule sections by CLSM (data not shown). The higher bacterial cell densities in larger granules decrease the distances between bacteria and increase interspecies transfer of metabolites. Therefore, single bacteria benefit from forming granules by association with other bacteria. When different substrates were used for SMA assay, SMA (gCH_4-COD/gVSS·day) shown by total granules is as follows: glucose, 0.46 $\pm$ 0.07; acetate, 0.48 $\pm$ 0.07; VFA mixture, 0.64 $\pm$ 0.11 (data not shown).

Effect of environmental variables on specific methanogenic activities

Anaerobic process of wastewater treatment includes complex dynamics of both biological and physicochemical factors that are interrelated. Wastewaters often contain physicochemical factors that could change optimum operational conditions of a UASB reactor. Anaerobic processes are sensitive to sudden change in environmental conditions such as temperature, pH, and toxicant concentration, and often show decreased methane production [13, 14, 15]. Compared to suspended cells, sludge granules show more resistance to such environmental stresses in anaerobic process [1].

SMA of each granule group was examined to determine the effects of environmental variables (e.g., pH, temperature, and toxicants) on its acetoclastic activities. Fig. 4 shows relative value of SMA under various environmental conditions in percent of each group's SMA under optimum conditions (pH 7 and 35°C) as shown in Fig. 3. Temperature change greatly reduced acetoclastic methanogenic activities of granules (Fig. 4). When 35°C-grown granules were subjected to SMA assay at 50°C, the activity was not detected from all groups. Group 1 showed the highest sensitivity at 20°C, showing only 36% of acetoclastic activity compared to that at 35°C (Fig. 3). The adverse effect of temperature drop on acetoclastic methanogenic activity was decreased as granule diameter increased.

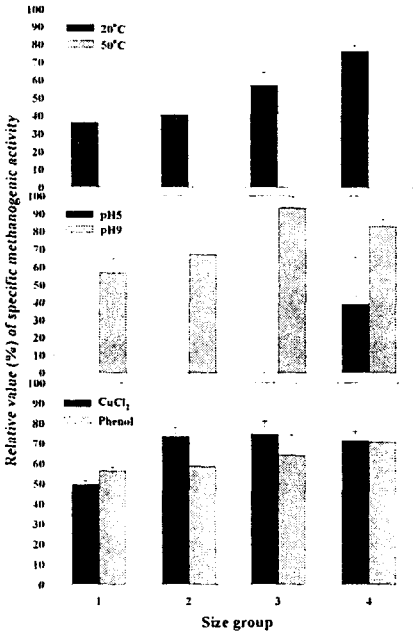

Fig. 4. Relative values of SMA of each granule group under various environmental conditions. Relative value of SMA represents the percent of the SMA under optimum conditions (pH 7 and 35°C) shown in Fig. 3.

Change in pH also adversely affected acetoclastic methanogenic activity of the granules especially at acidic pH. Groups 1, 2, and 3 did not show the activity at pH 5. However group 4 still showed 39% of acetoclastic activity at pH 5 (Fig. 4) compared to that at pH 7 (Fig. 3). Acetoclastic methanogenic activity at pH 9 did not decrease as significantly as that at pH5, suggesting possible neutralization by organic acids produced by fermentative bacteria in the granules. Compared to the activity at pH 7, the activities of groups 3 and 4 at pH 9 reduced by 7% and 17%, respectively, while group 1 showed the most reduced activity. From the practical point of view, anaerobic process is prone to build up organic acids due to different growth rates of fermentative bacteria and methanogens. Buildup of acids can damage methanogenic activity of granules. Therefore maintenance of proper alkalinity in UASB reactor is critical to achieve successful wastewater treatment.

Many toxicants showed inhibitory effects on acetoclastic methanogenic activity of granules [6, 13, 16]. This study conducted the SMA assay with 26 mg/l of phenol or 0.5 mg/l of $CuCl_2$ that was previously reported to be inhibitory to anaerobic digestion [17]. Acetoclastic methanogenic activity decreased as granule diameter decreased when phenol was added in the SMA assay (Fig. 4). In case of $CuCl_2$, acetoclastic activity of group 1 was reduced by 50%. However groups 2, 3, and 4 showed only 25 – 28% of reduction in the activity. Since acetoclastic methanogens are located inside granules [3, 4], bacteria in the outer layer seem to serve as a barrier to protect methanogens to certain extent. This protective effect seems to be more evident

as the granule diameter increases. Taken together, Fig. 4 suggested that cells consisting of developed granules could defend collectively against toxicants.

Comparative analysis of element content

Table 1 shows element content (dry weight %) of each group. Iron was most abundant and manganese was least abundant among the tested elements. Although the content of potassium increased with granule diameter, contents of most tested elements (Fe, Ca, P, Zn, Ni, and Mn) decreased with granule diameter. Highest contents of magnesium and sodium were observed in groups 1 and 2, respectively while lowest contents of them were found in group 4. The decreasing trend of the tested elements with granule diameter suggested that they played important roles in initial granulation.

Table 1. Content of selected elements from each granular sludge group as determined by inductively coupled plasma atomic emission spectroscopy.

Size group of granular sludge	Element (dry weight %)								
	Ca	Fe	K	Mg	Mn	Na	Ni	P	Zn
1	1.163	4.204	0.256	0.210	0.010	0.330	0.022	1.771	0.942
2	0.897	2.993	0.285	0.191	0.007	0.461	0.018	1.593	0.654
3	0.751	2.176	0.332	0.200	0.005	0.297	0.014	1.274	0.452
4	0.565	1.539	0.362	0.188	0.003	0.270	0.013	1.109	0.283

Calcium, potassium, and iron are known to be the main inorganic components of granules [1]. Grotenhuis et al [18] reported that calcium and potassium precipitates in sludge granules stabilized granule structure. Some divalent cations (e.g., Ca^{2+}, Fe^{2+}, Mg^{2+}, etc) were reported to bridge negatively charged bacteria together, thereby promoting initial bacterial aggregation or stabilizing integration of granular structure [18, 19, 20, 21].

4. REFERENCES

1. Schmidt, J.E. and Ahring, B.K., *Biotechnol. Bioeng.*, 49, 229-246 (1996).
2. Schmidt, J.E. and Ahring, B.K., *Appl. Environ. Microbiol.*, 42, 457-462 (1993).
3. Sekiguchi, Y., Kamagata, Y., Nakamura, K., Ohashi, A., and Harada, H., *Appl. Environ. Microbiol.*, 65, 1280-1288 (1999).
4. Rocheleau, S., Greer, C.W., Lawrence, J.R., Cantin, C., Laramee, L., and Guiot, S.R., *Appl. Environ. Microbiol.*, 65, 2222-2229 (1999).
5. Imachi, H., Sekiguchi, Y., Kamagata, Y., Ohashi, A., and Harada, H., *Appl. Environ. Microbiol.*, 66, 3608-3615 (2000).

6. Donlon, B.A., Razoflores, E., Field, J.A., and Lettinga, G., *Appl. Environ. Microbiol.*, 61, 3889-3893 (1995).
7. Grotenhuis, JTC, Kissel, J.C., Plugge, C.M., Stams, A.J.M., and Zehnder, A.J.B., *Water. Res.*, 25, 21-27 (1991).
8. Alphenaar, P.A., Perez, M.C., and Lettinga, G., *Appl. Microbiol. Biotechnol.*, 39, 276-280 (1993).
9. Ohtsuki, T., Tominaga, S., Morita, T., and Yoda, M., *Proc. of the 7th Int. Symposium on Anaerobic Digestion*. Jan. 1994, Int. Association on Water Quality, Cape Town, South Africa pp. 348-357 (1994).
10. Ahn, Y., Lee, Y.J., Kim, H.S., and Park, S., *Biotechnol. Lett.*, 22,1591-1596 (2000).
11. Yukselen, M.A., *J. Environ. Sci. Health*, A32, 2069-2076 (1997).
12. Kitsos, H.M., Roberts, R.S., Jones, W.J., and Tornabene, T.G., *Biotechnol. Bioeng.*, 39, 1141-1146 (1992).
13. Lin, C.-Y. and Chen, C.-C., *Wat. Res.*, 33, 409-416 (1999).
14. Lau, I.W.C. and Fang, H.H.P., *Wat. Res.*, 31, 2626-2632 (1997).
15. van Lier, J.B., Rintalar, J., Sanz Martin, J.L., and Lettinga, G., *Wat. Sci. Tech.* 22, 183-190 (1990).
16. Hwu, C-S. and Lettinga, G., *Enz. Microbiol. Technol.*, 21, 297-301 (1997).
17. Parkin, G.F. and Owen, W.F., *J. Environ. Eng.*, 112, 867-920 (1986).
18. Grotenhuis, J.T.C., van Lier, J.B., Plugge, C.M., Stams, A.J.M., and Zehnder, A.J.B., *Appl. Microbiol. Biotechnol.*, 36, 109-114 (1991).
19. Yu, H.Q., Tay, J.H., and Fang, H.H.P., *Wat. Res.*, 35, 1052-1060 (2001).
20. Schmidt, J.E. and Ahring, B.K., *Enz. Micrbiol. Technol.*, 15, 304-310 (1993).
21. Lettinga, G., van Velsen, A.F., Hobma, S.W., de Aeeuw, W., and Klapwyk, A., *Biotechnol. Bioeng.*, 22, 699-734 (1980).