J. Microbiol. Biotechnol. (2002), **12**(6), 901–908

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Increased Microbial Resistance to Toxic Wastewater by Sludge Granulation in Upflow Anaerobic Sludge Blanket Reactor

BAE, JIN-WOO, SUNG-KEUN RHEE[1], IN S. KIM[2], SEUNG-HOON HYUN[2], AND SUNG-TAIK LEE*

Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Taejon, Korea
[1]*Environmental Sciences Division, Oak Ridge National Laboratory, TN, U.S.A.*
[2]*Department of Environmental Science and Engineering, Kwangju Institute of Science and Technology, Gwangju, Korea*

Received: August 8, 2002
Accepted: September 27, 2002

Abstract The relationship between the layered structure of granules in UASB reactors and microbial resistance to toxicity was investigated using disintegrated granules. When no toxic materials were added to the media, the intact and disintegrated granules exhibited almost the same ability to decrease COD and to produce methane. However, when metal ions and organic toxic chemicals were added to a synthetic wastewater, the intact granules were found to be more resistant to toxicity than the disintegrated granules, as determined by the methane production. The difference in resistance between the intact and disintegrated granules was maximal, with toxicant concentrations ranging from 0.5 mM to 2 mM for trichloroethylene with toluene and 5 mM to 20 mM for metal ions (copper, nickel, zinc, chromium, and cadmium ions). The augmented COD removal rate by granulation compared to disintegrated granules was also measured in the treatment of synthetic and real wastewaters; synthetic wastewater, - 2.6%; municipal wastewater, 2.8%; swine wastewater, 6.4%; food wastewater, 25.0%; dye works wastewater, 42.9%; and landfill leachate, 61.8%. Continuous reactor operation also demonstrated that the granules in the UASB reactor were helpful in treating toxic wastewater, such as landfill leachate.

Key words: COD, granules, microbial resistance, toxic wastewater, UASB

The environmental study of bioreactors is primarily motivated by finding an optimal wastewater treatment process. As such, a number of attempts have been made not only to design and model bioreactors, but to enhance the activity of microorganisms as well [14, 16, 18, 20, 21, 22, 26, 27, 31]. In particular, the immobilization or flocculation of the biomass has been used to improve the performance of microorganisms in degrading toxic chemicals [13, 16, 28, 34]. As with wastewater components, toxic organic chemicals and metal ions are known to inhibit the operation of an anaerobic digester [1, 10, 23], however, the structural characteristics of bacterial aggregates and high biomass retention can be used to increase the tolerance level of anaerobic bacteria, thereby overcoming the toxicity of the compounds [1, 5, 7, 9].

Among the anaerobic bioreactors, the upflow anaerobic sludge blanket (UASB) reactor has received a great deal of attention, because of its ability to degrade high concentration of toxicants [23]. Several reports have indicated that the high resistance of granules in a UASB reactor to toxic chemicals and metal ions is due to their layered structure [8, 21]. Earlier studies on internal structure have suggested that there are essentially three layers within a granule, each with a different bacterial composition [25]. Acidogens plus H_2-producing and H_2-consuming organisms are located in the outer layer, while methanogens are included in the inner layer [33].

Although the toxicity level of metal ions and organic toxic chemicals on granules has already been evaluated in the acetoclastic step in a defined wastewater [2, 3, 4], there have been no reports on the resistance of granules to the complex wastewater with toxic materials. Therefore, we investigated the microbial resistance of UASB granules by comparing the treatability of complex wastewater with intact layered and disintegrated granules. This is the first comprehensive study on the utility of granules to suspended sludge, comparing with the conventional anaerobic digester. Understanding the resistance of granules would facilitate the applicability of UASB reactors, and more importantly, provide more information on the improved microbiological conditions for the treatment of toxic wastewater.

*Corresponding author
Phone: 82-42-863-5617; Fax: 82-42-863-5617;
E-mail: stlee@sorak.kaist.ac.kr

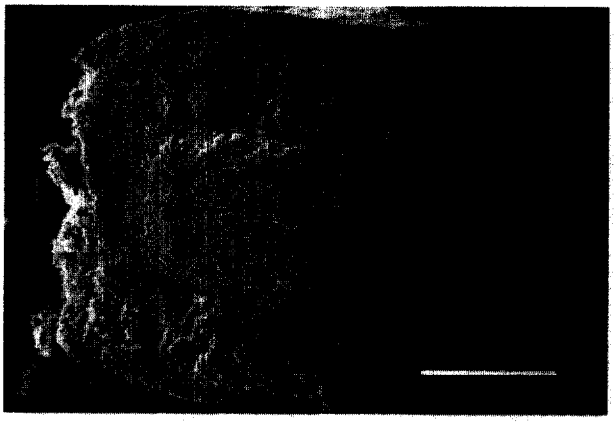

Fig. 1. Micrograph of split granules; White bar indicates 0.5 mm.

MATERIALS AND METHODS

Granules

Sludge granules were sampled from a 1,000 m^3 UASB reactor which treats beer-brewery wastewater with a chemical oxygen demand (COD) of 3,000-4,000 mg/l. The reactor had been in operation for 2 years at a hydraulic retention time of 10 h while removing as much as 95% of the influent COD. The granules were approximately 2 mm in diameter and brownish black in color. Split granules revealed the layered structure (Fig. 1). In order to obtain a nonlayered sludge with the same microbial population as the intact granules, the granules were blended with a homogenizer (Nihonseiki Kaisha Ltd., Tokyo, Japan) at 10,000 rpm for two minutes [2, 3]. One mM Na_2S was added to the sampled granules before blending. The disintegrated granules were then sieved by using a 0.5 mm mesh to remove any undisintegrated granules prior to the experiment. The prepared sludge samples were all washed three times with 0.1 M KH_2PO_4/K_2HPO_4 buffer (pH 7.0) before all the experiments. The sludge had not been previously acclimated to any toxicants. All procedures were conducted in an anaerobic atmosphere.

Wastewaters

The synthetic wastewater, 5,000 mg COD/l, used in the anaerobic resistance assay contained the following (in milligrams per liter): KH_2PO_4, 30; K_2HPO_4, 70; $CaCl_2$, 100; $MgCl_2$, 100; KCl, 70; NH_4Cl, 260; Na_2SO_3, 50; Yeast extract, 100; $C_6H_{12}O_6$, 4,500. The final pH of the medium ranged from 7.0 to 7.5. Different kinds of real wastewater were sampled from various locations in Korea (Table 1). The pH of the real wastewater was not adjusted.

Table 1. pH and sampling locations of real wastewaters taken in Korea.

Wastewater	pH	Sampling spot
Swine wastewater	8.3	Chungnam Hongsung pigpens
Landfill leachate	6.5	Taejon Kumgo-dong waste landfill
Dyeworks wastewater	5.2	Taegu Dye works industrial complex.
Food wastewater	3.8	University restaurant attached to KAIST
Municipal wastewater	6.2	Taejon Municipal wastewater treatment

Batch Assay of Sludge to Toxic Wastewater

The specific wastewater degradation activities were measured by using 120 ml of glass serum bottles sealed with a 12 mm-thick butyl rubber septa. The granular sludge (30 ml of wet volume, 1.65 g dry weight, 1.20 g volatile suspended solid) was transferred to vials containing 70 ml of the synthetic wastewater. Two toxic organic chemicals, i.e. trichloroethylene (TCE) and toluene, in addition to five heavy metals commonly found in electroplating effluent [8], i.e. cadmium ($CdCl_2$), chromium [$CrK(SO_4)_2$], copper ($CuCl_2$), nickel ($NiCl_2$), and zinc ($ZnCl_2$), were selected for this study. Unit concentrations of the toxicants, i.e. 1 mM (106 µl/l of toluene and 90 µl/l of TCE) of the organic chemicals and 2 mM heavy metals (0.1-0.12 g/l), were added to the synthetic wastewater. The assay medium was then adjusted to pH 7.0±0.1 and flushed for 2 min with argon gas which is used as the chromatography carrier gas. The mixtures were incubated at 37°C with shaking at 150 rpm. During the incubation periods, the gas phase and liquid phase were sampled to determine the microbial activity.

Analytical Methods

The COD was measured by the method of Lange modified by Wacheux *et al.* [35], which is modified from the open reflux method of the Standard Method. The gas contents in the headspace of the assay bottles were determined by using a pressure meter and their compositions calculated by a gas chromatograph (GC). The gas contents were modulated according to the pressure and temperature, however, the dissolved contents were not estimated. The GC was equipped with a steel column (1.83 m, 2.125 cm) that was packed with 80/100 mesh size. The temperatures of the column, injection port, and thermal conductivity detector were 35°C, 60°C, and 100°C, respectively.

Degree of Granulation Utility in Toxic Wastewater

To demonstrate the positive effect of the layered structure on resistance to toxic chemicals, the utility degree was calculated as follows:

Utility (%)=100 [(specific COD removal rate of intact granules/specific COD removal rate of disintegrated granules)- 1]

Utility represents the performance of the UASB process compared to traditional anaerobic digestion when treating toxic wastewater, as determined by COD removal.

Reactor Design and Operation

Four acrylic UASB reactors with an active volume of 7.6 l and 0.88 l of headspace were used for the experiments.

Stainless steel and glass tubing were used for the connections to minimize any wastewater adsorption or evaporation. The reactor systems were checked for abiotic loss of methane before the granules were added to the reactors. The reactors were then operated continuously with an organic loading rate increasing step-wise from 0.5 kg COD/m^3·day to 4 kg COD/m^3·day. Samples were taken from sample ports 1 and 2, representing the reactor influent and effluent, respectively. The samples were then analyzed for COD and methane. All sample ports were closed with Teflon-lined butyl septa. The reactors were kept in a dark room and operated at a constant temperature (30°C). The reactors were filled with 3 l of wet intact and disintegrated granules, gassed with N_2-CO_2 (4:1, v/v), and filled with the wastewater.

RESULTS AND DISCUSSION

Comparison of Intact and Disintegrated Granules for Treatment of Synthetic Wastewater

As a preliminary experiment to investigate the effect of the layered structure of the granules on toxic wastewater treatment, disintegrated and intact granules were incubated with defined wastewater containing toxic organic chemicals and heavy metals (Fig. 2). When glucose was used as the anaerobic digestion substrate, about 80% of the glucose COD was transformed into methane. The synthetic wastewater treatment was not affected by the disintegration of the granules: Both the intact and disintegrated granules completely decreased 5,000 mg/l of COD in 30 h at about the same rate as in the absence of toxicants. Therefore, the disintegrated granules without a layered structure exhibited approximately the same microbial activity and produced approximately the same amount of methane as with the intact granules.

Schmidt and Ahring [32] reported that granule disintegration by a vortex mixer or ultrasound has no effect on methane production, whereas breaking by a blender or syringe decreases the methane production by 54%. In fact, this could be due to different conditions existing during the granule disintegration; for example, in the present study we used 1 mM Na_2S as a reducing agent and an anaerobic chamber to ensure anaerobic condition during granule disintegration.

Theoretically, difficulties in interspecies hydrogen transfers in disintegrated granules could decrease the COD removal

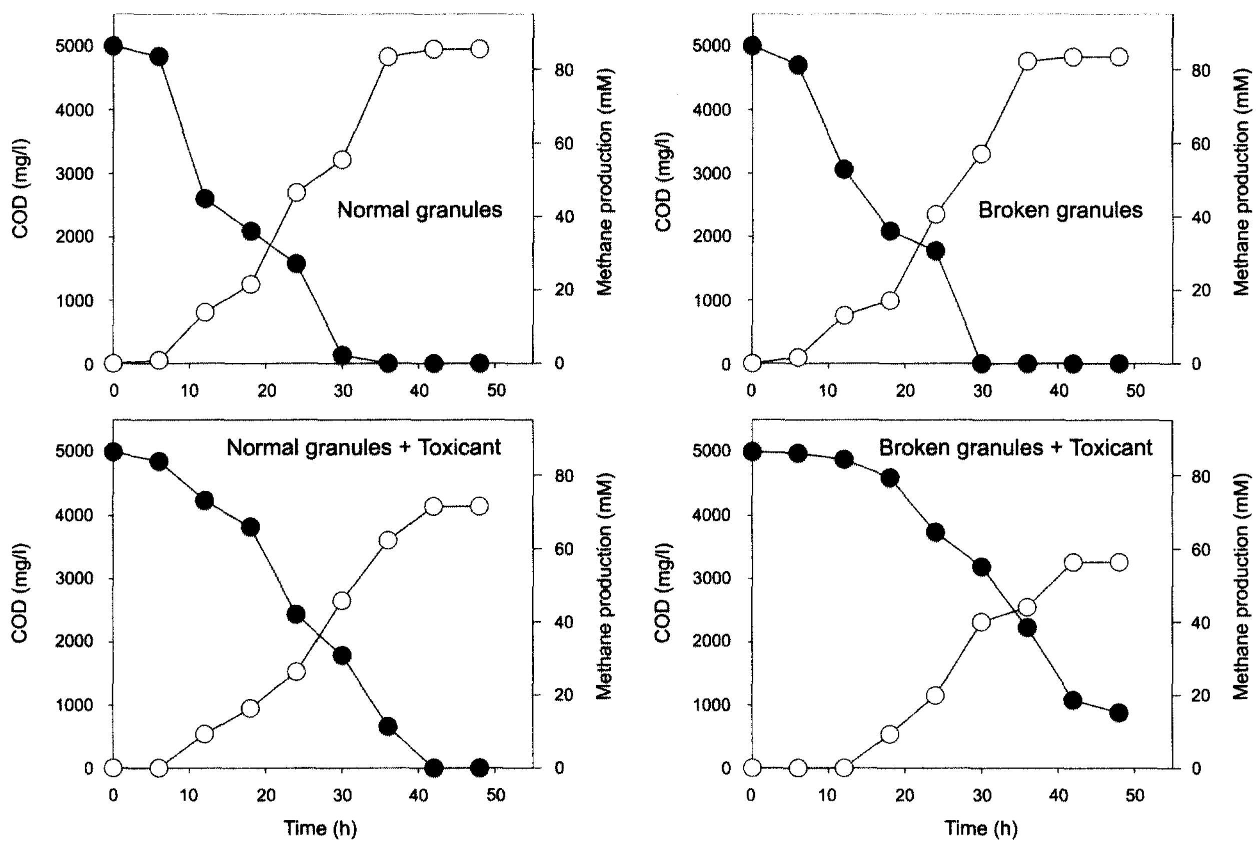

Fig. 2. Removal of COD and production of methane by intact and disintegrated granules in media containing 0 and 1 unit toxicant concentrations.
(●) COD, (○) methane production.

rate. Conversely, disintegrated granules could equally increase COD removal due to lower diffusion limit. In fact, when treating chemicals with the same number of cells, suspended cells have been found to be more active in degrading chemicals when compared with immobilized cells within a certain range of toxic chemical concentrations [7, 30]. Accordingly, the fact that the same removal rate was observed in this test indicates that the granules lost some of their microorganism activity during the disintegration process, and decrease of the COD removal due to the disintegration of granules was compensated by the increase of diffusion.

Degradation of Glucose by Intact and Disintegrated Granules in Presence of Toxicants

When one unit volume (1 mM trichloroethylene and toluene, and 10 mM cadmium, chromium, copper, nickel, and zinc) was added to the media, the COD removal and methane production rates of the disintegrated granules decreased by 64% and 68%, respectively, whereas the rates of the intact granules decreased by 34% and 42%, respectively. Since glucose degradation under anaerobic conditions is a multi-step process carried out by fermentative/acidogenic bacteria, acetogens, and methanogens, the disintegrated granules are more sensitive to the toxicants than the intact granules in both the glucose degradation and methane production steps [3]. Furthermore, in the current study, two-fold more acetic acid and propionic acid were accumulated in the media containing 500 mg/g-VSS copper ions (data not shown). This result implies that the methanogenic activity appeared to be more sensitive to the metal ions than the fermentative activity. It is generally known that methanogens are extremely vulnerable to the toxicity of metal ions [12, 24].

Effect of Toxicant Concentration on Treatment of Synthetic Wastewater by Intact and Disintegrated Granules

To determine the effect of toxicant concentrations on the degradation of synthetic wastewater, synthetic wastewater containing various unit concentrations of toxicants (0–4 units) was applied to intact and disintegrated granules with no prior exposure to toxicant (Fig. 3). When the toxicant concentration was less than 0.25 units, there was no decrease in the COD removal rate (166.7 mg/l/h) or methane production rate (1.88 mM/h), regardless of granule homogenization. However, when the toxicant concentration increased to 0.5 units, there was a significant decrease in the COD removal and methane production rates due to the disintegration of granules by 34% and 46%, respectively. Meanwhile, with this same toxicant concentration (0.5 units), the activity of the intact granules exhibited no significant change (94% and 92% of the control, respectively). With a one unit toxicant concentration, both the COD removal

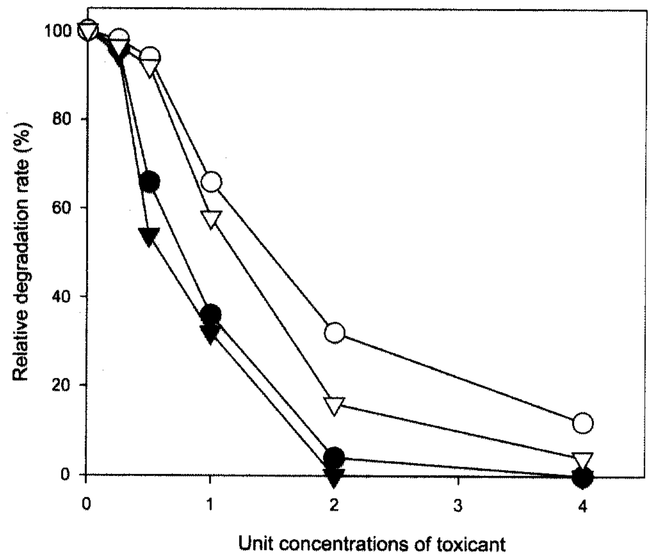

Fig. 3. Relative COD removal and methane production rates by intact and disintegrated granules in media containing various unit toxicant concentrations.
(●) COD removal with intact granules, (○) COD removal with disintegrated granules, (▼) Methane production with intact granules, (▽) Methane production with disintegrated granules.

and methane production rates by the disintegrated granules were about 33% of the control, whereas the rates by the intact granules remained at about 66% of the control. A further increase in the toxicant concentration to two units resulted in no COD removal by the disintegrated granules, yet 33% of the control by the intact granules.

There was a significant difference in the COD removal activity between the intact and disintegrated granules with toxicant concentrations ranging from 0.5 to 2.0 units. In particular, 0.5 units toxicant concentration produced the most significant difference in the activity between the intact and broken granules. Landfill leachate contains high concentrations of inhibitory compounds, such as aromatic compounds, chlorinated solvents, and cyanide compounds as well as diverse heavy metals [11, 29]. Therefore, it is likely that these compounds could be toxic to disintegrated granules. Considering these results, UASB reactors containing granule-structured sludge would appear to be more advantageous for the treatment of toxic wastewaters such as landfill leachate.

Chemical toxicity is closely correlated to the sludge surface area with a larger surface area yielding an increased toxicity [36]. Furthermore, it seems that the inner part of the granules composed of methanogens, which are the most sensitive to toxicants, is shielded from the harsh environment by the layered structure. Consequently, since the toxicant diffusion is limited, the toxicant can be degraded or adsorbed by the surface layer. This also explains why the anaerobic digestion by the microbial community becomes more sensitive to toxicity when the granules are disintegrated.

Although so far ignored, additional factors like death and decay rate of the organisms also play a significant role in the quality of immobilized anaerobic sludge [17]. Similarly, the sensitivity of suspended cells has also been reported in immobilized cell experiments related to the treatment of toxic chemicals [7].

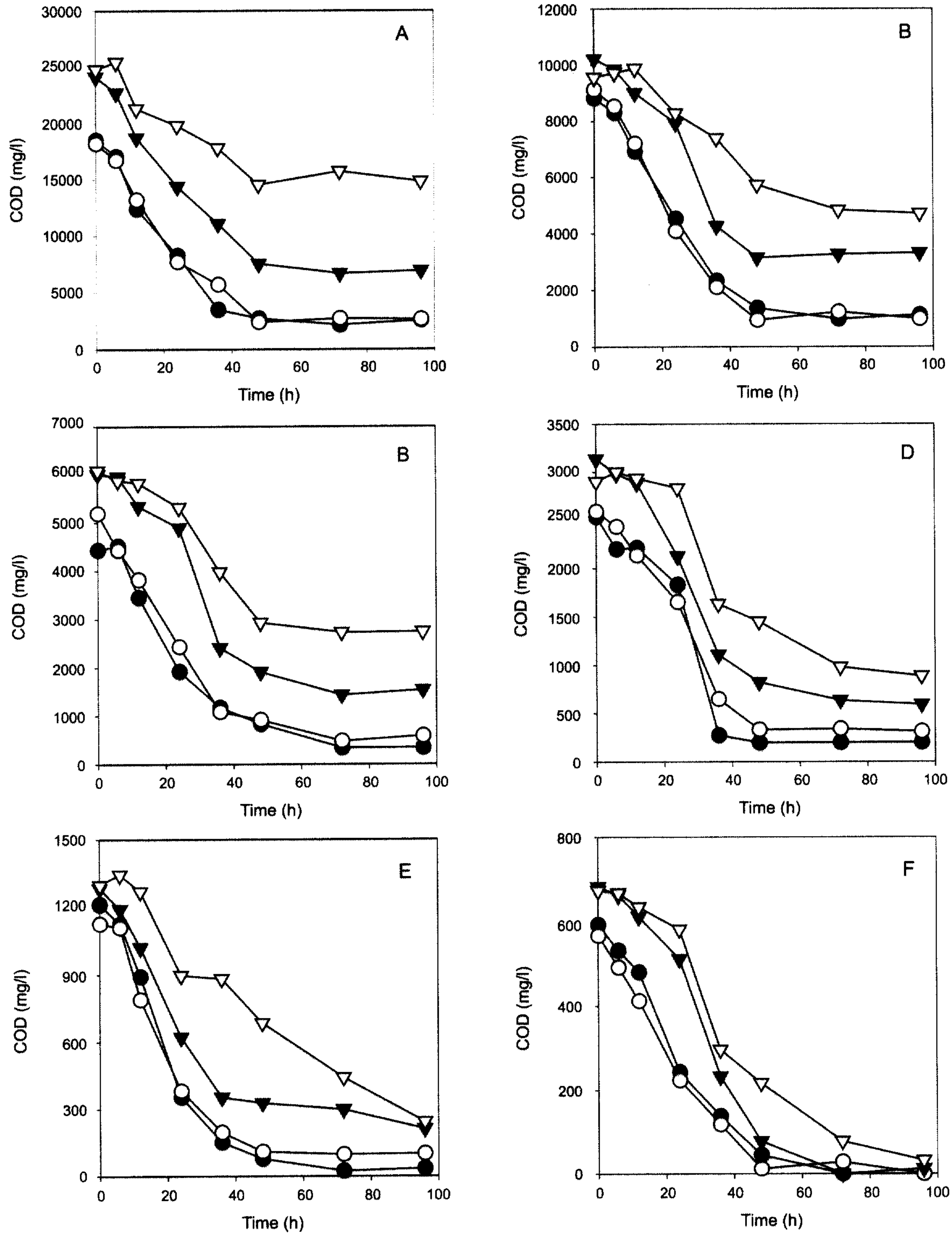

Fig. 4. Decrease in COD degree from landfill leachate and swine wastewater treated by intact and disintegrated granules with (A) 2^0, (B) 2^1, (C) 2^2, (D) 2^3, (E) 2^4, (F) 2^5 dilution.
(●) Swine wastewater treated by intact granules, (○) Swine wastewater treated by disintegrated granules, (▼) Landfill leachate treated by intact granules, (▽) Landfill leachate treated by disintegrated granules.

Treatment of Real Wastewater with Intact and Disintegrated Granules

To study the resistance of granular sludges in the treatment of real wastewater, swine wastewater and landfill leachate were treated with intact and disintegrated granules (Fig. 4). Although the initial COD and toxicant concentrations were found to be different in each wastewater, the intact granules treated the swine wastewater and landfill leachate at the same rate. Significant differences between the intact and disintegrated granule activities were observed only in the treatment of landfill leachate, thus indicating that the landfill leachate contained more toxicant level than the swine wastewater. In the landfill leachate treatment, when the wastewater was less diluted, more differences in the COD removal rates were observed between the intact and disintegrated granules, indicating that the microbial populations without granulation were also able to endure the toxic chemicals up to certain concentrations.

However, over 2^5 dilutions of wastewater did not result in any further differences between the activities of the intact and disintegrated granules. Therefore, when the wastewater contained high toxicant concentrations, UASB granulation was clearly necessary to efficiently treat the toxic wastewater. Since the landfill leachate had smaller amounts of biodegradable ingredients [6], only 50-60% of the COD in the landfill leachate was decreased anaerobically. Nevertheless, this corresponded to a 91% BOD removal. As such, these results suggest that wastewater with a high ratio of COD/BOD is more likely to contain toxic components [19].

Utility of Granulation in Degrading Toxic Wastewater

Using the relative activity of the intact over the disintegrated sludge, an assessment was made of the advantages of an UASB process over traditional anaerobic digestion when treating toxic wastewater (Fig. 5). The advantage of the granular sludges was quite evident, when the utility degree (augmented COD removal rate by granulation compared to disintegrated sludges) was considered. The utility of the UASB granulation for synthetic wastewater degradation without toxicants was a minus degree; that is, the disintegrated granules were better at treating the synthetic wastewater used in the current study. However, in the tests with landfill leachate and dye works wastewater, the granulation augmented the COD removal rate by 62% and 43%, respectively, indicating that the UASB granulation was able to endure the toxicants in the landfill leachate and dye works wastewater. The relative utility of the granulation on the treatment of each wastewater was in the order of landfill leachate > dye works wastewater > food wastewater > swine wastewater > municipal wastewater > synthetic wastewater. This tendency implies that the granule-containing reactors are more useful compared to conventional anaerobic reactors for the treatment of wastewaters containing inhibitory toxicants [19].

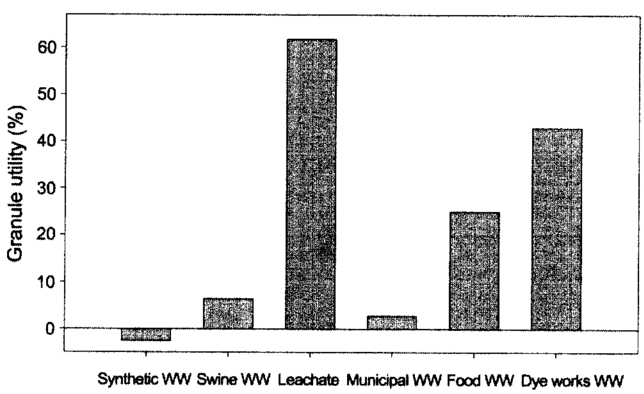

Fig. 5. Degree of granulation utility with various wastewaters (WW=wastewater; Leachate=landfill leachate).

Continuous Treatment of Wastewater with Intact and Disintegrated Sludges

To determine the activity of intact and disintegrated granules in treating inhibitory wastewater, synthetic wastewater and landfill leachate were continuously treated by using four UASB reactors. Figure 6 shows the temporal variations in the COD removal with an organic loading rate of 0.5-4 kg COD/m^3·day over the experimental period.

During the initial period of OLR 0.5 kg COD/m^3·day, the COD removal from the synthetic wastewater by the intact granules remained fairly consistent within a range of 81-87%. Meanwhile, during the same period of time under the same conditions, the broken granules achieved 76-83% COD removal, indicating that the granulation

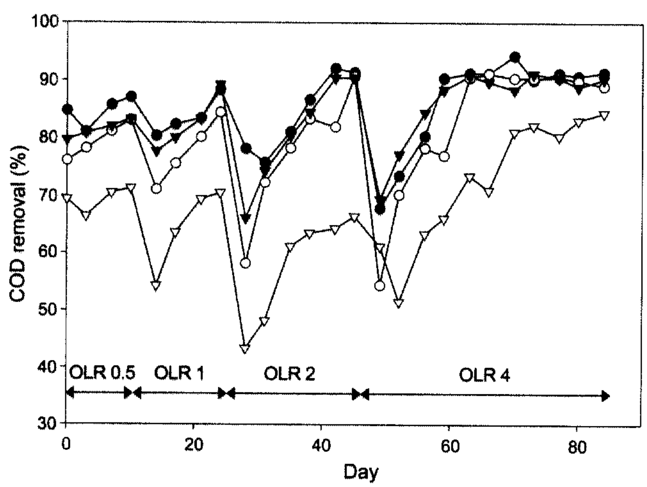

Fig. 6. Continuous operation of UASB reactor to treat landfill leachate and synthetic wastewater by increasing OLR (organic loading rate; kg COD/m^3·day).
(●) Synthetic wastewater treated by intact granules, (○) Synthetic wastewater treated by disintegrated granules, (▼) Landfill leachate treated by intact granules, (▽) Landfill leachate treated by disintegrated granules.

also had no significant effect on the anaerobic treatment of nontoxic wastewater. However, when the landfill leachate was treated, the difference in the COD removal between the intact and disintegrated granules was significant. The COD removal from the landfill leachate wastewater by the intact granules was the same as that from the synthetic wastewater, yet the COD removal by the disintegrated granules was significantly decreased by approximately 70%.

Immediately after each change of the organic loading rate (OLR), a loading shock, that is a sudden decrease in the COD removal rate, was observed in all the reactors, irrespective of the granular state and type of wastewater. The time necessary to recover the COD removal rate was proportional to the OLR.

After operating the UASB reactors for 70 days, flocculation of the disintegrated granules became apparent and their COD removal rate approached that of the intact granules. The flocculation occurred over the period of OLR 4, during which the influent wastewater was diluted at COD 5,000 mg/l, i.e. a quarter of the COD of the real landfill leachate. This phenomenon indicates that the layered structure of granular sludge is the major factor in determining resistance in anaerobic toxic wastewater digestion.

In conclusion, it was found that the layered structure of granular sludge provides resistance against toxicants, and therefore, the granulation of a UASB reactor would appear to be an appropriate method for treating toxic wastewater.

Acknowledgment

This work was supported by the Brain Korea 21 Project in 2002 from the Ministry of Education & Human Resources Development of Korea.

REFERENCES

1. Angelidaki, I. and B. K. Ahring. 2000. Methods for increasing the biogas potential from the recalcitrant organic matter contained in manure. *Water Sci. Technol.* **41:** 189-194.
2. Bae, J.-W. and S.-T. Lee. 1999. Layered structure of UASB granules gives microbial population resistance to toxic chemicals. *Biotech. Lett.* **21:** 159-162.
3. Bae, J.-W., S.-K. Rhee, S.-H. Hyun, I. S. Kim, and S.-T. Lee. 2000. Layered structure of granules in upflow anaerobic sludge blanket reactor gives microbial populations resistance to metal ions. *Biotech. Lett.* **22:** 1935-1940.
4. Bae, J.-W., S.-K. Rhee, A. Jang, I. S. Kim, and S.-T. Lee. 2002. Copper ion toxicity causes discrepancy between acetate degradation and methane production in granular sludge. *J. Microbiol. Biotechnol.* **12:** 849-853.
5. Blum, J., R. Hergenroeder, G. F. Parkin, and R. E. Speece. 1986. Anaerobic treatment of coal conversion wastewater constituents: Biodegradability and toxicity. *J. Water Pollut. Control Fed.* **58:** 122-231.
6. Cliona, O., R. H. Freda, R. R. E. Sandra, L. H. Dennis, and S. J. Wilcox. 1999. Anaerobic and aerobic treatment of a simulated textile effluent. *J. Chem. Tech. Biotech.* **74:** 993-999.
7. Dwyer, D., M. L. Krumme, S. A. Boyd, and J. M. Tiedje. 1986. Kinetics of phenol biodegradation by an immobilized methanogenic consortium. *Appl. Environ. Microbiol.* **52:** 345-351.
8. Fang, H. H. P. 1997. Inhibition of bioactivity of UASB biogranules by electroplating metals. *Pure Appl. Chem.* **69:** 2425-2429.
9. Fedork, P. M. and S. E. Frudey. 1986. Anaerobic treatment of phenolic coal conversion wastewaters in semicontinuous cultures. *Water Res.* **20:** 113-122.
10. Galil, N. I. and D. Wolf. 2001. Removal of hydrocarbons from petrochemical wastewater by dissolved air flotation. *Water Sci. Technol.* **43:** 107-113.
11. Henry, J. G. and D. Prasad. 2000. Anaerobic treatment of landfill leachate by sulfate reduction. *Water Sci. Technol.* **41:** 239-246.
12. Hickey, R. F., J. Vanderwielen, and M. S. Sitzenbaum. 1989. The effect of heavy metals on methane production and hydrogen and carbon monoxide levels during batch anaerobic sludge digestion. *Water Res.* **23:** 207-218.
13. Hörber, C., N. Christiansen, E. Arvin, and B. K. Ahring. 1998. Improved dechlorinating performance of upflow anaerobic sludge blanket reactors by incorporation of *Dehalospirillum multivorans* into granular sludge. *Appl. Environ. Microbiol.* **64:** 1860-1863.
14. Hung, C. H. and S. G. Pavlostathis. 1999. Kinetics and modeling of autotrophic thiocyanate biodegradation. *Biotechnol. Bioeng.* **62:** 1-11.
15. Hwang, S.-S. and H.-G. Song. 2000. Biodegradation of pyrene by the white rot fungus, *Irpex lacteus*. *J. Microbiol. Biotechnol.* **10:** 344-348.
16. Kim, J. K., S.-K. Kim, and S.-H. Kim. 2001. Characterization of immobilized denitrifying bacteria isolated from municipal sewage. *J. Microbiol. Biotechnol.* **11:** 756-762.
17. Kleerebezem, R., M. Ivalo, L. W. pol Hulshoff, and G. Lettinga. 1999. High-rate treatment of terephthalate in anaerobic hybrid reactors. *Biotechnol. Prog.* **15:** 347-357.
18. Lee, H.-S. and K. Lee. 2001. Bioremediation of diesel-contaminated soil by bacterial cells transported by electrokinetics. *J. Microbiol. Biotechnol.* **11:** 1038-1045.
19. Lettinga, G. 1995. Anaerobic digestion and wastewater treatment systems. *Antonie Van Leeuwenhoek* **67:** 3-28.
20. Lettinga, G., S. Rebac, S. Parshina, A. Nozhevnikova, J. B. van Lier, and A. J. Stams. 1999. High-rate anaerobic treatment of wastewater at low temperatures. *Appl. Environ. Microbiol.* **65:** 1696-1702.
21. Lettinga, G., A. F. M. van Velsen, S. M. Hobma, W. de Zeeuw, and A. Klapwijk. 1980. Use of upflow sludge blanket (USB) reactor concept for biological wastewater treatment, especially for anaerobic treatment. *Biotechnol. Bioeng.* **22:** 699-734.

22. Lim, J. G. and D. H. Park. 2001. Degradation of polyvinyl alcohol by *Brevibacillus laterosporus*: Metabolic pathway of polyvinyl alcohol to acetate. *J. Microbiol. Biotechnol.* **11:** 928-933.
23. Lin, C. Y. 1993. Effect of heavy metals on acidogenesis in anaerobic digestion. *Water Res.* **27:** 147-152.
24. Lin, C. Y. and M. L. Lin. 1997. Effect of heavy metals on the upflow anaerobic sludge blanket process. *J. Environ. Sci. Health A* **32:** 1933-1944.
25. MacLeod, F. A., S. R. Guiot, and J. W. Costerton. 1990. Layered structure of bacterial aggregates produced in an upflow sludge bed and filter reactor. *Appl. Environ. Microbiol.* **56:** 1598-1607.
26. Mukhopadhyay, B., E. F. Johnson, and R. S. Wolfe. 1999. Reactor-scale cultivation of the hyperthermophilic methanarchaeon *Methanococcus jannaschii* to high cell densities. *Appl. Environ. Microbiol.* **65:** 5059-5065.
27. Murgel, G. A., L. W. Lion, C. Acheson, M. L. Shuler, D. Emerson, and W. C. Ghiorse. 1991. Experimental apparatus for selection of adherent microorganisms under stringent growth conditions. *Appl. Environ. Microbiol.* **57:** 1987-1996.
28. Park, D. H. and Y. K. Park. 2001. Bioelectrochemical denitrification by *Pseudomonas* sp. or anaerobic bacterial consortium. *J. Microbiol. Biotechnol.* **11:** 406-411.
29. Park, S., K. S. Choi, K. S. Joe, W. H. Kim, and H. S. Kim. 2001. Variations of landfill leachate's properties in conjunction with the treatment process. *Environ. Technol.* **22:** 639-645.
30. Rhee, S.-K., G.-M. Lee, J.-H. Yoon, Y.-H. Park, H.-S. Bae, and S.-T. Lee. 1997. Anaerobic and aerobic degradation of pyridine by a newly isolated denitrifying bacterium. *Appl. Environ. Microbiol.* **63:** 2578-2585.
31. Ryeom, T.-K., I.-G. Lee, S.-Y. Son, and T.-Y. Ahn. 2000. Degradation of phenanthrene by *Sphingomonas* sp. 1-21 isolated from oil-contaminated soil. *J. Microbiol. Biotechnol.* **10:** 724-727.
32. Schmidt, J. E. and B. K. Ahring. 1991. Acetate and hydrogen metabolism in intact and disintegrated granules from an acetate-fed, 55C, UASB reactor. *Appl. Microbiol. Biotechnol.* **35:** 681-685.
33. Schmidt, J. E. and B. K. Ahring. 1996. Granular sludge formation in upflow anaerobic sludge blanket (UASB) reactor. *Biotech. Bioeng.* **49:** 229-246.
34. Schmidt, J. E. and B. K. Ahring. 1999. Immobilization patterns and dynamics of acetate-utilizing methanogens immobilized in sterile granular sludge in upflow anaerobic sludge blanket reactors. *Appl. Environ. Microbiol.* **65:** 1050-1054.
35. Wacheux, H., S. da Silva, and J. Lesavre. 1993. Inventory and assessment of automatic nitrate analyzers for urban sewage works. *Water Sci. Technol.* **28:** 11-12.
36. White, C., J. A. Sayer, and G. M. Gadd. 1997. Microbial solubilization and immobilization of toxic metals: Key biogeochemical processes for treatment of contamination. *FEMS Microbiol. Rev.* **20:** 503-516.