Biotechnology Letters **21:** 159–162, 1999.

Layered structure of UASB granules gives microbial populations resistance to toxic chemicals

Jim-Woo Bae & Sung-Taik Lee*
Department of biological science, Korea Advanced Institute of Science and Technology, Kusong-dong 373–1, Yusong-ku, Taejon 305–701, South Korea
**Author for correspondence (Fax: +82-42-869-2610; E-mail: stlee@sorak.kaist.ac.kr)*

Received 3 December 1998; Accepted 11 January 1999

Key words: granules, UASB, resistance, toxic chemicals, layered structure

Abstract

To investigate the effect of granular structure on resistance to toxic chemicals in UASB (Upflow Anaerobic Sludge Blanket) reactors, normal and broken granules were examined for their ability to degrade acetate with and without the addition of toluene or trichloroethylene as a toxic chemical. Without a toxic chemical, both normal and broken granules degraded the acetate at the same volumetric degradation rate (3.21 mM h^{-1}). However, when 500 $\mu l\ l^{-1}$ of toluene or trichloroethylene was added, the acetate-degradation rate of the broken granules was about a third of the rate with normal granules. Therefore, the layered structure of the UASB granules seems to give microbial populations the ability to resist toxic chemicals.

Introduction

Anaerobic digestion was one of the first biological unit-processes used for the treatment of high-concentration and complex wastewater (Lettinga *et al.* 1980). Over the decades, many kinds of anaerobic reactors have been developed (Seghezzo *et al.* 1998). The UASB (Upflow Anaerobic Sludge Blanket) reactor is a recently developed anaerobic process. Because of their ability to retain high biomass concentrations, UASB reactors have received a lot of attention (Schmidt & Ahring 1996).

After 1–6 months of UASB reactor operation, suspended sludge is slowly flocculated and granulated. Special conditions are necessary to promote the aggregation of microbial populations. This is caused by the extracellular matrix produced by acidogens, methanogens and H_2-consuming microorganisms. Granular sludge is generally seen in two or three layers. Acidogens and H_2 producing- and H_2 consuming-organisms are located in the outer layer, and methanogens at the center (Schmidt & Ahring 1996).

A few reports have indicated that the high resistance of granules to toxic chemicals is attributable to their layered structure (Fang 1997, Lin 1993). However, there has been no report on the effect of granulation using broken granules as a control for normal granules. In this paper, we describe the relationship between the layered structure of granules and their resistance to toxic organic compounds by comparing normal layered and broken granules.

Materials and methods

Granules and sludge

Sludge granules were sampled from a 1000 m^3 UASB reactor treating beer brewery wastewater having a COD (Chemical Oxygen Demand) of 3,000–4,000 mg l^{-1}. The reactor has been in operation for 2 years at the hydraulic retention time of 10 h while removing 95% of influx COD. The granules were about 2 mm in diameter and were brownish black. To obtain non-layered sludge with the same ability to degrade carbon sources and microbial populations as normal granules, the granules were blended with a homogenizer (Ace Homogenizer, Nihonseiki Kaisha LTD., Japan) at 10,000 rpm for two minutes. The broken granules were sieved with a mesh size of 0.5 mm prior to experiment to remove unbroken granules. All the sludge samples prepared were washed three times with 0.1 M KH_2PO_4/K_2HPO_4 buffer before the experiments. All the procedures were conducted in an anaerobic atmosphere (Controlled Atmosphere Chamber. Lancing, Mich. Co.).

Medium

Toluene and trichloroethylene (HPLC grade, Sigma) were used as the toxic chemicals. The basal medium 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; and 40 mM of sodium acetate in a 125 ml serum bottle (pH 7.0). Toluene and trichloroethylene were added to media containing 30 ml of wet granules (1.65 g dry cell, 1.20 g volatile suspended solid). The mixtures were purged by N_2 to maintain the anaerobic condition and incubated at 37 °C with shaking at 150 rpm.

Analytical methods

The acetate concentration was determined by isocratic HPLC (Millipore Waters, Redford, Mass., USA) with an ion exchange column (Aminex HPX-87H, 300 × 7.8 mm) and an organic acid analysis kit. The acetate was detected at 210 nm with a Waters 441 UV detector (Blake *et al.* 1987). Degassed 4 mM H_2SO_4 was used as an eluant.

Determination of acetate degradation rate

The rate constant of initial volumetric acetate degradation, *k*, was defined as follows:

$$-k = \frac{[\text{Ace}]_{12} - [\text{Ace}]_0}{t}$$

t denotes the reaction time, 12 h, and $[\text{Ace}]_0$ and $[\text{Ace}]_{12}$ denotes the initial concentration of acetate and the concentration of acetate of 12 h after starting, respectively.

Results and discussion

In order to obtain broken granules without a layered structure, normal granules were blended with a homogenizer. Both the normal and broken granules completely degraded 40 mm of acetate in 24 hours at the same rate (Figure 1). The volumetric degradation rates of normal and broken granules after 12 h were 3.21 mM h^{-1} and 3.22 mM h^{-1}, respectively.

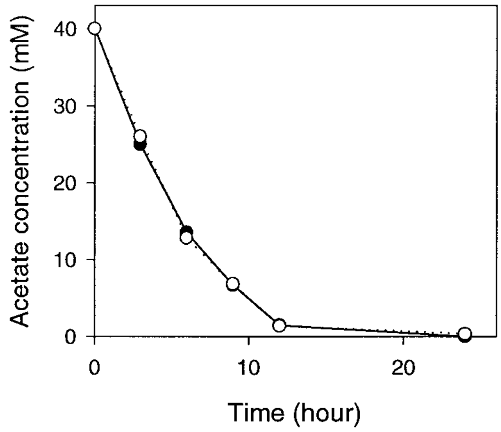

Fig. 1. Acetate degradation by normal and broken granules; (●) normal granules, (○) broken granules.

This result differed from what was previously reported by Schmidt & Ahring (1991). In this report, the granules disintegration, by vortex mixer or by ultrasound, had no effect on methane production but breaking by blender or syringe decreased the production of methane by 54%. This could be caused by the difference in methane production and acetate degradation because the granules contained not only methanogens but also a large number of acetate-degrading organisms (Schmidt & Ahring 1996). The disintegration of the granules might cause abruption in metabolizing the acetate to methane (Schmidt & Ahring 1991).

Thus, we were able to disintegrate granules without a layered structure but with the same microbial population and ability to degrade acetate.

In previous studies (Fang 1997, Lin 1993) of the effect of toxicants on the ability of granules, flocculated digestion sludge was used as the control for the granules. However, the digestion sludge had a slightly different microbial population from that of the UASB granules (Bhatti *et al.* 1996). In our study, the granules were blended by the homogenizer to eliminate the normal layered structure from the granules.

To investigate the influence of the layered structure on granule resistance to the toxic chemical of toluene and trichloroethylene, intact and disintegrated granules were incubated in media containing toxic compounds in the range from 50 $\mu l\ l^{-1}$to 10000 $\mu l\ l^{-1}$. Figure 2 shows a significant interdependence among

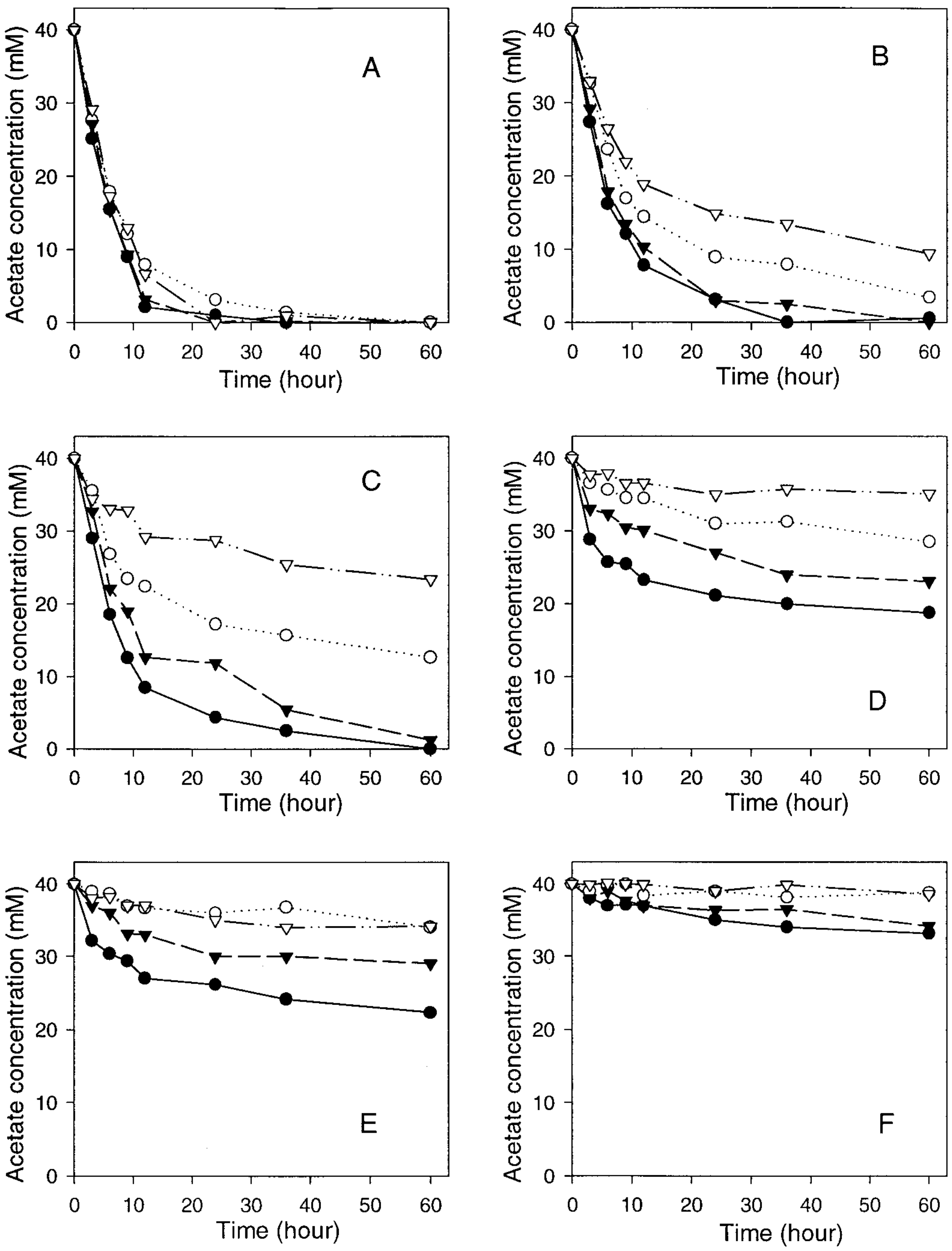

Fig. 2. Acetate degradation by normal and broken granules in media containing (A) 50 $\mu l\, l^{-1}$, (B) 100 $\mu l\, l^{-1}$, (C) 500 $\mu l\, l^{-1}$, (D) 1000 $\mu l\, l^{-1}$, (E) 5000 $\mu l\, l^{-1}$, (F) 10000 $\mu l\, l^{-1}$ of toxic chemicals; (●) normal granules with toluene, (○) broken granules with toluene, (▼) normal granules with trichloroethylene, (▽) broken granules with trichloroethylene.

the degradation rate, the granules state and the kind of toxic organic compounds.

When the initial concentration of toxic compounds was less than 50 $\mu l\ l^{-1}$, the granules degraded acetate completely regardless of their structure. However, when the concentration of toxic organic chemicals was increased more than 100 $\mu l\ l^{-1}$, broken granules could not completely degrade the acetate within 60 h. When the concentration of toxicants was further increased to 500 $\mu l\ l^{-1}$, the acetate-degradation rate of the broken granules was about a third of the rate with normal granules. The degradation rates of normal and broken granules in media containing 500 $\mu l\ l^{-1}$ toluene were 2.5 mM h^{-1} and 1.1 mM h^{-1}, and in media containing trichloroethylene 2.1 mM h^{-1} and 0.7 mM h^{-1}, respectively. When the initial concentration of toxic compounds was higher than 1000 $\mu l\ l^{-1}$, the acetate was not completely degraded by either type of granule within 60 h. However, normal granules had more tolerance to toxic organic chemicals than broken granules even up to concentrations of 5000 $\mu l\ l^{-1}$. When the toxicant concentration was further increased to 10,000 $\mu l\ l^{-1}$, no degradation of acetate by granules was observed.

The rate constant of acetate degradation, *k*, of each granule state and the initial concentrations of toluene and trichloroethylene were plotted logarithmically in Figure 3 in order to show clearly the positive effect of the layered structure on resistance to toxic chemicals. When the concentration of toxicants were in the range from 500 $\mu l\ l^{-1}$ to 1000 $\mu l\ l^{-1}$, the degradation rates, *k*, decreased significantly. This indicated that toluene and trichloroethylene significantly influenced the activities of the granules in this range.

The inhibitory effect of trichloroethylene on the activity of both granules was greater than that of toluene. The volumetric degradation rate of the granules in toluene was an average of 1.4 times higher than that in trichloroethylene. However, the difference in degradation rates between normal granules and broken granules was same regardless of the toxic chemical. According to these results, the improvement in the granule tolerance of the toxic chemicals through the granulation appeared to be the result of internal diffusional resistance (Schmidt & Ahring 1991).

It was observed that broken granules as well as normal granules completely degraded acetate, irrespective of the state of the granules in media without toxic organic compounds. However, in a comparison of normal and broken granules in toxic organic compound media, improvement in microorganisms' resistance by granular structure in UASB reactor was observed. In conclusion, a UASB reactor, in which the microbial population is in a granular state, is more feasible for the treatment of the wastewater containing toxic compounds compared to traditional anaerobic digestion.

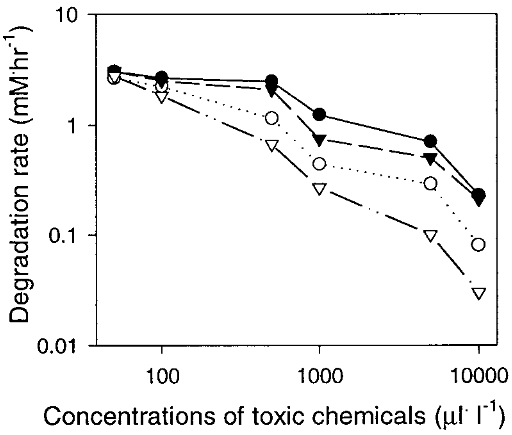

Fig. 3. Change of rate constant of acetate degradation, *k*, by increasing the concentration of toxic chemicals; (●) normal granules with toluene, (○) broken granules with toluene, (▼) normal granules with trichloroethylene, (▽) broken granules with trichloroethylene.

Acknowledgements

The authors wish to thank Jinro Coors for the generous supply of the granules. This work was supported by grants of the STEPI (Science & Technology Policy Institute) for the project of National R&D Program (98-N1-05-02-A-03).

References

Bhatti ZI, Furukawa K, Fujita M (1997) *Pure. Appl. Chem.* **69**: 2431–2438

Blake JD, Clarke ML, Richards GN (1987) *J. Chrom.* **398**: 265-277.

Fang HHP (1997) *Pure. Appl. Chem.* **69**: 2425–2429.

Fang HHP, Hui HH (1994) *Biotechnol. Lett.* **16**: 1091–1096.

Kosaric N, Blaszczyk R (1990) *Adv. Biochem. Eng. Biotechnol.* **42**: 27–62.

Lettinga G, van Velsen AFM, Hobma SM, de Zeeuw W, Klapwijk A (1980) *Biotechnol. Bioeng.* **22**: 699–734.

Lin CY (1993). *Water Res.* **27**: 147–152.

Schmidt JE, Ahring BK (1991). *Appl. Microbiol. Biotechnol.* **35**: 681–685.

Schmidt JE, Ahring BK (1996). *Biotechnol. Bioeng.* **49**: 229–246.

Seghezzo L, Zeeman G, Vanlier JB, Hamelers HVM, Lettinga G (1998) *Biores. Technol.* **65**: 175–190.

Speece RE (1983) *Environ. Sci. Technol.* **17**: 416–427.