Pure & Appl. Chem., Vol. 69, No. 11, pp. 2425–2429, 1997.
Printed in Great Britain.

Inhibition of bioactivity of UASB biogranules by electroplating metals

Herbert H. P. Fang

Environmental Engineering Research Centre, Department of Civil and Structural Engineering, The University of Hong Kong, Pokfulam Road, Hong Kong.

Abstract: Among the high-rate anaerobic wastewater treatment processes, the upflow anaerobic sludge blanket (UASB) reactor has attracted most commercial and research interests. In this study, the toxicity of five heavy metals commonly found in the electroplating effluent on the activity of anaerobic biogranules was investigated. Biogranules were sampled from UASB reactors treating four types of wastewaters containing, individually, acetate, propionate, starch and benzoate as the sole substrate. All reactors were operated at 37°C for over six months at a loading rate of 10 g $COD^{-1}d^{-1}$. The methanogenic activity of biogranules treating each type of wastewater decreased with the increase of metal concentration. The toxicity of each metal to the biogranules was measured by the reduction of methanogenic activity of biogranules in serum vials, as compared to the controls. Results show that, in general, acetate- and propionate-degrading biogranules exhibited higher resistance to electroplating metals than the starch- and benzoate-degrading ones. Acetate conversion is a one-step process conducted by acetotrophic methanogens alone. Propionate degradation, on the other hand, is a two-step process conducted by acetogens and methanogens; whereas, benzoate and starch degradations are multi-step processes conducted jointly by fermentative/acidogenic bacteria, acetogens and methanogens. Results of this study imply that heavy metals from electroplating effluent, in general, inhibit the bioactivities of fermentative/acidogenic bacteria more than those of methanogens and acetogens. For the latter two groups of bacteria, the toxicity of the heavy metals were in the following descending order: zinc > nickel > copper > cadmium > chromium.

Keywords: anaerobic, biogranule, inhibition, metal, UASB

INTRODUCTION

Anaerobic technology has become widely accepted in the past decade for the treatment of industrial wastewater containing concentrated organic pollutants. Among the recently developed high-rate processes, the upflow anaerobic sludge blanket (UASB) reactor (ref. 1) has probably attracted most commercial and research interests. Hundreds of full-scale reactors have been installed worldwide for treating wastewaters from food/beverage and agricultural industries. The success of this process relies on the agglutination of biomass, forming granules with high settleability and bioactivity. In addition, recent studies showed that UASB biogranules were also capable of degrading aromatic pollutants, such as benzoate (ref. 2) and phenol (ref. 3). Since many complex aromatic compounds degrade via benzoate and phenol, these findings suggest that anaerobic technology could likely be extended to the treatment of wastewater from many chemical industries.

Wastewater from electroplating industry containing high levels of heavy metals is the major polluter in many countries. Although in trace amount they are the essential elements for the microbial growth (ref. 4), heavy metals at elevated concentrations often become inhibitory and toxic to the anaerobic degradation of organic matter (ref. 5). In municipal wastewater treatment, high concentration of heavy metals in sewage could also lead to upset and failure of the anaerobic sludge digestion process (refs. 6,7). It is thus of great interest to investigate the effect of heavy metals from the electroplating effluent on the bioactivity of different UASB biogranules.

MATERIALS AND METHODS

Five heavy metals commonly found in the electroplating effluent, i.e. cadmium, chromium, copper, nickel and zinc, were selected for this study. Biogranules were sampled from four UASB reactors (refs.2,8,9 & 10) treating wastewaters individually containing acetate, propionate, starch and benzoate as the sole substrate at a steady COD (chemical oxygen demand) loading rate of 10 g $CODl^{-1}d^{-1}$ for over six months at 37°C.

The activities of biogranules were measured by the maximum rates of methane production in 157ml serum vials using the method of Dolfing and Mulder (ref. 11) modified from the one developed by Owen, *et al.*, (ref. 12). To each vial was added granules equivalent to 100mg of VSS (volatile suspended solids). The exact amount of VSS in each vial was later measured (ref. 13) at the end, after methane production data had been obtained. To each vial was also added 100ml of synthetic wastewater containing individual substrate at the dosage equivalent to 300mg of COD, plus nutrients, vitamins and trace minerals; in addition, individual selected heavy metals over a wide range of concentration were also dosed to the serum vials. All vials were then capped with butyl rubber and submerged in 37°C water in a shaking bath. Transfers of wastewater and granules to the serum vials were conducted under an anaerobic environment.

The biogas production from each vial was monitored every few hours using a syringe; the methane contents in the biogas were measured using a gas chromatograph (Hewlett Packard, model 5890A) equipped with a thermal conductivity detector and a 2m x 2mm (inside diameter) stainless-steel column packed with Porapak N (80-100 mesh). Injector and detector temperatures were kept at 130°C while column temperature was 50°C. Each bioactivity measuring program lasted about one week after the methane production had levelled off. The initial slope of specific methane production represented the specific methanogenic activity (SMA) of the biogranules using the corresponding substrate. All SMA measurements were conducted in duplicate. The toxicity of an individual metal at a given concentration was indicated by the decrease of SMA relative to the controls.

RESULTS AND DISCUSSION

Anaerobic degradation is a multi-step process (14,15). Complex organic substrates are first hydrolyzed by enzymes forming soluble acids and sugars, which are then degraded by acidogenic bacteria into volatile fatty acids (VFA). These intermediate VFA are further degraded by acetogenic bacteria forming acetate, formate, carbon dioxide and hydrogen. These final intermediates are ultimately converted to methane by the methanogenic bacteria. Fang, *et al.* (ref. 16) conducted a series of experiments to investigate the performance of UASB reactor in treating wastewaters individually containing several model substrates and key intermediates; in addition, they also examined the bacterial population dynamics and microstructure of the biogranules. Results showed that biogranules' bacterial populations and microstructures were strongly dependent on the nature of the substrate (ref. 16). Acetate conversion is a one-step process conducted by acetotrophic methanogens alone. Propionate degradation, on the other hand, is a two-step process conducted by acetogens and methanogens; whereas, benzoate and starch degradations are multi-step processes conducted jointly by fermentative/acidogenic bacteria, acetogens and methanogens.

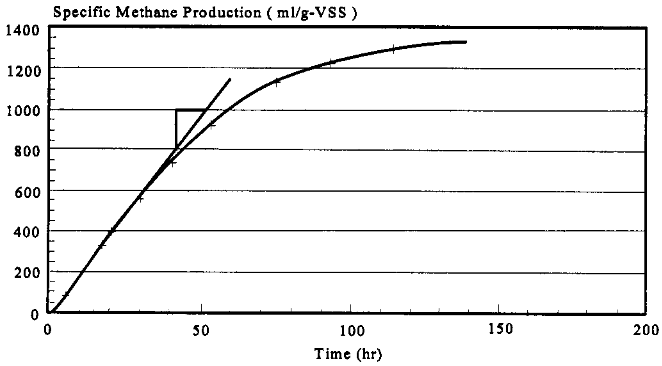

Fig.1. Specific Methane Production of Starch-Degrading Biogranules

Fig. 1 illustrates the typical specific methane production of control starch-degrading biogranules. The initial slope in Fig. 1 indicates that, in the absence of any heavy metals, the SMA of the biogranules for the degradation of starch was 1.19 g methane COD (g VSS day)$^{-1}$. The SMA of the biogranules decreased with the increase of heavy-metals concentration in the solution.

Fig. 2 illustrates that the SMA of starch-degrading granules decreased with the increase of heavy metal concentration, except for cadmium. Treating wastewater containing 300-550 mg l^{-1} of cadmium, the SMA of starch-degrading biogranules was reduced only by 20%; and there was no sign indicating that the SMA will be further decreased with the increase of cadmium concentration. The toxic effect on methanogenic activity of starch-degrading biogranules was in the following order: zinc (most toxic) > nickel > copper > chromium > cadmium (least toxic). Similar figures were also plotted to illustrate the toxic effect of individual metals over a wide range of concentration on the methanogenic activities of the other three types of biogranules.

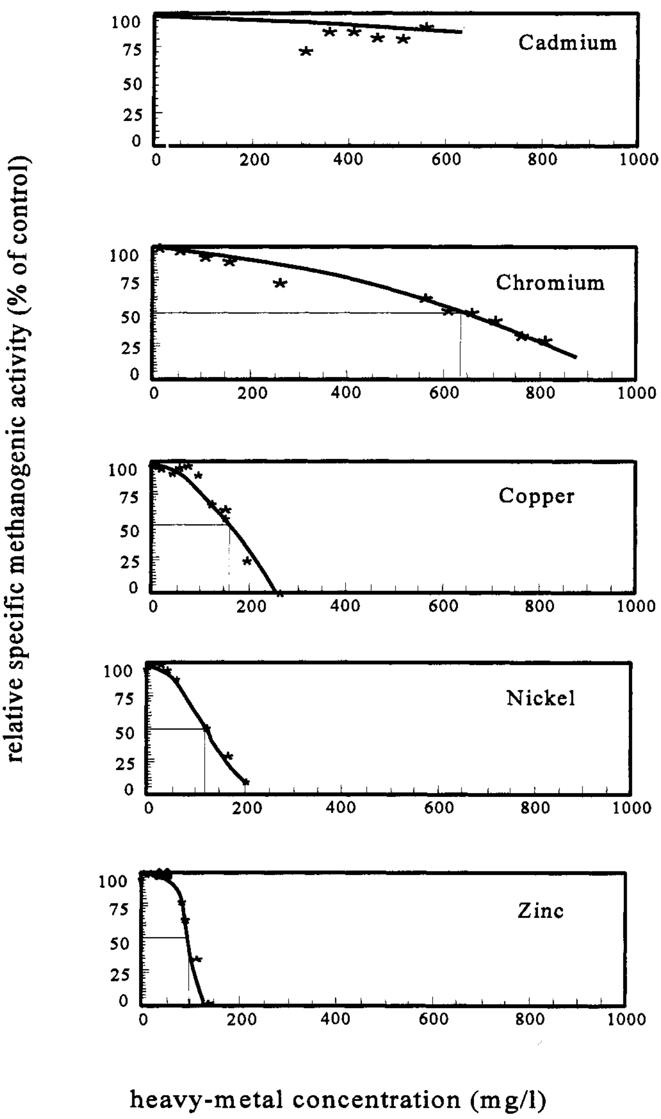

Fig.2. Reduction of specific methanogenic activity with increased metal concentration

The toxicity of each metal can be expressed by a simple $C_{I,50}$ value, which represents the metal concentration at which level the SMA of the biogranules was reduced to 50% of the control. Higher $C_{I,50}$ values mean that the biogranules exhibited better resistance to metal toxicity. Fig. 2 illustrates that the respective $C_{I,50}$ values for starch-degrading biogranules were >400 mg Cd l^{-1}, 630 mg Cr l^{-1}, 158 mg Cu l^{-1}, 118 mg Ni l^{-1} and 97 mg Zn l^{-1}. Similar data were also obtained for the other three types of biogranules.

Fig. 3 compares the $C_{I,50}$ values of heavy metals for the four anaerobic biogranules investigated in this study. The starch-degrading granules exhibited high resistance to the cadmium with $C_{I,50}$ > 400 mg Cd l^{-1}; the exact $C_{I,50}$ value was not obtained, and thus not shown in the figure. Fig. 3 illustrates that, in general, acetate- and propionate-degrading biogranules exhibited higher resistance to heavy metals than the starch- and benzoate-degrading ones.

It is of interest to note that the acetate- and propionate-degrading biogranules were composed of methanogens and acetogens only, without the presence of fermentative/acidogenic bacteria, while the starch- and benzoate-degrading biogranules were composed of all three types of bacteria. Results illustrated in Fig. 3, therefore, imply that, in general, methanogens and acetogens have better resistance toward heavy-metals toxicity than the fermentative/acidogenic bacteria. Although it was generally believed that methanogens are vulnerable to toxicity, Hickey, *et al.*, (ref. 17) speculated that some other bacteria in the anaerobic consortia might be inhibited more severely by the pulse addition of heavy metals than the methanogens. Lin (ref. 18), on the other hand, also showed that acidogens were more sensitive to heavy-metals toxicity than methanogens. The findings of this study provide further evidence that methanogens and acetogens are not the groups of bacteria most sensitive to heavy-metals toxicity in anaerobic degradation.

For the acetate- and propionate-degrading biogranules, the toxicity of the heavy metals were in the following

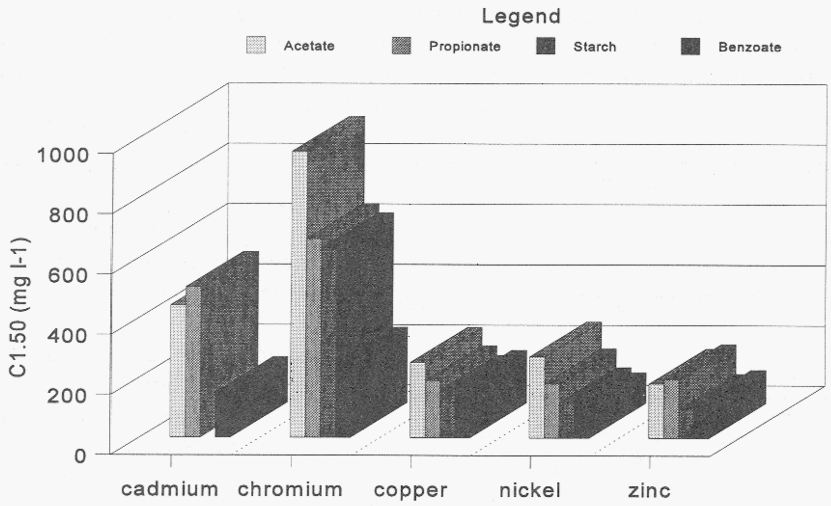

Fig.3. $C_{1.50}$ values of heavy metals for various biogranules

order: zinc (most toxic) > nickel > copper > cadmium > chromium (least toxic). Methanogens and acetogens exhibited high resistance toward chromium and cadmium, even though both of which have been commonly perceived as highly toxic.

CONCLUSIONS

Results of this study show that, in general, acetate- and propionate-degrading biogranules exhibited higher resistance to heavy metals than the starch- and benzoate-degrading ones. This implies that methanogens and acetogens have better resistance toward heavy-metals toxicity than the fermentative/acidogenic bacteria. For the acetate- and propionate-degrading biogranules, the toxicity of the heavy metals were in the following order: zinc (most toxic) > nickel > copper > cadmium > chromium (least toxic). Methanogens and acetogens exhibited high resistance toward chromium and cadmium, the two heavy metals commonly perceived as highly toxic.

ACKNOWLEDGEMENT

The author would like to thank the Hong Kong Research Grants Council for the financial support of this study.

REFERENCES

1. Lettinga, G., van Velsen, A. F., Hobma, S. M., de Zeeuw, W. & Klapwijk, A. (1980). Use of upflow sludge blanket (USB) reactor concept for biological wastewater treatment. *Biotech. Bioengrg.*, **22**, 699-734.
2. Li, Y. Y., Fang, H. H. P., Chui, H. K. & Chen, T. (1995). UASB treatment of wastewater with concentrated benzoate. *J. Environ. Engrg.* **121(10)**, 748-751.
3. Fang, H. H. P., Chen, T., Chui, H. K. & Li, Y. Y. (1996) Removal of phenol from wastewater in an anaerobic upflow reactor. *Wat. Res.*, **30(6)**, 1353-1360.
4. Speece, R. E. (1983). Anaerobic biotechnology for the industrial wastewater treatment. *Environ. Sci. Tech.*, **17(9)**, 416A-427A.
5. Kugelman, I. J. & Chin, K. K. (1971). Toxicity, synergism, and antagonism in anaerobic waste treatment processes. In: *Anaerobic Biological Treatment Processes* (Pohland, F. G., ed.), *Am. Soc. Chem. Engrg., Adv. Chem.* 105: 53-90.
6. Coker, E. G. & Matthews P. J. (1983). Metals in sewage sludge and their potential effects in agriculture. *Wat. Sci. Technol.* 15: 209-225.
7. Swanwick, J. D., Shurben, D. G. & Jackson, S. (1969). A survey of the performance of sewage sludge digestion in Great Britain. *J. Wat. Pollut. Control Fed.* 68: 639-651.
8. Fang, H. H. P. & Chui, H. K. (1993). Maximum COD loading capacity in UASB reactors at 37°C. *J. Environ. Engrg.*, **119(1)**, 103-119.

9. Fang, H. H. P., Li, Y. Y. & Chui, H. K. (1995a). Performance and sludge characteristics of UASB process treating propionate-rich wastewater. *Wat. Res.*, **29(3)**, 895-898.
10. Fang, H. H. P. & Kwong, T. S. (1995). Anaerobic degradation of starch particulates in an upflow sludge blanket filter reactor. *Environ. Tech.*, **16**, 13-23.
11. Dolfing, J. & Mulder, J. W. (1985). Comparison of methane production rate and coenzyme F_{420} content of methanogenic consortia in anaerobic granular sludge. *Appl. Environ. Microbiol.*, **49(5)**, 1142-1145.
12. Owen, W. F., Stuckey, D. C., Healy, J. B., Young. L. Y. & McCarty, P. L. (1979). Bioassay for monitoring biochemical methane potential and anaerobic toxicity. *Wat. Res.*, **13**, 485-492.
13. APHA, (1985). *Standard Methods for the Examination of Water and Wastewater*, 16th Ed., American Public Health Association, Washington D.C.
14. Gujer, W. & Zehnder, A. J. B. (1983). Conversion Processes in anaerobic digestion. *Wat. Sci. Tech.*, **15(8/9)**, 127-167.
15. Thiele, J. H. & Zeikus, J. G. (1988). Control of interspecies electron flow during anaerobic digestion: the role of formate versus hydrogen transfer during syntrophic methanogenesis in flocs. *Appl. Envir. Microbiol.*, **54(1)**, 20-29.
16. Fang, H. H. P., Chui, H. K. & Li, Y. Y. (1995b). Effect of degradation kinetics on the microstructure of biogranules. **30(6)**, 1353-1360.
17 Hickey, R. F., Vanderwielen, J. & Switzenbaum M. S. (1989). The effect of heavy metals on methane production and hydrogen and carbon monoxide levels during batch anaerobic sludge digestion. *Wat. Res.*, **23(2)**, 207-218.
18. Lin, C-Y. (1993). Effect of heavy metals on acidogenesis in anaerobic digestion. *Wat. Res.*, **27(1)**, 147-152.