Microbial distribution in UASB granules and its resulting effects

H.H.P. Fang

Centre for Environmental Engineering Research, Department of Civil Engineering, The University of Hong Kong, Pokfulam Road, Hong Kong, China

Abstract Microscopic (SEM and TEM) examinations of biogranules sampled from various UASB (Upflow Anaerobic Sludge Blanket) reactors indicated that microbes are densely packed. The microbial distribution is strongly dependent upon the degradation thermodynamics and kinetics of individual substrates. Biogranules degrading carbohydrates exhibited typically a layered distribution with a surface layer populated with hydrolytic/fermentative acidogens, a mid-layer comprising syntrophic colonies and an interior comprising acetotrophic methanogens. On the other hand, those substrates having a rate-limiting hydrolytic/fermentative step did not exhibit any layered pattern; instead, bacteria were interwined and distributed evenly. These observations have two implications. Biogranules are developed through evolution instead of random aggregation of suspended microbes. Furthermore, biogranules should be less vulnerable to the changes of mixed liquor condition, because the large majority of microbes inside the biogranules are shielded from the hostile mixed liquor environment. The latter is supported by experimental evidence that biogranules are more resistant than suspended sludge to the toxicity of hydrogen sulfide, heavy metals and aromatic pollutants in wastewater.

Keywords Anaerobic; biogranule; distribution; microbial; syntrophic; thermodynamics

Water Science and Technology **Vol 42 No 12 pp 201–208** © IWA Publishing 2000

Introduction

The upflow anaerobic sludge blanket (UASB) has become widely accepted as a wastewater treatment technology (Lettinga *et al.*, 1980). Its applications used to be limited to the treatment of high-strength industrial wastewater containing alcohols and carbohydrates (Lettinga and Hulshoff Pol, 1991). But more recently, they have been broadened to the treatment of wastewaters containing recalcitrant aromatic pollutants (Li *et al.*, 1995; Fang *et al.*, 1996; Zhou and Fang, 1997; Fang and Chan, 1997), and low strength wastewaters (Kato, 1994; van Haandel and Lettinga, 1994). A UASB reactor consists of a built-in gas-liquid-solid separator, which effectively retains the biomass. Having a long retention time in the reactor, the biomass gradually developed into granules, normally about 1–3 mm in size.

Biogranules have two obvious advantages over the suspended biomass in the mixed liquor. A sludge bed of biogranules may contain 50 g/L of volatile suspended solids (VSS), considerably higher than that in a suspended-sludge bed (Fang and Chui, 1993). Furthermore, biogranules settle better, due to larger sizes than the suspended sludge in the reactor, and thus have less tendency of being washed out.

Anaerobic degradation is a complex process (Gujer and Zehnder, 1983). It may be roughly divided into three steps. Organic substrates, such as proteins and carbohydrates, are first hydrolyzed by enzymes of bacteria forming soluble amino acids and sugars, which are then degraded by acidogenic bacteria into volatile fatty acids (VFA). Many aromatic compounds, on the other hand, are converted to benzoate by other bacteria (Knoll and Winter, 1989). Both VFA and benzoic acid are further degraded to acetate, formate, carbon dioxide and hydrogen by acetogenic bacteria. This final group of intermediates is ultimately converted to methane. Thus, a complete degradation of complex substrates involves three specific groups of bacteria, namely acidogens, acetogens and methanogens.

A biogranule is itself a microenvironment, in which complex pollutants are converted ultimately into methane by these three groups of bacteria. The microbial distribution in a biogranule is naturally dependent upon the degradation nature of the substrate. A series of studies was recently conducted to investigate the performance of a UASB reactor in treating wastewaters individually containing several model substrates and key intermediates (Fang and Chui, 1993; Chui *et al.*, 1994; Fang and Kwong, 1994; Fang *et al.*, 1994a; Fang *et al.*, 1995a, b; Li *et al.*, 1995). The microstructures of individual biogranules were reported in these papers, along with discussions of other parameters, such as COD (chemical oxygen demand), removal efficiency, maximum substrate utilization rate, sludge yield, etc. This paper is to summarize the microbial distribution patterns in typical biogranules, based on information obtained in these studies. It is hoped that such an analysis would lead to a better understanding on the biogranules' characteristics, formation mechanisms and potential applications.

Development of biogranules and microscopic analysis

Biogranules treating brewery effluent were obtained from a full-scale UASB reactor. Other biogranules were sampled from a number of laboratory UASB reactors (2.8 and 8.5 litres in size), each of which had been operated steadily at a COD loading rate of about 10 g/(l.d) for at least five months. All laboratory reactors were seeded with suspended sludge obtained from the anaerobic digester of a local sewage treatment plant. Suspended biomass in the reactors gradually developed into biogranules. Discrete biogranules became visible normally in 2–3 months. Biogranules were sampled from each reactor for examinations for the distribution of methanogens using light microscopy (Chui and Fang, 1994), and of the bacterial morphology using scanning and transmission electron microscopies (SEM and TEM). Details of the reactor conditions, biogranule characteristics and the respective preparation procedures are available elsewhere (Fang and Chui, 1993; Chui *et al.*, 1994).

Results and discussion

It is impossible to identify all the bacteria involved in the degradation of complex substrates simply by cell morphologies illustrated in SEM micrographs or by cell ultrastructures illustrated in TEM micrographs. However, detailed information on many methanogens and some hydrogen-producing acetogens have been well documented, including *Methanosaeta concilii* (Patel and Sprott, 1990), *Methanospirillum hungatei* (Zeikus and Bowen, 1975), *Methanobrevibacter arboriphilus* (Mah and Smith, 1981), *Syntrophobacter wolinii* (Boone and Bryant, 1980), *Syntrophomonas wolfei* (McInerney *et al.*, 1981), *Syntrophus buswellii* (Mountfort *et al.*, 1984) etc. Based upon this information, the distribution of certain bacteria inside a biogranule could be estimated with a certain degree of confidence from SEM and TEM micrographs.

Layered microbial distribution

Microscopic examinations indicated that biogranules treating brewery (Fang *et al.*, 1995c), sucrose and starch-containing wastewaters exhibited a three-layer microstructure. Similar observations were reported by MacLeod *et al.* (1990) for sucrose-degrading biogranules, and by Guiot *et al.* (1992) for glucose-degrading biogranules. All of these biogranules were treating carbohydrate substrates.

Figure la illustrates a biogranule sampled from a UASB reactor treating carbohydrate-rich wastewater, whereas Figure lb illustrates the section of biogranule (Chui and Fang, 1994) under epi-fluorescent excitation. Because all methanogens contain F_{350} and F_{420} cofactors, they can be recognized by the emitted fluorescence (Edwards and McBride, 1975), under epi-fluorescent excitations at 350 nm and 420 nm. Figure lb illustrates that the

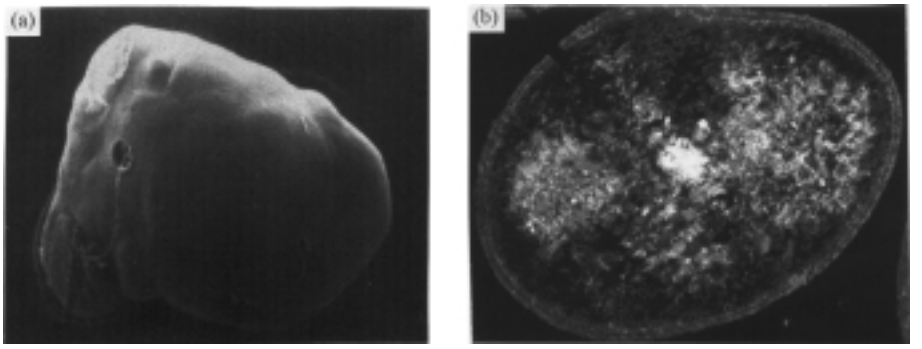

Figure 1 (a) biogranule observed under SEM, and (b) a section of biogranule observed under epifluorescent excitation

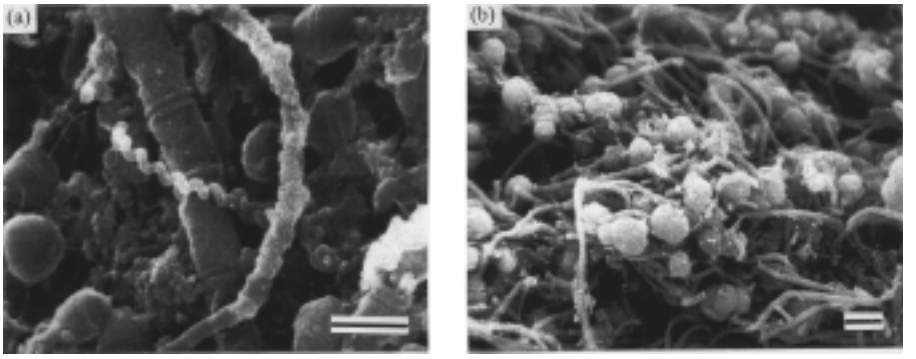

Figure 2 Bacteria of diverse morphologies in the surface layer of (a) sucrose- (bar=1 μm), and (b) peptone-degrading biogranules (bar=4 μm)

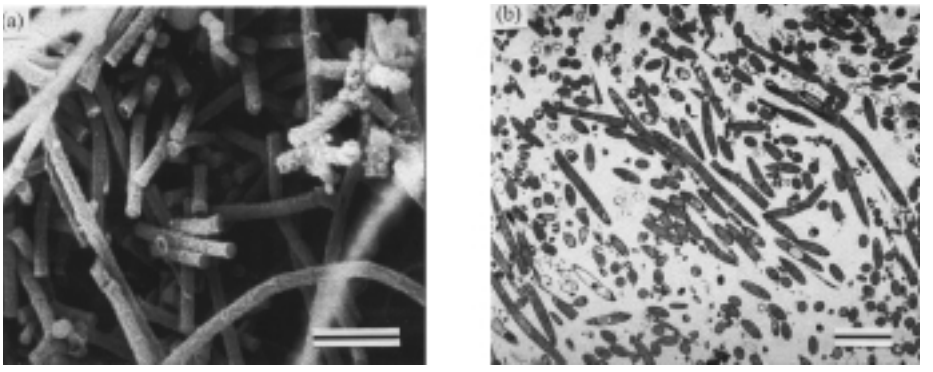

Figure 3 Methanosaeta-like bacteria in the interior of biogranules under (a) SEM, and (b) TEM. (bar=4 μm)

granule had a dense surface layer of 20–40 μm and an interior mostly composed of methanogens. The surface layer, in which hydrolysis and fermentation were taking place, was composed of a wide variety of bacteria, as illustrated in Figure 2a for a sucrose-degrading biogranule and in Figure 2b for a peptone-degrading biogranule. Most of the methanogens in the biogranule interior were mainly the acetotrophic *Methanosaeta* (previously known as *Methanothrix*; Huser,*et al.*, 1982), as illustrated in Figures 3a (SEM micrograph) and 3b (TEM micrograph). The middle layer was mostly populated with syntrophic colonies comprising acetogens which converted VFA, the intermediate products of

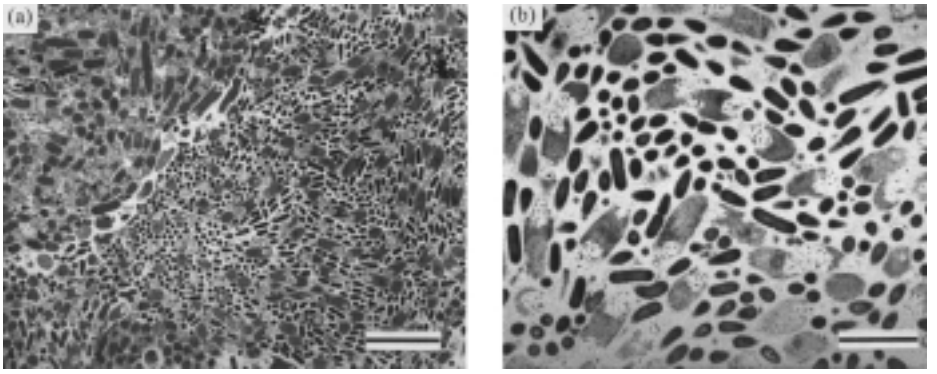

Figure 4 Colonies in the mid-layer of a brewery-treating biogranule: (a) two syntrophic colonies (bar=4 μm), and (b) juxtapositioned syntrophic association. (bar=2 μm)

acidogenic bacteria in the surface layer, into acetate and hydrogen. Acetate was then diffused further inward to be consumed by the *Methanosaeta*, whereas hydrogen was utilized by hydrogenotrophic methanogens readily.

Syntrophic associations and thermodynamic analysis

Figure 4a illustrates that, in the middle layer of a biogranule treating brewery wastewater, a *Methanosaeta*-like colony and a two-bacterium colony are in juxtaposition. Figure 4b further illustrates that inside the latter colony, *Syntrophobacter*-like bacteria were in juxtaposition with *Methanobrevibacter*-like bacteria. These two figures illustrate that the three bacteria were syntrophically associated. One could visualize that propionate, a product of acidogenic bacteria in the surface layer, was diffused to this colony in the mid-layer to be converted by *Syntrophobacter*-like bacteria producing hydrogen and acetate (Reaction 1). Hydrogen was then readily utilized by the juxtapositioned *Methanobrevibacter*-like bacteria to produce methane (Reaction 2), whereas acetate was diffused to the adjacent colony to be converted to methane (Reaction 3) by the *Methanosaeta*-like bacteria.

$$CH_3CH_2COO^- + 3H^2O \rightarrow CH_3COO^- + 3H_2 + HCO_3^- + H^+ \quad \Delta G^{O'} = +76.0\ kJ \quad (1)$$

$$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O \quad \Delta G^{O'} = -135.4\ kJ \quad (2)$$

$$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^- \quad \Delta G^{O'} = -31.0\ kJ \quad (3)$$

where $\Delta G^{O'}$ represents the change of standard Gibbs free energy at pH7. The positive sign of $\Delta G^{O'}$ for Reaction 1 and its magnitude indicate that the acetogenesis of propionate is thermodynamically infeasible, unless products are kept at very low concentrations. The $\Delta G^{O'}$ for Reaction (1) is particularly sensitive to hydrogen, because it is dependent on the cube of the hydrogen concentration. Thus, hydrogen-producing *Syntrophobacter*-like bacteria have to closely attach to the hydrogen-utilizing *Methanobrevibacter*-like bacteria, as illustrated in Figure 4, so that hydrogen produced by the former can be readily consumed by the latter without a buildup of hydrogen concentration. The overall reaction taking place in the syntrophic colony becomes feasible thermodynamically as shown in the following:

$$CH_3CH_2COO^- + 0.75H_2O \rightarrow CH_3COO^- + 0.75CH_4 + 0.25HCO_3^- + 0.25H^+ \quad \Delta G^{O'} = -25.45\ kJ \quad (4)$$

Similar patterns of syntrophic colonies were common in the mid-layer of many biogranules. In some colonies, butyrate-degrading *Syntrophomonas wolfei*-like bacteria (Figure

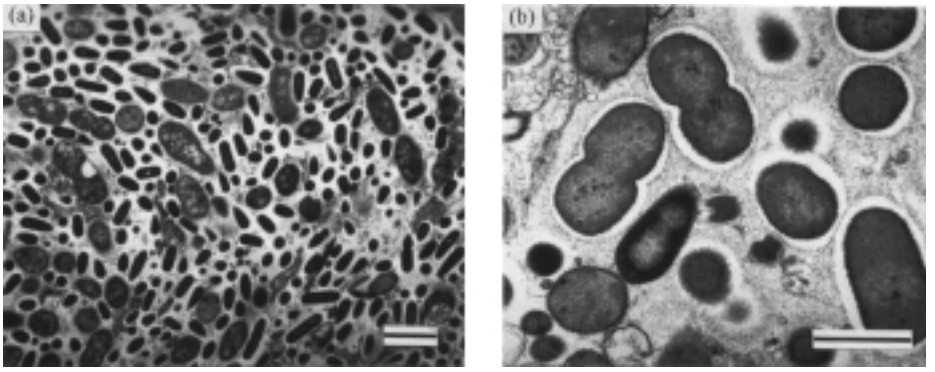

Figure 5 Juxtapositioned syntrophic association in (a) butyrate- (bar = 2 μm), and (b) benzoate-degrading biogranules. (bar = 1 μm)

5a) and benzoate-degrading *Syntrophus buswellii*-like bacteria (Figure 5b) were also juxtapositioned with *Methanobrevibacter*-like bacteria.

Like propionate, conversions of butyrate and benzoate to acetate are thermodynamically unfavorable, as indicated by the positive $\Delta G^{O'}$ values of the following reactions:

$$CH_3CH_2CH_2COO^- + 2\,H_2O \rightarrow 2CH_3COO^- + 2H_2 + H^+ \quad \Delta G^{O'} = +48.1\ kJ \qquad (5)$$

$$C_7H_5O_2^- + 6H_2O \rightarrow 3CH_3COO^- + 3H_2 + CO_2 + 2H^+ \quad \Delta G^{O'} = +58.3\ kJ \qquad (6)$$

It is thus critical for the hydrogen-producing *Syntrophomonas*- and *Syntrophus*-like bacteria juxtapositioned with the hydrogen-utilizing *Methanobrevibacter*-like bacteria.

Infrequently, three syntrophically associated groups of bacteria were found in juxtaposition. Figure 6 illustrates such a case observed in biogranules treating wastewater containing mixed VFA (Fang *et al.*, 1995d). The three juxtapositioned bacteria resemble the propionate-degrading *Syntrophobacter*, *acetotrophic Methanosaeta* and *hydrogenotrophic Methanospirillum*.

Non-layered microbial distribution

On the other hand, biogranules treating propionate (Fang *et al.*, 1995b), peptone (Fang *et al.*, 1994a) and glutamate (Fang *et al.*, 1994b) as substrates did not exhibit any layered microbial distribution. These biogranules were packed with bacteria of different morphologies interwined randomly throughout the cross-section. Similar observations were reported by Grotenhuis *et al.* (1991) for biogranules treating propionate, ethanol, and sugar refinery wastewaters. It is believed that, during the degradation of these substrates, the initial step of degradation, i.e. acctogenesis of propionate (Fang *et al.*, 1995b), hydrolysis of protein (Gujer and Zehnder, 1983) and acidogenesis of glutamate, were rate-limiting. Therefore, a considerable fraction of substrate diffused toward the biogranule interior before being degraded. As a consequence, there was little substrate concentration gradient in the cross-section, resulting in a rather uniform microbial distribution in these biogranules.

Relationship of degradation kinetics and microstructure

Thus the biogranule microstructure is dependent on the rate of the initial degradation. As illustrated in Figure 7a substrates which can be readily fermented/hydrolyzed are mostly degraded in the surface layer while the intermediate products, such as VFA, are converted to acetate and H_2/CO_2 in the mid-layer. Lastly, acetate and H_2/CO_2 are converted to methane. Thus, biogranules degrading substrates, which can be readily fermented/hydrolyzed exhibit a multi-layered microstructure.

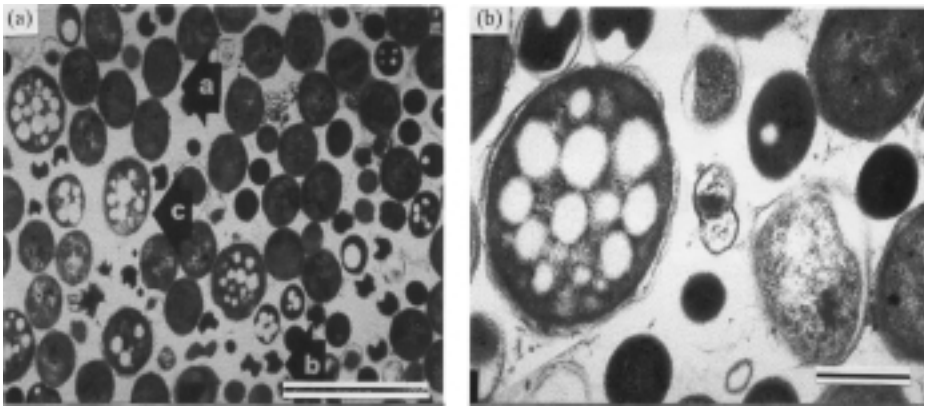

Figure 6 Juxtapositioned syntrophic association in VFA-degrading biogranules. (a) bar = 2 µm; (b) bar = 0.5 µm

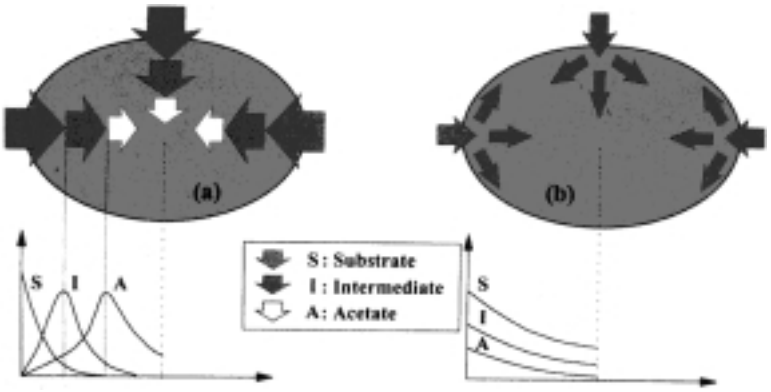

Figure 7 Effect of degradation kinetics on biogranule microstructure

On the other hand, biogranules treating substrates of which the fermentation/hydrolysis step was slow exhibit a complex, but uniform microstructure. Substrates would diffuse toward the biogranule interior without being fermented/hydrolyzed near the surface, as illustrated in Figure 7b.

Formation mechanism of biogranules

Observations summarized in this report show that microbial distribution in biogranules is strongly dependent upon the thermodynamics and kinetics of substrate degradation. The evidence of layered microbial distribution in many biogranules strongly suggests that biogranules are not developed by the random aggregation of suspended bacteria in the mixed liquor. Instead, biogranules are probably developed by evolution as bacteria searched for strategic positions for supply of substrates as well as for removal of products. Once the essential bacteria aggregate to form a nucleus, a biogranule will grow in size as bacteria proliferate. What the conditions are which trigger the formation of nuclei is not yet clear. However, as a biogranule grows in size, bacteria inside would orient themselves for optimal growth, such as formations of layered distributions and juxtapositioned syntrophic colonies. On the other hand, the overall interfacial area between bacteria and the mixed liquor decreases as a biogranule grows in size. This becomes a constraint to how big a biogranule can grow, because lowering the interfacial area limits the initial fermentation/hydrolysis taking place at the biogranule surface.

Toxicity resistant characteristics of biogranules

The microbial distribution seems to suggest that biogranules should be less vulnerable than the suspended sludge to the chemical toxicity of pollutants in wastewater. On one hand, only a limited number of bacteria near the biogranule surface are directly exposed to the toxic chemicals in the mixed liquor, whereas most of the bacteria are located in the interior and are thus protected. On the other hand, it has been widely reported that methanogens are sensitive to the pH fluctuation and chemical toxicity; however, in a biogranule, the vulnerable methanogens as found in this study are mostly located in the interior, and are thus shielded by the hydrolytic/fermentative acidogens near the surface.

Recent experimental results seem to support such an argument. It was found (Fang *et al.*, 1997) that bioactivity of benzoate-degrading biogranules were unaffected by the toxicity of sulfide in the mixed liquor, up to 769 mg/l of soluble sulfide and up to 234 mg/l of H_2S. For comparison, Maillacheruvu *et al.* (1993) reported that 150–200 mg/l of soluble sulfide and 60–75 mg/l of H_2S caused stress in chemostat reactors treating acetate and propionate; higher concentrations could result in system failure.

It was also found that biogranules were able to tolerate heavy metals and toxic organics much better than the suspended sludge. The degree of inhibition can be expressed by the $C_{I,50}$ value, which represents the concentration of a toxic chemical at which the methanogenic activity of sludge is reduced by 50%. The higher the $C_{I,50}$ value, the lower the toxicity. Fang (1997) reported that the $C_{I,50}$ values of several heavy metals, including cadmium, chromium, copper and zinc., for biogranules treating a variety of substrates were one order of magnitude lower than those for the suspended anaerobic sludges. In addition, biogranules also exhibited a high degree of tolerance to the toxic phenolic pollutants (Fang and Chan, 1997). UASB reactors were capable of treating wastewater containing toxic phenol/cresol at concentrations up to 1000 mg/l without any other co-substrate (Fang *et al.*, 1996; Zhou and Fang, 1997). Although activity of biogranules deteriorated drastically at higher concentrations of phenols in continuous treatment, it could be fully recovered in a few weeks when the phenol concentration was lowered to the tolerable levels.

Conclusion

Microbial distribution in a biogranule is strongly dependent upon the degradation nature of the substrate. Biogranules degrading carbohydrates exhibited a layered distribution with a surface populated with hydrolytic/fermentative acidogens, a mid-layer comprising of syntrophic colonies and an interior populated with acetotrophic methanogens. On the other hand, those substrates having a rate-limiting hydrolytic/fermentative step did not exhibit any layered pattern; instead, bacteria were interwined and distributed evenly. These observations imply that biogranules are developed through evolution instead of random aggregation of suspended microbes. Furthermore, only a limited amount of bacteria near the surface are exposed, biogranules are less vulnerable to toxic chemicals in the wastewater.

Acknowledgement

The author wishes to thank the Hong Kong Research Grants Council for the partial financial support of this study, and to the Electron Microscope Unit of the University of Hong Kong for the technical assistance.

References

Boone, D.R. and Bryant, M.P. (1980). Propionate-degrading bacterium, *Syntrophobacter wolinii* sp. nov. gen. nov., from methanogenic ecosystems. *Appl. Environ. Microbiol.*, **40**(3), 626–632.

Chui, H.K. and Fang, H.H.P. (1994). Histological analysis of the microstructure of UASB granules. *J. Envir. Engrg., ASCE*, **120**(5), 1322–1326.

Chui, H.K., Fang, H.H.P. and Li, Y.Y. (1994). Removal of formate from wastewater by anaerobic process. *J. Envir. Engrg., ASCE*, **120**(5), 1308–1320.

Edwards, T. and McBride, B.C. (1975). New method for the isolation and identification of methanogenic bacteria. *Appl. Microbiol.*, **29**(4), 540–545.
Fang, H.H.P. and Chui, H.K. (1993). Maximum COD loading capacity in UASB reactors at 37ºC. *J. Envir. Engrg., ASCE,* **119**(1), 103–119.
Fang, H.H P., Chui, H.K., Li, Y.Y. and Chen, T. (1994a). Performance and granule characteristics of UASB process treating wastewater with hydrolyzed proteins. *Wat. Sci. Tech.*, **30**(8), 55–63.
Fang, H.H.P., Chui, H.K. and Li, Y.Y. (1994b). Microbial structure and activity of UASB granules treating different wastewaters. *Wat. Sci. Tech.*, **30**(12), 87–96.
Fang, H.H.P. and Kwong, T.S. (1994). Degradation of starch particulates in a hybrid reactor. *Wat. Sci. Tech.*, **30**(4), 97–104.
Fang, H.H.P. and Liu, Y. (1995). X-ray analysis of anaerobic granules. *Biotechnol. Techniques,* **9**(7), 513–518.
Fang, H.H.P., Chui, H.K. and Li, Y.Y. (1995a). Anaerobic degradation of butyrate in a UASB reactor. *Bioresource Technol.*, **51**, 75–81.
Fang, H.H.P., Li, Y.Y. and Chui, H.K. (1995b). Performance and sludge characteristics of UASB process treating propionate-rich wastewater. *Wat. Res.*, **29**(3), 895–898.
Fang, H.H.P., Chui, H.K. and Li, Y.Y. (1995c). Microstructural analysis of UASB granules treating brewery wastewater. *Wat. Sci. Tech.*, **31**(9), 129–135.
Fang, H.H.P., Li, Y.Y. and Chui, H.K. (1995d). UASB treatment of wastewater with concentrated mixed VFA, *J. Envir. Engrg., ASCE,* **121**(2), 153–160.
Fang, H.H.P., Chen, T., Chui, H.K. and Li, Y.Y. (1996). Degradation of phenol in wastewater in an upflow anaerobic sludge blanket reactor. *Wat. Res.*, **30**(6), 1353–1360.
Fang, H.H.P. and Chan, O.C. (1997). Toxicity of phenol towards anaerobic biogranules. *Wat. Res.*, **31**(9), 2229–2242.
Fang, H.H.P. (1997). Inhibition of bioactivity of UASB biogranules by electroplating metals. *Pure Appl. Chem.*, **69**(11), 2425–2430.
Fang, H.H.P., Liu, Y. and Chen, T. (1997). Effect of sulfate on anaerobic degradation of benzoate in UASB reactors. *J Envir. Engrg. ASCE,* **123**(4), 320–328.
Grotenhuis, J.T.C., Smit, M., Plugge, C.M., Xu Yuansheng, van Lammeren, A.A.M., Stams, A.J.M. and Zehnder, A.J.B. (1991). Bacteriological composition and structure of granular sludge adapted to different substrates. *Appl. Envir. Microbiol.*, **57**(7), 1942–1949.
Guiot, S.R., Pauss, A. and Costerton, J.W. (1992). A structured model of the anaerobic granule consortium. *Wat. Sci. Tech.*, **25**(7), 1–10.
Gujer, W. and Zehnder, A.J.B. (1983). Conversion processes in anaerobic digestion. *Wat. Sci. Tech.*, **15**(8/9), 127–167.
Huser, M.A., Wuhrmann, K. and Zehnder, A.J.B. (1982). *Methanothrix soehngenii*, gen. nov. sp. nov., a new acetotrophic non-hydrogen-oxidizing methane bacterium. *Arch. Microbiol.*, **132**, 1–9.
Kato, M.T. (1994). The Anaerobic Treatment of Low Strength Soluble Wastewaters. *PhD Thesis*, Agricultural University, Wageningen, The Netherlands.
Knoll, G. and Winter, J. (1989). Degradation of phenol via carboxylation to benzoate by a defined, obligate syntrophic consortium of anaerobic bacteria. *Appl. Envir. Microbiol.*, **30**, 318–324.
Lettinga. G. and Hulshoff Pol, L.W. (1991). UASB-process design for various types of wastewaters. *Wat. Sci. Tech.*, **24**(8), 87–107.
Lettinga, G., van Velsen, A.F.M., Hobma, S.M., de Zeeuw, W. and Klapwijk, A. (1980). Use of the upfiow sludge blanket (USB) reactor concept for biological wastewater treatment. *Biotech. Bioengrg.*, **22**, 699–734.
Li, Y.Y., Fang, H.H.P., Chen, T. and Chui, H.K. (1995). UASB treatment of wastewater containing concentrated benzoate. *J Envir. Engrg.*, **121**(10), 748–751.
MacLeod, F.A., Guiot, S.R. and Costerton, J.W. (1990). Layered structure of bacterial aggregates produced in an upfiow anacrobic sludge bed and filter reactor. *Appl. Envir. Microbiol.*, **56**(6), 1598–1607.
Mah, R.A. and Smith, M.R. (1981). The methanogenic bacteria. *The Prokaryotes*. Starr, M.P., Stolp, H., Turper, H.G., Balows, A., Schlegel, H.G. (eds.). **1**, 948–977.
Maillacheruvu, K.Y., Parkin, G.F., Chen, Y.P., Kou, W.C., Oonge, Z.I. and Lebduschka, V. (1993). Sulfide toxicity in anaerobic systems fed sulfate and various organics. *Wat. Envir. Res.*, **65**(2), 100–108.
McInerney, M.J., Bryant, M.P., Hespell, R.B. and Costerton, J.W. (1981). *Syntrophomonas wolfei* gen. nov. sp. nov., an anaerobic, syntrophic fatty acid-oxidizing bacterium. *Appl. Envir. Microbiol.*, **41**(4), 1029–1039.
Mountfort, D.O., Brulla, W.J., Krumholz, L.R. and Bryant M.P. (1984). *Syntrophus buswellii* gen. nov., sp. nov.: a benzoate catabolizer from methanogenic ecosystems. *Intl. J System. Bacteriol.*, **34**(2), 216–217.
Odom, J.M. (1993). *The Sulfate-Reducing Bacteria: Contemporary Perspectives.* Springer-Verlag, New York.
Patel, G.B. and Sprott, D. (1990). *Methanosaeta concilii* gen. nov., sp. nov. ("*Methanothrix concilii*") and *Methanosaeta thermoacetophil* nom. re., comb. nov. *Intl. J. System. Bacteriol.*, **40**(1), 79–82.
Van Haandel, A.C. and Lettinga, G. (1994). *Anaerobic Sewage Treatment*. Wiley, New York.
Zeikus, J.G. and Bowen, V.G. (1975). Fine structure of *Methanospirillum hungatii. J. Bacteriol.*, **121**(1), 373–380.
Zhou, G.M. and Fang, H.H.P. (1997). Co-degradation of m-cresol and phenol in a UASB reactor. *Biores. Tech.*, **61**(1), 47–52.