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X-RAY ANALYSIS OF ANAEROBIC GRANULES

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SUMMARY

Biogranules developed in the upflow anaerobic sludge blanket (UASB) reactors were analysed by X-ray spectrometry for the local concentration of individual inorganic elements and by X-ray dot mapping for the pictorial distribution of these elements in the cross-section of the biogranules. This information is of significance for the understanding of the sludge granulation mechanism and the role of inorganic elements.

INTRODUCTION

Among the high-rate anaerobic reactors developed in recent years, the upflow anaerobic sludge blanket (UASB) reactor (Lettinga, *et al.,* 1992; Fang and Chui 1993; Fang, *et al.*, 1995) has probably received most commercial interests, especially in Europe and, more recently, in Asia. In a UASB reactor, bacteria aggregate to form biogranules which have high activity as well as superb settleability. However the granulation mechanisms, particularly the effect of inorganic elements have not been fully understood. Lettinga *et al.* (1980) reported that high levels of Ca^{2+}, Mg^{2+}, and Ba^{2+} in wastewater had a positive effect on the granulation of anaerobic sludge, probably because their presence enhanced the mechanical strength and the settleability of the granules. Guiot *et al.* (1988), on the other hand, found that the presence of Ca^{2+} did not enhance sludge granulation, but the presence of other trace metals, such as Fe^{2+}, Ni^{2+}, Co^{2+} and Mn^{2+}, did. Dolfing *et al.* (1985) found about FeS contributed to 30% of the ash content of UASB granules, and, thus, hypothesized that FeS might also play a role in the granulation.

Ash analysis (Dubourguier, *et al.,* 1988; Hulshoff Pol, *et al.,* 1986) was commonly applied to biogranules for the overall contents of inorganic elements. This study was conducted to demonstrate that the more sophisticated X-ray analysis can also be applied to biogranules. The X-ray spectrometry elucidates the local concentration of individual inorganic elements and the X-ray dot mapping illustrates the distribution of these elements throughout the biogranule cross-section. This information is of significance for the understanding of the role of the inorganic elements in the granulation of anaerobic sludge.

MATERIALS AND METHODS

Biogranules from two UASB reactors were sampled for X-ray analysis. Both reactors treated wastewater containing 3000 mg/l of sodium benzoate plus the essential elements (Fang and Chui, 1993). One wastewater contained only 280 mg-sulphate/l, sufficient for the normal anaerobic degradation of benzoate, while the other contained 2000 mg/l of sulphate at which level sulphate-reducing bacteria would proliferate (Speece, 1983; Widdel, 1988). Both reactors were operated in parallel at 37°C and a COD (chemical oxygen demand) loading rate of 10 g/l·d steadily for over three months. The granule samples were washed three times in a 0.1 M phosphate buffer solution at pH 7.2, and then fixed in the phosphate buffer solution with 2.5% glutaraldehyde for 24 hours. The fixed granules were then sliced in the phosphate buffer solution exposing its interior. The sliced granules were dehydrated stepwise by immersing in a graded series of ethanol/water solutions, and then critical-point dried with carbon dioxide (Fang, *et al.*, 1994). The dried granules were mounted on a stud with colloidal carbon followed by sputter coating with gold and palladium, or vacuum coating with carbon, prior to being examined by the Scanning Electron Microscope (SEM, Cambridge Stereoscan 360) in conjunction with an X-ray microanalyser (Link Analytical, Model eXL).

Under SEM, the granule sample was bombarded with electrons, which caused X-ray to be emitted from the sample surface. Using an electron probe pointing at a specific location of the exposed granule interior, individual elements at the pointed location could be determined by an energy-dispersive X-ray spectrometer not only qualitatively (from the energy of emission), but also semi-quantitatively (from the intensity). In the X-ray dot-mapping analysis, the electron probe scanned the cross-section of the sliced granule and the emitted X-ray was used to produce a micrograph depicting the distribution of the corresponding element on the whole cross-section. A number of elements could be detected, according to the energy levels selected. The relative concentration of the element corresponding the specific energy level was indicated by the brightness (Goldstein, *et al.*, 1981).

RESULTS

The cross-section of both types of biogranules were analysed by X-ray dot-mapping for the spatial distribution of a number of elements, including metals (such as ferrous, copper, calcium, potassium, sodium, nickel, cobalt and manganese) as well as non-metals (such as sulphur, phosphorus, silicon and chloride). However, results showed that only three elements, namely ferrous, copper and sulphur, were present in significant quantities in the granules treating wastewater containing concentrated sulphate; the other elements appeared to be insignificant. Figure 1 illustrates microgranphs of the normal SEM image (1d) and those depicting the distribution of sulphur (1a), ferrous (1b), copper (1c) in a granule treating wastewater containing 280 mg/l of sulphate. Figure 2 illustrates the corresponding micrographs for a typical granule treating wastewater containing 2000 mg/l of sulphate.

Figure 1 illustrates that granules treating wastewater of low sulphate concentration exhibited a uniform structure (1d), and had noticeable amount of sulphur precipitates (1a) throughout the cross-section of the granules. Due to the anaerobic environment and the low solubility nature of many metal sulphides, sulphur was presumably precipitated as metal sulphides. Among the metals detected, ferrous was present at significant quantity (1b) while the presence of copper (1c) was barely noticeable. Furthermore, the distribution of inorganic elements was uniform throughout the cross-section of the granule. Figure 2, on the other hand, illustrates that the granules treating wastewater of high sulphate concentration

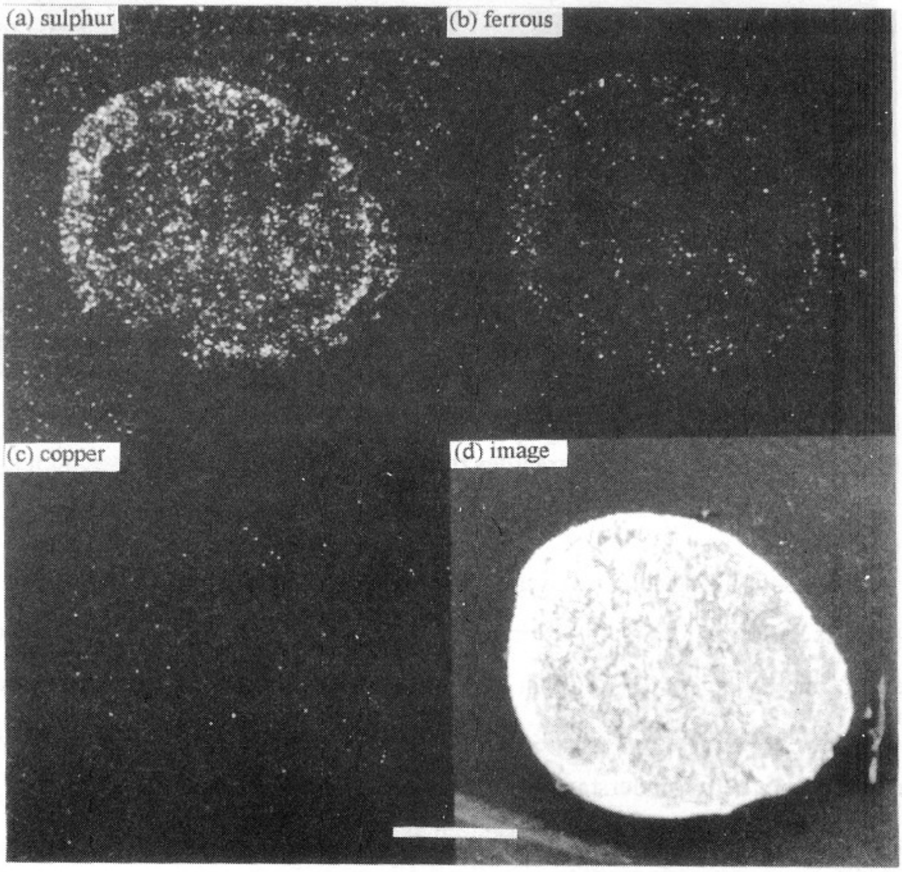

Figure 1: X-ray dot-mapping micrographs for (a) sulphur, (b) ferrous and (c) copper, plus (d) normal SEM image for biogranules treating wastewater containing 280 mg/l of sulphate concentration (bar = 500 μm).

exhibited a layered microstructure (2d). Sulphide precipitates noticeably in rings (2a) on the cross-section of the granules, unlike the former granules. Moreover, both ferrous (2b) and copper (2c) were precipitated in significant quantities, presumably in the forms of ferrous and copper sulphides.

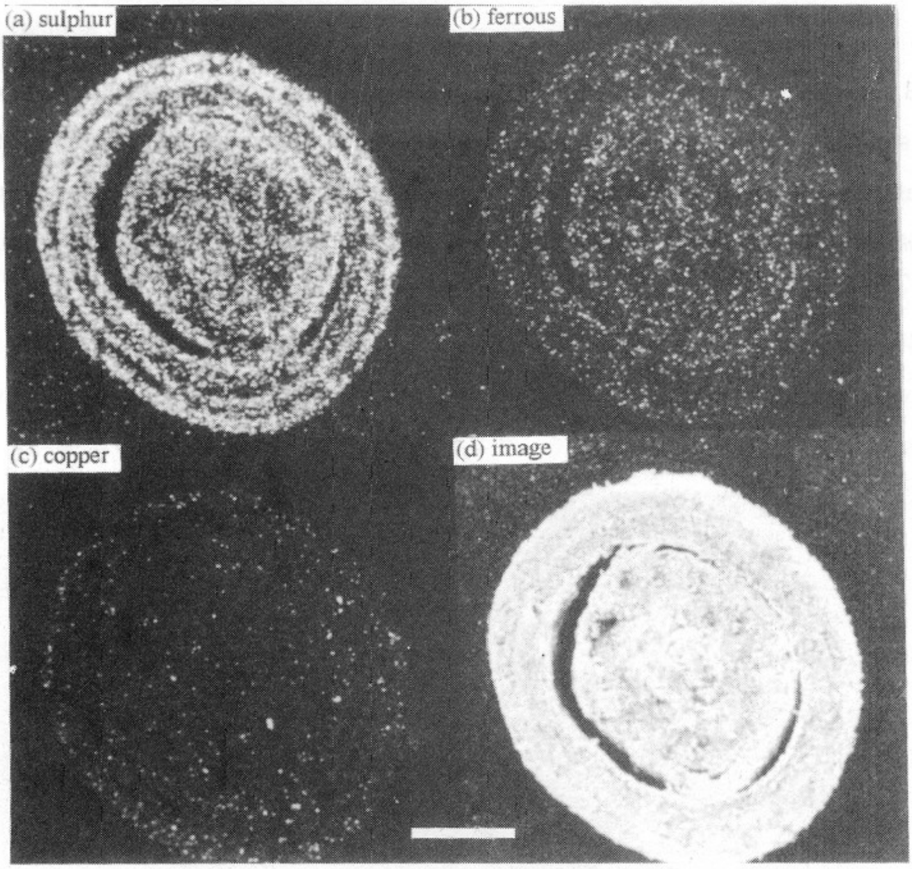

Figure 2: X-ray dot-mapping micrographs for (a) sulphur, (b) ferrous and (c) copper, plus (d) normal SEM image for biogranules treating wastewater containing 2000 mg/l of sulphate concentration (bar = 500 μm).

Recent studies using histological analysis (Chui and Fang, 1994; Fang, *et al.,* 1994) showed that UASB granules treating more complex organic substrates often exhibited a layered microstructure. However, no one has ever reported that high concentration of sulphate in wastewater could also result in layered biogranules. The finding of this study is intriguing and warrant further investigation which may lead to a better understanding of the granulation mechanism.

Comparison of bacterial species in both types of granules under SEM shows that *Methanothrix*, the acetotrophic methanogen, was predominant in the biogranules treating wastewater containing low concentration of sulphate; but its presence was drastically reduced in the biogranules treating 2000 mg/l of sulphate. This is because in the latter wastewater, sulphate-reducing bacteria competed with methane-producing anaerobes for organic substrate and hydrogen, a key intermediate product, resulting in a reduced production of methane. Some bacterial species appeared only in the latter granules, presumably the sulphate-reducing bacteria. In addition, the surface of these bacteria had concentrated crystalline precipitates (Figure 3a), which appeared to be copper sulphide according to the X-ray spectrum analysis (Figure 3b).

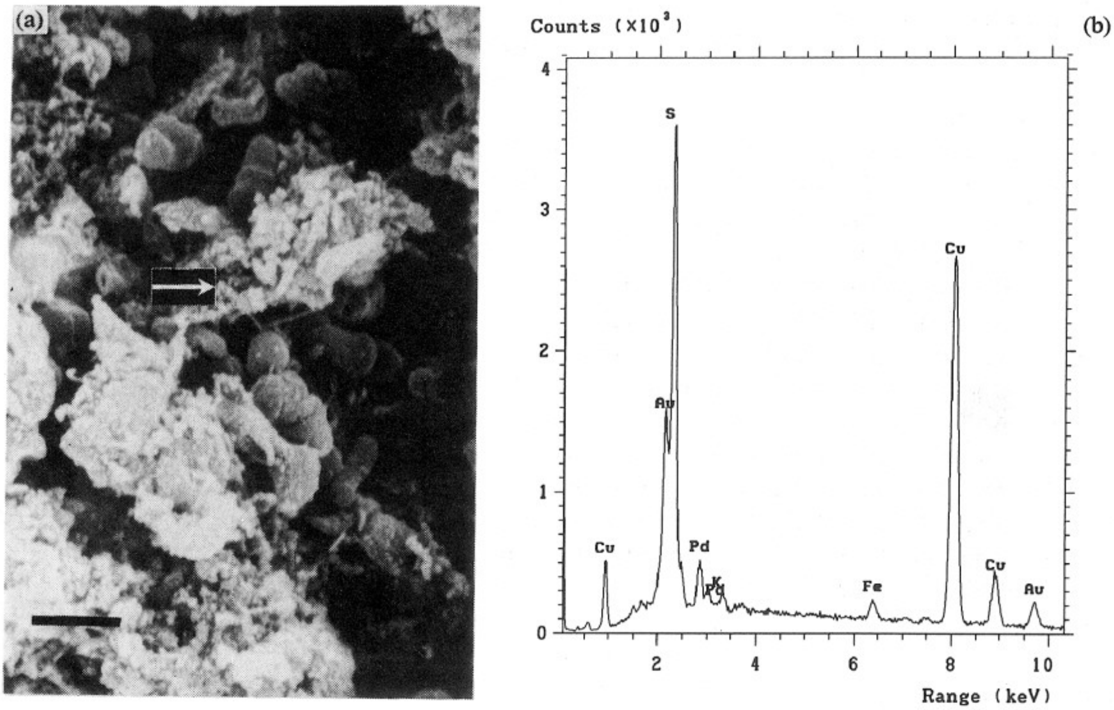

Figure 3 (a) Crystalline precipitates on the surface of sulphate-reducing bacteria (bar = 1 μm), and (b) X-ray spectrum analysis showing the crystals (marked by the arrow in (a)) consisted mostly of sulphur and copper.

CONCLUSION

Anaerobic biogranules were analysed by X-ray spectrometry for the local concentration of inorganic elements and by X-ray dot mapping for the pictorial distribution of these elements in the cross-section of the granules. This information is of significance for the understanding of the role of the inorganic elements in the granulation of anaerobic sludge.

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REFERENCES

Chui, H.K. and Fang, H.H.P. (1994). *J. Environmental Engineering,* **120(5)**, 1322-1326.

Dolfing, J., Griffioen, A., Van Neerven, A.R.W. and Zevenhuizen, L.P.M. (1985). *Can. J. Microbiol.,* **31**, 744-750.

Dubourguier, H.C., Buisson, M.N., Tissier, J.P., Prensier, G. and Albagnac, G. (1988). In: *Granular Anaerobic Sludge: Microbiology and Technology, Proceedings of the GASMAT-Workshop,* Lettinga, G., Zehnder, A.J.B., Grotenhuis, J.T.C. and Hulshoff Pol, L.W. (ed). 78-86, Wageningen, The Netherlands.

Fang, H.H.P. and Chui, H.K. (1993). *J. Environmental Engineering,* **119(1)**, 103-119.

Fang, H.H.P. Chui, H.K. and Li, Y.Y. (1994). *Water Science and Technology*, **30(12)**

Fang, H.H.P. Li, Y.Y. and Chui, H.K. (1995). *J. Environmental Engineering,* **121(2)**, 153-160.

Goldstein, J.I., Newbury, D.E., Echlin, P., Joy, D.C., Fiori, C. and Lifshin, E. (1981). In: *Scanning electron microscopy and X-ray microanalysis -- A text for biologists, materials scientists, and geologists,* 299-231, Plenum Press, New York.

Guiot, S.R., Gorur, S.S. and Kennedy, K.J. (1988). In: *Anaerobic Digestion, 5th International Symposium on Anaerobic Digestion,* Hall, E.R. and Hobson, P.N. (ed). 47-53, Pergramon Press.

Hulshoff Pol, L.W., van de Worp, J.J.M., Lettinga, G. and Beverloo, W.A. (1986). In: *Anaerobic Treatment, A grown-up technology, Aquatech'86,* 89-101, Amsterdam.

Lettinga, G. and Hulshoff Pol, L.W. (1992). In: *Design of anaerobic processes for the treatment of industrial and municipal wastes,* J.F.Malina and F.G.Pohland, (ed). 119-145, Technomic Publishing, Lancaster.

Lettinga, G., Van Velsen, A.F.M., Hobma, S.M., de Zeeuw, W. and Klapwijk, A. (1980). *Biotechnology and Bioengineering,* **22**, 699-734.

Speece, R. E. (1983). *Envir. Sci. Tech.*, **17**, 416A-427A.

Widdel, F. (1988). In: *Biology of anaerobic microorganisms*, Zehnder, A.J.B. (ed). 469-586.