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MICROSTRUCTURAL ANALYSIS OF UASB GRANULES TREATING BREWERY WASTEWATER

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ABSTRACT

The microstructure of granules from a full-scale UASB reactor treating brewery wastewater was examined using light microscopy as well as the scanning and transmission electron microscopies. The granules typically have a complex, layered structure. The outer layer of the granule was densely packed with various types of bacteria, including cocci, bacilli, *Methanosarcina*, and *Methanothrix*. The second layer was composed of a matrix of *Methanothrix* with two types of microcolony showing evidence of syntrophic associations between hydrogen-producing acetogens and hydrogen-consuming methanogens. The interior of the granule, on the other hand, was predominantly *Methanothrix* with scattered microcolonies of syntrophic associations, which became sparse and disappeared toward the centre core of the granule.

KEYWORDS

Brewery wastewater; methanogens; microstructure; syntrophic associations; UASB granules.

INTRODUCTION

As the fifth largest beer-consuming nation in the world, China produces over 6 million tonnes of beer and 120 million tonnes of brewery wastewater annually. Brewery wastewater, having about 2500-3000 mg/l of Chemical Oxygen Demand (COD), is one of the major sources of pollution in China. A simple and cost-effective method for the treatment of brewery wastewater is thus essential.

Since its introduction over a decade ago, the upflow anaerobic sludge blanket (UASB) process (Lettinga *et al.*, 1980) has been successfully applied to the treatment of various wastewaters (Campos *et al.*, 1986; Lettinga and Hulshoff Pol, 1991; Fang and Chui, 1993), including brewery effluent (Craveiro *et al.*, 1986; Hulshoff Pol and Lettinga, 1986; Fang *et al.*, 1990). The success of the UASB process relies on the formation of active and settleable sludge granules. It is thus of scientific and engineering significance to have a better understanding of the granulation mechanism (Hulshoff Pol and Lettinga, 1983) and the granular microstructure (Bochem *et al.*, 1982; Dolfing *et al.*, 1985; Dubourguier *et al.*, 1988). Yet, not much has been understood in both areas so far.

Only a small number of studies have been reported on the microstructures of UASB granules (Stams *et al.*, 1989). MacLeod *et al.* (1990) proposed a three-layered microstructure for the UASB granules treating sucrose as substrate, using scanning and transmission electron microscopies (SEM and TEM). Grotenhuis *et al.* (1991), on the other hand, found that there was no evidence of a layered structure for UASB granules treating propionate, ethanol, and sugar refinery wastewaters, using not only SEM and TEM, but also the immunoprobes. The propionate-degrading granules were found to be composed of two types of colony: one consisted of *Methanothrix* and the other consisted of the small electron-opaque *Methanobrevibacter arboriphilus* in juxtaposition with large, spherical electron-translucent bacteria. Furthermore, Macario *et al.* (1991), using a histochemical technique, found that granules treating mixed volatile fatty acids (VFA) under thermophilic conditions had a layered structure of methanogens; such a structure was however not observed when treating the same substrates under mesophilic conditions.

In this study, the microstructure of UASB granules treating brewery wastewater was examined not only by SEM and TEM, but also by a simple technique developed recently using light microscopy (LM) which requires histological preparation of the granules (Chui and Fang, 1994).

METHODS AND MATERIALS

Microstructures of granules obtained from a full-scale UASB reactor treating brewery wastewater were examined using a light microscope (Olympus, Model BH2), a scanning electron microscope (Cambridge Stereoscan 150), and a transmission electron microscope (JEOL 100SX). Procedures for preparing granules for LM and SEM examinations followed those reported previously (Chui and Fang, 1994; Fang and Chui, 1993).

For TEM examination, a granule was first fixed overnight at 4 °C in a 0.1 M cacodylate buffer solution (pH 7.4) containing 2.5% glutaraldehyde and 4% paraformaldehyde. After washing three times in the cacodylate buffer, the granules were then washed with cacodylate buffer and dehydrated in a graded series of ethanol. Ethanol was again cleared with two exposures of propylene oxide for 15 min. The samples were then transferred to a mixture (1:1) of propylene oxide and resin (TAAB 812 Resin, U.K.) for 3 hours and in the pure resin for 12 hours for infiltration. Finally, the granules were embedded in fresh resin and polymerised at 60 °C for 24 h. Ultrathin sections were cut with an ultramicrotome (Ultracut E, Reichert-Jung, Austria) using a diamond knife and collected on 200-mesh formvar-coated copper grids. Ultrasections were stained with uranyl acetate for 20 min and lead citrate for 10 min, and finally examined by a TEM at an accelerating voltage of 80 kV.

As all methanogens have cofactors F_{350} and F_{420}, which have not been found in any other bacteria, they fluoresce under excitations at 350 nm and 420 nm (Doddema and Vogels, 1978). As a consequence, methanogens could be distinguished from other bacteria when examined by LM with epifluorescence. Furthermore, two genera of methanogens, *Methanothrix* (Zehnder *et al.*, 1980; Huser *et al.*, 1982) and *Methanosarcina* (Mah and Smith, 1981) could be identified using SEM and TEM from their well defined morphologies.

The specific methanogenic activities of the granules using formate, acetate, and propionate, respectively, as the sole organic substrate, were measured in serum vials using the methodology Dolfing and Mulder (1985) and Hwang and Cheng (1990), modified from the one proposed by Owen *et al.* (1979). A gas chromatograph (Hewlett Packard, model 5890 series 2) equipped with a thermal conductivity detector was used for the analysis of methane content in the biogas. A 2 m x 2 mm (inside diameter) stainless-steel column packed with Porapak N (80-100 mesh) was used. Injector and detector temperatures were kept at 130 °C and 200 °C, respectively, and the oven temperature was 50 °C isothermal.

RESULTS AND DISCUSSION

Fig. 1a illustrates a typical brewery granule observed by LM under the bright field. The dark appearance of the core probably resulted from the sulfide precipitates. Fig. 1b illustrates the layered structure and the distribution of methanogens of the same granule observed under epifluorescent excitation. The outer layer, about 100 μm in thickness, had low fluorescent intensity, while the interior was composed of colonies with various fluorescent intensities.

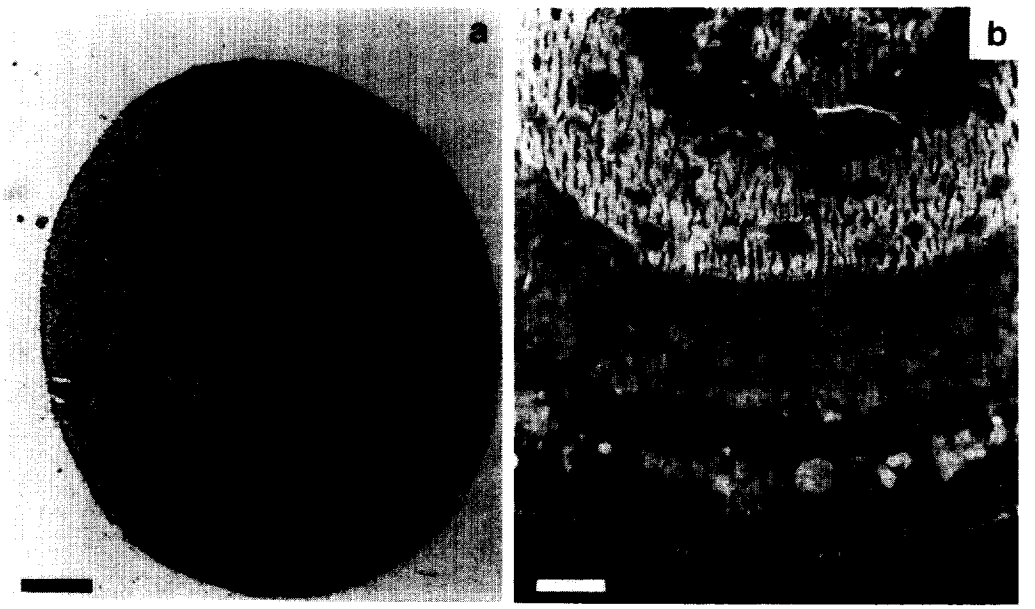

Figure 1. A granule treating brewery wastewater under LM. (a) under bright field, bar = 250 μm; (b) under epifluorescent excitation, bar = 100 μm.

Of the three-layered structure proposed by MacLeod *et al.* (1990), the outer layer was composed of acidogens and hydrogen-consuming bacteria. Complex substrate molecules in wastewater were first degraded by bacteria in this layer into lower molecular-weight VFA. These VFA diffused inward and were degraded by bacteria in the second layer, which was composed of hydrogen-producing acetogens and hydrogen-consuming methanogens. The syntrophic association between these two groups of bacteria is essential for keeping the hydrogen concentration at low levels; otherwise, reactions converting certain intermediates, such as butyrate and propionate, to acetate become thermodynamically infeasible. In the case of propionate conversion to acetate, hydrogen has to be kept below a partial pressure of 10^{-4} atmospheres based on energetic analysis. In order to keep at such low concentrations, hydrogen-consuming methanogens have to be in juxtaposition to the hydrogen-producing acetogens to avoid any local accumulation of hydrogen (Thiele and Zeikus, 1988). Acetate was eventually converted to methane in the interior of the granule which was mainly composed of *Methanothrix*.

SEM and TEM micrographs of this study also illustrated a similar, although not identical, three-layered structure for granules treating brewery wastewater. The surface and the outer layer, as illustrated in Fig. 2a and b, respectively, were composed of various types of bacteria including bacilli, cocci and filaments. The outer layer was mainly composed of acidogenic bacteria, as illustrated by its dim fluorescence under epifluorescent excitation. However, unlike MacLeod's structural model, it had small number of scattered colonies of methanogens. Fig. 2c is the SEM micrograph taken at the outer layer illustrating a colony of *Methanothrix*, whereas Fig. 2d is the TEM micrograph illustrating two neighbouring colonies of

Methanothrix and *Methanosarcina*; the latter colony could also be observed by LM under epifluorescent excitation, as illustrated in Fig. 2e.

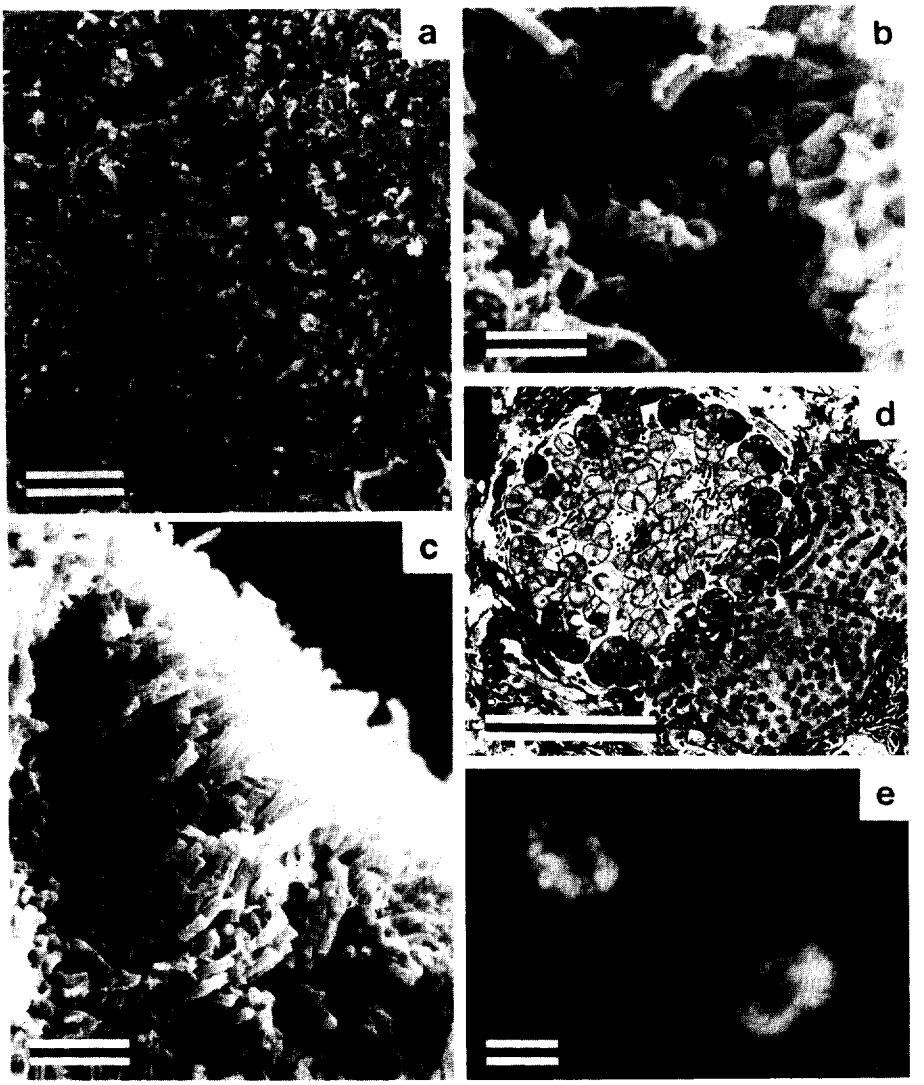

Figure 2. Microstructure of the surface layer of a granule (a) surface under SEM, bar = 10 μm; (b) the outer layer under SEM, bar = 5 μm; (c) a colony of *Methanothrix* under SEM, bar = 5 μm; (d) two neighbouring colonies of *Methanothrix* and Methanosarcina under TEM, bar = 10 μm; (e) a *Methanosarcina* colony under epifluorescent excitation, bar = 10 μm.

The second layer was dominated by colonies of small, electron-opaque rods in juxtaposition with large, electron-translucent bacteria similar to those reported by Dubourguier *et al.* (1988) and Grotenhuis *et al.* (1991). Fig. 3a illustrates these specific spatial arrangements under SEM. Thorough examination of TEM micrographs revealed that there were two distinct types of microcolony with juxtapositioned syntrophic association, as illustrated respectively in Fig. 3b and c. In Fig. 3b and c, the hydrogen-consuming *Methanobrevibacter*-like bacteria (0.3 μm in diameter and 0.8-1.5 μm in length) were in juxtaposition with

the larger rod-shaped hydrogen-producing bacteria (0.8-1.2 μm in diameter). The two microcolonies could be distinguished by their differences in the morphology of the hydrogen-producing bacteria and the interspecies distance.

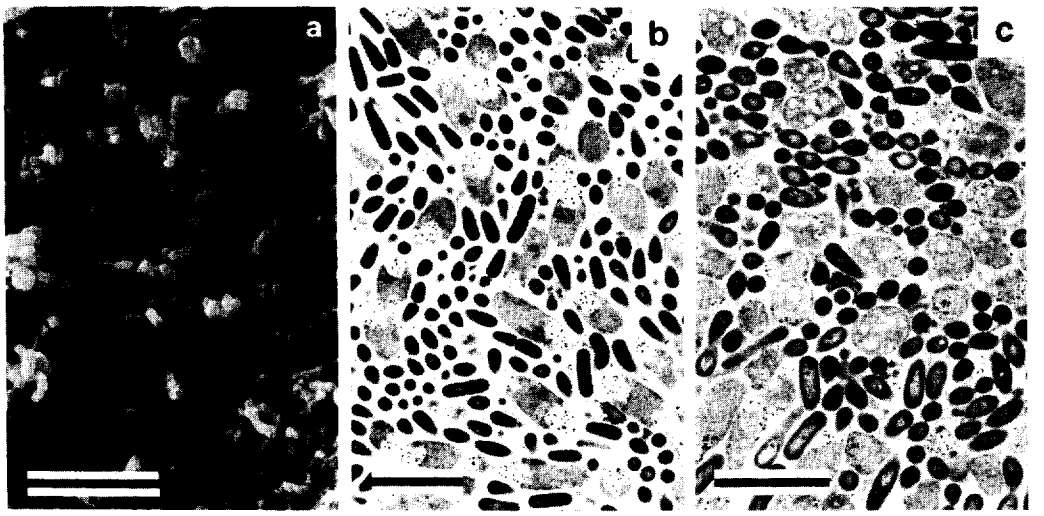

Figure 3. Juxtapositioned syntrophic associations; (a) under SEM, bar = 5 μm; (b) with wider interspecies distance under TEM, bar = 2 μm; (c) with closer interspecies distance under TEM, bar = 2 μm.

The interior of the granule is mainly composed of the acetoclastic *Methanothrix*, as illustrated in Fig. 4a and b, which could be readily identified by its size (0.7 μm in diameter and 1.8-2.0 μm in length) and its bamboo-shaped morphology.

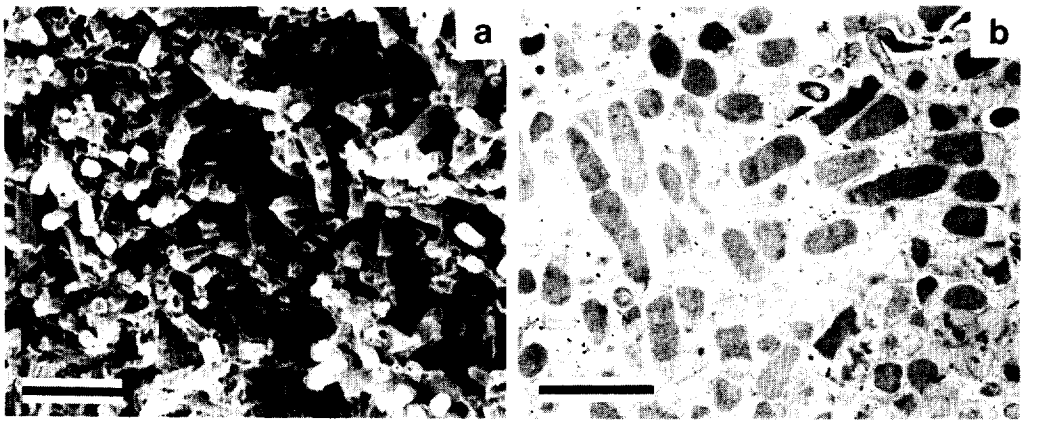

Figure 4. Methanothrix in the granule interior; (a) under SEM, bar = 5 μm; (b) under TEM, bar = 2 μm.

The specific methanogenic activities of the granules for the degradation of formate, acetate, and propionate were found to be 13.7, 5.3 and 1.4 μmol-methane/g-VSS/min, respectively. They were comparable to those reported for granules degrading sugar or sucrose, as shown in Table 1.

Table 1. Specific methanogenic activities of granules (in μmol-methane/g-vss/min) using fatty acids as substrate

Type of wastewater treated by UASB	Substrate for activity test			Sources
	formate	acetate	propionate	
brewery	13.7	5.3	1.4	this study
sucrose	8.2	3.5	2.4	MacLeod et al, 1990
sugar		6.3	2.5	Dolfing, 1985
sugar	11.0	4.1	2.8	Dolfing and Mulder, 1985
sugar	11.0	10.0	4.5	Dolfing and Bloemen, 1985
sugar		3.3	3.4	Grotenhuis et al, 1991

CONCLUSION

Microscopic examinations of granules treating brewery wastewater revealed that they had a three-layered structure. The outer layer, being dominated with acidogens with scattered *Methanosarcina* and *Methanothrix* colonies, was mainly responsible for the hydrolysis of complex substrates in the wastewater to form VFA. The second layer was mainly composed of two types of microcolony with juxtapositioned syntrophic associations in the matrix of *Methanothrix*. This layer was responsible for the conversion of VFA to acetate. The interior of the granule was composed mainly of *Methanothrix*, along with a few microcolonies with syntrophic associations, responsible for the conversion of acetate to methane.

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