HISTOLOGICAL ANALYSIS OF MICROSTRUCTURE OF UASB GRANULES

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ABSTRACT: A histological method was demonstrated for the microscopic analysis of granules from upflow-anaerobic-sludge-blanket (UASB) reactors. Granules were first fixed with formaldehyde and embedded in paraffin, before being sectioned by a microtome to a thickness of 3–5 μm. The section was then mounted on a glass slide for microscopic examination using phase contrast and epi-fluorescent excitation. Using this simple method, the microstructure of the granules and the distribution of methanogenic bacteria could be analyzed. Over 100 granules from UASB reactors treating sucrose wastewaters were examined. Microscopic photos showed that the granules had a surface layer comprised of hydrogen-consuming methanogenic cocci and bacilli, and a loosely packed interior, mainly comprised of acetotrophic *Methanothrix*. This simple method provides engineers with a practical tool for studying the microstructure of the granules, the mechanisms of their formation, and kinetic modeling.

INTRODUCTION

The upflow-anaerobic-sludge-blanket (UASB) process has become popular as an effective means for the treatment of high-strength wastewaters. Over 300 full-scale UASB reactors have been built worldwide (Lettinga and Hulshoff Pol, 1992) in the past decade. The success of this process relies on the formation of sludge granules with high settleability and bioactivity. The study of the microstructure of the granules may lead to a better understanding of the mechanisms of the formation of granules with superior settleability. In addition, knowledge of microstructure formation may lead to a better understanding of various substrates' degradation and diffusion rates, as well as factors that would affect interspecies substrate transfer. All of these factors are of vital importance to the development of kinetic models (McCarty and Mosey 1991).

Many researchers have studied the microstructure of the UASB granules using different methods, such as scanning electron microscopy (SEM) and transmission electron microscopy (TEM) (MacLeod et al. 1990), immunological probes (Grotenhuis et al. 1991), and immunohistochemistry (Macario et al. 1991). All of these sophisticated methods require expensive equipment and skilled operating techniques. For most wastewater-treatment practitioners, it would be simpler to use an optical microscope. However, the use of an optical microscope has been limited, because the granules often disintegrate when being sliced into thin sections for microscopic analysis.

A simple technique is described in this technical note by using histological methods to prepare granule samples for the analysis of microstructure and the distribution of methanogens. The microscope should be equipped with phase contrast and epi-fluorescent attachments. Images of unstained bac-

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Note. Discussion open until March 1. 1995. To extend the closing date one month, a written request must be filed with the ASCE Manager of Journals. The manuscript for this technical note was submitted for review and possible publication on October 27, 1992. This technical note is part of the ***Journal of Environmental Engineering***, Vol. 120, No. 5, September/October, 1994. ©ASCE, ISSN 0733-9372/94/0005-1322/ $2.00 + $.25 per page. Technical note No. 5016.

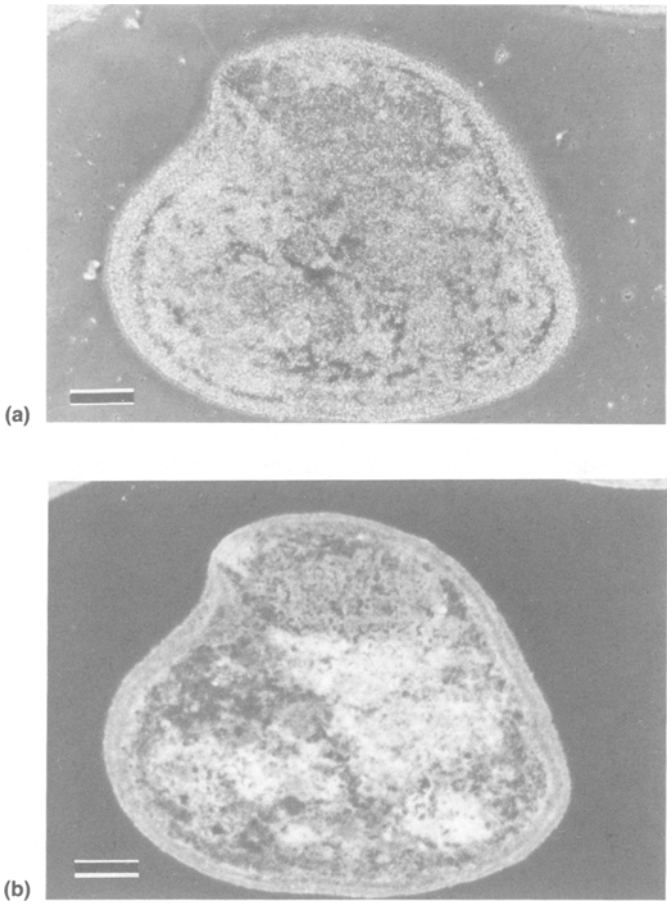

FIG. 1. Section of Typical Granule Treating Sucrose Wastewater: (*a*) under Phase Contrast; and (*b*) under Epi-Fluorescent Excitation; Bar = 100 μm

teria are normally of superior quality under phase contrast, compared to those under the normal bright field. All methanogens have F_{350} and F_{420} cofactors, and thus can be readily identified by their emitted fluorescence (Edwards and McBride 1975), under epi-fluorescent excitations at both 350 nm and 420 nm [ultraviolet (UV) and violet (V), respectively].

MATERIALS AND METHODS

UASB granules from a bench-scale reactor treating sucrose wastewater at 37°C and a COD loading rate of 20 $gL^{-1}d^{-1}$ (Fang and Chui 1993) were examined in this study. The sludge granules were washed three times in a 0.1 M phosphate buffer solution at pH 7.2. They were then fixed in a phosphate buffer solution with 10% formaldehyde for 4 h (Bancroft and Stevens 1990). Dehydration was carried out stepwise by immersing the fixed granules in a graded series of ethanol/water solutions increasing in ethanol content, from 50%, to 75%, 95%, and finally to 100%. The ethanol was

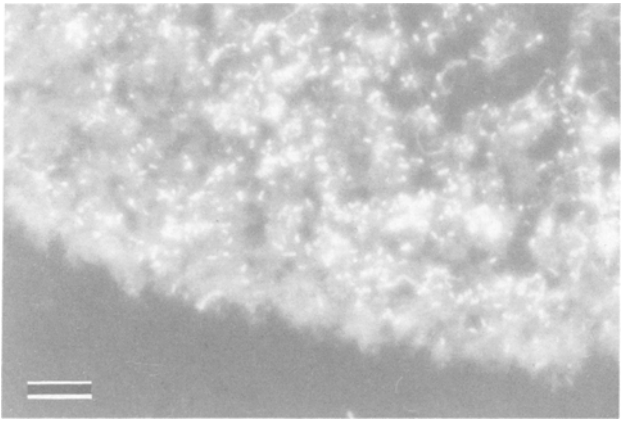

FIG. 2. Hydrogen-Consuming Methanogenic Cocci and Bacilli in Surface Layer of Typical Granule Treating Sucrose Wastewater under Epi-Fluorescent Excitation; Bar = 10 μm

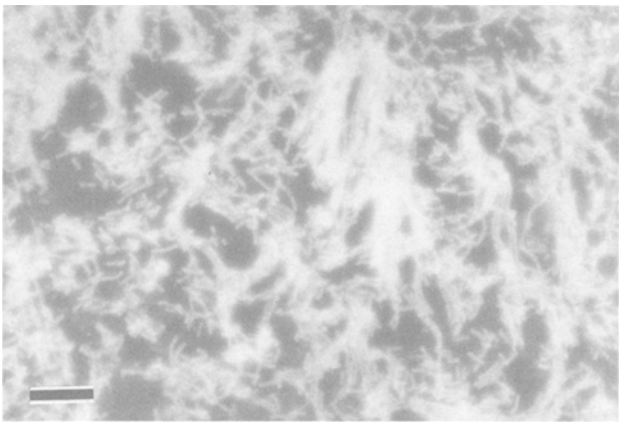

FIG. 3. Acetotrophic *Methanothrix* in Interior of Granule Treating Sucrose Wastewater under Epi-Fluorescent Excitation; Bar = 10 μm

then replaced with xylene by immersing the granules in a graded series of xylene/ethanol solutions, similarly gradually increasing the xylene content from 50% to 100%. The granules saturated with xylene were immersed in molten paraffin (melting point 45–55°C) at 60°C–65°C overnight, allowing the paraffin to penetrate into the granules. The paraffin-embedded granules were cooled to room temperature in peel-off molds, before being sliced in sections of 3–5 μm in thickness by a microtome (Leitz Model 1400). The thin paraffin section was then allowed to float on a waterbath and placed on a glass slide. The slide was dried in air, put in an oven at 70°C for 10 min, to melt the paraffin, and finally dewaxed with xylene. The slides were examined by a microscope (Olympus Model BH2) under phase contrast, and epi-fluorescent (UV and V) excitations.

RESULTS

Over 100 granules taken from various height levels of the UASB reactor were examined by the described method. There was no noticeable variations on the microstructure of these granules. Fig. 1 illustrates the section of a typical granule under phase contrast [Fig. 1(*a*)] and epi-fluorescent excitation [Fig. 1(*b*)]. The granule had a dense surface layer with a thickness of 20–40 μm and a loosely packed interior. Both the surface layer and the interior were of bright fluorescence under the UV and V excitations. Fig. 2 illustrates a higher degree of magnification of the surface layer, that consisted of a variety of hydrogen-consuming methanogenic cocci and bacilli, under epi-fluorescent excitation. The granule's interior was mainly composed of bundles of acetotrophic *Methanothrix*, that appeared as fluorescent filaments, illustrated in Fig. 3.

The granules' microstructure observed was consistent with the prior SEM observations of granules from the same UASB reactor (Fang and Chui 1993).

CONCLUSION

Knowledge of the microstructure of UASB granules can be of importance for the basic understanding of the granulation mechanism and its effect on settleability, and for kinetic modeling. A simple method, using an optical microscope and histological methods was demonstrated in this study. The method allows observation of the microstructure of granules, and identification of the methanogens within the granules. Although other methods, such as SEM and TEM, have been used, they require expensive instruments and skilled operating techniques. The simple histological method demonstrated in this study could be a very practical and useful tool.

ACKNOWLEDGMENT

The writers are grateful to the Croucher Foundation for the financial assistance of this study, to Yu-You Li for his helpful discussion, and to Y. C. Mok and W. S. Lee for their technical assistance.

APPENDIX. REFERENCES

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