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Microbial diversity in UASB reactors

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Abstract: Upflow anaerobic sludge blanket (UASB) process is now widely used for the treatment of industrial wastes. Immobilized methanogenic granules play a vital role in such a process. In this study, microbial diversity of these granules from five different full-scale and laboratory UASB reactors operating under different conditions was investigated. The results were categorized on the basis of four different cases of operational and environmental conditions. Predominant methanogens in granules operating under a specific set of operational or environmental conditions were generally different from each other. The results indicated that microorganisms resembling those of the genera *Methanobacterium*, *Methanobrevibacter*, *Methanothrix* and *Methanosarcina* dominated the granules. However, a variety of other species co-existed with the dominant methanogens. The presence of diverse bacterial groups manifested a unique property of the granules of seeding and fast start-up of other UASB reactors.

Key words: UASB, microbial diversity, methanogens, scanning electron microscopy, operational and environmental conditions.

INTRODUCTION

High rate anaerobic wastewater treatment processes like the upflow anaerobic sludge blanket(UASB), expanded/fluidized bed, and anaerobic filter have many advantages over conventional aerobic wastewater treatment processes. Being simple in operation and low in cost, their applicability is particularly feasible in developing countries. The UASB process is the most widely used anaerobic wastewater treatment process especially for stabilizing high strength industrial organic wastes. Hundreds of full-scale UASB plants are reported to be operating worldwide (refs. 1&2).

The UASB process utilizes the principle of bacterial self-immobilization known as granulation. The success of UASB reactors to treat high-strength industrial organic wastes generally depends upon the formation of these stable granules. The mechanism governing formation of granular sludges was not clearly understood till recently and many questions remain unanswered. We have recently provided an insight into granule formation (ref. 3). Because of their diverse nature, there has been great interest in the microbial characterisics of granular sludges (refs 4,5,6 & 7).

This study focuses on the investigation of microbial diversity in granular sludges obtained from five different full-scale and laboratory UASB reactors treating industrial wastes under different operational and environmental conditions. The results are categorized on the basis of four such cases.

MATERIAL AND METHODS

Granular sludges were obtained from the full-scale UASB reactors under maximum possible anaerobic conditions. Samples of granular sludges from the laboratory UASB reactors were obtained under a nitrogen environment. All samples were preserved in anaerobic conditions at 4°C until the time of preparing the samples

for scanning electron microscopy (SEM). SEM was performed on a number of individual granules chosen at random from each sample and was carried out according to the procedures elaborated previously (ref. 8). All other analyses were carried out according to Standard Methods (ref. 9).

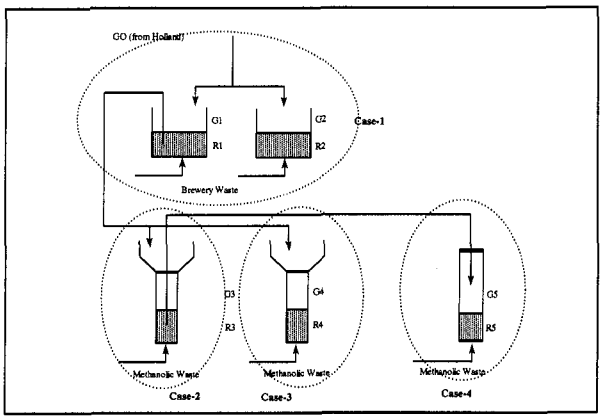

Fig.1. Schematic Diagram of the Reactors and their Sequence of Seeding

RESULTS AND DISCUSSION

Laboratory UASB studies were carried out over a period of three years. Laboratory UASB reactors were either seeded with the granules obtained from a full-scale UASB reactor or with the granules transferred from one laboratory UASB reactor to the other. The full-scale reactors had been treating brewery waste for a long period prior to our laboratory studies. The reactors and the sequence of seeding is schematically illustrated in Fig. 1. Some physical and chemical properties of the granular sludges from these reactors are listed in Table 1.

TABLE 1. Some Physical and Chemical Characteristics of the Granular Sludges.

Granular sludge sample	Avg. size (mm)	Avg. settling velocity ($cm.s^{-1}$)	Ash content (%)[a]	Color	Solids content (%)	Carbohydrates (% TSS)	Nucleic acids (% TSS)	Proteins (% TSS)
G1	2~3	4.19	20.6	black	12.2	1.7	2.5	13.2
G2	2~3	2.10	11.0	brown	11.8	2.7	5.1	16.3
G3	2~3	-[b]	-	blackish	-	-	-	-
G4	2~3	-	-	black	-	-	-	-

[a]All percentile/values expressed as w/w
[b]No data available (-)

Granules from all the reactors were obtained and subjected to scanning electron microscopy. The five UASB reactors were operated under different conditions so as to investigate the relative microbial changes. The operational and environmental conditions of the reactors are given in Table 2.

TABLE 2. Summary of Operational and Environmental Conditions in the UASB Reactors

Case No	Type of reactor	Volume of reactor	Period of operation	Substrate		Operational conditions			Seed granules	Modified granules
				Seed granules	Test granules	pH	Temp (°C)	Organic loading rate ($Kg.COD..m^{-3}.d$)		
1	Full-scale (R1)	-	Years	Alcoholic waste	Brewery waste	~7.0	~30	16.8	G0	G1
	Full-scale (R2)	3800 m^3	Years	Alcoholic waste	Brewery waste	7.7-7.9	~30	6.4	G0	G2
2	Lab-scale (R3)	6.5 l	410 days	Brewery waste	Methanolic waste	7.0	37	15.0 (avg)	G1	G3
3	Lab-scale (R4)	4.25 l	250 days	Brewery waste	Methanolic waste	5.5-6.0	20-30	7.0-8.0 (avg)	G1	G4
4	Lab-scale (R5)	2.5 l	180 days	Methanolic waste	Methanolic waste	7.0	37	13.0 (avg)	R3 floc	G5

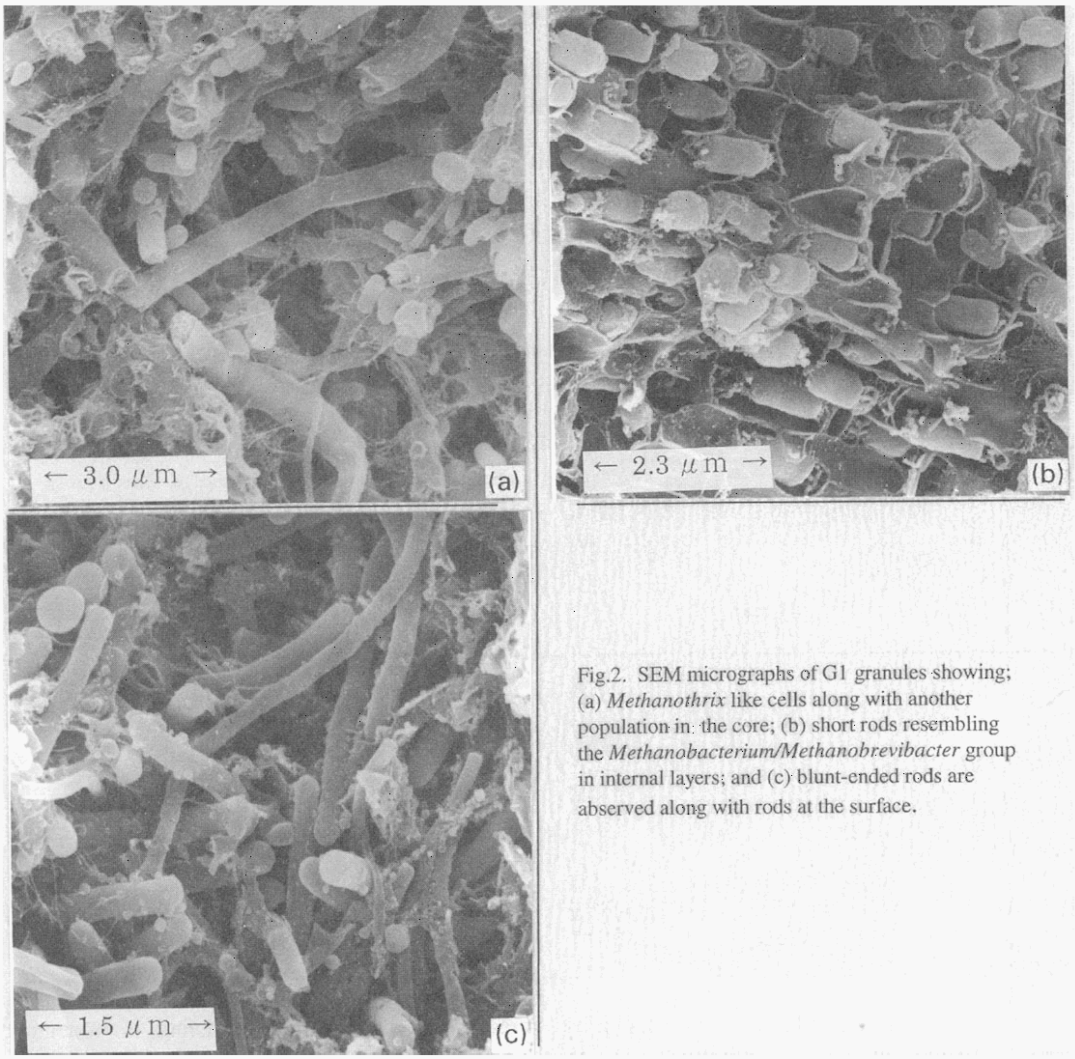

Fig.2. SEM micrographs of G1 granules showing; (a) *Methanothrix* like cells along with another population in the core; (b) short rods resembling the *Methanobacterium/Methanobrevibacter* group in internal layers; and (c) blunt-ended rods are observed along with rods at the surface.

Microbial Diversity

Microbial populations were investigated in the core and exterior layers and on the surface of the granules. Characteristics of the 4 case studies are prsented in Table 2.

Case 1. Full scale reactors, called R1 and R2, were seeded with the same granular sludge imported from Holland. Both the reactors treated brewery waste under almost identical environmental conditions. However, R2 was operated at a low organic loading rate. After years of operation, G1 and G2 granules from reactors R1 and R2 respectively showed different characteristics as listed in Table 1. Dominant microbial populations also differed as shown in the SEM micrographs of Figs. 2 and 3.

G1 granules were dominated by *Methanothrix* like cells (Fig. 2a) in the core and at the surface (Fig. 2c) but by *Methanobacterium* resembling cells in the interior (Fig. 2b). On the contrary, G2 granules were dominated by blunt-ended sheathed rods consistent with the appearance of *Methanothrix* species in the core and the interior (Fig. 3a and b). Heterogeneous population was only observed at the surface (Fig. 3c) which included short and long rods, filaments and cocci.

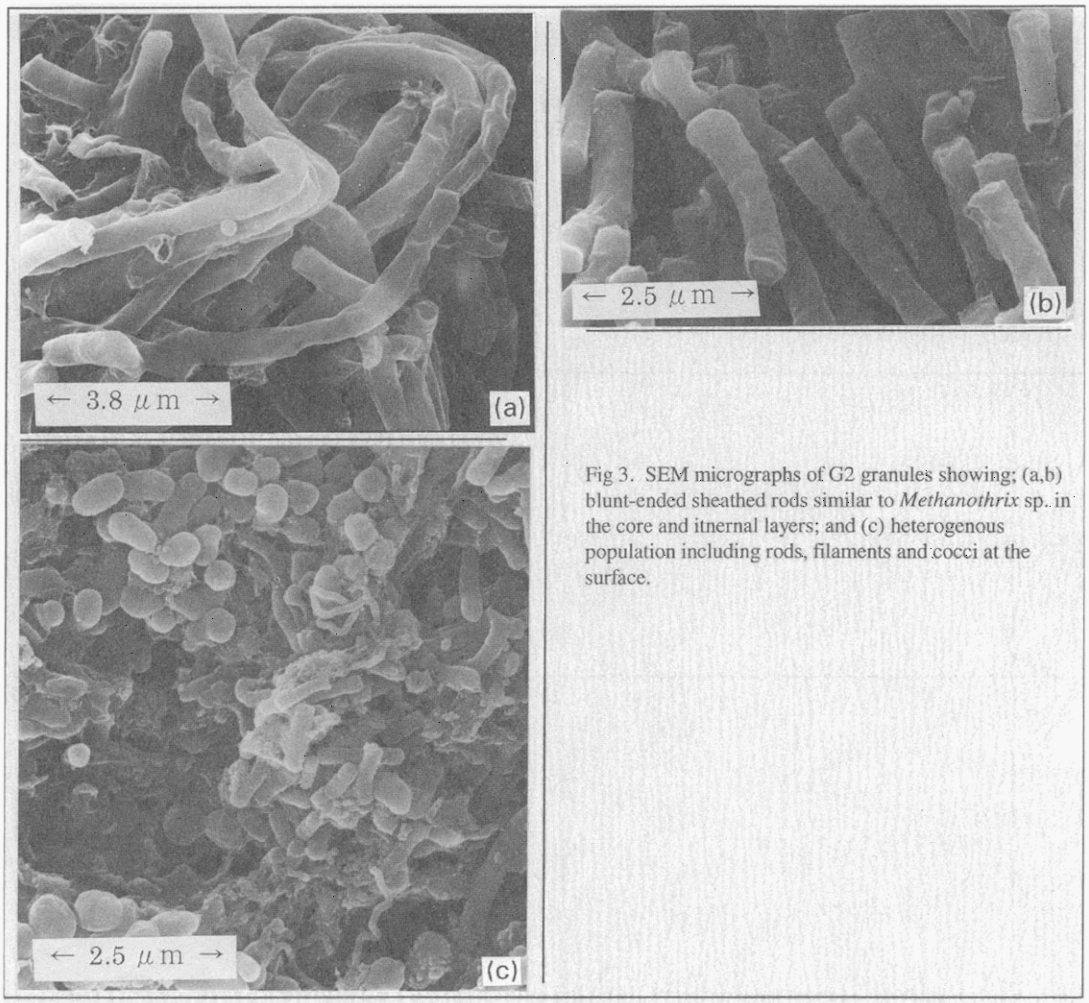

Fig 3. SEM micrographs of G2 granules showing; (a,b) blunt-ended sheathed rods similar to *Methanothrix* sp. in the core and itnernal layers; and (c) heterogenous population including rods, filaments and cocci at the surface.

The bulk of granules in G1 was dominated by round-ended short rods resembling in appearance *Methanobacterium* and/or *Methanobrevibacter* species. In contrast, G2 granules were dominated by *Methanothrix* like microorganisms. This difference in microbial population was attributed to the difference in organic loading rate since no other apparent difference in operational or environmental conditions existed.

Case 2 In Case 2, the effect of substrate on the microbial diversity was investigated. G1 granules from R1 were seeded in a laboratory scale reactor (R3) which was operated on synthetic methanolic waste. After 410 days of continuous operation, the microbial population had changed in the seeded granules. These altogether different granules were called G3. Other new granules were also formed which resembled to G3 in microbial diversity. The *Methanobacterium/Methanobrevibacter* like bacteria in G1 were replaced by single or paired cocci in the interior layers and at the surface as seen in the electron micrographs of Fig. 4a and b respectively. Their number was less in the core as observed in Fig. 4c. Except for a small core region, G3 granules were dominated by coccal forms of organisms resembling in appearance *Methanosarcina* species. It was, therefore, concluded that different substrate resulted in different microbial populations.

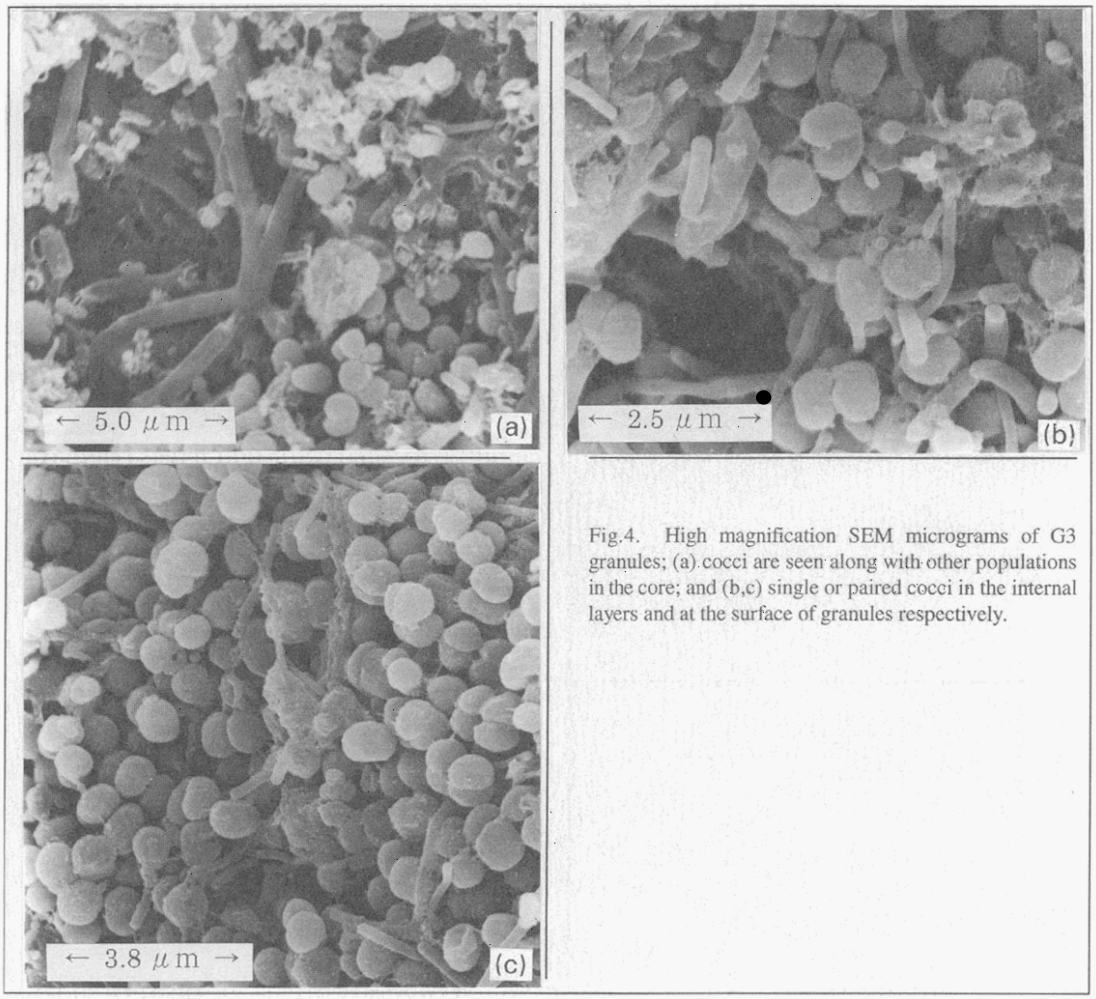

Fig.4. High magnification SEM micrograms of G3 granules; (a) cocci are seen along with other populations in the core; and (b,c) single or paired cocci in the internal layers and at the surface of granules respectively.

Case 3. In Case 2, UASB reactor was operated on a methanolic waste at a pH value around 7.0. In case 3, another UASB reactor (R4) was operated on the same substrate, i.e. methanolic waste but at lower pH values for about 250 days of continuous operation. This reactor was seeded with granular sludge obtained from reactor R1. Thus the difference in environmental conditions between R3 and R4 was mainly the difference in the pH value. In this reactor, no external alkali was added to maintain neutral pH conditions. After an initial start-up period, the reactor maintained a self-operating pH range of 5.5-6.0. SEM performed on the granules (G4) obtained from this reactor manifested the complete dominance of round-ended short rods resembling in appearance *Methanobacterium* and/or *Methanobrevibacter* species throughout the granule as observed in electron micrographs of Fig. 5 a and b. However, only the surface of these granules showed long rods in addition to short rods (Fig. 5c). Though the substrate was the same, a comparison of G3 and G4 granules indicated that these granules were dominated by an altogether different species, i.e. cocci in G3 and rods in G4. This result suggested that a pH difference resulted in different dominating species and may furthersuggest the prevalence of different substrate degradation pathways.

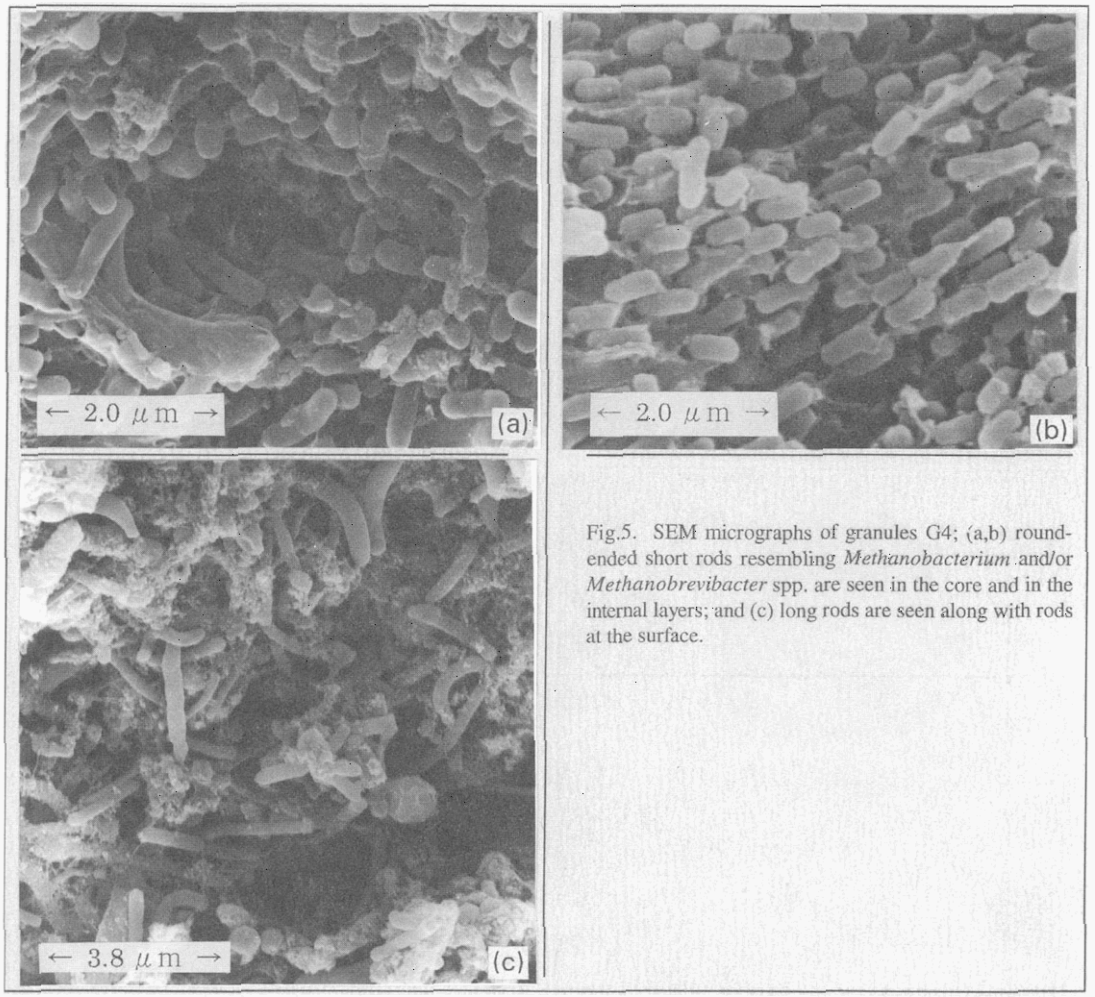

Fig.5. SEM micrographs of granules G4; (a,b) round-ended short rods resembling *Methanobacterium* and/or *Methanobrevibacter* spp. are seen in the core and in the internal layers; and (c) long rods are seen along with rods at the surface.

Case 4. The mechanism of granulation was investigated in Case 4 by cultivating new granules. Supernatant sludge from R3 consisting of fine flocs was seeded into a 2.5 liter laboratory reactor called R5. The substrate was a synthetic methanolic waste. 1~2 mm discrete granules developed after 180 days of continuous operation. These granules (G5) were dominated by single and paired cocci both in the core and at the surface, as observed in the SEM micrographs of Fig. 6a, b and c respectively. Some large granules 4~5 mm in diameter were also formed in this reactor but because of their low settling velocity, washed out of the reactor. These granules contained bunches of coccoidal microorganisms resembling of *Methanosarcina*, as seen in Fig. 7a. Some of these granules consisted of paired cocci linked by some form of extracellular, polymeric material as observed in Fig. 7b.

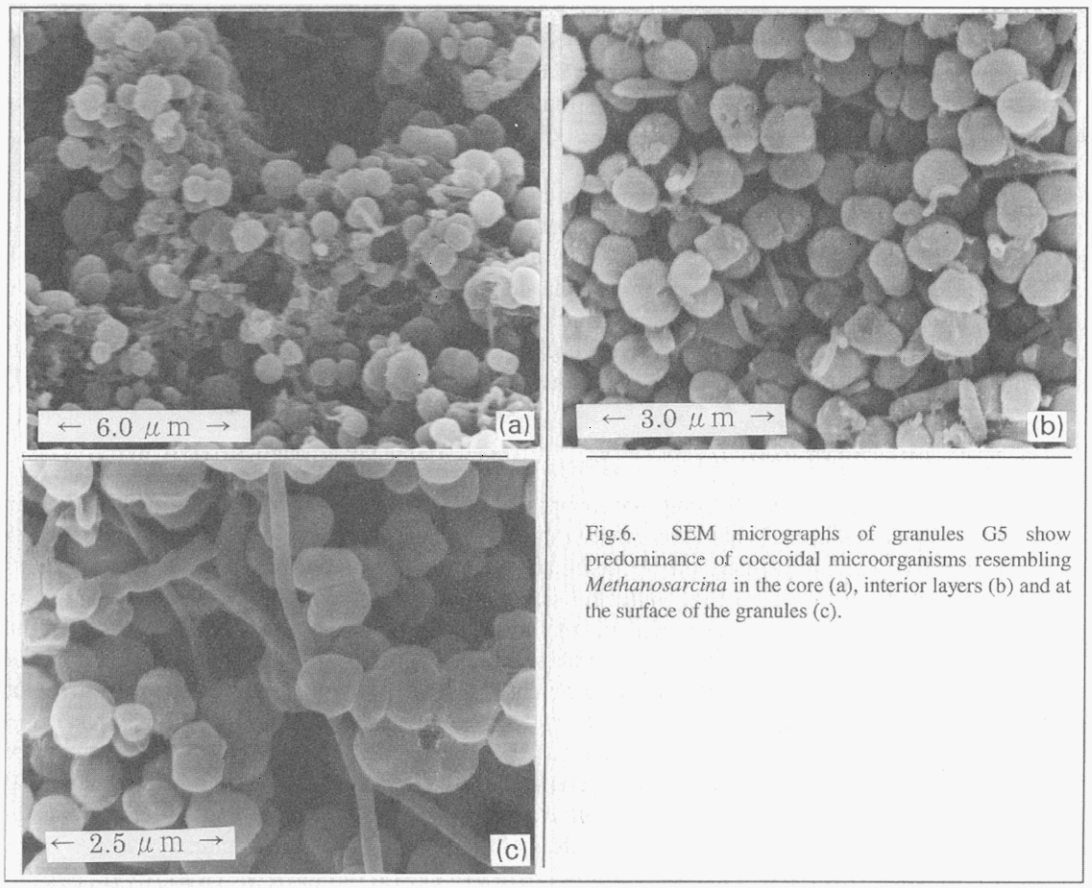

Fig.6. SEM micrographs of granules G5 show predominance of coccoidal microorganisms resembling *Methanosarcina* in the core (a), interior layers (b) and at the surface of the granules (c).

In all the cases, granules were dominated by different genera of methanogens although a variety of species co-existed with the dominant methanogens. In general, the predominating genera of methanogens were those resembling in appearance *Methanothrix*, *Methanobacterium*, *Methanobrevibacter* and *Methanosarcina*. The

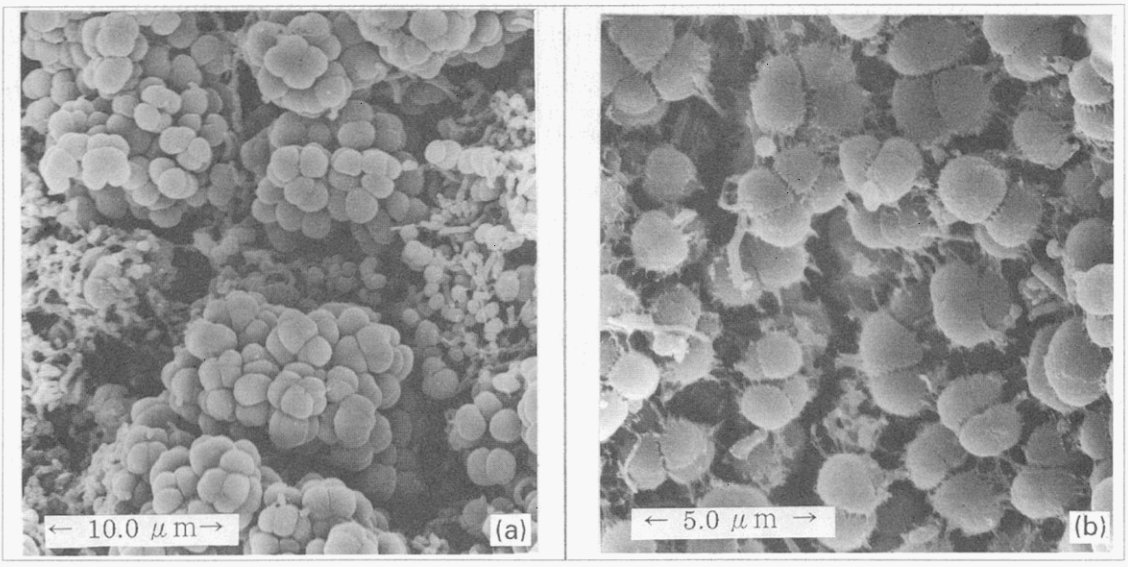

Fig 7. SEM micrographs of big granules washed out of the reactor show bunches of coccoidal organisms resembling *Methanosarcina* (a) and paired cocci, also resembling *Methanosarcina* linked by extracellular polymeric material (b)

dominating methanogens resembled each other in G1 and G4 granules although the substrate was different. The dominant microbial population in G3 resembled that of G5 granules having developed on the same substrate and under almost identical environmental conditions. The non-resemblance of microbial populations of G1 and G2 granules indicated a strong effect of organic loading rate whereas similarities between G3 or G5 and G4 suggested a strong effect of pH.

The granular sludges consisted of diverse bacterial groups. The results suggested that specific microorganisms were selected according to the prevailing operational and environmental conditions. Where optimum conditions existed, the organisms grew at a fast rate and eventually dominated the granules. In all the cases seeding of unacclimatized granules neither slowed the stabilization process nor resulted in process failure, but, on the contrary, enhanced the fast start-up of reactors. This phenomenon suggested a unique property of granular sludges to quickly acclimatize to different operational and environmental conditions. Thus, a major disadvantage of late start-up in anaerobic reactors seem to be overcome by granular sludges.

CONCLUSIONS

From the results of this study, the following conclusions were drawn: (i) Granular sludges grown on different waste streams or under different operational or environmental conditions show different characteristics and microbial diversity; (ii) In general, the predominant genera of methanogens in granular sludges are *Methanobacterium*, *Methanobrevibacter*, *Methanothrix* and *Methanosarcina*; (iii) Organic loading rate has a strong effect on the selection of methanogenic microorganisms. Differences in the organic loading rate with the same substrate may result in different microbial populations; (iv) Granular sludges operating under same substrate and same pH values will manifest no change in microbial populations provided that other conditions are also identical; (v) pH has a strong effect on the dominating methanogens. Different pH values, even with the same substrate, will tend to alter the bacterial populations; (vi) Diverse bacterial groups co-exist in granular sludges. Favorable operational and environmental conditions will select the dominant group bacteria. This is a unique and useful property of granular sludges to seed different UASB reactors treating different waste streams. This property can reduce the start-up periods of UASB reactors.

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