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ORGANIC REMOVAL AND MICROBIOLOGICAL FEATURES OF UASB-REACTOR UNDER VARIOUS ORGANIC LOADING RATES

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Abstract

The performance of a laboratory upflow anaerobic sludge blanket reactor (UASB-reactor) fed synthetic wastewater was investigated under organic loading rates (OLRs) ranging from 3·4 to 44·9 g COD/l·day. The distribution of substrates, intermediate byproducts, pH, VSS, specific sludge activities (acidogenic, acetoclastic, lithotrophic) and the number of different groups of microorganisms at different heights in the reactor were monitored. The conditions for active granular biomass formation were found for the OLRs investigated. When using high-flow UASB-reactors, attention should be paid to the efficient retention of the most active biomass circulating in the sludge blanket zone.

The numbers of methanogens were one to two orders of magnitude higher in the lower part of the reactor (sludge bed zone) than in the upper part (sludge blanket zone). Electron micrographs of the granules showed that the predominant microbial biomass was Methanothrix. *However, increasing the OLR led to a substantial increase of* Methanosarcina *in the granules. Significant amounts of other bacteria were found distributed within the granules between* Methanothrix *filaments. Copyright © 1996 Elsevier Science Ltd.*

Key words: UASB-reactor, granular anaerobic biomass, kinetics, acidogenic, acetoclastic and lithotrophic sludge activities, electron microscopy, *Methanothrix*.

INTRODUCTION

One of the most promising types of reactor used for anaerobic treatment of wastewater is the UASB-reactor (Lettinga *et al.*, 1980; Hulshoff Pol *et al.*, 1983), High efficiency of this reactor is achieved by maintaining high concentrations of active biomass in the reactor. As a result of recent investigations on the nature and structure of biomass granules, the principal groups of microorganisms involved in granules formation and structure have been elucidated (Hulshoff Pol *et al.*, 1983; Wiegant, 1988; Hulshoff Pol, 1989; MacLeod *et al.*, 1990; Forster, 1991; Kalyuzhnyi *et al.*, 1991).

However, kinetic data of UASB-reactors under varying OLRs, passage from one quasi-steady-state regime to another, and data on stratification of different groups of microorganisms within the reactor are scarce in the literature. Such information is very important for creation of fundamental ideas of anaerobic conversion as a whole, for elucidation of the process of granule formation and function and for quick start-up and stable operation of the UASB-reactors.

This work investigates the kinetic and microbiological features of a laboratory UASB-reactor fed synthetic wastewater under various OLRs. Data on substrate and byproduct concentrations, specific sludge activities and distribution of various microorganisms throughout the reactor height are presented.

METHODS

Reactor

Investigations were carried out on a laboratory UASB-reactor (diameter 10 cm, height 85 cm, total working volume 2·7 l) made from transparent plastic and equipped with six sampling ports along the reactor height. The reactor was inoculated with 850 ml of suspended sludge from a pilot-scale anaerobic reactor treating milk industry wastewater. As a model wastewater, the mineral medium described by Varfolomeyev and Kalyuzhnyi, 1989 with the addi-

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tion of glucose (2 g/l) and potassium acetate (from 2 to 5 g/l as acetic acid) mixture as organic substrates was used. The influent pH was 6·6–6·7, operating temperature was 35–37°C.

Analyses

Volatile fatty acids (VFA), methane and carbon dioxide concentrations were measured using gas chromatography, as previously described (Varfolomeyev & Kalyuzhnyi, 1989). COD and volatile suspended solids (VSS) analyses were performed according to *Standard Methods* (APHA, AWWA, WPCF, 1975). Glucose was determined spectrophotometrically using the glucose oxidase–peroxidase method (Berezin *et al.*, 1977).

Specific sludge activity tests

Sludge samples for these tests and for enumeration of bacteria were taken from the different points along the reactor height when the reactor achieved quasi-steady-state under chosen OLR. Before inoculation, sludge samples were softly pounded under the flow of nitrogen gas in the cylindrical glass mortar to obtain a homogeneous suspension. The inoculations and transfers were carried out with syringes.

The activity tests were performed in 0·5 l glass bottles sealed with a rubber septum retained with a screw-cap. Each bottle contained 150 ml of mineral medium (pH 7·0) (Varfolomeyev & Kalyuzhnyi, 1989), corresponding substrate and a known amount of sludge VSS (Lettinga & Hulshoff Pol, 1990). The following substrates were used to determine different sludge activities: glucose (1 g/l) — for acidogenic activity; sodium acetate (3 g/l) — for acetoclastic methanogenic activity; mixture of hydrogen and carbon dioxide (80:20) — for lithotrophic methanogenic activity. At the start of each experiment the gas phase of the bottles was flushed with N_2/CO_2 (70:30) mixture when organic substrates were used and H_2/CO_2 (80:20) when lithotrophic activity was determined. The bottles were then placed in a thermostat at 35°C. All the activity tests were carried out in three replicates.

Determination of specific acidogenic activity

The glucose concentration in each bottle was monitored every 2–3 h. From the slope, S_g, of a plot of the glucose concentration against time, and the amount of VSS, the specific acidogenic activity, A_g, was calculated according to the equation:

$$A_g = \frac{S_g}{\text{VSS}} \qquad (1)$$

Determination of specific methanogenic activities

After overnight preincubation, the gas phase of the bottles was flushed. Two hours after this procedure, the methane concentration of the head space of each bottle was monitored every 2–3 h. From the slope, S_m, of a plot of the relative methane concentration (%) against time, and the amount of VSS, the specific acetoclastic or lithotrophic methanogenic activities, A_m, were calculated according to the equation:

$$A_m = \frac{S_m \cdot V \cdot f_m}{100 \cdot \text{VSS}} \qquad (2)$$

where V — volume of head space (ml); f_m — conversion factor from ml CH_4 to mg CH_4 (f_m =0·633, 35°C).

Enumeration of bacteria

For cultivation and determination of the number of anaerobic bacteria, the bicarbonate buffered Pfennig medium with 0·002% of resazurin to control the redox potential was used (Pfennig, 1965); 0·2 g/l of yeast extract, 2 ml/l of trace element Lippert solution (Pfennig & Lippert, 1966), 10 ml/l of vitamin solution (Wolin *et al.*, 1963) were also added. Corresponding substrates were used to cultivate different physiological groups of anaerobic bacteria: casein (5 g/l) — for proteolytic bacteria, glucose (1 g/l) — for saccharolytic bacteria, sodium acetate (3 g/l) — for acetate-utilizing methanogens, mixture of hydrogen and carbon dioxide (80:20) — for hydrogen-utilizing methanogens.

The cultivation was carried out at 35°C in 50 ml serum bottles hermetically sealed with rubber stoppers and aluminum caps. Twenty millilitres of corresponding medium were poured into the bottles flushed with N_2/CO_2 mixture when organic substrates were used and H_2/CO_2 when lithotrophic methanogens were grown.

The method of twelve-fold serial dilutions was used to determine the number of anaerobic bacteria belonging to different physiological groups. After 2 and 4 weeks of cultivation, bacterial suspensions were examined with phase-contrast microscopy, morphological forms were described and predominant organisms were noted. When necessary, the methane production was examined with gas chromatography.

Detailed morphological and cytological characteristics of the granular active sludge were studied by means of scanning and transparent thin-section electron microscopy.

Scanning microscopy

Granules under investigation were placed into 0·15 M phosphate buffer (pH 7·0) with 5% glutaraldehyde for 16–18 h at 4°C for preliminary fixation. Extra glutaraldehyde was removed by washing with the same buffer. Dehydration of the fixed material was accomplished using a series of ethanol solutions (50, 60, 70 and 96%) for 20 min in each solution, then twice for 20 min in absolute acetone. Dehydrated

samples were dried at 50°C, adhered to the objective table by electroconductive glue, sprayed with gold in an installation for ionic spraying, Spitter IFC-1100, and observed using an ISM-1300 microscope (Jeol, Japan).

Transparent thin-section electron microscopy

For ultra-thin section preparation, the granules were fixed as described above. Strong fixation was carried out with osmic acid. Then granules were washed once with acetate–veronal buffer (pH 7·0), placed into 30% ethanol with 3% uranyl-acetate for 2 h for contrasting, dehydrated in a set of ethanol solutions with increasing concentrations (50, 70, 80 and 96%) for 15 min, followed by treatment with absolute acetone twice for 15 min, and transferred into a mixture of epoxy waxes. Thin sections were cut using a microtome (LKB, Sweden), contrasted with 3% water solution of uranyl acetate and lead citrate. Preparations were observed using an IEM-100C microscope (Jeol, Japan).

RESULTS AND DISCUSSION

Investigation of reactor performance under different OLRs

After start-up, the reactor was fed continuously. The initial OLR was 3·4 g COD/l·day. In 2 weeks, the reactor achieved quasi-steady-state, effluent COD concentrations decreased practically to zero and removal efficiency was above 98%. The term 'quasi-steady-state' was used in this study because a true steady-state for UASB-reactors could not be achieved in practice.

The OLR was increased by increasing the influent flow rate or the influent COD concentration. Since the growth rates of methanogenic bacteria are low (the doubling time for *Methanosarcina* is 20–30 h and for *Methanothrix* is 200-300 h), low flow rates were initially used to avoid biomass wash out. The OLR was increased slowly to prevent disturbances in reactor performance.

Results of our investigation of the UASB-reactor performance under different OLRs are presented in Table 1. This study showed that quasi-steady-state performance of the UASB-reactor can be achieved over a wide range of OLR changes. Immediately after changing the OLR some disturbances in the reactor performance were observed (for example, appearance of large gas bubbles in the sludge bed zone, partial sludge flotation, destruction of granules). This was probably the result of increased biomass lysis caused by temporary overloading of the reactor. However, after a period of time (3–6 hydraulic retention times, HRTs), this undesirable phenomenon was generally eliminated. However, there was still a rather high COD concentration in the effluent and removal efficiency did not exceed 65–70%. Finally, the reactor performances became more stable with a removal efficiency above 96% after 12–15 HRTs. In the cases where the OLRs were lower than 21 g COD/l·day, the quasi-steady-state performance of the UASB-reactor could be reached at 8–10 HRTs. At the OLR of 44·9 g COD/l·day, 30 HRTs were required to increase the COD removal efficiency from 69 to 97%.

Thus, this study shows that granular biomass can relatively quickly adapt to gradual increases of OLR and this results in a high conversion efficiency of the process. Slow OLR increase (during 4 months) leads to an increase of the mean biomass concentration up to 28–38 g VSS/l of total reactor volume and up to 60–70 g VSS/l in the sludge bed zone (Fig. 1). At the OLR of 44·9 g COD/l·day with the HRT of 4 h, a COD removal efficiency of 97% (Table 1) and methane production rate of 14·7 l/l reactor per day (methane yield of 0·327 l/g COD added) were achieved. This is a very high methane production rate for this type of anaerobic reactor.

When the OLRs were lower than 11 g COD/l·day, a clear division of the reactor into a lower (sludge bed) zone and upper (sludge blanket) zone was observed at the level of the fourth sampling port from the bottom. Further OLR increase led to a

Table 1. Operation performance of UASB-reactor under different OLRs (quasi-steady-state)

OLR, g COD/l·day	HRT, h	Influent, g COD/l	Effluent, g COD/l	Removal efficiency, %
3·4	22·5	3·21	<0·1	>98
4·4	17·6	3·21	<0·1	>98
5·5	18·6	4·28	<0·1	>98
6·4	20·0	5·35	<0·1	>98
6·7	19·2	5·35	<0·1	>98
10·2	12·6	5·35	<0·1	>98
14·2	12·7	7·49	0·31	96
17·6	10·2	7·49	0·29	96
20·4	8·8	7·49	0·21	97
23·3	7·7	7·49	0·23	97
28·5	6·3	7·49	0·21	97
31·5	5·7	7·49	0·30	96
33·9	5·3	7·49	0·22	97
44·9	4·0	7·49	0·25	97

partial erosion of the boundary between the zones (Fig. 1).

Typical biomass, substrate, byproduct and pH distributions along the reactor height under quasi-steady-state conditions are shown in Fig. 2. Biomass concentration was highest at the bottom of the reactor and decreased with reactor height. Glucose was digested quickly and was not detected even in the first sampling port. The concentrations of butyric and propionic acids were at a maximum in the first sampling port and then decreased quickly. Acetic acid concentration was negligible in the middle of the sludge bed zone (second sampling port) and its concentration at this level was only slightly higher than the acetic acid concentration in the sludge blanket zone. The VFA accumulation and decomposition caused the pH fluctuation through the reactor height.

Figure 3 gives a comparison of VFA and pH reactor height distributions obtained immediately after increasing the OLR and after achieving quasi-steady-state [removal efficiencies of 65 and 98% for Fig. 3(a) and (b), respectively]. After disturbing quasi-steady-state some fluctuations of VFA concentration along the reactor height were observed [Fig. 3(a)], but VFA concentration generally decreased as the reactor height increased. However, as far as achieving quasi-steady-state is concerned, the VFA concentrations observed in the upper part of the sludge bed zone were comparable with those in the effluent [Fig. 3(b)].

Distribution of specific sludge activities through reactor height

Tables 2–4 show distribution of mean values of specific sludge activities (acidogenic, acetoclastic, lithotrophic) through the reactor height at different OLRs (relative standard error of determination of these values varied from 15 to 33%). All data correspond to reactor performances under quasi-steady-state conditions. As was mentioned above, the zone boundary was approximately at the level of the fourth sampling port from the reactor bottom.

In the sludge bed zone, when the OLRs were lower than 29 g COD/l·day, the highest specific

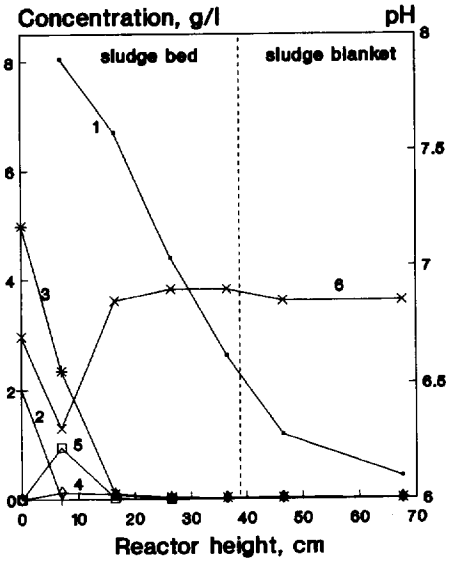
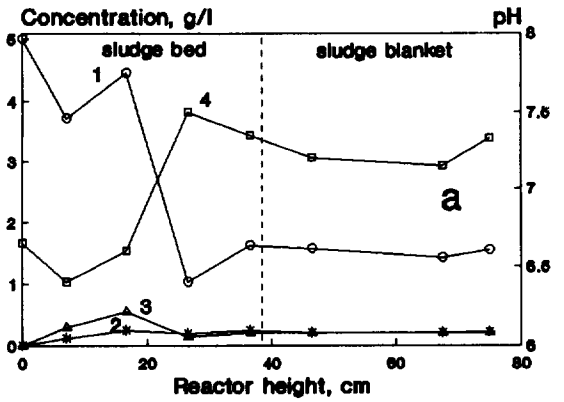


Fig. 2. Distribution of the concentrations through the reactor height at the OLR of 20·4 g COD/l.day: 1 — VSS·10^{-1}; 2 — glucose; 3 — acetic acid; 4 — propionic acid; 5 — butyric acid; 6 — pH (quasi-steady-state).



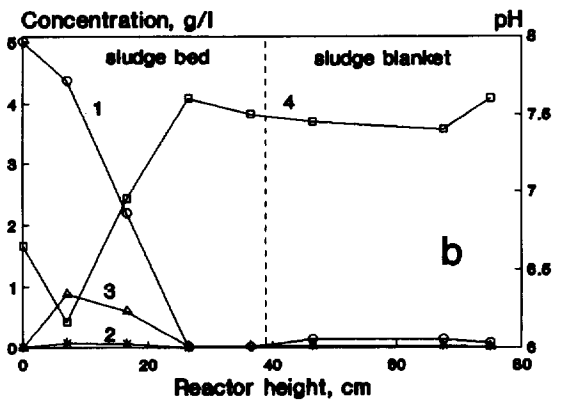


Fig. 3. Distribution of VFA concentration and pH through the reactor height at initial (a) and final (b) stages of operating at the OLR of 33·9 g COD/l.day: 1 — acetic acid; 2 — propionic acid; 3 — butyric acid; 4 — pH.

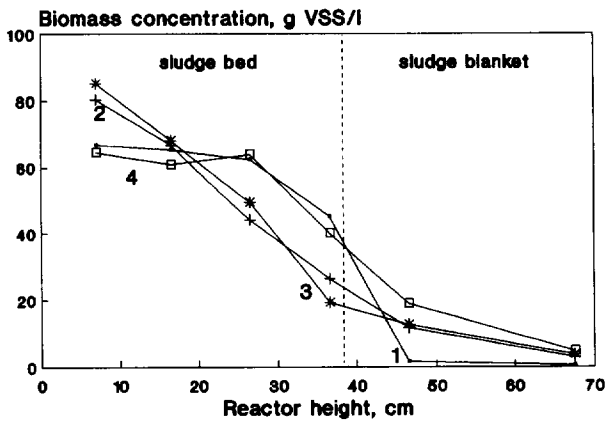


Fig. 1. Distribution of biomass concentration (g VSS/l) through the reactor height at different OLRs (g COD/l·day): 1 — 10·2; 2 — 20·4; 3 — 28·5; 4 — 44·9 (quasi-steady-state).

Table 2. Specific acidogenic sludge activity (g glucose/g VSS·day) through the reactor height under different OLRs[a]

Height, cm	OLR, g COD/l·day			
	10·2	20·4	28·5	44·9
Sludge bed				
7·0	3·56±0·57	3·48±0·50	2·84±0·43	2·59±0·39
16·5	0·92±0·14	1·67±0·27	1·42±0·27	2·07±0·37
26·5	0·37±0·06	1·27±0·25	0·89±0·18	2·05±0·39
36·5	0·13±0·03	2·35±0·50	0·96±0·20	3·43±0·70
Sludge blanket				
46·5	10·41±2·08	8·15±2·45	3·29±0·79	5·09±1·27
67·5	10·42±2·61	8·27±2·56	3·51±0·91	6·91±1·80

[a] Results expressed as means±standard error.

Table 3. Specific acetoclastic sludge activity (mg CH_4/g VSS·day) through the reactor height under different OLRs[a]

Height, cm	OLR, g COD/l·day			
	10·2	20·4	28·5	44·9
Sludge bed				
7·0	55±12	33±7	26±8	45±9
16·5	51±9	39±8	45±12	42±8
26·5	33±9	34±7	25±8	37±10
36·5	34±8	46±9	129±29	33±9
Sludge blanket				
46·5	212±42	54±12	141±39	116±35
67·5	406±122	255±50	320±95	200±61

[a] Results expressed as means±standard error.

Table 4. Specific lithotrophic sludge activity (mg CH_4/g VSS·day) through the reactor height under different OLRs[a]

Height, cm	OLR, g COD/l·day			
	10·2	20·4	28·5	44·9
Sludge bed				
7·0	2·4±0·4	2·4±0·5	5·8±1·8	14·1±3·0
16·5	3·2±0·5	3·1±0·8	4·3±1·2	5·7±1·8
26·5	3·4±0·6	3·9±1·0	6·2±1·8	4·5±1·0
36·5	2·3±0·6	5·9±1·9	19·4±3·9	7·3±2·4
Sludge blanket				
46·5	12·1±2·1	9·5±2·9	31·6±5·9	6·8±2·3
67·5	15·2±3·2	19·4±4·5	34·2±8·5	10·7±2·6

[a] Results expressed as means ± standard error.

acidogenic activity occurred in the lowest sampling port (Table 2), probably because the glucose concentration was highest where the influent entered the reactor. Taking into account the statistical significance of the data for the OLR of 44·9 g COD/l·day, one can say that specific acidogenic activity did not vary through the sludge bed height in this case. The same statistical significance analysis of the data in Table 3 showed that the sludge throughout all the lower reactor part had the same specific acetoclastic activity at all the OLRs investigated, except the fourth sampling port value at the OLR of 28·5 g COD/l·day. Specific lithotrophic activity at the OLR of 44·9 g COD/l·day was highest at the lowest sampling port of the sludge bed zone (Table 4), where the most hydrogen was formed by acidogenic and obligate proton-reducing bacteria. At the next three sampling ports, lithotrophic activity decreased to some extent at this OLR. Under lower OLRs, lithotrophic activity (from the point of view of statistical significance) did not change with sludge bed zone height (except the fourth sampling port values at the OLRs of 20·4 and 28·5 g COD/l·day) and was comparable to the lowest activities for the OLR of 44·9 g COD/l·day (Table 4).

In the sludge blanket zone (two sampling ports from the top of the reactor), an unexpected phenomenon was observed (Tables 2–4). Namely, all

three specific sludge activities measured were up to one order of magnitude higher than their respective activities in the sludge bed zone. Thus, there was a simultaneous sludge concentration decrease and specific sludge activities increase between the two zones.

Thus, in order to improve the efficiency of high-rate UASB-reactors, attention should be paid to retaining the most active biomass circulating in the sludge blanket zone. This is already being practised in the so called hybrid reactor (Guiot *et al.*, 1984).

Microbiological investigation of biomass

The granules in the lowest part of sludge bed zone were mainly large and black or grey (diameter 1–2 mm), whereas those taken higher were smaller and lighter in colour. Often the granules were covered by slime. The biomass from the sludge blanket zone was amorphous, light flocks and very rarely small, light granules. Increasing the OLR up to 44·9 g COD/l·day caused an almost complete disappearance of lighter granules in the reactor.

Electron micrographs of biomass samples taken at the OLR of 10·2 g COD/l·day showed that throughout the heart of the reactor the granules were dense and well formed (spherical and irregular), with friable ones only in the upper reactor zone. The granules mainly consisted of filaments of *Methanothrix* cells. Other microorganisms were rarely present and *Methanosarcina* was practically absent. Some granules probably contained iron sulfide, giving them a black colour. Small daughter granules were observed on the surface of many large granules (Fig. 4).

Granules from the reactor operated at the OLR of 20·4 g COD/l·day varied in microbial composition depending on the sampling point. In the granules from the sludge bed zone, *Methanothrix* cells predominated and *Methanosarcina* cells (large cocci) were also present but in smaller amounts [Fig. 5(a)], while in the samples taken from the sludge blanket zone, *Methanosarcina* cells were absent [Fig. 5(b)]. This is probably related to the low concentration of acetate in the sludge blanket zone. The predom-

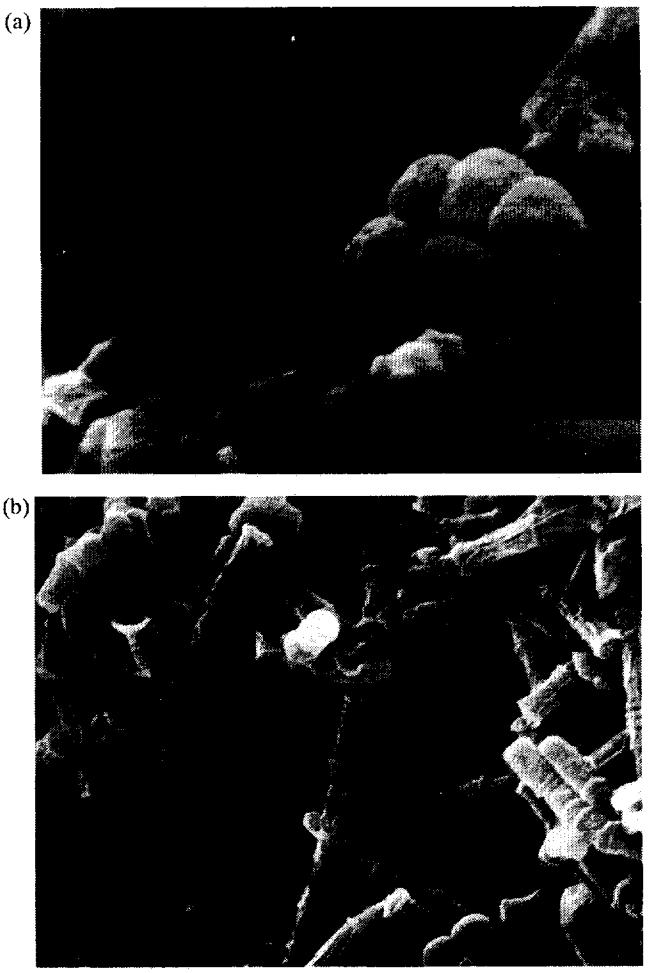


Fig. 5. Electron scanning photography of the granule surface at the OLR of 20·4 g COD/l·day: (a) the lower part of the reactor (10 mm=0·63 μm); (b) the upper part of the reactor (10 mm=1 μm).

Fig. 4. Electron scanning photography of the granule surface with daughter formations at the OLR of 10·2 g COD/l.day in the lower part of the reactor (10 mm=22 μm).

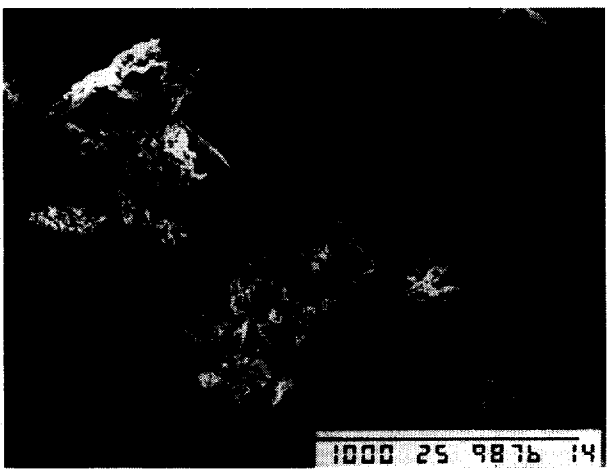


Fig. 6. The role of crystals on granule formation at the OLR of 28·5 g COD/l·day in the lower part of the reactor (10 mm=200 μm).

Table 5. The number of microorganisms through the reactor height under various OLRs

OLR, g COD/l·day	Sample taking zone	Substrate	Number of cells per ml	
			Total	Methanogens
10·2	Sludge bed	Glucose	10^8–10^9	Not determined
		Casein	10^8	Not determined
		Acetate	10^4	Not determined
		H_2+CO_2	10^{10}	10^9
	Sludge blanket	Glucose	10^7	Not determined
		Casein	10^7	Not determined
		Acetate	10^4	Not determined
		H_2+CO_2	10^9	10^8
20·4	Sludge bed	Glucose	10^9	10^8
		Casein	10^{11}	10^{10}
		Acetate	10^6	10^6
		H_2+CO_2	10^{10}	10^{10}
	Sludge blanket	Glucose	10^8	10^7
		Casein	10^{10}	10^8
		Acetate	10^6	10^6
		H_2+CO_2	10^9	10^9
28·5	Sludge bed	Glucose	10^8	10^8
		Casein	10^{12}	10^8
		Acetate	10^6	10^6
		H_2+CO_2	10^{10}	10^{10}
	Sludge blanket	Glucose	10^7	10^6
		Casein	10^{11}	10^6
		Acetate	10^6	10^2
		H_2+CO_2	10^7	10^7

inance of *Methanothrix* in the sludge blanket zone was probably caused by its ability to use acetate in significantly lower concentrations compared to *Methanosarcina*.

Under the OLR of 28·5 g COD/l·day practically all of the granules had irregular forms, appearing to be more friable than the granules from the lower OLRs. *Methanothrix* predominated in the granules, although *Methanosarcina* and other rods and cocci were also present in significant amounts, including the granules from the sludge bed zone.

The granules formed under different OLRs showed the presence of crystal inclusions (Fig. 6), resulting from precipitation of mineral medium components. The surfaces of these crystals were overgrown by biomass (Fig. 6) and they apparently became the centres of granule formation.

Table 5 shows that there were higher cell counts in the sludge bed zone than in the sludge blanket zone for each group of microorganisms enumerated. These data correspond well to the results of VSS determination (Fig. 1). A high concentration of proteolytic bacteria both in the lower (10^8–10^{12} cells/ml) and the upper part of the reactor (10^7–10^{11} cells/ml) should be noted. It is evidently related to the presence of significant amounts of bacteriolytic microorganisms. Short thin rods and small cocci were the predominant proteolytic bacteria.

Saccharolytic microorganisms in the reactor ranged from 10^7 to 10^9 cells/ml in both reactor zones. As the OLR increased, the number of saccharolytic bacteria did not change markedly (Table 5) because the glucose fermentation was not the limiting step of the overall process of the methanogenesis.

In contrast, increasing the OLR from 10·2 to 28·5 g COD/l·day led to an increase in the number of acetoclastic methanogens from 10^4 to 10^6 cells/ml (Table 5).

The number of methanogenic bacteria that grew on the H_2/CO_2 mixture was rather high (10^{10} cells/ml in the sludge bed zone and 10^7–10^9 cells/ml in the sludge blanket zone, Table 5).

In general, the overall quantity of methanogenic bacteria in the sludge bed zone was 2–3 orders of magnitude higher than in the sludge blanket zone (Table 5).

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