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Population dynamics on anaerobic sludge granulation in UASB reactors

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Abstract— Three UASB reactors were operated to investigate the population dynamics of anaerobic sludge granulation. It is found that the increase of bacterial population relates to the bacterial status in anaerobic food chain and relates to sludge organic loading rates. In order to form granular sludge, it is necessary to have sufficient amount of different groups of bacteria in the sludge. In our experiment, the population of fermentative bacteria, propionate degraders, butyrate degraders and methanogens is about 10^{7-8}, 10^{5-7}, 10^{5-7} and 10^{5-7} cells/ml respectively at the appearance of granular sludge. Filamentous methanogenic bacteria are found to be the important species in sludge granulation. Based on the results obtained, a descriptive model is proposed to describe the microbial characteristics of granulation.

Keywords: UASB reactor; granular sludge; sludge granulation.

1 Introduction

The up-flow anaerobic sludge blanket (UASB) reactor is one of the widely used anaerobic wastewater treatment systems. Besides the well-known merits of anaerobic digestion, the UASB process is also characterized by its high organic loading capacity and excellent performance. It is a preferential choice to treat wastewaters such as brewery wastewater and potato processing wastewater. At present, one of the main problems is how to detain large amount of sludge with high bioactivity and to prevent it from being washed out from reactors during the start up and sometimes in stable operation (Lettinga, 1985; 1987). Anaerobic sludge granulation is one way to attain such a goal. Research work has been conducted worldwide which mainly focused on the conditions of cultivating granular sludge and the factors affecting its formation (Dolfing, 1986; Hullshoff, 1987; Tang, 1990). But all of these touch little on the microbiological aspects during the process of sludge granulation.

Microbes that take part in anaerobic digestion can be classified into four groups according to their physiological functions: fermentative bacteria, H_2-producing acetogens, methanogenic bacteria (methanogens) and homoacetogenic bacteria (Bryant, 1979; Zeikus, 1979; Fig. 1). Organics such as sugars were fermented to such fatty acids as propionic acid, butyric acid and alcohols by fermentative bacteria, H_2-producing acetogens degrade the acids formed to acetic acid and H_2 and CO_2, then methanogenes utilize acetate, H_2 and CO_2 to produce methane. Homo-acetogenic bacteria is a group of bacteria producing acetic acid from H_2 and CO_2. Recent studies show that the amount of acetate produced from H_2 and CO_2 accounts for 5% of the total amount of acetate in anaerobic surroundings (Liu, 1991). The functions of this group of bacteria remain unclear.

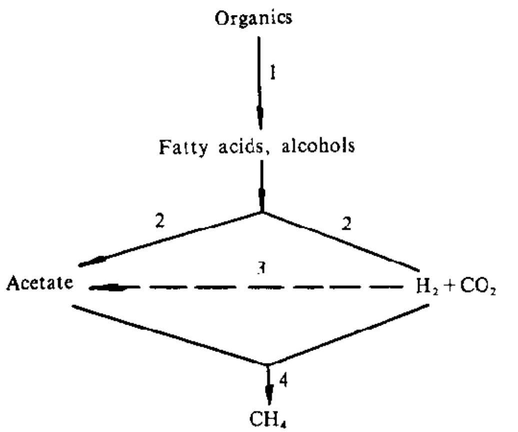


Fig. 1 Mechanism of anaerobic digestion
1. Fermentative bacteria; 2. H_2-producing acetogens; 3. Homoacetogenic bacteria; 4. Methanogens

The paper presented here is mainly focused on the behavior and functions of different groups of anaerobic bacteria during the process of sludge granulation.

2 Materials and methods

2.1 UASB system

Three UASB reactors of 5 L capacity each, made of polyvinyl chloride pipe were used in our experiments. Fig. 2 sketchily showed the outline of reactors and at the flowchart. Reactors were placed in a wooden box and operated at 35 ℃.

2.2 Components of wastewater

Industrial glucose wastewater was used to cultivate granular sludge. Crude glucose was used as carbon source, and NH_4Cl and KH_2PO_4 were used as nitrogen and phosphorus source respectively. The wastewater had a ratio of COD:N:P = 200:5:1, with COD concentration ranging from 1000 to 5000 mg/L, and the influent pH ranged from 6.0 to 7.5.

2.3 Analytical method

COD: by standard method; pH; measured with a pHs-2 type meter; Gas: a 102-c type gas chromatograph was used to determine the CH_4 and CO_2 in biogas.

2.4 Microbial population analysis

All media used in our experiments were prepared strictly by Hungate anaerobic technique and sterilized at 101.3 kPa for 20 min. Culture medium components are as follows.

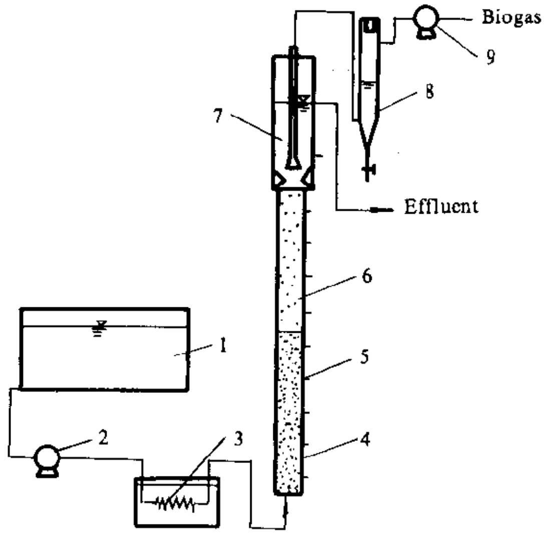


Fig. 2 Bench scale UASB reactor system

1. Feed tank; 2. Influent pump; 3. Heat exchanger; 4. Sludge bed; 5. Sampling port; 6. Blanket zone; 7. Settling zone; 8. Water seal; 9. Gas meter.

2.4.1 Fermentative bacteria

Glucose 10g; peptone 5g; beef extract 3g; NH_4Cl 1g; K_2HPO_4 0.4g; KH_2PO_4 0.4g; L-cysteine 0.5g; resazurine 0.002g; distilled water 1000ml; pH 7.0 – 7.3

2.4.2 H_2-producing acetogenic bacteria

Yeast extract 2g; $MgCl_2$ 0.1g, NH_4Cl 1g; K_2HPO_4 0.4g; KH_2PO_4 0.4g; L-cysteine 0.5g; resazurine 0.002g; distilled water 1000 ml; pH 7.0 – 7.3; 2.9 g of sodium propionate and 3.3 g of sodium butyrate were added respectively for propionate degrading bacteria and for butyrate degrading bacteria.

2.4.3 Methanogens

Sodium acetate 5 g; sodium forma e 5g; NH_4Cl 1 g; $MgCl_2$ 0.1g; K_2HPO_4 0.4g; KH_2PO_4 0.4g; L-cysteine 0.5g; resazurine 0.002 g; distilled water 1000ml; pH 7.2 – 7.3; 80% H_2 and 20% CO_2 (v/v) for gas phase.

Media were dispensed anaerobically into anaerobic tubes. After sterilization, the tubes were inoculated with dilutions of disrupted anaerobic sludge which was sampled from the UASB reactors. In order to obtain single cells, sludge was disrupted by a homogenizer at 6000 r/min for 1 min. Most proble number (MPN) method was used to enumerate population levels of different physiological groups of bacteria. The positive growth indexes of each group of bacteria are listed in Table 1.

Table 1 Positive growth indexes for each group bacteria

Groups	Phenomena
Fermentative bacteria	Culturing medium becoming turbid and pH declined
H_2-producing acetogens	H_2 and acetate produced, bacterial cells observed under microscopes, butyrate or propionate degraded
Methanogens	CH_4 produced

2.5 Properties of seed sludges and inoculation of the reactors

Three kinds of seed sludges were utilized in our experiments. They are the digested sewage sludge from Beijing Jiuxianqiao Municipal Wastewater Plant. The anaerobically digested sludge from Beijing Weigou Pig Farm and the sludge from the settling tank of anaerobic digester effluent from Beijing Tiancun Fermented Bean Curd Plant. Some properties of the seed sludges are listed in Table 2.

Table 2 Some properties of seed sludges

No	Source of sludge	ss[1]	vss/ss	Umax. CH_4[2]	Umax. COD[3]
1	Jiuxianqiao Plant	20.7	0.41	–	–
2	Weigou Pig Farm	15.6	0.39	0.38	0.03
3	Tiancun Plant	25.2	0.72	0.47	0.04

1. g/L; 2. mmol/gvss. h; 3. gCOD/gvss. h; gvss is the abbreviation of gram volative suspended solids.

The three UASB reactors were inoculated with the above three kinds of seed sludges respectively. Before inoculation, the seed sludges were passed through meshes of 0.2mm diameter to remove large particles. The amount of seed sludge for each reactor was 10−15 gvss/L. After inoculation for 1 day, wastewater was pumped into each reactor continuously or semicontinuously.

3. Results

3.1 Start up of reactors and granulation of sludge

Reactor 1

Reactor 1 was inoculated with digested sludge from Beijing Jiuxianqiao Municipal

Wastewater Plant. Since the seed sludge had low methanogenic activity, it was impossible to raise the organic loading rate at the beginning of the start up stage. Hence the volumetric loading rate was kept at 0.75 – 1.5 kg $COD/m^3 \cdot d$ for 18 days. Both COD removal efficiency and biogas production were low during this period. The performance is summarized in Table 3.

Table 3 Performance of reactor 1

Operation stage	Lag stage	Raise-up organic loading stage	Mature stage of granules
Operation period	1st – 18th day	19th – 60th day	61st – 100th day
Influent COD, mg/L	751 – 1018	1020 – 3548	3560 – 4214
Effluent COD, mg/L	320 – 619	620 – 887	860 – 893
COD removal rate, %	57.9 – 40.0	41.5 – 75.2	75.8 – 84.2
Volumetric COD loading rate, $kgCOD/m^3$. d	0.75 – 1.57	1.57 – 4.37	4.38 – 12.7
Sludge loading rate, kgCOD/kgvss.d	0.08 – 0.16	0.19 – 0.45	0.45 – 1.37
HRT, h	23.8 – 14.7	14.7 – 19.5	15.0 – 8.0
Alkalinity, $mgCaCO_3/L$	388 – 786	695 – 1168	937 – 1346
Effluent pH	6.5 – 7.2	6.7 – 7.3	7.0 – 7.2
Gas production, L/d	0.3 – 1.3	1.3 – 6.6	6.2 – 16.0
CH_4 Content, %	60.8 – 68.4	64.5 – 67.4	58.4 – 68.1
VFA of effluent, mg/L, CH_3COOH	151 – 310	125 – 310	160 – 470

The granulation process of reactor 1 might be divided into 3 stages, as indicated in Table 3, i. e. lag stage, raise up organic loading stage and mature stage of granular sludge. Lag stage was the stage which occurred at very low organic loading, for example, lower than 1.5 kg COD/m^3.d. It was caused by lack of diverse anaerobic bacteria and by the microbes adjusting to the wastewater. The time lasted for this stage is related to the population and the species in seed sludge. Generally, the larger the population and the more the species in seed sludge, the shorter the lag stage lasted. In our experiment, this stage took 18 days, including the first 12 days with semicontinously feeding and the following 6 days with continuously feeding. In the raise up organic loading stage, the performance of reactor became more and more stable and COD removal rate stabilized at high level although the organic loading increased continuously. Such excellent performance is due to the increase of microbial population and species. In our experiment, from 19th day to 60th day, the volumetric loading rate raised from 1.57 to 4.37 kg COD/m^3.d, the sludge loading rate raised up from 0.19 to 0.45 kg COD/kgvss.d, and COD removal efficiency increased from 40% to 75.2%. Anaerobic sludge granules were discovered by sampling at the bottom of the reactor on the 60th day. The granules is black, with diameters of about 2mm. In order to speed up sludge granulation, we raised volumetric loading rate from 4.8 to 12.7 kg COD/m^3.d in about 40 days, the sludge loading rate raised from 0.45 to 1.37 kg COD/kgvss.d. This enriched the nutrition for microbes in the reactor and indeed speeded up granulation. The performance of the reactor also became better, COD removal efficiency reached 80% at volumetric loading rate of 12.7 kg COD/m^3.d.

Reactor 2

Reactor 2 was inoculated with anaerobically digested sludge from Beijing Weigou Pig Farm. Biogas production was found on the first day of inoculation and feeding was started on the second day. At the beginning, the COD concentration of influent was 840 mg/L, the volumetric loading rate was 1.7 kg COD/m^3.d. Compared with the reactor 1 this loading rate was higher. Experiment showed that the performance of the reactor was good and after 10 days of operation, the COD removal efficiency reached 60% at volumetric loading rate of 2.6 kg COD/m^3.d. As the seed sludge had higher methanogenic activity, it was very successful to increase the organic loading rate and after 23 days of operation the reactor volumetric loading rate reached 3.8 kg COD/m^3.d (sludge loading rate, 0.47 kgCOD/kgvss.d). Anaerobic sludge granules were discovered at the bottom of sludge bed but not in the upper part. This indicated that the granulation was not yet completed. Then we raised the organic loading rate to a higher level as long as the COD removal efficiency was higher than 70% and pH of effluent was above 7.2. When the reactor volumetric loading rate reached 11.5 kg COD/m^3.d on the 60th day, the COD removal efficiency is about 90%. Granules were found at the top part of sludge bed, indicating the completion of granulation. The performance and the operation data of reactor 2 are listed in Table 4.

There was no obvious lag stage in the process of start up for reactor 2, the start up could be divided into two stages, i. e., raise up organic loading stage and mature stage of granular sludge. The time from inoculation to the day of the appearance of granules took 23 days. Characteristics of the raise up organic loading stage were: performance of reactor became better and better; gas production increased gradually. The mature stage of granular sludge took 37 days in this experiment. Compared with reactor 1, reactor 2 had no lag stage, and the sludge granulation took shorter time.

Table 4 Operation condition of reactor 2

Operation stage Operation period	Raise up stage 1st - 23rd day	Mature stage of granules 24th - 60th day
Influent COD, mg/L	840 - 1310	1108 - 4080
Effluent COD, mg/L	143 - 327	332 - 420
COD removal rate, %	50.8 - 74.8	70.0 - 89.7
Volumetric loading, $kgCOD/m^3.d$	1.7 - 3.8	4.1 - 11.5
Sludge loading rate, kgCOD/kgvss.d	0.24 - 0.47	0.48 - 0.85
Alkalinity, $CaCO_3$mg/L	685 - 1240	834 - 1568
Effluent pH	6.5 - 7.5	6.6 - 7.3
Gas production, L/d	2.7 - 6.5	3.6 - 14.8
CH_4 content, %	60.3 - 70.3	60.9 - 67.4
HRT, h	11.9 - 8.3	6.4 - 8.8
VFA of effluent, mgHAc/L	240 - 470	120 - 290

Reactor 3

Reactor 3 was inoculated with settled sludge of anaerobic digester effluent from Beijing Tiancun Fermented Bean Curd Plant. The seed sludge had high methanogenic activity but poor settling property. The performance and operation data are shown in Table 5.

Table 5 Performance of reactor 3

Operation stage Operation period	Raise up stage 1st - 11th day	Mature stage of granules 12th - 32nd day
Influent COD, mg/L	3240 - 3120	3150 - 3180
Effluent COD, mg/L	930 - 330	480 - 445
COD removal rate, %	71.3 - 81.4	84.8 - 86.0
Volumetric loading rate, $kgCOD/m^3.d$	2.8 - 8.2	8.2 - 11.8
Sludge loading rate, kgCOD/kgvss.d	0.21 - 0.76	0.76 - 0.98
Alkalinity, $CaCO_3$mg/L	1430 - 1500	1200 - 1450
Effluent pH	7.0 - 7.5	7.0 - 7.5
Gas production, L/d	4.9 - 15.7	16.4 - 21.5
CH_4 content, %	60.0 - 65.3	61.0 - 67.5
HRT, h	8.5 - 18.0	7.5 - 10.5
VFA of effluent, mgHAc/L	540 - 270	470 - 280

In view of sludge granulation, the performance of reactor 3 was similar to that of reactor 2. Continuous feeding was started on the second day after inoculation. The granular sludge were discovered on the 11th day at the bottom of sludge bed. The volumetric and sludge loading rate were found to be 8.2 kgCOD/m^3. d and 0.76 kg COD/kgvss.d, respectively. Compared with the other two reactors, sludge washout was observed for reactor 3 (about 50 ml sludge liquor every day for several days). But this made no obvious detrimental impact on sludge granulation. Completion of sludge granulation was observed on the 32nd day when volumetric loading rate was 11.8 kgCOD/m^3. d and COD removal efficiency was 86%. Sludge washout disappeared.

3.2 Morphological characteristics of sludge during granulation

The sludge granulation and performance of three reactors were described above. From the point of technology the sludge granulation was divided into three stages: lag stage, raise up organic loading stage and mature stage of granular sludge. The sludge morphological features under microscopes were observed.

3.2.1 Lag stage

Among the three reactors, only reactor 1 had lag stage. In this stage, bacteria in seed sludge grandually adapted to wastewater (inducing enzyme for metabolism or modifying their functions). Microscopic observations indicated that there was no specific morphology of sludge and dispersed bacteria existed. They had the tendency to grow aggregatively.

3.2.2 Raise up organic loading stage

The reactor performance became better and better as its organic loading raised up in this stage. Generally, the volumetric loading rate was 2 – 5 kgCOD/m^3.d or higher. At the beginning of this stage filamentous bacteria grew quickly and its population became large obviously. The bacteria grew interwined or in bundles, forming spherical particles with dimensions of 50 – 100 μm.

3.2.3 Mature stage of granular sludge

The volumetric loading rate was generally higher than 5 kg COD/m^3.d and the sludge loading rate was higher than 0.45 kgCOD/kgvss.d at this stage. The sludge in reactor finally turned to granules from flocculent flocs. Under microscopes, we observed granular sludge was formed by aggregation of small particles, several or dozens of small particles aggregated together or interwined by filamentous bacteria, forming nascent granules.

3.3 Population dynamics during sludge granulation

3.3.1 Population level of different groups of bacteria during granulation

Anaerobic sludge granulation naturally is a evolution process of anaerobic microbial population, the development of predominant bacteria and the establishment of their relationship to other bacteria. Quantitative study on population dynamics of fermentative bacteria, propionate degraders, butyrate degraders and methanogens was made. The results obtained are listed in Table 6.

Table 6 Population of different groups of bacteria during sludge granulation

Reactor No.	Time	Sludge loading, kgCOD/kgvss.d	A	B	C	D
	1st day	–	2.5×10^4	6.5×10^2	4.0×10^2	2.0×10^1
1	24th day	0.17	2.5×10^7	1.5×10^3	4.5×10^5	9.5×10^2
	54th day	0.42	4.5×10^8	9.5×10^6	4.5×10^6	2.5×10^5
	86th day	1.20	2.5×10^9	9.5×10^6	4.5×10^7	7.5×10^7
	1st day	–	2.0×10^5	4.5×10^3	2.5×10^4	6.5×10^4
2	20th day	0.42	6.5×10^6	6.5×10^5	4.5×10^5	1.6×10^5
	44th day	0.63	1.5×10^8	7.5×10^6	6.5×10^6	2.5×10^6
	60th day	0.85	7.5×10^9	6.5×10^7	6.5×10^7	1.5×10^8
	1st day	–	1.5×10^5	6.5×10^3	4.5×10^4	6.5×10^4
3	11th day	0.76	6.5×10^7	7.5×10^5	9.5×10^6	4.0×10^6
	22nd day	0.80	7.5×10^8	1.6×10^7	4.5×10^7	6.5×10^7
	32nd day	0.98	9.5×10^9	7.5×10^7	1.5×10^8	4.0×10^8

A. Fermentative bacteria, cells/ml; B. Propionate degraders, cells/ml;

C. Butyrate degraders, cells/ml; D. Methanogen, cells/ml.

Based on the results obtained above, we discovered that the changes of different groups of bacteria during granulation were similar, although the seed sludge was different for the three reactors: (1) the fermentative bacteria grew quickly at the beginning of start up, the growth rate was higher than that of other groups. For reactor 1, the number of fermentative bacteria increased from 2.5×10^4 to 2.5×10^7 cells/ml; (2) at raise up organic loading stage, all groups of bacteria grew quickly and reached certain level to meet the needs of granulation at the end of this stage. The levels of propionate degraders, butyrate degraders and methanogens were about 10^{5-6}, 10^{5-7} and 10^{5-7} cells/ml. respectively. Fermentative bacteria was about 10^{7-8} cells/ml. The increase of the number of methanogenic bacteria (mostly filamentous bacteria when observed under microscopes) indicated that the filamentous methanogenic bacteria took an important role during granulation; (3) at the granular sludge mature stage, fermentative and methanogenic bacteria increase greatly again. This might be due to the increase of organic loading.

3.3.2 Relationship between microbial population and organic loading rate

Some researchers found that sludge loading rate was an important factor affecting sludge granulation. It was reported that granulation only took place when sludge loading rate was higher than 0.3 kgCOD/kgvss.d. In our experiment, granulation took place at 0.45, 0.48 and 0.76 kg COD/kgvss. d, respectively. Fig. 3 – 5 show the relation between sludge loading rate and microbial population.

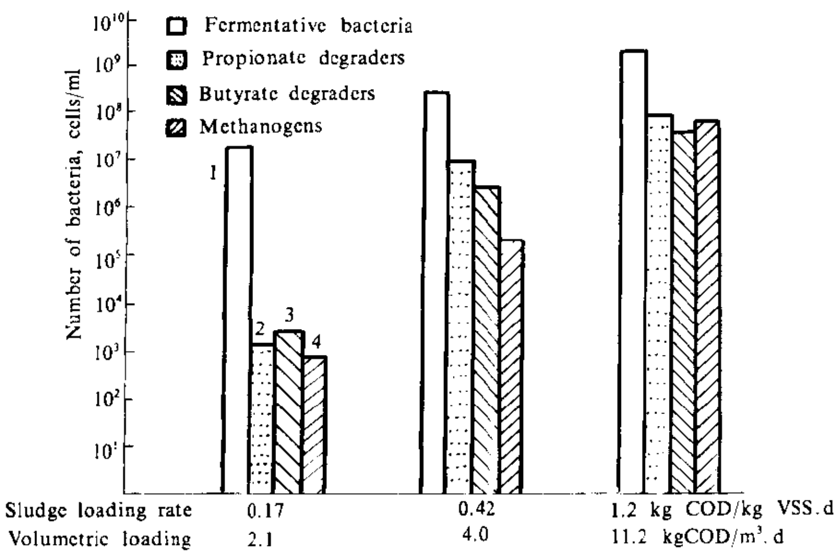


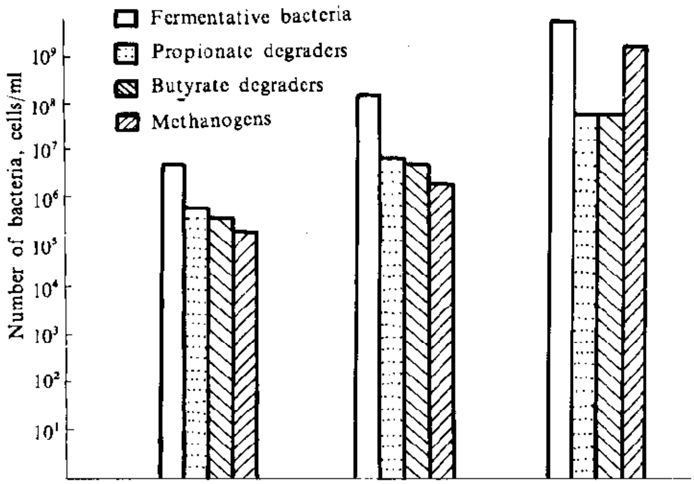
Fig. 3 Relation between sludge loading rate and microbial population for reactor 1

These results showed that (1) the total number of bacteria in sludge increased with the loading rate; (2) as organic loading increased, the ratio between different groups of bacteria changed, the ratioes of H_2-producing acetogens and of methanogens to fermentative bacteria increased. This indicated the predominant population changed from fermentative bacteria to H_2-producing acetogens and methanogens.

4 Discussions

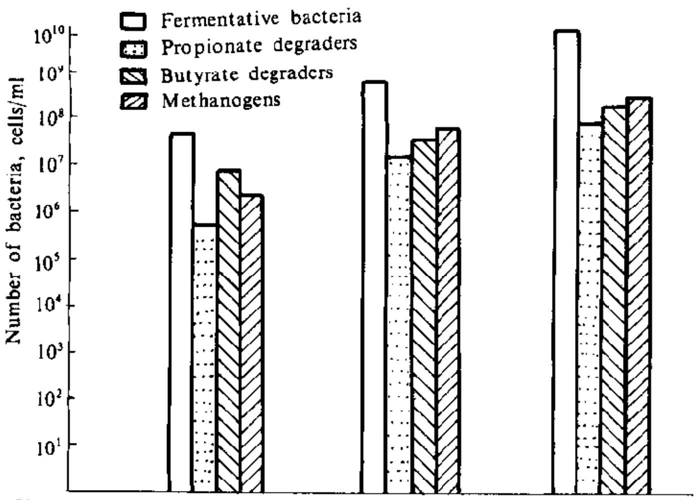
4.1 Microbiological aspect during sludge granulation

Several strains were isolated in our experiments from granules. Among the methanogenic strains, one is the mentioned methanogenic filamentous bacteria. Its single cell is rod shaped, with dimensions being $0.5 \times 1.0 - 2.0$ μm. According to its morphological and physiological properties, the filamentous bacteria are characterized to be the genus of *Methanothrix* strains of fermentative bacteria wer also isolated



Sludge loading 0.42 0.63 0.85 kg COD/kg VSS. d
Volumetric loading 3.5 7.4 11.5 kg COD/m^3. d

Fig. 4 Relation between sludge loading rate and microbial population for reactor 2



Sludge loading 0.76 0.80 0.98 kgCOD/kg VSS. d
Volumetric loading 8.2 9.1 11.8 kgCOD/m^3 · d

Fig. 5 Relation between sludge loading rate and microbial population for reactor 3 from granular sludge.

Based on the results mentioned previously, we speculated about the microbial process of granulation as follows: fermentative bacteria grow first when the reactor begin to start up, as they are the first member of anaerobic food chain and have the short double time. When fermentative bacteria population becomes large enough, their metabolites increased. This makes the bulk concentration of acetate, propionate, butyrate and other substrates increasing which are substrates for H_2-producing acetogens and for acetoclastic bacteria. Therefore, the populations of H_2-producing acetogens and of acetoclastic methanogens became larger. Filamentatous methanogenic bacteria will be predominant in sludge in this period. The granular sludge is formed by the cooperation of anaerobic bacteria in sludge and the environmental factors (e. g., hydraulic loading, biogas production and so on).

4.2 Descriptive model for granulation

Based on the population dynamics and morphological characteristics of sludge during granulation, as discussed above, basically five steps could be recognized. The five steps are shown in Fig. 6.

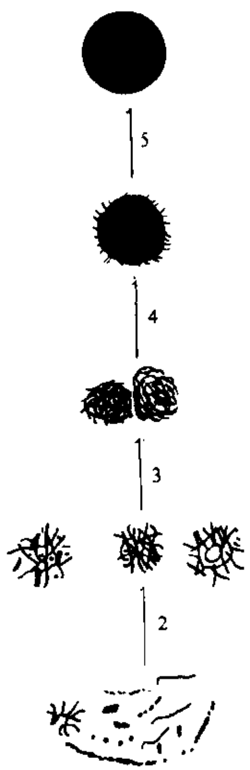


Fig. 6 Descriptive model for granulation

1. Growth of bacteria; 2. Formation of small bioparticles; 3. Aggregation of small bioparticles; 4. Formation of nascent granules; 5. Mature granules

4.3 Microbiological effect of sludge loading on granulation

As shown in Table 6, the reactor organic loading rates for each reactor were similar when granules formed, the volumetric loading rate was about 4 kg $COD/m^3 \cdot d$ and sludge loading rate was about 0.45–0.48 kg COD/kgvss.d. The reactor 3 had higher organic loading rate than the other two reactors when granulation took place. Under such condition, granulation in reactor 3 took place in short time. This indicates that granulation could be speed up under high organic loading as long as the reactor is operated stably.

Based on these results, it may be concluded: (1) the high organic loading rate makes it possible to reach high population for anaerobes, which results in formation of granules; (2) under low organic loading rate, nutritive materials are absorbed by fermentative bacteria, other group of bacteria has to keep low growth rate for the lack of nutritive materials; (3) without sufficient microbes and proper ratio between different bacteria group, granular sludge could not be formed.

References

Bryant MP. J Animal Sci, 1979; 48(1):193

Dolfing J. Appl Microbiol Biotech, 1985; 22:77

Dolfing J. Wat Sci Tech, 1986; 18(12):12

Mackie RI, MP Bryant. Appl Environ Microbiol, 1981; 41:1363

Lettinga G, W de Zeeuw, L Hushoff Pol, W Wiegant, A Rinzema. Proc 4th int symp on anaerobic digestion, Guangzhou, China, 1985:279

Lettinga G, AJB Zender. Proc of the gasmat-workshop, Luntern, 1987:140

Liu SJ. A microbiological study on the process of sludge granulation in UASB reactors, Ph. D. dissertation, Tsinghua University, China, 1991

Tang Y. Studies on the influence of alkalinity and hydraulic loading on sludge granulation in UASB reactors, Master thesis, Tsinghua University, China, 1990

Zeikus JG. Anaerobic Digestion, 1979; 2:66

(Received November 1, 1991)