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ENHANCED GRANULATION IN UASB REACTORS BY PRODUCING EXTRACELLULAR POLYMER AT OVER LOADING

Weili ZHOU[1], Tsuyoshi IMAI[2], Masao UKITA[3], Masahiko SEKINE[4] and Takaya HIGUCHI[5]

[1]Member of JSCE, M. Eng., Graduate Student, Dept.of Civil Eng. Yamaguchi University
E-mail: b3644@yamaguchi-u.ac.jp
[2]Member of JSCE, Dr. Eng., Associate professor, Dept.of Civil Eng. Yamaguchi University
Email: imai@yamaguchi-u.ac.jp
[3]Member of JSCE, Dr. Eng., professor, Dept.of Civil Eng. Yamaguchi University
Email: Mukita@yamaguchi-u.ac.jp
[4]Member of JSCE, Dr. Eng., Associate professor, Dept.of Civil Eng. Yamaguchi University
[5]Member of JSCE, Dr. Eng., Assistant, Dept.of Civil Eng. Yamaguchi University
(Dept. of Civil Engineering, Yamaguchi University, 2-16-1, Tokiwadai, Ube, Yamaguchi, 755-8611, Japan)

The method of producing extracellular polymer (ECP) at over loading to enhance the process of granulation was evaluated in lab scale UASB reactor with glucose as substrate. Granules formed within 39 days, following the emergence of ECP and the accumulation of sludge particles. The ECP content increased at every increase of organic loading rate (OLR). The experiment indicated that the increase of OLR, ECP and granulation are closely related to each other. The results showed that the treatment performance could be recovered from the unbalance between foodstuff and biological requirement. Besides, SEM observation revealed that different bacteria species predominated in the granules formed in different running conditions.

Key Words: *enhanced granulation, extracellular polymer (ECP), over loading, methanogen*

1. INTRODUCTION

With the rapid development of anaerobic technology in industrial wastewater treatment, more and more attention has been paid on the granulation of biomass in the anaerobic reactors. Granulation is a process in which the individual microorganisms are intimately associated with each other and form discrete pellets. It is widely accepted that after the granules appear in the reactor, higher treatment efficiencies and capacity can be expected. Since the granules are characterized with higher specific gravity and better settling ability than flocculent sludge, sludge washout can be prevented more efficiently and much higher biomass can be available in the reactor.

However, one of the major practical problems encountered with UASB-like reactors is the long start-up period required for the development of granules [1), 2)]. In lab scale UASB reactors, generally it takes 2-3 months or even more to granulate with glucose substrate. Therefore enhanced granulation is highly desirable in order to shorten this period. In this paper, a method of enhanced granulation by producing extracellular polymer (ECP) at over loading was studied and discussed.

It is commonly regarded that the ECP plays an important role in the process of anaerobic granulation [3), 4), 5), 6)] although up to now the mechanism of granulation is still not very clear. The ECP is excreted by the microbial cells and exposed at their surfaces under suitable physiological conditions. Because of its position and chemical characteristics, the polymer will affect the surface properties of the bacterial flocs. With its viscosity and the surface charge [7)] and other surface functions[3)], the ECP materials help to adhere and

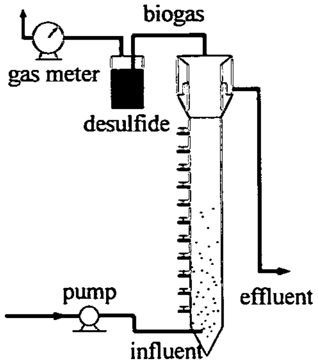

Fig. 1 experiment layout

connect more and more flocculent sludge particles together so that granules are developed. In the past research, some researchers ever succeeded in using man-made water absorbing polymer (WAP) to enhance the process of granulation [8], some others [9), 10)] also found it helpful to add the inert particles into the inoculated sludge in order to enhance the process of granulation.

The focus of this study was producing ECP biologically in order to achieve a more economical running and to avoid diminishing the valid volume of the reactor caused by the use of inert particles. Based on the consideration that the over-supplied substrate would stimulate the microorganisms to excrete more extracellular polymer in a shorter time and furthermore, would promote the granulation, the over loading was exerted and the characters of the sludge were studied in the lab scale experiment. For comparison, the low loading start-up experiment was also performed at the same time.

2. MATERIALS AND METHODS

(1) Experiment layout

The lab scale experiment was performed with two UASB reactors shown in **Fig.1**. The reactor consisted of a cylindrical glass tube with the total valid volume of 10.52 L, height of 1.5m and inner diameter of 92mm. 11 sampler pores were set along the height of the column to inspect the running condition inside. Each of the two metherphilic (35 ℃) reactors was inoculated with 6 liters of digester sludge from the municipal wastewater treatment plant. Glucose solution was used as the substrate to feed the bacteria. Nutrients such as N, P, Cl, S, and the necessary trace elements such as Fe, Co, Ni were added into the substrate solution (shown in **Table 1**). Because the tap water is acidic (its pH is about 6.0), more buffering agents were used. The influent pH was maintained about 7.2. The effluent was collected and sampled every day and the biogas produced was removed of sulfide and counted through the wet gas meters.

Table 1 Composition of the substrate

Glucose	9.4 (g/L)
A	2.0 (ml/L)
B	10.0 (ml/L)
C	1.0 (ml/L)
$NaHCO_3$	4.0 (g/L)
K_2HPO_4	4.0 (g/L)
Yeast	0.1 (g/L)

(10000mg COD/L)

In which,

A	$(NH_4)_2HPO_4$	350.0 (g/L)
B	KCl	75.0 (g/L)
	$MgCl_2.6H_2O$	81.0 (g/L)
	NH_2Cl	85.0 (g/L)
	$MgSO_2.7H_2O$	25.0 (g/L)
	$FeCl_3.6H_2O$	42.0 (g/L)
	$CoCl_2.6H_2O$	1.8 (g/L)
C	$CaCl_2.6H_2O$	150.0 (g/L)

(2) Analytic Method

UASB influent and effluent were sampled and analyzed for Chemical Oxygen Demand (COD), volatile fatty acid (VFA), MLSS and MLVSS. The sludge was sampled and analyzed for MLSS, MLVSS, and ECP content (including polysaccharide and protein). COD, MLSS, MLVSS were analyzed according to the standard method [11)]. Two gas chromatography apparatuses (GC-8APF/FID) were used for VFA and biogas analysis, respectively. Besides, pictures of sludge appearance were taken with an Olympus DP10-B microscope, and microorganism composition of the sludge was observed with a Hitachi-2300 Scanning Electronic Microscope (SEM).

There is no universal method for the extraction of the extracellular polymer yet. Many attempts were made including steaming, centrifugal stripping, alkali stripping and cooling extraction method. In this study, the cooling extraction method, which is described as follows, was confirmed to be effective[12)].

For ECP analysis, 2ml of sludge was centrifuged, removed of supernatant, added with 10ml of 0.85%

Table 2 Running Parameter of UASB Reactor

(a) Over-loading operation

Stage	Time	Influent COD	Flow Rate	OLR
	(d)	(mg/l)	(L/d)	KgCOD /m^3.d
1	0-3	2000	5	1
2	4-7	5000	5	2.5
3	8-10	10000	5	5
4	11-14	20000	5	10
5	15-17	30000	5	15
6	18-21	10000	15	15
7	22-24	10000	25	25
8	25-39	10000	30	30

(b) Low-loading operation

Stage	Time	Influent COD	Flow Rate	OLR
	(d)	(mg/l)	(L/d)	KgCOD /m^3.d
1	0-14	500	5	0.25
2	14-28	500	10	0.5
3	29-40	1000	10	1
4	41-56	2000	10	2
5	57-70	2000	20	4
6	71-90	4000	20	8

NaCl solution and 60ml Formalin, and then the ECP in the mixed liquid was extracted with ultrasonication while being cooled in the ice water. After being centrifuged at 12,000 rpm for 30min, the supernatant was analyzed for carbohydrate ECP and proteinaceous ECP, which were regarded as the main part of ECP materials and were measured as polysaccharide and protein. Polysaccharide ECP was determined by Sulfuric Acid-Anthron method and proteinaceous ECP was analyzed according to Lowry Folin method [13].

3. RESULTS AND DISCUSSION

Table 2 shows the operational conditions of the UASB reactors. After a simple domestication for 3 days, the Organic Loading Rate (OLR) was increased rapidly from 1kgCOD/m^3.d to 30kgCOD/m^3.d within 30 days, in the over loaded UASB reactor. For the low loading one, the maximum OLR, 8kgCOD/m^3.d, wasn't exerted until the 71st day.

(1) Sludge Appearance

Fig.2 shows the variation of the sludge appearance in both reactors. In the over loaded UASB, some white, flocculent, nuclei-like material (later convinced to be polymer) appeared in the sludge on the 7th day of running (at the OLR of 2.5 KgCOD/m^3.d) (**Fig.2b**). Then with the increase of OLR and the development of this kind of material, more and more tiny sludge particles accumulated around the white nuclei (**Fig.2c**). Finally, at the OLR of 30 KgCOD/m^3.d, a lot of granules were found in the reactor (**Fig.2d**), which indicated that the progress of granulation could be achieved within 39 days at over loading.

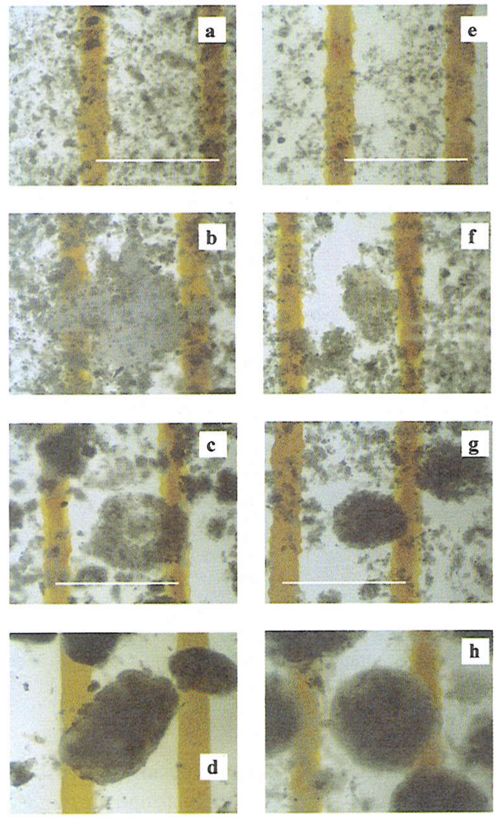

Fig.2 Variation of sludge appearance (bar: 1mm). **a,b,c,d** over-loading operation on the 1st, 7th, 21st, 38th day. **e, f, g, h** low loading operation on the 1st, 48th, 62nd, 90th day

It was very interesting to find the sludge in the low loading reactor also changed in almost the same way although it took much more time due to the slow increase of the OLR. The white ECP materials also emerged at the OLR of about 2KgCOD/m^3.d (**Fig.2f**). Small sludge particles were also found to accumulate around them (**Fig.2g**), and granules appeared finally as the result of the accumulation (**Fig.2h**).

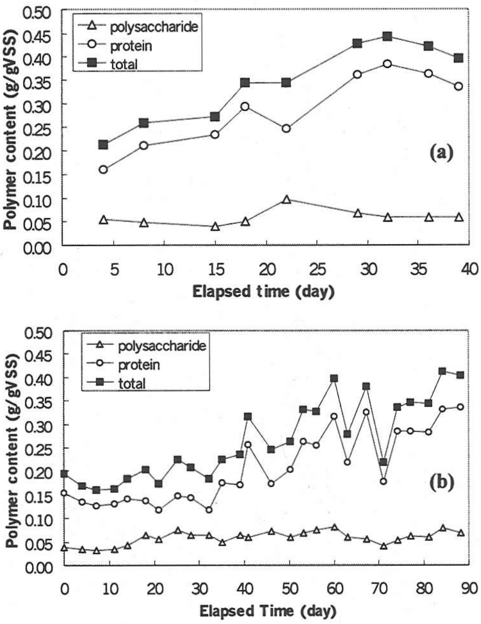

Fig.3 Variation of ECP content in the sludge. **(a)** Over loading operation. **(b)** Low loading operation

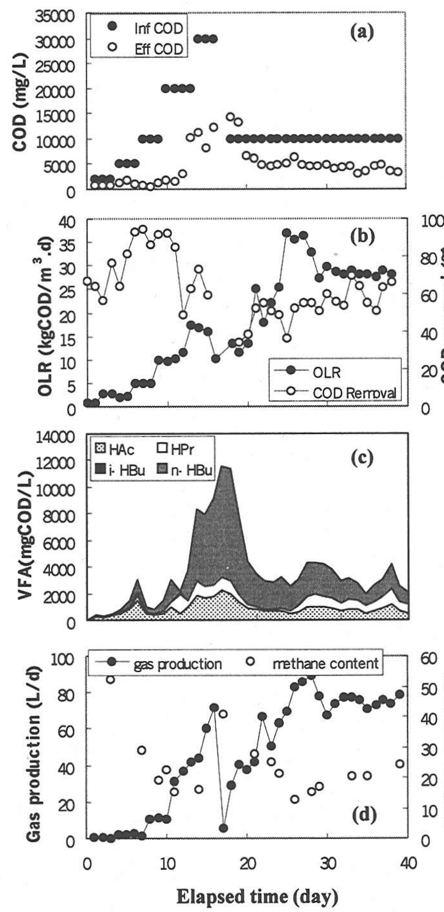

Fig.4 Running performance of the over loaded UASB reactor. **(a)** Influent and effluent COD. **(b)** OLR and COD removal **(c)** VFA concentration in the effluent. **(d)** Biogas production and Methane content

The repeatability of the experiment was well verified for times. It was clearly found through the experiments that granulation always followed the emergence of ECP materials and both of them were closely related to the increase of OLR. The authors also thought it difficult for the flocculent anaerobic sludge to granulate when OLR was too low, even after a long period of running under very good treatment performance.

(2) Polymer content in the Sludge

Although the precise mechanism of granulation and polymer development is still incompletely understood, the role attributed to extracellular polymers with respect to the granulation progress is well established [3), 14)]. In the anaerobic digestion, the carbohydrates are firstly converted into soluble sugars in the function of extracellular enzymes of the fermentative bacteria, then the soluble sugars are taken up into the cells of fermentative bacteria and turned into some simple organic compounds such as volatile fatty acids (VFA), hydrogen and carbon dioxide. In the next methanogenesis step, the VFAs are decomposed into acetate, which is finally consumed and converted into methane and carbon dioxide. In this simplified progress, ECP are a kind of biopolymer secreted by the cells of microorganism during anabolism and metabolism [3)]. With plentiful substrate, microorganisms tend to produce more ECP because of the increased anabolic activity [15), 16)].

In this study, when food was over-supplied, the variation of the ECP content in the sludge is shown in **Fig.3a**. After the higher loads were exerted on the 4th, 8th, 11th, 15th, 22nd and 25th day, evident increases of ECP content were always detected. Finally it showed an obvious declination after the loading rate was fixed for a longer time, which might be explained as the naturally made ECP materials having been consumed partly when the unbalance between the foodstuff supply and microorganisms requirement was eliminated gradually.

In the low loading operation (**Fig.3b**), the content of ECP remained almost the same level in the first 40 days when OLR was less than 1kgCOD/m^3.d. Then

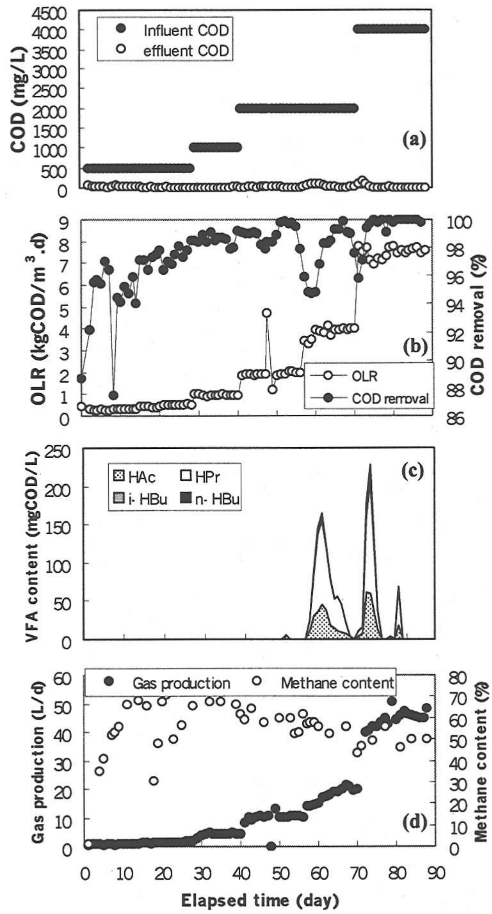

Fig.5 Running performance of the low loading UASB reactor. **(a)** Influent and effluent COD. **(b)** OLR and COD removal **(c)** VFA content in the effluent. **(d)** Biogas production and Methane content

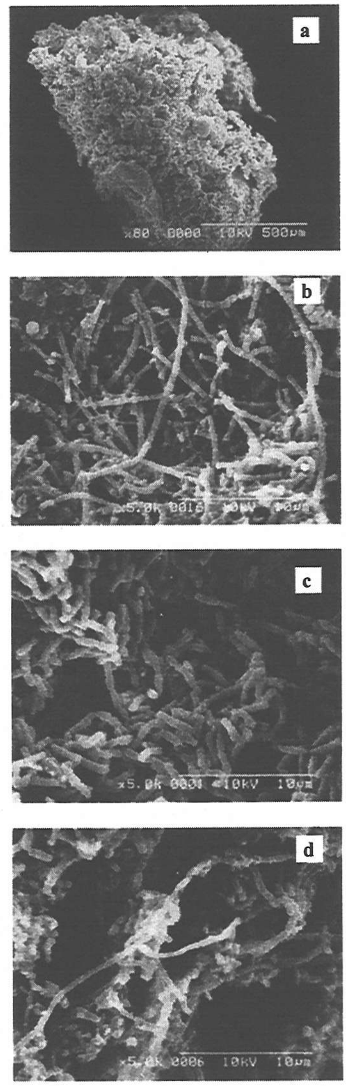

Fig.6 SEM observation of the granules. **(a)** Accumulation of sludge particles results in granules. **(b)** Methnothrix-like filaments predominated in the granules. **(c)** Rod-type Methanothrix-like bacteria came to be the main methnogens in the granules. **(d)** Small amount of Methanothrix-like filaments were also found in the granules formed in over loading operation.

with the OLR increase on the 41st, 57th, and 71st day, ECP content also increased correspondingly and then, a few days later, partly decreased.

Whether in over loading or low loading operation, ECP content showed the same variation trend with the change of OLR. The OLR increase always caused the temporary increase of ECP, but if the OLR was fixed for long enough, part or all of the ECP materials were consumed by the bacteria for the menergy and/or the organic carbon, thus the ECP concentrations were reduced later. This is consistent to some reported observations in activated sludge and in anaerobic culture [15].

Fig.3 revealed that the over supplied substrate promoted the biological activity and stimulated the microorganisms to secrete more ECP materials, as the response. And because the OLR was increased so rapidly that microorganisms could not consume the ECP in time, the concentrations of ECP could achieve a relatively higher value in a shorter period, which enhanced the process of granulation more efficiently.

(3) Running Performances of the Reactors

At over loading, the running performance such as the effluent quality, COD removal efficiency and biogas production would be an important consideration since the final objective of granulation is to achieve higher treatment capacity and better effluent. **Fig.4** and **Fig.5** show the treatment performance of the UASB reactors running at over loading and low loading conditions, respectively. In low loading UASB, the treatment performance was very good **(Fig.5)**, but it took a long time (about 3 months) to start up and its treatment capacity was much lower. For the over loaded UASB reactor, when OLR was increased rapidly from 5kgCOD/m^3.d to 10kgCOD/m^3.d and then 15kgCOD/m^3.d, there was indeed a period of low removal and relatively worse effluent **(Fig.4b)**, accompanied with the drastic accumulation of VFA **(Fig.4c)** and abrupt decrease of methane content in the biogas **(Fig.4d)**. But with the change of the influent COD in the next stage, the reactor was recovered step by step although the OLR was kept unchanged. In the following two stages, OLR was increased again. The running performance remained relatively stable. The reason might be that the microorganisms multiplied themselves rapidly so that the unbalance between food supply and requirement was abated gradually. In the final stage, as the OLR, influent COD, and flow rate were kept constant for long, the removal rate returned to about 70% **(Fig.4b)** and VFA accumulation declined **(Fig.4d)**. Although the treatment performance is still not very satisfying at this time, considering the higher biomass and the rapider microorganism multiplication at higher OLR, it is reasonable to believe that the treatment performance will be improved rapidly in the following days. And compared with the low loading process, because the reactor has the higher treatment ability at over loading, less dilution or easier posttreatment is needed to discharge the same total volume of wastewater during the start-up period.

Throughout the over loading experiment, effluent pH remained between 6.4 and 7.2 (data not shown). No severe acidification was found, but the methane content in the biogas was lower than that in the normal anaerobic treatment **(Fig.4c)**.

(4) Microorganism composition of the granules

The scanning electronic microscope (SEM) observation of the granules is shown in **Fig.6**. In **Fig.6a**, many tiny sludge particles can be clearly seen on the surface of the granule, which verified that granulation was resulted from the accumulation of the particles in this study.

In low loading operation **(Fig.6b)**, Methnothrix-like filaments predominated in the granules. While in over loaded reactor, rod-type Methanothrix-like bacteria came to be the main methnogen in the granules **(Fig.6c)**. Small amount of Methanothrix-like filaments were also found **(Fig.6d)** in the granules formed in over loading operation.

It is still not clear why the predominant methanogen species were so different although the same glucose substrate and inoculums were used for both of the reactors. The pH surroundings might be one of the reasons. At over loading, the pH value in the reactor was about 6.4-7.2, which may be compatible for rod-type Methanothrix-like bacteria. And in low loading operation, pH value fluctuated between 7.0 and 7.5, which probably nurtured the filaments bacteria very well. On the other hand, the residual VFA may also explain the difference between these two processes since Methnothrix-like filaments are often found to be predominant in low VFA media.

4. CONCLUSIONS

Through the comparison between the over loading and low loading operation, we can draw the following conclusions:

(1) The granulation process can be enhanced by producing extracellular polymer at over loading. In the 39-day-running, with the over loading exerted, the ECP materials emerged in a relatively shorter time, which was followed by the accumulation of sludge particles and formation of granules.

(2) The content of extracellular polymer increased as the response of OLR increase in both of the low loading and over loading reactors. The study confirmed that ECP content was closely related to OLR change.

(3) The running performance of the over loading reactor was studied and the results convinced that the reactor could be recovered from the unbalance between foodstuff and biological requirement as the microorganisms developed gradually.

(4) SEM observations showed the different composition of the bacteria in the granules. Methnothrix-like filaments predominated in the granules from the low loading UASB reactor. While in the over loaded reactor, rod-type Methanothrix-like bacteria came to be the main bacteria in the granules. This phenomenon might be due to the different pH surroundings and the VFA concentrations under the different running conditions.

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過負荷運転によるUASB法におけるグラニュールの形成促進に関する研究

周偉麗・今井剛・浮田正夫・関根雅彦・樋口隆哉

本研究では，グルコースを基質としたラボスケールのUASBにおいて，反応器内を過負荷状態維持することにより，細胞外ポリマーを早期に蓄積させ，そのことによる，グラニュールの形成促進効果について実験的に検討した．実験結果から，過負荷運転と細胞外ポリマーの蓄積，及びグラニュールの形成に密切な関係があることが明らかてなった．また，過負荷状態の保持により，グラニュール形成期間を約40日に短縮できた．過負荷運転を行った場合，グラニュール形成までの期間は処理性能は低下するが，グラニュールが形成され始めると速やかに回復した．SEMによる観察結果から，負荷のかけ方によって微生物相が異なることが確認された．