Extracellular Polymers in Biogranules Treating Brewery and Acetate Wastewaters*

JIA Xiao-shan[1], FANG Herbert H P[2]
(1. *Department of Environmental Engineering*, *Sun Yat-sen University*, *Guangzhou* 510275, *China*;
2. *Department of Civil Engineering*, *The University of Hong Kong*, *Hong Kong*, *China*)

Abstract: Biogranules treating brewery wastewater were considerably stronger in resistance to attrition and dissolution, due to the higher content of ECP, than the acetate-degrading biogranules. Each mg of the latter biogranules, which were predominantly *Methanothrix*, had an average 0.09 mg of ECP, whereas the former biogranules had 0.15 mg because of the high ECP contents in the hydrolytic/fermentative bacteria on the surface and in the syntrophic bacteria of the mid-layer.

Key words: anaerobic; biogranules; extracellular polymers (ECP)
CLC number: X172 **Document code**: A **Article ID**: 0529-6579(2003)S1-0141-03

Anaerobic degradation of pollutants is a complex process involving at least three groups of bacteria. Hydrolytic and fermentative bacteria convert complex pollutants into simple fatty acids, which are then further converted by acetogenic bacteria into acetate and hydrogen. Finally, methanogenic bacteria convert acetate and H_2/CO_2 into methane. In the past decade, anaerobic technology has been broadly applied to the treatment of many high-strength wastewaters, to a very large extent, due to the successful development of UASB (upflow anaerobic sludge blanket) process. In a UASB reactor, bacteria aggregate into forming biogranules which have high bioactivity and superb settleability[1]. Recent studies have shown that UASB biogranules exhibit different microstructures, depending upon the chemical nature of the substrate in wastewater and the degradation kinetics[2].

Although the granulation mechanism of anaerobic bacteria has not been yet fully understood, it has been speculated that the extracellular polymers (ECP) could likely play an important role[3]. ECP are metabolic products of bacteria which accumulate on the cell surface[4]. They protect cells from the harsh external environment, and provide cells with energy and carbon when there is a food shortage. It has been reported by many researchers that ECP is related to the flocculation of bacterial cells in aerobic wastewater treatment system[5].

In a recent study[2], UASB biogranules treating brewery wastewater were found to have a layered microstructure with different bacterial population in each layer. Hydrolytic/fermentative bacteria formed a dense outer layer, whereas syntrophic colonies proliferated in the mid-layer and acetotrophic methanogens were predo-minant in the interior core. On the other hand, biogranules treating acetate exhibited a simple, uniform microstructure predominantly populated by *Methanothrix*[6]. It is thus of great interest to analyse the ECP content in these two types of biogranule, and to determine the correlation, if any, between the ECP and the biogranules bacterial distribution.

1 Materials and methods

Biogranules treating brewery wastewater were obtained from a full-sized UASB reactor. Biogranules from the same reactor had previously been analysed for microstructure and bacterial distnoution[2]. Acetate-degrading biogranules were, on the other hand, obtained from a 2.8 L UASB laboratory reactor[7] treating wastewater containing 2800 mg/L of acetate over a nine-month period. Individual biogranules were hand-picked for this study, so that all biogranules were near spherical with sizes ranging 1.9 ~ 2.1 mm and a mean of 2.0 mm. The total numbers of biogranules picked for this study were 140 for the brewery biogranules, and 100 for the acetate-degrading biogranules

The two types of biogranules were put in separate 157 mL glass vials, each of which contained 50 mL of 0.05% of sodium chloride solution. The solution in each vial was then mixed using a Teflon-coated magnetic bar at 500 r/min. The average size of the biogranules was gradually reduced over time due to surface attrition and dissolution; some of the granules were even disintegrated as the mixing proceeded. After a certain time interval, the solution was decanted. The average size of the biogranules

* 收稿日期：2003-01-15
基金项目：香港RGC资助项目
作者简介：贾晓珊（1962年生），男，教授，博士生导师；E-mail：eesjxs@zsu.edu.cn

was measured and the number of biogranules was counted. The decanted solution was then measured for its VSS (volatile suspended solids) and ECP contents. Subsequently, the biogranules were put back to the glass vial, which was again filled with 50-mL of fresh sodium chloride solution, and the attrition/dissolution process was repeated. The process was repeated until all the biogranules became totally disintegrated at the end.

The VSS content in the decanted solution was measured according to the Standard Methods. ECP was extracted in a formaldehyde solution at 80 ℃[8] followed by 30 minutes of high-speed centrifugation (15 000 G/min at 25 000 G; model Jouan-KR22i). The carbohydrate content of the ECP(ECP_c) in the extracted solution was measured by the phenol/suiphuric-acid method, and the protein content (ECP_p) by the folin method. The sum of ECP_c and ECP_p was the total ECP.

2 Results and discussion

Acetate-degrading biogranules appeared to have little resistance to the surface attrition/dissolution caused by the stirring action. Within 20 hours, all the 100 biogranules in the glass vial were totally disintegrated. Because it happened unexpectedly in such a short period, there was no solution sample collected for analysis during the intermediate. Analysis of the final solution showed that each mg of acetate-degrading biogranules (as measured by VSS) contained an average of 0.09 mg of ECP.

On the other hand, brewery biogranules exhibited much stronger resistance toward attrition/dissolution. Only two out of the 140 biogranules were disintegrated in the first 23 hours. After 47 hours, two additional biogranules were further disintegrated. Eventually, all the biogranules were totally disintegrated after 110 hours. Fig. 1 illustrates that the average size of the remained biogranules was reduced over time due to attrition/dissolution. The average radius was reduced linearly from the initial 1.00 mm to 0.78 mm after 84 hours. But all biogranules were disintegrated within the following 26 hours.

At the end of a given time interval, the ECP and VSS in the decanted solution represented those of the bacterial cells on the biogranule surface being removed by attrition/dissolution during that time interval. Table 1 shows that the ECP/VSS ratio increased from 0.16 ~ 0.17 mg – ECP/mg – VSS near the surface (r = 0.87 ~ 1.00 mm) to 0.27 ~ 0.23 mg – ECP/mg – VSS in the middle layer (r = 0.78 ~ 0.87 mm). But, in the interior core (r < 0.78 mm), the ratio was only 0.11 mg – ECP/mg – VSS. The variation of ECP/VSS ratio seemed to agree well the microscopic observations of a layered microstructure for the brewery biogranules. Based on results of this study, hydrolytic/fermentative bacteria in the outer layer appeared to have lesser amount of ECP than the syntrophic bacteria which were found populated in the mid-layer. Whereas, acetotrophic methanogen, *Methanothrix*, which were the predominant species in the granule interior had the least amount of ECP. The low ECP content in *Methanothrix* was likely the reason for the rapid disintegration of the acetate-degrading biogranules within 20 hours, and for the rapid disintegration of brewery biogranules after 84 hours when most of the hydrolytic/fermentative and syntrophic bacteria in the outer layers had been removed from the biogrannles. These results indicate that ECP is responsible to the integrity of the biogranules and thus may play an important role to the granule formation.

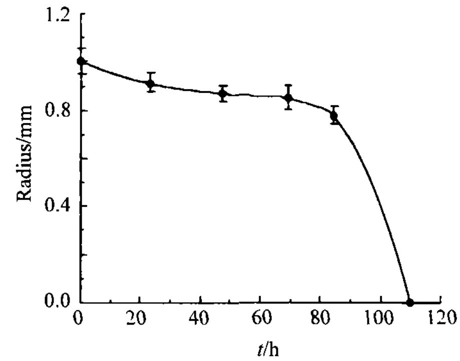

Fig.1 Size reduction of brewery biogranules due to attrition and dissolution

Tab.1 ECP content in each mg of VSS from bacteria at various radius of the biogranules

r/mm	ECP/mg
1.00 ~ 0.91	0.16
0.91 ~ 0.87	0.17
0.87 ~ 0.85	0.27
0.85 ~ 0.78	0.23
< 0.78	0.11
overall average	0.15

4 Conclusions

Biogranules treating brewery wastewater were significantly stronger in resistance to attrition and dissolution than the acetate-degrading biogranules. This was likely due to the higher ECP content in the former. Hydrolytic/fermentative bacteria near the surface of the former granules contained 0.16 ~ 0.17 mg of ECP for each mg of VSS; the ratio was increased to 0.27 ~ 0.23 mg – ECP/mg – VSS for the mid-layer populated with syntrophic bacteria. However, the ratio for bacteria in the interior of the brewery biogranules, mostly *Methanothrix*, contained only 0.11 mg(ECP)/mg(VSS). Acetate-degrading biogranules were also mainly composed of *Methanothrix*, and had a similarly low ECP/VSS ratio of 0.09. These results indicate that ECP is responsible to the integrity of the biogranules and thus may play an important role to the granule formation.

Acknowledgment: The authors wish to thank the Hong Kong Research Grants Council for the partial financial support of this study.

References:

[1] LETTINGA G, van VELSEN A F M, HOBMA S M, et al. Use of the upflow sludge blanket (USB) reactor concept for biological wastewater treatment[J]. Botech Bioengrg, 1980, 22:699-734.

[2] FANG H H P, CHUI H K, LI Y Y. Micro-structural analysis of UASB granules treating brewery wastewater[J]. Wat Sci Tech, 1995, 31(9): 125-135.

[3] JIA X S, FURUMAI H, FANG H H P. Extracellular polymers of hydrogen-utilizing methanogenic and sulfate-reducing sludges[J]. Wat Res, 1996, 30: 1439-1444.

[4] MORGAN J W, FORSTER C F, EVISON L. A comparative study of the nature of biopolymers extracted from anaerobic and activated sludge[J]. Wat Res, 1990, 24(6): 743-750.

[5] ROSS W R. The phenomenon of sludge pelletisation in the anaerobic treatment of a maize processing waste[J]. Water SA, 1984, 10: 197-203.

[6] ZEHNDER A J B, HUSER B A, BROCK T D, et al. Characterization of an acetate-decarboxylating non-hydrogen-oxidizing methane bacterium[J]. Arch Microbiol, 1980, 124: 1-11.

[7] FANG H H P, LI Y Y, CHUI H K. UASB treatment of wastewater with concentrated mixed VFA [J]. J Environ Engrg, 1995, 121(2): 153-160.

[8] FANG H H P, JIA X S. Extraction of extracellular polymers from anaerobic sludges[J]. Biotechnology Techniques, 1996, 10: 803-808.

处理酿酒厂和乙酸废水生物颗粒中的细胞外聚合物

贾晓珊[1], 方汉平[2]

(1. 中山大学环境工程系, 广东 广州 510275;
2. 香港大学土木工程系, 香港)

摘 要: 处理酿酒厂废水的生物颗粒与乙酸的生物颗粒相比，具有相当强的抗损耗和溶解能力是因为较高含量的 ECP。每 mg 由 *Methanothrix* 细菌组成的后者平均只含有 0.09 mg 的 ECP。但是前者含有 0.15 mg 的 ECP，主要是因为高含量的 ECP 存在于位于生物颗粒表面的 hydrolytic/fermentative 细菌和中间层的 syntrophic 细菌之中的缘故。

关键词: 厌气; 生物颗粒; 细胞外聚合物 (ECP)

中图分类号: X172 **文献标识码**: A **文章编号**: 0529-6579 (2003) S1-0141-03