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EXTRACTION OF EXTRACELLULAR POLYMER FROM ANAEROBIC SLUDGES

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SUMMARY

Two types of anaerobic sludge were analyzed for ECP (extracellular polymers) content under five extraction conditions. Results showed that EDTA was more effective than formaldehyde as an extractant. Increase of temperature and addition caustic also enhanced the extraction. The ratio between carbohydrate and protein fractions of ECP for both acetate- and benzoate-degrading sludge was 0.16-0.18. The former sludge had only 40-45% of ECP as in the latter sludge.

INTRODUCTION

Extracellular polymers (ECP) are metabolic products of bacteria which accumulate on the cell surface (Morgan, *et al.* 1990). They not only protect the cells from the harsh external environment and provide with energy and carbon when food is in short supply, but also play an important role in the flocculation of bacterial cells in wastewater treatment (Tenney and Stumm 1968). A number of studies have been reported on the bioflocculation mechanisms (Tenney and Stumm 1968; Busch and Stumm 1968; Ryssov-Nielson 1975; Eriksson and Hardin 1984; Sutherland, 1985; Eriksson and Alm 1991) and the chemical nature of ECP (Forster and Clarke, 1983 and Morgan *et al.* 1990). But, most of these studies have been limited to aerobic microorganisms.

More recently, many reported that ECP is also critical for the granulation of anaerobic sludge (Ross 1984; Jia *et al.* 1991), which is key element for the success of the UASB (upflow anaerobic sludge blanket) wastewater treatment technology (Lettinga *et al.* 1980; Li *et. al.* 1995). Although several ECP extraction methods have been reported (Pavoni *et al.*, 1971; Tezuka, 1973; Nishikawa *et al.*, 1968; Sutherland *et al.*, 1971), there is not yet a standard method universally

accepted by researchers studying ECP. As a result, comparison and interpretation of published results are difficult.

The sludge ECP measurement is strictly dependent upon the extraction method. This study was conducted to compare a number of ECP measurements for two types of anaerobic sludge.

MATERIALS AND METHODS

Anaerobic sludge were sampled from two UASB reactors (Li *et. al.* 1995) treating synthetic wastewater which contained benzoate and acetate, respectively, as sole substrate. Both reactors were operated at 37°C under steady state condition for over six months at a COD (chemical oxygen demand) loading rate of 10 g/(l·day). In UASB reactors, granulated sludge settle to the reactor bottom, whereas dispersed sludge remain on top forming a blanket layer. Because granular sludge have a densely packed microstructure (Fang *et al.* 1995) which may affect the rate of ECP extraction, only the dispersed sludges from the blanket zone of the reactors were sampled for analysis in this study.

In each analysis, a 10ml sludge sample was washed twice using de-ionized water, before being re-suspended in 10ml of 0.85% sodium chloride solution. The suspended sample was then extracted for ECP following five different procedures. After extraction, supernatant was separated from the residual sludge using a high-speed centrifuge (Jouan, KR22i) at $15x10^3$rpm, corresponding to $25x10^3$G. The protein content of supernatant ECP (ECP_p) was measured by the folin method (Lowry *et al.* 1951) and the carbohydrate content (ECP_c) by the phenol/sulfuric-acid method (Dubois *et al.* 1956). In addition, the VSS (volatile suspended solids) content in the residual sludge was also measured following the standard method (APHA 1985). Each sample was analyzed in quadruplicate.

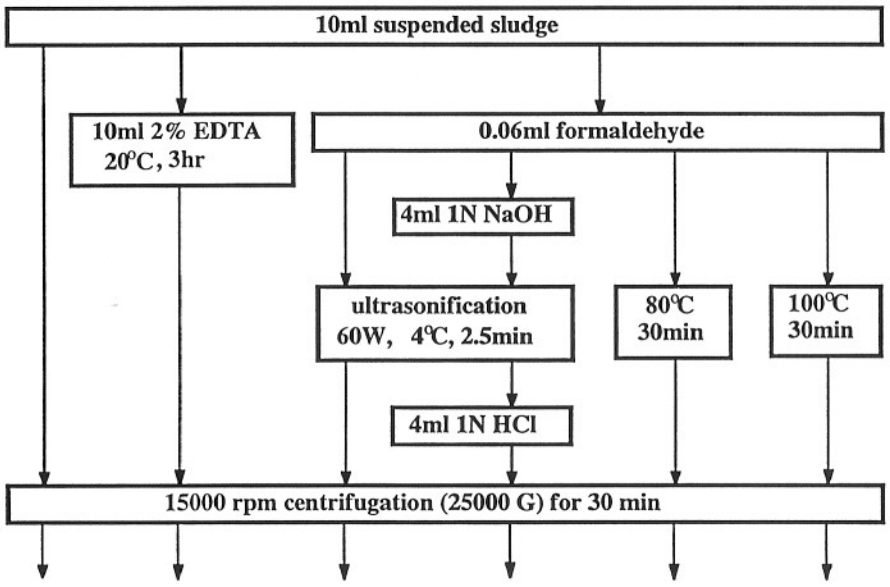

Figure 1. Extraction Procedures

The procedures of the five ECP extraction methods were illustrated in Figure 1. A sixth set of samples were treated by high-speed centrifugation alone, without adding any extractant, serving as control (Pavoni *et al.* 1971). Among the five extraction methods, one used EDTA (ethylenediaminetetraacetic acid) as extractant (Nishikawa *et al.* 1968) (20°C, 3hrs) and four used formaldehyde (Sutherland *et al.* 1971). For those using formaldehyde as extractant, two used thermal treatment (80°C and 100°C, respectively, for 30min) and the other two used ultrasonification (60W at 4°C for 2.5min), in one of which caustic (4ml of 1N NaOH) was also dosed to enhance the extraction (Tezuka 1973). An ultrasonic homogenizer (Cup Horn, Model 4710) was used for ultrasonification.

Two additional tests were also conducted to examine effects of extraction time and the VSS content on the ECP extraction of benzoate-degrading sludge.

RESULTS AND DISCUSSION

Comparison of ECP_c and ECP_p by Five Extraction Procedures

Table 1 summarizes the mean ECP_p and ECP_c contents in each gram of benzoate-degrading sludge. As compared to the extractant-free control, both EDTA and formaldehyde were able to extract significant amount of ECP. The former was, however, a much stronger extractant than the latter. EDTA extracted 204.1 mg of ECP_p from each gram of benzoate-degrading sludge, much higher than those extracted by formaldehyde using either thermal or ultrasonic treatment. For those using formaldehyde as extractant, more ECP_p was extracted at 100°C (138.3 mg) than at 80°C (119.2 mg), both were in turn more than that extracted by ultrasonification at 20°C (101.8 mg). However, the combination of ultrasonification and caustic addition considerably increased the extraction of ECP_p to 190.8 mg.

Table 1. Average ECP_c and ECP_p Extracted from Each Gram of Benzoate-Degrading Sludge

Extractant and Condition	ECP_p (mg)	ECP_c (mg)	ECP_c/ECP_p ratio
Control	32.1	14.8	---
EDTA	190.8	33.7	0.18
Formaldehyde			
100°C	138.3	22.6	0.16
80°C	119.2	19.6	0.16
ultrasonification	101.8	18.3	0.18
ultrasonification plus caustic	190.8	33.7	0.18

Table 1 also shows that, regardless of the extraction method, the extracted ECP_c amounted to only 16-18% of ECP_p. The relative effectiveness of different methods for ECP_c was similar to that for the extraction of ECP_p.

Table 2 summarizes the extracted data of ECP_p and ECP_c in each gram of acetate-degrading sludge. The relative effectiveness among various extraction methods was the same as that observed for the benzoate-degrading sludge. The ECP_c/ECP_p ratio of acetate-degrading sludge was similarly 0.17-0.18, regardless of the extraction method. However, acetate-degrading sludge contained only 40-45% amount of ECP_p and ECP_c as the benzoate-degrading sludge.

Table 2. Average ECP_c and ECP_p Extracted from Each Gram of Acetate-Degrading Sludge

Extractant and Condition	ECP_p (mg)	ECP_c (mg)	ECP_c/ECP_p ratio
Control	13.4	5.2	---
EDTA	93.7	16.0	0.17
Formaldehyde			
100°C	63.4	10.6	0.17
80°C	52.6	8.8	0.17
ultrasonification	45.0	8.2	0.18
ultrasonification plus caustic	88.8	15.0	0.17

One might speculate that a sludge with a higher ECP content would have a better tendency to agglutinate into granules. This was confirmed by comparing the size of granules sampled from the two reactors in this study. The benzoate-degrading granules ranging 1-3mm were considerably bigger the acetate-degrading granules averaging 0.5mm.

Effects of Extraction Time and VSS Concentration

Figure 2a illustrates that, using formaldehyde at 80°C, ECP_p and ECP_c were mostly extracted within the first 15min. Prolonged treatment would increased the extracted amount, but only slightly. For example, extending the extraction from 30min to 60min only increased the amount of extracted ECP from 300mg to 360mg for each liter of sludge sample. Figure 2b illustrates a similar trend for ECP extraction by formaldehyde in combination with ultrasonification. Most of ECP was extracted within the first 2.5min, and extending to 5min and 10min only increased

extracted ECP_p from 250 mg/l to 280 mg/l and 400 mg/l, respectively.

Figure 3 illustrates that the amount of ECP extracted at a given condition was proportional to the amount of VSS at low VSS concentrations. Above 1200 mg/l VSS, ECP was extracted from VSS at a lower proportion. This was observed for formaldehyde extractions using either thermal treatment at 80°C (Figure 3a) or ultrasonic treatment (Figure 3b).

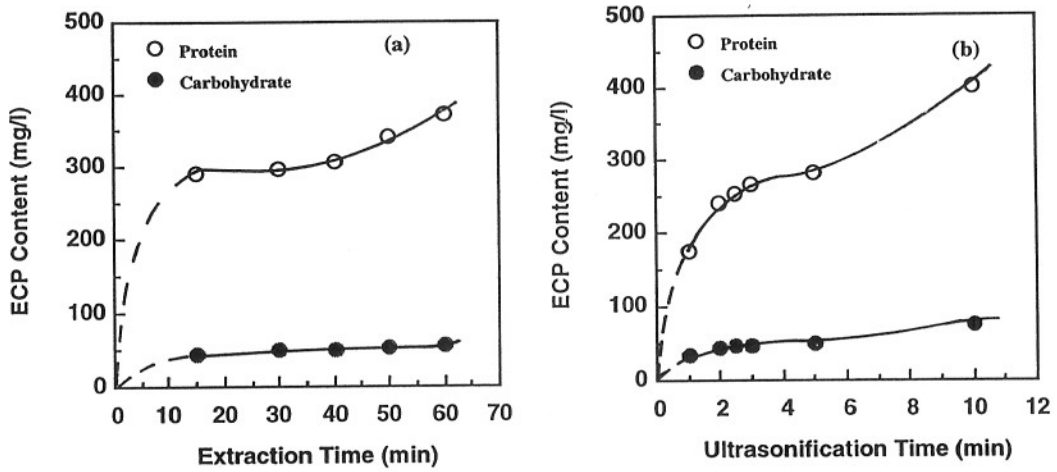

Fig 2. Extracted ECP_c and ECP_p by formaldehyde vs time: (a) at 80°C and (b) by ultrasonification

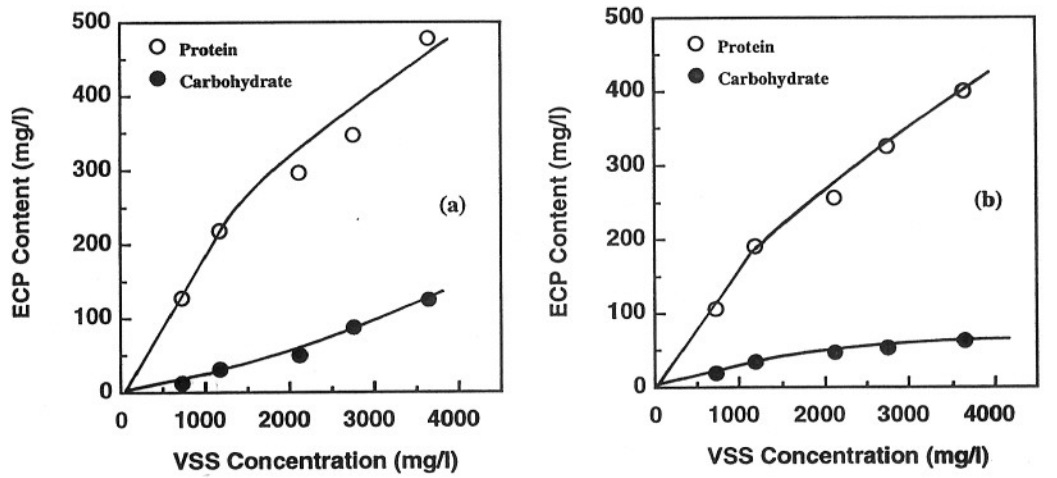

Fig 3. Extracted ECP_c and ECP_p by formaldehyde vs VSS: (a) at 80°C and (b) by ultrasonification

CONCLUSIONS

Results of this study show that amount of ECP extracted from anaerobic sludge is strictly

dependent upon the extractant and the extraction procedure. EDTA was more effective than formaldehyde as an extractant. Increase of temperature and the addition caustic also enhanced the extraction. Regardless of the extraction methods, the ratio of ECP_c/ECP_p for both acetate- and benzoate-degrading sludge was consistently 0.16-0.18. Under the same extraction condition, the amount of ECP extracted from the acetate-degrading sludge consistently amounted to 40-45% of those extracted from the benzoate-degrading sludge. This may likely be the reason that the latter sludge formed superior quality of granular sludge in UASB reactors.

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