Pergamon

Wat. Sci. Tech. Vol. 34, No. 5–6, pp. 309–316, 1996.
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0273–1223/96 $15·00 + 0·00

PII: S0273-1223(96)00660-9

SURFACE CHARGE AND EXTRACELLULAR POLYMER OF SLUDGE IN THE ANAEROBIC DEGRADATION PROCESS

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ABSTRACT

Changes of surface charge and extracellular polymer (ECP) content were investigated in batch experiments for three anaerobic sludges, each of which had been enriched at 35°C and pH 6.9-7.3 for more than 40 batches using propionate, butyrate and glucose, individually, as the sole substrate. Results showed that both ECP and the negative surface charge were dependent on the growth phase of microorganisms. They increased at the beginning of all batches when the microorganisms were in the prolific-growth phase, having high substrate concentration and food-to-microorganisms ratio. Both later gradually returned to their initial levels when the microorganisms were in the declined-growth phase, as the substrate became depleted. The negative surface charge increased linearly with the total-ECP content in all series with slopes of 0.0187, 0.0212 and 0.0157 meq/mg-total-ECP for sludge degrading propionate, butyrate and glucose, respectively. The change of surface charge for the first two sludges was mainly due to the increase of proteinaceous fraction of ECP; but, for glucose-degrading sludge, that could be due to the increases of both proteinaceous and carbohydrate fractions of ECP. The negative-charged nature of anaerobic sludge implies that cations should be able to promote granulation of anaerobic sludge. Copyright © 1996 IAWQ. Published by Elsevier Science Ltd.

KEYWORDS

Anaerobic; carbohydrate; extracellular polymer; protein; sludge granulation; surface charge.

INTRODUCTION

In conventional biological wastewater treatment processes, bacteria tend to agglutinate forming flocs and films. As a consequence, reactors are able to retain an increased amount of biomass and lower the tendency of bacteria washout. Although the precise mechanism for such bioflocculation processes has not been fully understood, the extracellular polymers (ECP) produced by the bacteria are believed to play a critical role for the flocculent activated sludge (Tenney and Stumm, 1968; Busch and Stumm, 1968; Ryssov-Nielson, 1975; Sutherland, 1985). Activated sludge having high levels of ECP content exhibited poor settleability (Magara et al., 1976). Furthermore, it is well known that surface charge is also critical to the coagulation of colloid in water and wastewater treatment (Weber, 1972). Flocs in activated sludge usually carry negative charge at

neutral pH, usually between -10 to -20 mV (Horan and Eccles, 1986). This is due to the ionization of the anionic functional groups, such as carboxylic and phosphate, on the sludge surface.

On the other hand, anaerobic technology has become mature in the past two decades for industrial wastewater treatment, due to the successful development of high-rate reactors. Among them, the anaerobic filter (Young and McCarty, 1969) and upflow anaerobic sludge blanket (UASB) reactor (Lettinga *et al.*, 1980; Fang and Chui, 1993) are most popular. Anaerobic bacteria agglutinate to form biofilm in the former reactor, and form biogranules with superb settleability in the latter reactor. Several recent studies (Ross, 1984; Harada *et al.*, 1988; Jia *et al.*, 1991) showed that ECP influences the formation of UASB granules. Morgan et al. (1990) found that each gram of suspended solids in UASB granules contained less ECP (10-20 mg) than those in activated sludge (70-90 mg); furthermore, the former carried less negative charge than the latter.

This study was conducted to investigate the changes of ECP and surface charge of different types of anaerobic sludges during substrate degradation in batch reactors. It was hoped that results of this study would lead to a better understanding on the nature of the anaerobic sludge and the formation mechanisms of biofilm and biogranule.

MATERIALS AND METHODS

Three types of anaerobic sludge were first enriched using propionate, butyrate and glucose, individually, as the sole substrate (Jia et al., submitted). Over forty batches of enrichment were carried out until reaching steady state condition. Experiments were then conducted in duplicate in 135 ml glass serum vials, also using the same individual substrates: propionate (Runs 1 and 1a), butyrate (Runs 2 and 2a) and glucose (Run 3 and 3a). About 100 ml of individual enriched sludges were added to the vials, respectively containing 99 mg of VSS (volatile suspended solids) for the propionate-degrading sludge, 90 mg for the butyrate-degrading sludge, and 145 mg for glucose-degrading sludge. Five ml of feed solution was then added to each vial. Each solution contained individual substrate equivalent to 50 mg of COD (chemical oxygen demand), plus nutrients and trace metals, including K_2HPO_4 10000 mg/l, $NiCl_2 \cdot 6H_2O$ 500 mg/l, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ 100 mg/l, $CuCl_2 \cdot 2H_2O$ 20 mg/l, $MgCl_2$ 1000 mg/l, H_3BO_3 100 mg/l, KI 100 mg/l, $CoCl_2 \cdot 6H_2O$ 4000 mg/l, $FeCl_2 \cdot 2H_2O$ 4000 mg/l, $AlCl_3 \cdot 6H_2O$ 100 mg/l, $MnCl_2$ 500 mg/l, $ZnCl_2$ 100 mg/l, EDTA 100 mg/l, and concentrated HCl 1 ml/l. The pH of the feed solutions and the mixed liquors were kept at pH 6.9-7.3.

After adding the enriched sludge and feed solution, each vial was capped with butyl rubber stopper and the vapor phase was flushed with inert nitrogen. The vial was then kept at a constant temperature of 35°C in a water bath sitting on a reciprocal shaker table (35mm x 125 strokes/min) for gentle mixing. At several time intervals, the volume of biogas produced was monitored using a syringe and its methane content was analyzed, while the mixed liquor was sampled for the analyses of residual substrate, proteinaceous ECP (ECP_P) and carbohydrate ECP (ECP_C), as well as surface charge. The methane content in the biogas was analyzed by a gas chromatograph equipped with a thermal conductivity detector (Shimadzu GC-8APT) using nitrogen as the carrier gas. The reaction was terminated after 36 hours when the methane production was levelling off.

The substrate content in the mixed liquor was analyzed by another gas chromatograph equipped with a flame ionization detector (Shimadzu GC-8APF) using argon as the carrier gas. ECP were extracted from the sludge samples using the cold aqueous extraction techniques (Sutherland and Wilkinson, 1971; Jia *et al.*, 1991). Each sludge sample was washed twice with de-ionized water followed by low speed (3500 rpm) centrifugation. The centrifuged biomass was then re-suspended in a 5 ml 8.5% sodium chloride solution containing 0.22% formaldehyde. The solution was subsequently chilled in ice and mixed using an ultrasonic homogenizer at 40 watts for 3 minutes, during which the ECP of bacteria were extracted into the solution. After removing the residual solids by high speed centrifugation (12000 rpm for 30 minutes), the ECP_C in the extracted solution was measured using the phenol/sulfuric-acid method and the ECP_P using the folin method (Lowry et al., 1951). The sum of ECP_P and ECP_C represents the total ECP of the sludge.

Sludge surface charge was measured by the colloid titration technique (Tiravanti et al., 1985 and Morgan et al., 1990). Polybrene and polyvinyl sulfate kalium (PVSK) were used as the standard cationic and anionic colloids, respectively. A given volume of the mixed liquor sample was diluted by de-ionized water and mixed with excess 0.002N polybrene before being titrated against 0.001N PVSK using toluidine blue as the indicator. Titration was terminated when electrical neutrality was reached, as indicated by the change of color from blue to pink. An equal volume of polybrene in distilled water was used as the blank.

RESULTS AND DISCUSSION

Changes of ECP and surface charge during substrates degradation

Figure 1 illustrates (1a) percent of substrate remained, (1b) changes of ECP_P and ECP_C content, and (1c) sludge surface charge for the batch experiments using propionate as the sole substrate. The corresponding plots for those using butyrate and glucose as the sole substrate were respectively illustrated in Figures 2 and 3. Figure 1b illustrates that, in the batch degradation of propionate, the ECP_P content increased gradually from the initial 16 mg/g-VSS to 20 mg/g-VSS after 26 hours, at which time the mixed liquor still had about 60% of unreacted substrate as illustrated in Figure 1a. As more substrate was further depleted, the ECP_P content was reduced to its initial level. On the other hand, the ECP_C content in the propionate-degrading sludge was nearly unchanged at the 5 mg/g-VSS level, which was significantly lower than the ECP_P content. Figure 1c illustrates that the negative charge of the sludge surface increased slightly from the initial -0.75 meq/g-VSS to -0.80 meq/g-VSS after 26 hours, and, like the ECP_P content, returned to its initial level as substrate was depleted.

Figure 1 illustrates that the ECP content of propionate-degrading sludge and its surface charge were dependent on the concentration of available substrate, which in turn affects the food-to- microorganisms (F/M) ratio and the growth phase of the bacteria. At the beginning of the batch experiment when the substrate concentration and, thus, the F/M ratio were high, bacteria were presumably at the prolific-growth phase. At this phase, both the ECP content and the negative surface charge tended to increase as substrate was degraded. But they gradually returned to their initial levels as substrate became depleted later, during which the F/M ratio was lower and bacteria were in declined-growth phase. Similar trends were also observed for the degradation of butyrate and glucose, as illustrated in Figs 2 and 3, except that the changes of ECP_C content and surface charge were even more prominent.

ECP are biopolymers produced by microorganisms during anabolism and metabolism (Morgan et al., 1990). With plentiful substrate available, microorganisms tend to produce more ECP because of increased anabolic activity, as observed not only in pure culture but also in activated sludge (Magara et al., 1976; Gulas et al., 1979; Kurane et al., 1986a, b; Characklis and Marshall 1990). The phenomenon was also confirmed in this study. The initial increase of ECP in all batches could be attributed to the prolific-growth of bacteria, because of high substrate concentration and high F/M ratio.

However, as the substrate became depleted, some ECP were metabolized by bacteria for energy and/or carbon, and thus the ECP concentrations were reduced to their initial levels, as illustrated. This is consistent to some reported observations in activated sludge and in anaerobic culture. Magara et al. (1976) and Gulas et al. (1979) found that the ECP content was lower in activated sludge operating at low F/M ratios. On the other hand, Ryssov-Nielson (1975) found that, under anaerobic conditions, biopolymers may be readily degraded by bacteria, forming CO_2 and CH_4 as byproducts.

Activated sludge tends to form better flocs when bacteria are in the declined-growth phase, i.e. at low F/M ratios (Busch and Stumm, 1968). Magara et al. (1976) investigated the relationships between the F/M ratio and the physicochemical properties, such as sludge volume index (SVI) and sludge electrophoretic mobility. They found that at low F/M ratios, sludge tended to have lower SVI and zeta potential, due to the reduced negative surface charge. Similarly, Figures 1c, 2c and 3c also illustrate that, for all three sludges, the

negative charge was reduced as substrate concentration and, thus, F/M ratio became lower as degradation proceeded toward completion.
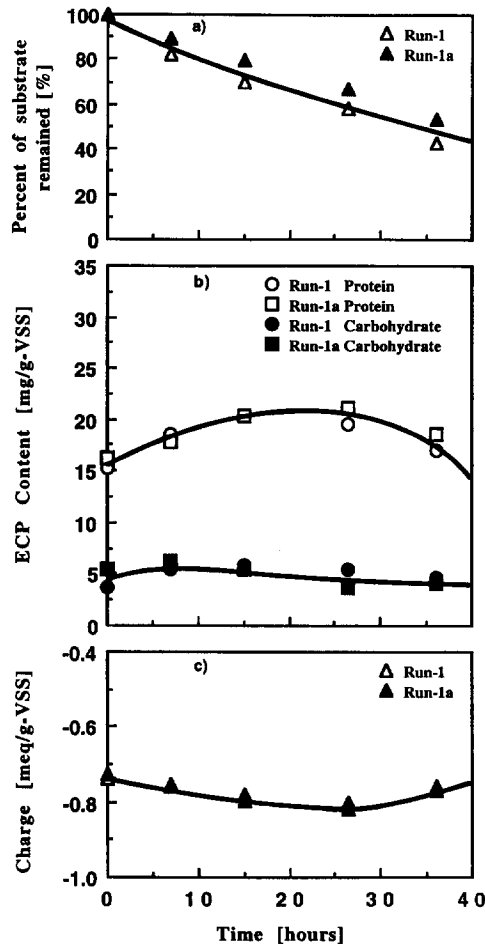

Figure 1. (a) percent of substrate remained, (b) ECP_P and ECP_C contents, and (c) surface charge during batch degradation of propionate.

Relationship between sludge surface charge and ECP

Since ECP are biopolymers accumulated on the surface of microorganisms, it is most likely that the surface charge is due to the ECP's functional groups, which carry either positive or negative charge depending on the nature of the groups and the pH (Marshall, 1967). At neutral pH, functional groups such as carboxylic and phosphate carry negative charge, while those like amino groups carry positive charge. Therefore, surface charge of sludge is strongly dependent on the ECP's chemical composition and concentration, as reported by Magara *et al.* (1976) for the activated sludge.
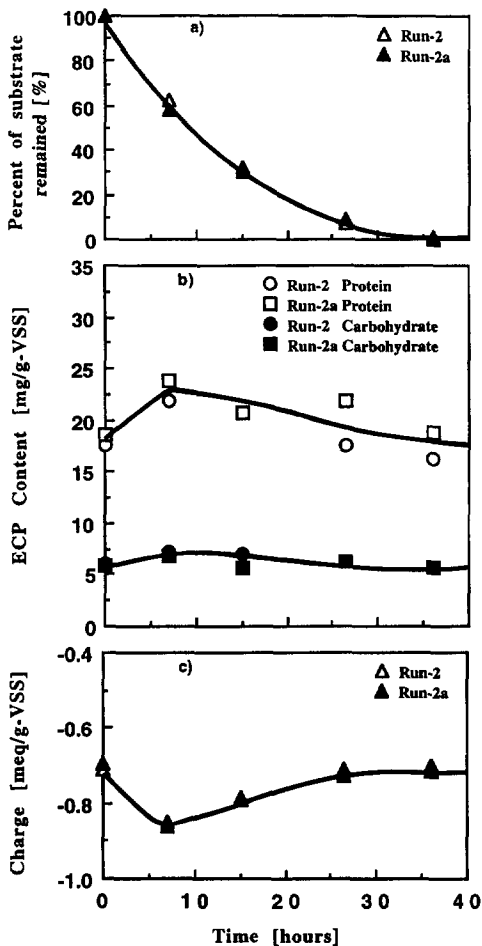
Figure 2. (a) percent of substrate remained, (b) ECP$_P$ and ECP$_C$ contents, and (c) surface charge during batch degradation of butyrate.
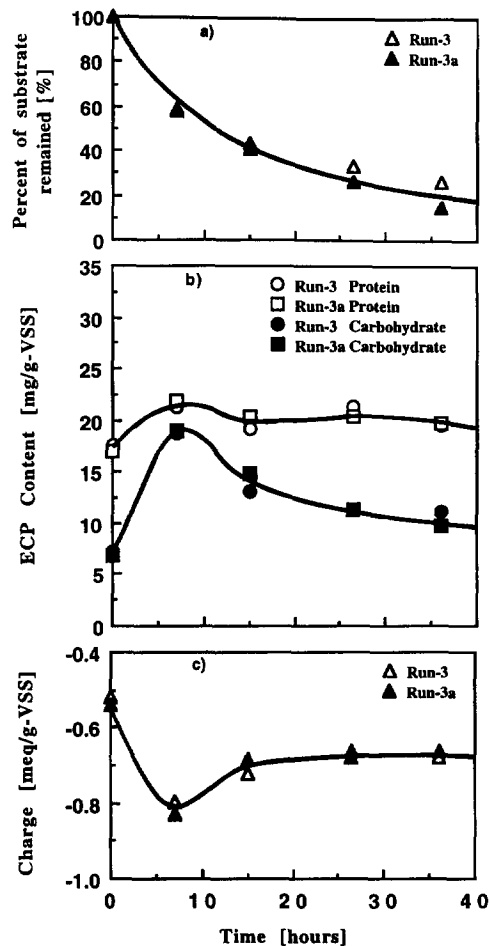
Figure 3. (a) percent of substrate remained, (b) ECP$_P$ and ECP$_C$ contents, and (c) surface charge during batch degradation of glucose.

Figure 4a and b illustrate that for propionate- and butyrate-degrading anaerobic sludges the negative surface charge increased linearly with the ECP$_P$ with a slope of 0.0182 meq/mg-ECP$_P$ for the former and 0.0256 meq/mg-ECP$_P$ for the latter. This suggests that ECP$_P$ of both sludge had an isoelectric pH below 6.9-7.3, like most globular proteins. On the other hand, Dolfing et al. (1985) and Dubourgier et al. (1988) found that the ECP$_C$ of anaerobic granules contained uronic acid, $CHO(CHOH)_nCOOH$, a principle ionogenic component of polysaccharides. ECP$_C$ is, therefore, likely to carry negative charge at neutral pH because of the carboxylic group in uronic acid. However, the effect of ECP$_C$ was not noticeable for these two sludges because the ECP$_C$ concentrations remained almost unchanged throughout the degradation. For the glucose-degrading sludge, the increase of negative charge could be linearly related to not only the ECP$_P$ but also ECP$_C$, as illustrated in Figure 4c.

Nevertheless, Fig. 5 illustrates that for all three sludges, the negative surface charge increased linearly with the total ECP content, regardless of the individual contents of ECP$_P$ and ECP$_C$. The slopes were 0.0187,

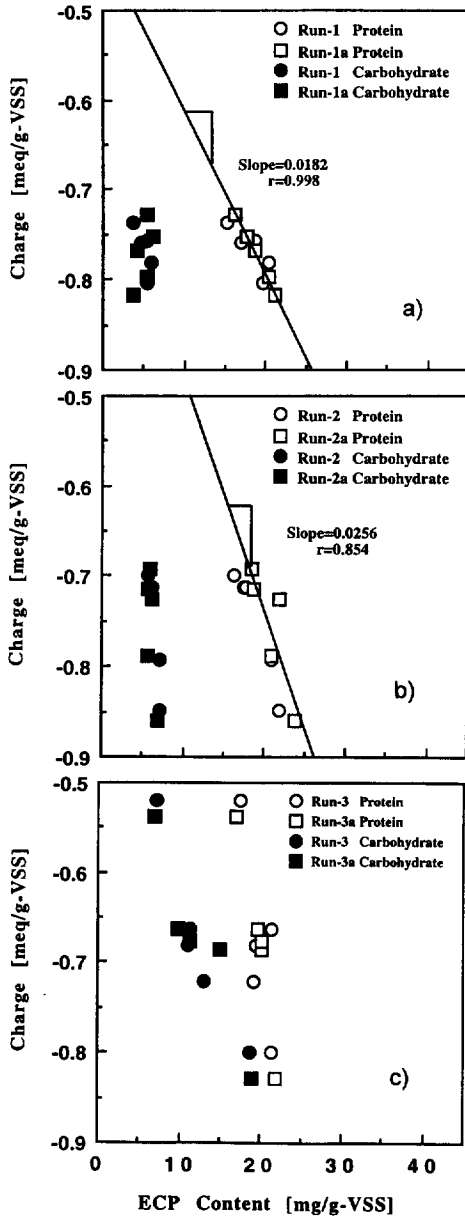0.0212 and 0.0157 meq/mg-total-ECP, respectively, for propionate-, butyrate- and glucose-degrading sludges.

Figure 4. Surface charge at various levels of ECP_P and ECP_C for sludges degrading:
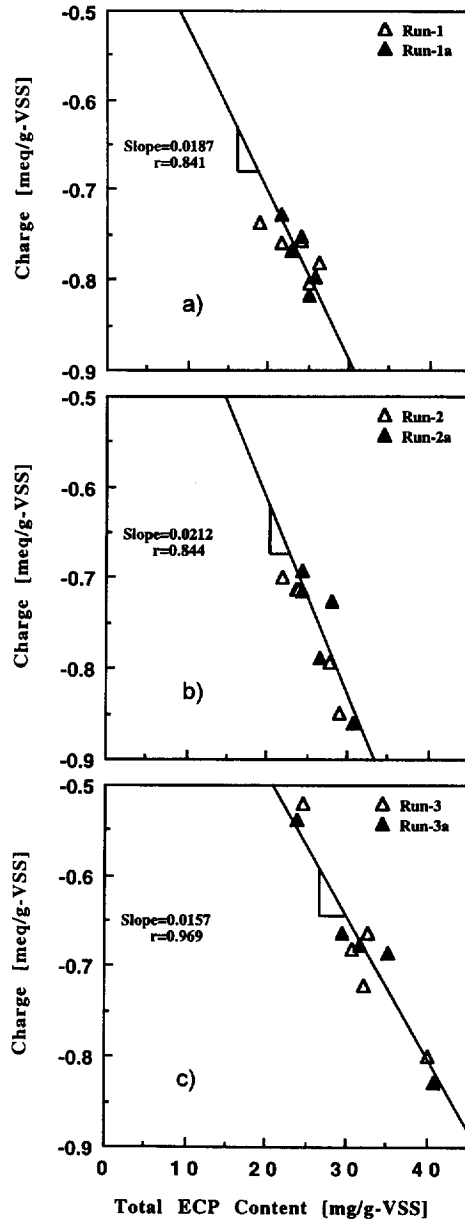(a) propionate, (b) butyrate, and (c) glucose.

Figure 5. Surface charge at various levels of total-ECP for sludges degrading:
(a) propionate, (b) butyrate, and (c) glucose.

Effect of cations

Many reported that addition of divalent cations, such as calcium, could enhance the flocculation of activated sludge (Kurane *et al.*, 1986a,b; Kakii *et al.*, 1986). Others reported that addition of cations, in particular

calcium, also promoted the granulation of anaerobic sludge in UASB reactors (Lettinga *et al.*, 1980; Hulshoff Pol *et al.*, 1983; Mahoney *et al.*, 1987; Harada *et al.*, 1987; Jia *et al.*, 1990). Tachibana *et al.* (1990) used ECP extracted from anaerobic granules as the coagulant for the precipitation of kaoline and dispersed sludge. They found that coagulation was enhanced by the addition of calcium, probably due to its capability to bridge the negative-charged ECP of adjacent cells. The negative-charged nature of the ECP of anaerobic sludge was confirmed in this study. This implies that the addition of cations would likely enhance the granulation of anaerobic sludge in UASB reactors. Furthermore research on this area is warranted.

CONCLUSIONS

Results of this study showed that both ECP and the negative surface charge of anaerobic sludge were dependent on the growth phase of the microorganisms. They increased at the beginning of all batches when the microorganisms were in the prolific-growth phase having high substrate concentration and F/M ratio. Both later gradually returned to their initial levels when the microorganism were in the declined-growth phase, as the substrate became depleted. The surface charge increased linearly with the total-ECP content in all series with slopes of 0.0187, 0.0212 and 0.0157 meq/mg-total-ECP for sludge degrading propionate, butyrate and glucose, respectively. The change of surface charge for the first two sludges was mainly due to the increase of proteinaceous fraction of ECP; but, for glucose-degrading sludge, that could be due to the increases of both proteinaceous and carbohydrate fractions of ECP. The negative-charged nature of anaerobic sludge implies that cations should be able to promote granulation of anaerobic sludge.

ACKNOWLEDGMENT

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