Effect of Carbohydrates to Protein Ratio in EPS on Sludge Settling Characteristics

Hang-Sik Shin*, Seok-Tae Kang, and Se-Yong Nam

Environmental Technology Research Laboratory, Department of Civil Engineering, KAIST, 373-1 Kusong-dong, Yusong-gu, Taejon 305-701, Korea

Abstract Extracellular polymeric substances (EPS) are believed to play a role in the binding and formation of microbial flocs. However, the precise role is not well known. Sludge settling characteristics and the carbohydrate to protein ratio in EPS were tested with various airflow rates in this study. Sludge was collected from three modified sequencing batch reactors (SBRs), which were operated at 16°C with an airflow rate of 0.8 L/min, 3 L/min and 6 L/min, respectively. During the operation, the reactor operated at an airflow rate of 0.8 L/min showed sludge volume index (SVI) of 80 to 90 mL/g and a constant ratio of carbohydrate to protein in the EPS, while a significant increase in the SVI was seen in the other reactors. Sludge bulking increased the amount of carbohydrate in the EPS, while kept protein almost constant in the airflow rate of 3 L/min and 6 L/min. Surface charge also increased with increases in the carbohydrate to protein ratio in the EPS, which weakens the attraction between the EPS and multivalent cations. The ratio of carbohydrate to protein in the EPS was inferred to be essential for bioflocculation.

Keywords: extracellular polymeric substance, SVI, sludge bulking, surface charge, sequencing batch reactors

INTRODUCTION

The settling characteristics of microorganisms, defined in terms of the sludge volume index (SVI), depend upon various conditions. Poorly settling sludges are said to be "bulked" and are caused by two main factors. One is the growth of filamentous organisms such as *Sphaerotilus natans* or organisms that can grow in a filamentous form under adverse conditions. The other is due to bound water, in which the bacterial cells composing the floc swell by absorbing water to the extent that their density is reduced and they will not settle [1-4]. Jenkins [4] reported that non-filamentous, or viscous bulking, is most probably related to the morphological characteristics of the floc and the presence of large amount of extracellular slime.

Several hypotheses have been suggested to explain flocculation. They show broad agreement on certain points *i.e.* that the chemical nature of the sludges surface influences the magnitude of the surface charge, which in turn affects the settlement properties of sludge [5-8]. A number of floc characteristics could be expected to exert some direct or indirect influence on sludge settling process, extracellular polymeric substances (EPS) have long been considered as playing an essential role in bioflocculation [3,9,10].

EPS are defined as any polysaccharide or peptidoglycan structure of bacterial origin lying outside the cell membrane [11]. EPS are believed to mediate the linkage in biofilms, flocs and granules [12]. However in the membrane bioreactor (MBR), EPS can easily attach to the membrane surface because of its negative charge, and it is a major source of cake resistance during membrane bioreactor operations [13]. The quantity of EPS was found to be essential for solid floc formation under both aerobic and anaerobic conditions, and many researchers have reported a higher protein content in anaerobic granular sludge [7,11,12].

Cell surface charge may also play a role in the hydrophobicity of the bacteria. Cells with a largely negative surface charge will appear more hydrophobic since they can easily associate to positively charged inorganic particles such as Ca^{2+}, Mg^{2+} [9].

There is some evidence that the composition and properties (*e.g.* hydrophobicity and surface charge) of the EPS is more important in sludge settling than the amount of EPS produced [3]. However, the precise role of EPS in relation to sludge is unknown.

In this study, SBR reactors were operated with various cycles and airflow rates to examine the relationship between physico-chemical properties of the EPS and the sludge settling characteristics.

*Corresponding author
Tel: +82-42-869-3613 FAX: +82-42-869-3610
e-mail: hangshin@kaist.ac.kr

MATERIALS AND METHODS

Reactor Operations

Microbial flocs were obtained from bench-scale modified sequencing batch reactors (SBRs). Three SBRs, which were acryl reactors with cylindrical volume of 4.5 L, were fed with synthetic wastewater. The influent contained 140 mg/L (150 mg/L as COD) glucose, 50 mg/L NH_4Cl, 10 mg/L KH_2PO_4 (COD: N:P ratio of 100:10:2), 86 mg/L $NaHCO_3$ (100 mg/L as $CaCO_3$), and other trace metals; 10 mg/L $CaCl_2$, 50 mg/L $MgSO_4 \cdot 7H_2O$, 0.038 mg/L $MnSO_4$, 0.035 mg/L $ZnSO_4$, 0.375 mg/L $FeCl_2$, 50 mg/L yeast extract. The experiment was performed in two stages using two airflow rate. During the first 4 weeks, three reactors were operated under the same conditions; a MLSS of 2,500 mg/L, SRT of 10 days, pH between 6.7 and 7.3, a temperature of 20°C and an air flow rate of 0.8 L/min. The SBR cycle consisted of a 20 min fill, 20 min mixing, 90 min reaction, 65 min mixing, 30 min settle and 15 min withdraw/idle period for a total cycle time of 4 h. In the second stage, the cycle was changed to 20 min fill, 20 min mixing, 155 min react, 30 min settle and 15 min withdraw/idle period, and each reactor was operated with different airflow rates; 0.8 L/min (SBR 1), 3 L/min (SBR 2) and 6 L/min (SBR 3), respectively.

Physicochemical Properties of Microbial Floc

Sludge volume indices (SVI) were determined in accordance with 2710 D of Standard Method [14] using the SBR sludge in the settling phase. Microscopic examinations were conducted using a Zeiss MC 100 florescence microscope.

The charge of the sludge and the EPS was measured using the colloid titration technique described by Morgan *et al.* [7]. Hexadimethrine bromide (polybrene) and polyvinyl sulfate (PVSK) were used as standard cationic and anionic colloids. Toluidine was used to indicate the endpoint where a subtle color change from blue to pink/purple occurred. An equal volume of polybrene in a distilled water was used as blank. The colloid charge was calculated using *Eqn* (1).

$$\text{Charge (meq/L)} = \frac{(A-C)\times B\times 100}{V} \quad (1)$$

where

A = volume of PVSK added to the sample, mL
B = normality of PVSK, eq/L
C = volume of PVSK added to blank, mL
V = volume of sample used, mL

Extraction and Analysis of EPS

EPS in the mixed liquor was extracted using the steaming extraction method [15]. Only carbohydrate and protein fractions in the EPS extract were analyzed although other macromolecules such as phospholipids, DNA, RNA *etc.* may be found in the extracellular organic matrix of the floc. The total carbohydrates in the EPS were determined using the colorimetric method described by Dubois *et al.* [16], with a Beckman UV-Visible spectrophotometer (USA) at 480, 484 and 490 nm. Glucose was used as a standard in the range 0-50 mg/L. Protein was measured by the method of Smith *et al.* [17] with bovine serum albumin (BSA) as the standard and using a wave length of 562 nm.

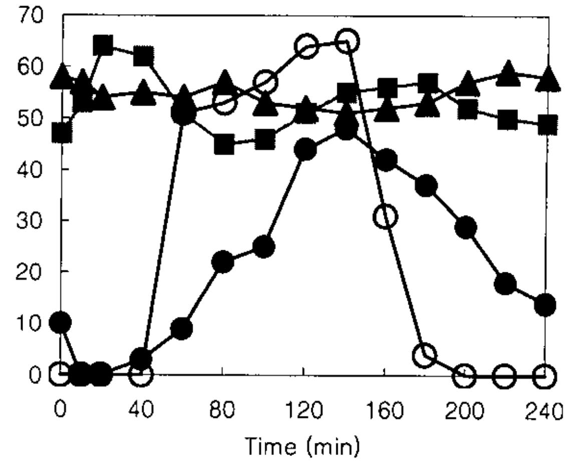

Fig. 1. Variations of EPS and nitrate concentration: O DO (×10), ■ EPS-carbohydrate (mg/g VSS), ▲ EPS-protein (mg/g VSS), ● nitrate (mg/L).

RESULTS AND DISCUSSION

Performance of Reactors

COD conversion in all reactors was very high and stable during the first stage. COD conversion from SBR 1 to SBR 3 was greater than 95% when the airflow rate was 0.8 L/min. SVI was between 80 and 90 mL/g in all reactors.

SVI and Total EPS in One Cycle

The concentrations of nitrate, dissolved oxygen (DO) and EPS at the end of the first stage are presented in Fig. 1. In one cycle, it was apparent that the EPS changed according to changes in the oxygen concentrations. Oxygen limitation generally caused decrease in the carbohydrate concentration in the EPS, especially during the settling phase in Fig. 1. It was reported that bioflocculation did not occur until the microorganisms have entered into an endogenous growth phase [18]. In the endogenous phase, the amount of proteic EPS increased while that of carbohydrate decreased. As the result, carbohydrate to protein ratios were decreased as shown in Fig. 2. This indicates that the negative charge of the cell surface increases since the protein in the EPS is the main source of negative charge on the cells surface [8].

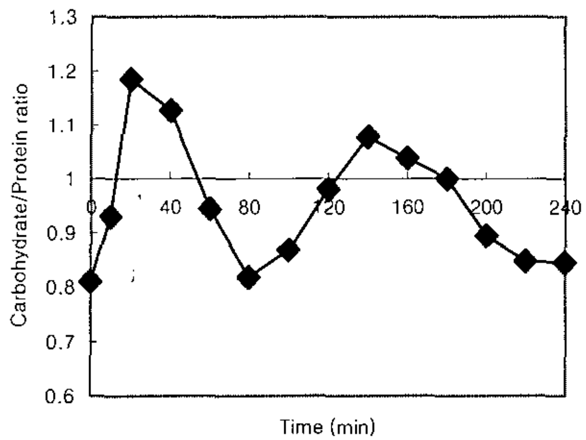

Fig. 2. Variations of carbohydrate to protein ratio of EPS.

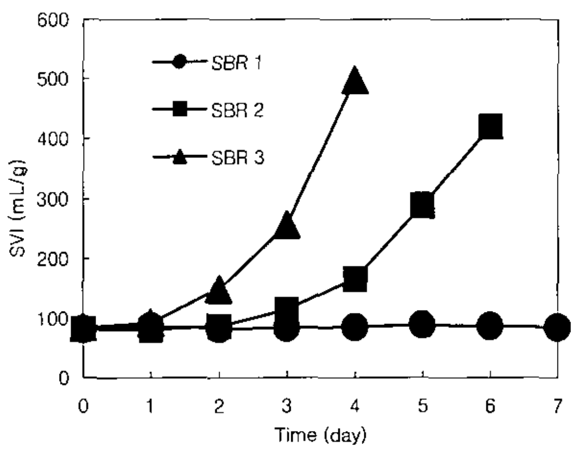

Fig. 3. Sludge settling characteristics (SVI) at various airflow rates.

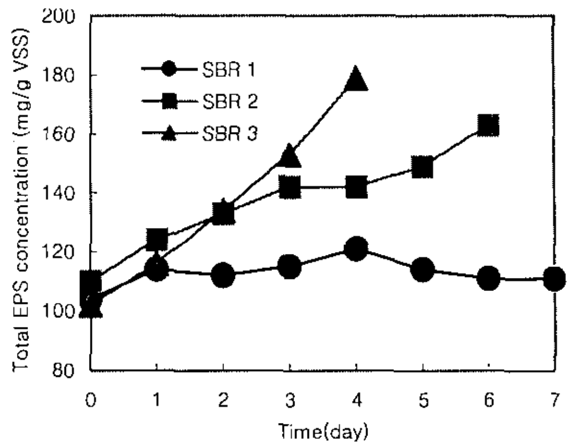

Fig. 4. Changes of total EPS concentration at various airflow rates.

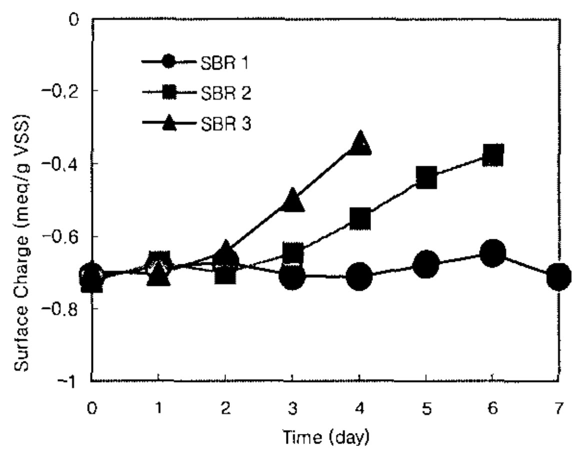

Fig. 5. Changes of surface charge at various airflow rates.

SVI and Total EPS with Various Airflow Rate

During the second stage, in which the three bench-scale SBRs were examined with different aeration rates, the settling characteristics and EPS of the sludge were examined. The SVI in the three reactors is dramatically changed as shown in Fig. 3. Sludge produced with an aeration rate of 0.8 L/min (SBR 1) had good settling characteristics as indicated by an SVI of 100 mL/g. The sludges in the reactor with aeration rates of 3 L/min (SBR 2) and at 6 L/min (SBR 3) settled poorly and were washed out. In SBR 2 and SBR 3, the SVI values were increased to 500 mL/g while microscopic examination of sludge samples showed no significant numbers of filamentous microorganisms. This indicates that the high SVI values reflect non-filamentous bulking or viscous bulking by the absorbed water in the flocs.

In the Fig. 4, the quantity of EPS in SBR 2 and SBR 3 dramatically increased from 100 mg/g VSS to 163 mg/ g VSS and 179 mg/g VSS, respectively, during the second stage. However settling of the sludge was unfavorable as shown in Fig. 3. This demonstrates that the SVI is related to the amount of EPS, which is contradiction to the previous researches showing that large amounts of EPS is favorable for bioflocculation because biopolymers played a central role in absorbing and bridging of the cells [6]. It indicates that the floc characteristics and the settling problems are more complex than previously thought and other factors (*e.g.* composition and properties) of the EPS are more important in sludge settling than the amount of EPS produced .

Surface Properties of the Microbial Floc

Andreadakis [3] reported that the composition and properties (*e.g.* hydrophobicity and surface charge) of the EPS are more important in sludge settling than the amount of EPS produced. For this reason, the charge on the cell surface was examined by a colloid titration technique using PVSK and polybrene. The change in the measured surface charge was found to be more pronounced when the sludge bulking was seen (Fig. 5). Surface charges of SBR 2 and SBR 3 were increased from −0.7 to −0.3 while that of SBR1 was constant. Increased

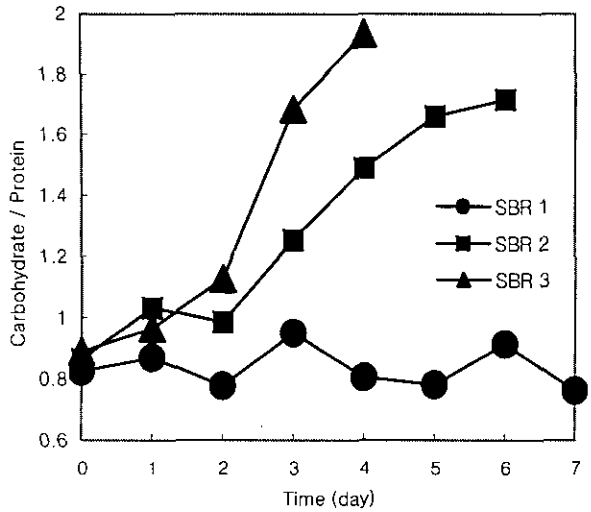

Fig. 6. Changes of carbohydrate to protein ratio in the EPS.

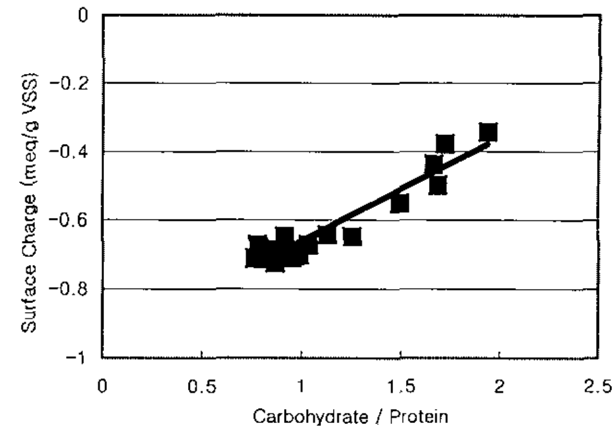

Fig. 7. Relationship between carbohydrate to protein ratio in the EPS; (Y = 0.3021X − 039623, R^2 = 0.90).

surface charge may weaken the bridging force between divalent cations such as Ca^{2+} and microbial flocs, and finally, induces absorbing more waters by weak hydrogen bonding.

It indicates that the surface charge of the cell is another important factor in viscous bulking. Urbain *et al.* [9] proposed that the overall floc structure is negatively charged and is the result of physico-chemical interactions between microorganisms (mainly bacteria), inorganic particles (silicates, calcium phosphate and iron oxides), EPS and multivalent cations. Since bacterial surfaces and EPS provide negatively charged adsorption sites for divalent cations such as Ca^{2+} and Mg^{2+}, a strong negative charge is more favorable for bacterial aggregation. Between the extracellular organic constituents of the flocs, divalent cations may act as bridging agents. As shown in Fig. 3 and Fig. 5, the strong negative charge on the cell surface was favorable for settling which was represented by the low SVI values. Urbain *et al.* [9] also showed that a weak negative surface charge may not be inconsistent with good settling conditions.

The important factor determining the charge of the cell surface is the ratio of carbohydrates to protein in the EPS. In the Fig. 5 and Fig. 6, the negative surface charge was decreased as the ratio of carbohydrate to protein in the EPS increased. It indicated that the surface charge of cells was affected by the composition of EPS. The amount of carbohydrates in both SBR 2 and SBR 3 was significantly greater than that in SBR 1, while the amount of protein was almost constant in all the reactors. This resulted in an increase in the ratio of carbohydrates to protein which inhibited floc formation by increasing cell surface charge or decreasing hydrophobicity of the cells. The cell surface charge was shown to be related with the carbohydrate to protein ratio in the EPS (Fig. 7).

As well, since the protein represents one of the nitrogenous constituent in the extracellular organic matrix in activated sludge, the COD/N:P ratio of the influent is the another factor that may affect sludge bulking. Bura *et al.* [10] reported that the COD:N:P ratio influenced the hydrophobicity, surface charge, bound water and EPS composition of the microbial flocs. It means that addition of nitrous or phosphorus compounds can be the one of the controlling method for sludge bulking.

CONCLUSION

In this study, sludge settling characteristics at the various airflow rate were tested in three SBR reactors. During the SBR cycle, the amount of EPS changed according to the oxygen concentration. At the various airflow rates, SVI did not correlate with the amount of EPS when sludge settling became poor. The surface charge of cells was a closely related with bioflocculation indicating that the highly negative charge was favorable for the floc formation. The correlative approach to microbial floc studies revealed that the surface charge of the cell was positively related with carbohydrates to protein ratio in the EPS.

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