Korean J. Chem. Eng., **23**(5), 767-772 (2006)

Effect of clinoptilolite addition and solids retention time on the extracellular polymeric substances composition and soluble chemical oxygen demands in activated sludge

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(*Received 20 February 2006 • accepted 4 May 2006*)

Abstract−This study was carried out to compare EPS (Extracellular Polymeric Substances) composition between conventional activated sludge (AS) and activated sludge dosed with clinoptilolite (CAS). Additionally, those were compared with organic removal efficiency in the effluent in conjunction with EPS concentrations. The experiments were conducted at SRT (Solids Retention Time) ranging from 5 to 100 d. For the CAS, proteins were more readily observed for SRT 20 and 100 d compared to that of the AS. Polysaccharide concentration in the sludge was greatly increased for the CAS, but it was significantly diminished when the SRT was extended. The level of EPS concentration observed from the effluent had the same pattern of variation for the two different types of systems. Regardless of type of reactor, the ratio of proteins for sludge versus effluent was independent of SRT, but the ratio of polysaccharides diminished as SRT increased. In the long run, the degree of protein synthesis directly ascribed to concurrent enhancement of SCOD removal efficiency was slightly more in the CAS. It was decided that clinoptilolite added system could be more reliably retrofitted to a conventional activated sludge process.

Key words: Nutrient Removal, EPS, Activated Sludge, Clinoptilolite

INTRODUCTION

EPS is generally produced as by-product or final product when microbes are decomposing organic nutrients. It is largely comprised of protein, polysaccharides, DNA, lipids, and humic substances [Jahn and Nielsen, 1998]. Proteins and polysaccharides are the dominant compounds of EPS [Sponza, 2002].

Dudman [1977] and Boyd and Chakrabarty [1994] asserted that there was no biochemically utilized EPS as a carbon source, but Pirog et al. [1997] found that enzymes produced from specific microbes could completely degrade it, and isolated an *Acinetobacter* sp. from soil specimen, that would use EPS as a carbon source. During wet and dry cycle of microbial synthesis, Roberson and Firestone [1992] found that the amount of polysaccharides diminished upon wetting immediately after dry cycle since bacteria consumed polysaccharide while producing protein.

Among EPS, carbohydrate and protein could be readily decomposed [Zhang and Bishop, 2003]. They also addressed greater utilization rate of EPS in suspended bioflocs in activated sludge than in biofilms. EPS was directly correlated to [Urbain et al., 1993] or inversely proportion to SVI (sludge volume index) [Goodwin and Forster, 1985]. EPS may be attributable to releasing SCOD or SBOD to effluent [Kasapgil et al., 2000; Park et al., 2003a]. Boreo et al. [1996] demonstrated that unknown organic carbon present in the effluent largely originated from EPS constituents rather than the original influent.

Lately, a simple technique of upgrading conventional AS process has been introduced such that powdered minerals, e.g., zeolite, clay and talc, were added to the aeration tank with a view to improving microbial concentration and sludge settling property [Chudoba and Pannier, 1994; Lee et al., 2002; Park et al., 2003b]. Park et al. [2002] also reported that the employment of clinoptilolite powder as bio-carrier in AS process could augment nitrification and organic compounds removal efficiency with enhanced biomass concentration. In addition, Kim et al. [2003] and Yoon et al. [2004] suggested that greater cationic exchange property of clinoptilolite should comparably provide high concentration of ammonium available for microorganisms attached around the carriers as compared to those in solution; moreover, the ion exchange capacity could give attached microorganisms driving force to overcome mass transfer resistance through biofilm. However, there was no any investigation of the effects of clinoptilolite on EPS while it was being used as micromedia for attached microorganisms.

This study was conducted to investigate effect of SRT and clinoptilolite treatment on the characteristics of EPS. The property of EPS production was compared between the AS and the CAS. It will also provide a comparison of organic compounds removal efficiency between two processes in association with EPS contents.

MATERIALS AND METHODS

1. Activated Sludge

The lab scale experimental apparatus included two 8.2 L aeration basins and two 3.5 L sedimentation basins as shown in Fig. 1, where reactor A at the left was a CAS and reactor B at the right was an AS. The structural characteristics of clinoptilolite were obtained by X-ray diffraction (Philips X'pert MPD). A 4,000 mg/l of clinoptilolite in the aerator was maintained at different SRT. Table 1 shows the experimental conditions for three different experimental runs with SRT of 5, 20 and 100 d.

Excess amount of sludge was regularly withdrawn from the sedimentation basin and quantified. SRT was estimated ignoring amount of SS washout through the effluent. During three runs of experi-

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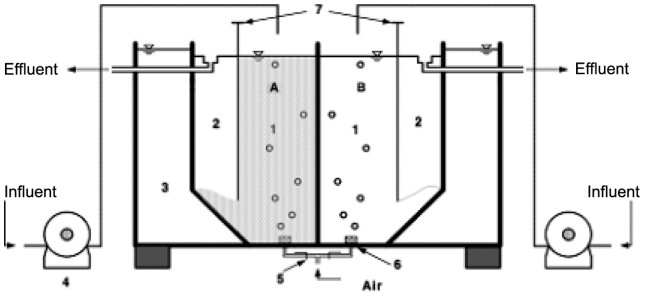

Fig. 1. Schematic diagram of activated sludge and clinoptilolite added sludge systems.

A. AS adding clinoptilolite
B. A conventional AS
1. Aeration basin (8.2 L×2)
2. Sedimentation basin (3.5 L×2)
3. Water jacket
4. Tubing pump
5. Air control valve
6. Air diffuser
7. Baffle

Table 1. Experimental conditions on three different runs

		Phase 1	Phase 2	Phase 3
Influent COD_{Cr} (mg/L)			250 to 265*	
HRT (hr)			8*	
SRT (hr)		5	20	100
DO (mg/L)			4.5 to 5.5*	
pH			7.0 to 7.5*	
Temperature (°C)			20±1*	
SVI (ml/g)	AS	234	91.3	128.3
	CAS	25	23.2	59.5
**VSS (mg/*l*)	AS	4.9	5.5	8.3
	CAS	3.4	5.1	7.3
***TSS (mg/*l*)	AS	5.1	5.7	8.6
	CAS	6.4	5.3	8.2
MLSS (mg/*l*)	AS	1055.8	2841.7	5398.7
	CAS	6092.5	7499.2	7661.7
MLVSS (mg/*l*)	AS	974.2	2375	4244.2
	CAS	1335.8	2872.5	3559.3

*The given condition was identically implied to all three phases.
**VSS: Volatile Suspended Solids.
***TSS: Total Suspended Solids.

ments, HRT (Hydraulic Retention Time) was unvaryingly fixed at 8 hr. Synthetic wastewater used in this study was prepared as given by Sponza [2002]. During experimental period, microbial variations were occasionally monitored by SEM (S-4300, Hitach) especially when sludge bulking was observed.

2. Preparation of Samples and Quantification of EPS Concentration

Polysaccharides present in the effluent and the sludge were separated by using RCF (Regular centrifugation with formaldehyde) extraction, whereas proteins were extracted using steaming extraction [Zhang et al., 1999]. For preparation of sample determining concentration of polysaccharides, 500 ml of effluent was taken, which was then filtered with 45 mm GF/C filter, which was further filtered with 0.45 μm membrane. Filtrate was eventually taken into a 30 ml glass tube, which was stored at 4 °C until quantification being initiated.

For determining concentration of polysaccharides in the sludge, a 20 ml sludge was taken into a 50 ml Falcon tube, which was then centrifuged at 4,000 rpm for 10 min. Supernatant was discarded and thickened sludge was re-suspended with 20 ml of 8.5% NaCl+0.22% HCHO in an aid of shaker (MS3000, Mtops, Korea) and vortexed (Vortex-2 Genie, Scientific Industries, USA) for 30 min, which was then centrifuged at 4,000 rpm for 10 min. Aqueous extract was temporarily stored at a 50 ml glass tube. In the following, thickened sludge was re-extracted with the separation procedures given above. From two consecutive extraction procedures, a total of 40 ml of aqueous extract was finally obtained, which was then filtered with 1 μm GF/C, and further filtered with 0.45 μm membrane, which was then stored at 4 °C until quantification procedures being initiated.

For quantification of protein concentration in the effluent, the extraction process was the same as given for determining polysaccharide concentration. To isolate protein from sludge in the aeration basin; however, 20 ml of sludge was taken into 50 ml Falcon tube, which was then centrifuged at 4,000 rpm for 10 min. The supernatant was discarded and thickened sludge was re-suspended with 20 ml MilliQ water, which was being shaken (MS3000, Mtops, Korea) and vortexed (Vortex-2 Genie, Scientific Industries, USA) for 30 min which was again centrifuged at 4,000 rpm for 10 min. Aqueous extract was temporarily stored at 50 ml of glass tube. Secondly, centrifuged sludge was again re-suspended with 20 ml MilliQ, which was being shaken (MS3000, Mtops, Korea) and vortexed (Vortex-2 Genie, Scientific Industries, USA) for 30 min. The sample in a 50 ml Falcon tube was autoclaved at 1 atm, 80 °C for 20 min, which was cooled down to room temperature and then centrifuged again at 4,000 rpm for 10 min. Previously obtained extract was tipped to the currently obtained supernatant; therein a total of 40 ml of extract was obtained, which was filtered with 1 μm GF/C and then again with 0.45 μm membrane filter. It was finally stored at 4 °C until quantification procedures being initiated.

Quantification was implemented within 20 hr after sample taken from the reactor to minimize potential interference resulting from storage and temperature variation. For the verification of this, the amount of polysaccharide and protein were compared before and after 20 hr of storage. Polysaccharides concentration was measured by the Dubois Method [Dubois et al., 1956], while protein concentration was quantified by the Hartee-Lowry method [Hartee, 1972] using UV-spectrometer (HP-agilent 8453). To avoid UV scattering due to residual clinoptilolite in the sample, the residual amount of clinoptilolite was centrifuged at 10,000 rcf (Eppendorf, Centrifuge 5415D). The extract obtained for determining protein concentration was verified by HPLC (P/N SD-APLC-PLUS, Lab Alliance).

Adsorption capacity of clinoptilolite for proteins was measured by adding 20 ml of sludge from the aerator into a clean 50 ml of Falcon tube and mixed a nominal amount of clinoptilolite for 1 hr with a shaker at 80 rpm (SI-300R, Lab. Companion). The sample was then centrifuged at 4,000 rpm for 10min following by supernatant was filtered with 0.2 μm membrane, which was consequently used for a final sample for the determination of adsorption property of proteins.

RESULTS AND DISCUSSION

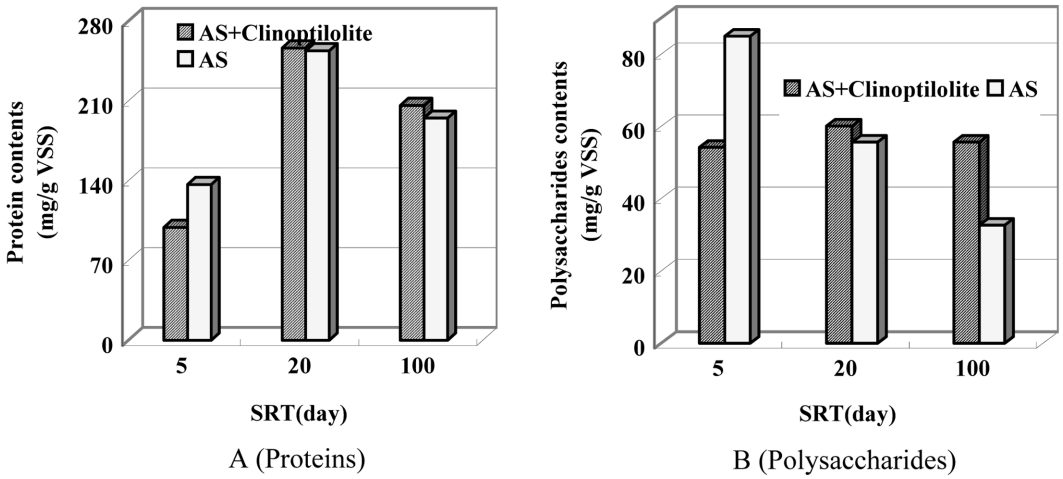

Fig. 2. Comparison of EPS in the sludge between AS and CAS systems according to SRT variation.

Powered clinoptilolite used in the experiment has 4 Å of interstitial spaces between layers (average of 19.75 μm diameter), which were represented by $M_xD_y[Al_{x+2y}Si_{n-(x+2y)}O_{2n}]_mH_2O$ (i.e., M: monovalent exchangeable alkaline metal, D: divalent exchangeable alkaline metal). Clinoptilolite has intrinsic cationic exchange capacity specific to NH_4^+, which was expected to adsorb amine mainly comprised of proteins [Lee et al., 1999; Kim et al., 2003]. Proteins from 40 ml activated sludge were observed at 302.39 mg/L, which was equivalent to 12.10 mg-proteins. Adsorbed amount of proteins on clinoptilolite was calculated at 13.52 mg-proteins/g-clinoptilolite. Cationic exchange capacity of clinoptilolite was 1.28 meq/g [Lee et al., 2002], which could adsorb the equivalent amount of either polysaccharides or protein. However, its adsorption property on clinoptilolite separately retrieved after completing EPS assay was not differing from that of the original clinoptilolite.

1. Comparison of EPS in the Sludge between CAS and AS According to SRT

Actual amount of proteins adsorbed onto clinoptilolite was estimated with regard to SRT as shown in Fig. 2A. At SRT 5 d, proteins from the AS was observed at 27.5% greater than that of CAS, but at SRT 20 d, its discrepancy has been diminished into 1%. At SRT 100 d, protein concentration for the CAS was 5.4% higher than that of the AS. At SRT 5 d, increased microbial death due to bulking presumably attributed to lysis of non-living bacteria and their excretion inducing to enhance release of EPS such as proteins, polysaccharides, and DNA, which in turn elevated the amount of proteins compared to that of the CAS, consistent with the findings addressed by Sponza [2002]. The bulking at SRT 5 d was ascribed to an incident temporarily occurred during sampling period of sludge from the aerator while a limited diversity of bulking microorganisms, *Sphaerotillus* and *Beggiatoa*, was occasionally found by SEM at a 9,000 magnification. It might consequently increase lysis of cells releasing escalated amount of proteins in the effluent, which led to attributing to the exceptional increase of the level of protein concentration for SRT 20 d compared to that of SRT 100 d.

Fig. 2B showed the difference of amount of polysaccharides between the CAS and AS with respect to SRT. At SRT 5 d, the polysaccharide concentration from the AS was 36.2% greater than that of the CAS due to bulking as previously described. For the AS, the polysaccharides concentration decreased from 85 to 32.68 mg/g-VSS when SRT was increased, whereas the concentration for the CAS did not vary. The linear decrease of polysaccharides content in the AS might lead to the decline of F/M ratio, which was further explained in Fig. 3.

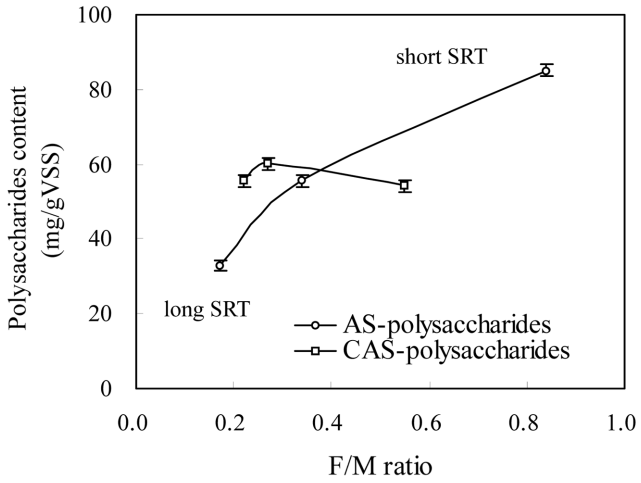

Fig. 3. Relationship between F/M ratio and polysaccharides concentration for the two different experimental conditions.

Fig. 3 presents relationship between F/M ratio and polysaccharides concentration for the two different experimental conditions. The error bars on each curve were the coefficient variance of triplicates for polysaccharides concentration. F/M ratio was simply estimated based on SRT in which organic loading was identical to the values in Table 1. For the duration of each SRT implied, F/M ratio correspondingly varied directly depending upon the changing environment either in CAS or in AS such that shown in Table 1, MLVSS was increased at the longer SRT. Fig. 3 shows that the decline of polysaccharides concentration in the AS led to the F/M ratio decreased. That is, F/M ratio decline at extended SRT caused starvation conditions where microbes may have consumed polysaccharides as carbon or energy sources. Other investigations have shown that polysaccharides stored as EPS could be utilized under a starvation environment [Hariss and Mitchell, 1972; Andreadakis, 1993].

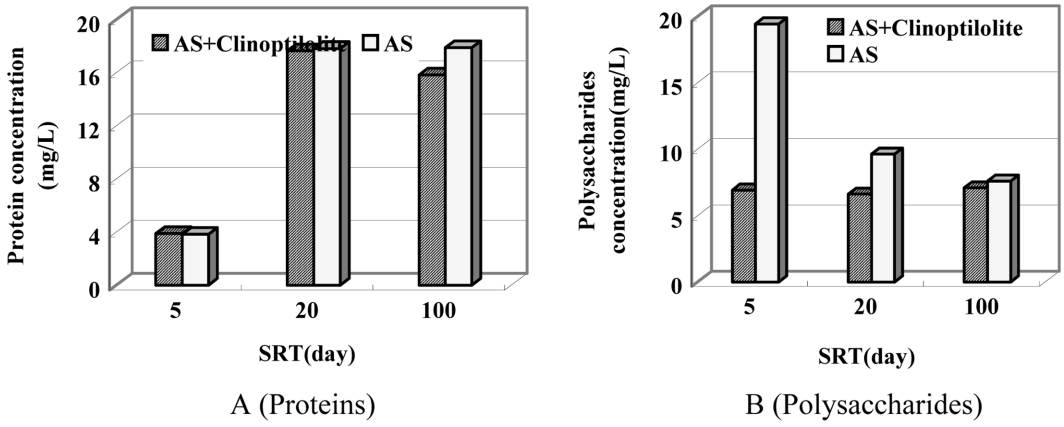

Fig. 4. Variations of effluent protein and polysaccharides concentration at different SRTs for the two different experimental systems.

In other words, polysaccharide concentration was linearly decreased as low as 32.7 mg/g-VSS because it was directly used for carbon or energy sources for microbial synthesis while F/M ratio was concurrently lowered from 0.34 to 0.17 F/M ratio. In comparison, levels of polysaccharides observed from the CAS was relatively independent of F/M ratio, which means that the greater extent of microbial concentration in the CAS could keep the balance of uptake of polysaccharides even though the nominal level of polysaccharides has been released. F/M ratio narrowly ranged from 0.22 to 0.55, so there was a relatively greater amount of substrate stably available than that of the AS.

2. Comparison of EPS in the Effluent between the CAS and the AS According to SRT

Fig. 4A shows proteins concentration in the effluent. At SRT 5 and 20 d, there was no difference of protein concentration in the effluent between the CAS and the AS. At SRT 100 d, the effluent from the AS showed 11.5% higher protein concentration than that of the CAS. Regardless of type of reactors, extent of proteins in the effluent has been increased by a factor of 4.6 as SRT was increased from 5 to 20 d; thereafter, there was no significant variation between SRT 20 and 100 d. It can be explained that as shown in Fig. 2A as increase as amount of proteins in the sludge ascribed to proportional amount of proteins flown out of the sedimentation basin.

Fig. 4B showed the polysaccharide concentration observed in the effluent of the two reactors upon changing SRT. Unlike the pattern of variation of the effluent protein concentration, effluent polysaccharides concentration from the CAS did not vary with SRT. In comparison, effluent polysaccharides concentration for the control varied with SRT. It was at the highest of 19.4 mg/L at SRT 5 d, which was then linearly diminished as extended as SRT showing 7.6 mg/L at SRT 100 d. It was well reflected from the pattern of its concentration variation previously observed for sludge upon SRT. At SRT 5 d, degree of EPS contents within sludge was increased due to bulking; thereby the level of EPS became increasingly washed out of sludge. However, upon increase of SRT the extent of polysaccharides dwindled since polysaccharides might have been utilized inducing decline of F/M ratio.

Fig. 5 showed ratio of EPS of sludge over EPS of effluent. The error bars on each curve were the coefficient variance of triplicates. At SRT 5 d the ratio of polysaccharides of sludge to effluent between two processes largely differed showing that the ratio for the AS exposed by a factor of 3 greater than that of the CAS. At SRT 100 d, the difference between them declined within ratio of 0.04. It indicated that polysaccharides could be rapidly utilized as a substrate compared to that of proteins as demonstrated by Zhang and Bishop [2003] addressing that microbes could more readily decompose polysaccharides rather than proteins. Adding clinoptilolite attributed to more decline of the ratio of EPS rather than that of the AS, which means that clinoptilolite was used as cationic adsorbent on protein and seed encapsulated with bio-film having enlarged specific surface area attracting polysaccharides around, so that its release from the bio-flocs should be minimized [Park et al., 2002].

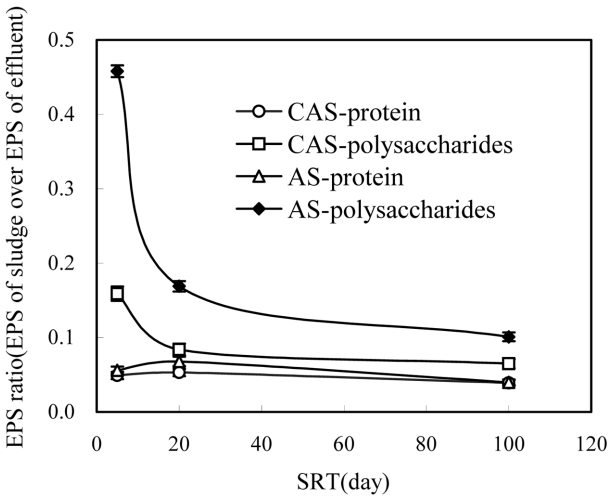

Fig. 5. Ratio of EPS of sludge over EPS of effluent for the two different systems.

Fig. 6 shows the relationship between EPS (e.g., polysaccharides, proteins) and SVI upon SRT in the AS. The error bars on each curve were the coefficient variance of triplicates. It will address how different EPS compositions have an effect on sludge settling property. Concentration of proteins has been inversely correlated with variation of SVI, but there was no relationship between polysaccharides content and SVI. It indicates that the formation of protein closely related to the settleability of sludge where the more protein, the more

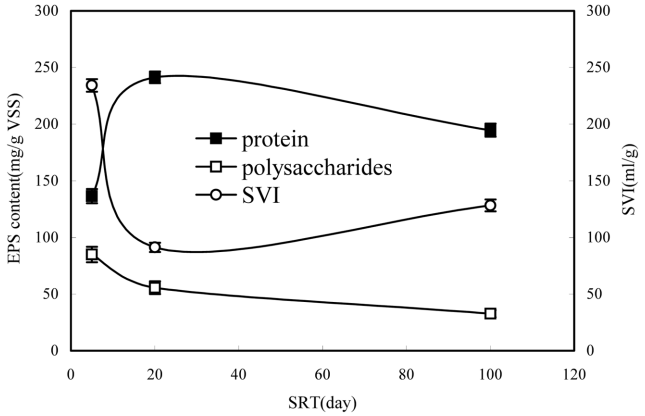

Fig. 6. Relationship between EPS and SVI upon SRT in the AS.

rapidly sludge forced to settle down. Sponza [2002] suggested that increase of proteins concentrations could be meaningfully used as a type of precursor indicating sludge settling property becoming improved.

Upon SRT 20 d, degree of proteins has been nevertheless increased as much as decline of polysaccharides concentration. It indicated that given microbial cultures could use polysaccharides to synthesize corresponded amount of proteins [Roberson and Firestone, 1992].

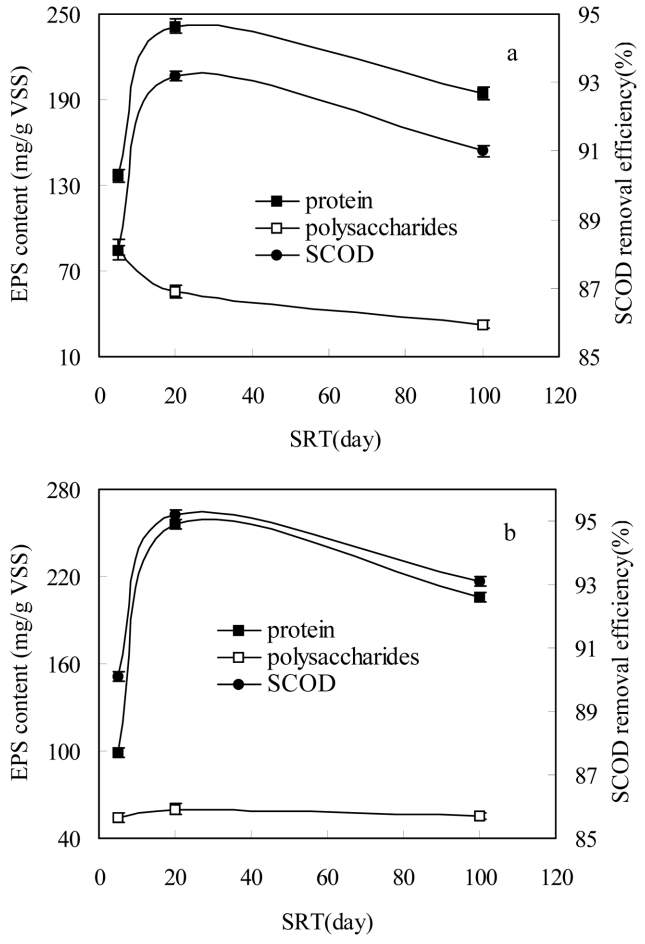

Fig. 7. Comparison of EPS content over SCOD removal efficiency at various SRT in a) AS and b) CAS.

3. Comparison of EPS Versus SCOD between Two Processes Upon SRT

In the long run, degree of removal efficiency for organic compound was assessed so that relationship of EPS versus SCOD was characterized at different SRT as described in Fig. 7. The error bars on each curve were the coefficient variance of triplicates. Fig. 7a shows that removal efficiency of SCOD in the AS has been increased at SRT 10 to 20 d, and then unlikely decreased to 91% at SRT 100 d. Such pattern of variation on removal efficiency of SCOD immediately correlated with proteins concentration, but not dependent on polysaccharides content. It simply means that protein contents are proportionally ascribed to producing increased SCOD in the effluent. Fig. 7b describes relationship between EPS and SCOD in the CAS with SRT. At SRT 20 d, removal efficiency of SCOD has been increased, but then diminished to 93% at SRT 100 d. It also shows that SCOD varied in a similar manner as for proteins concentration, but there was no relationship with polysaccharides content. Regardless of SRT variance, the removal efficiency of SCOD in the CAS was globally 2% greater than in the AS. It was concurrently reflecting SVI versus protein content influence on increased organic removal efficiency by enhanced settling process in the system.

CONCLUSIONS

From observing characteristics of EPS components in sludge, higher concentration of proteins was observed than that of polysaccharides regardless of type of reactor. However, for the AS, the polysaccharides concentration gradually decreased as SRT increased. It showed that decline of polysaccharides concentration was correlated to microbial decomposition under starvation environment of diminished F/M ratio as demonstrated by Hariss and Mitchell [1972] and Andreadakis [1993].

Effluent EPS concentration increased in response to the increase in SRT. For the ratio of EPS of sludge over effluent, the ratio on protein was independently constant upon variation of SRT, while the ratio on polysaccharides inversely dwindled as increasing as SRT. It was also supposed that polysaccharides excreted from sludge would be used for microbial synthesis of proteins. From the comparison of the ratio on EPS between two reactors, a relatively higher ratio was observed for the AS rather than that of the CAS, showing that clinoptilolite could be used for seed material reversibly adsorbing level of proteins. From the relationship between EPS and SVI, given microbial cultures could use polysaccharides to synthesize corresponding amount of proteins, which induces an increase in protein concentration in response to the decrease in polysaccharide concentration. It consequently revealed that effluent water quality for CAS could be enhanced to the highest of 95% of SCOD at SRT 20. The SCOD variance with different SRT was immediately reflected from the variation of protein concentration regardless of type of reactor.

ACKNOWLEDGMENT

This study was financially supported by Inha University Research Grant (30357) and RRC program.

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