Effect of EPS Content on Activated Sludge Reduction in Process of Predation by T. tubifex

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Abstract. A sludge reduction in a conventional activated sludge process combined with a membrane biofilm inoculated with T. tubifex was investigated. The influence of microbial extracellular polymeric substances (EPS) extracted in forms of LB-EPS and TB-EPS respectively on the surface properties of biomass was studied. Results showed that variations of polysaccharides and protein along with the increasing of EPS feeding would affect the existence of T. tubifex. When the amount of EPS varied from 10 to 50μg/mg, the specific resistance of a sludge suspension was obtained from 3.5×10⁷ to 1.4×10⁷ S²/g. Meanwhile, polysaccharides content in EPS was to be positively correlated with the SSR of sludge suspension whereas protein content would be not. Anyway, it can be argued that an increase in LB-EPS not TB-EPS may affect the performance of activated sludge reduction with efficiency about 40.1% to 31.6%.

1. Introduction

As one of the most widely used biological approaches to domestic and industrial wastewater treatment, the activated sludge process allows the transformation of dissolved organic pollutants into biomass, dioxide carbon and water, yet leaving excess sludge as main by-product. Taken the production principle of excess activated sludge into consideration, micro-fauna predation[1-2] was used to reduce excess sludge production. Generally the aquatic worms are often utilized during the microscopic investigation of activated sludge among which T. tubifex, a species of oligochaeta, is especially chosen to be a sludge predator[3-4]. Extracellular polymeric substances (EPS) are biosynthetic polymers localized at or outside the bacterial cell surface and are comprised of a variety of organic macromolecules with other nonpolymeric constituents of low molecular weight. As far as the macromolecule type is concerned, polysaccharides, proteins and nucleic acids are the largest fraction accounting nearly 70-80% of the components, providing a highly hydrated gel matrix in the microbial cells to allow the degradation of waste water treatment[5-7]. In this article, sludge reduction was carried out with a membrane biofilm on which T. tubifex was inoculated along with a sequencing batch reactor. The influence of concentration and properties of EPS and possible effect mechanism of T. tubifex predation on sludge settling were investigated.
2. Materials and methods

2.1. Microorganisms and culture medium

Sludge was collected on the day of the experiment and was aerated for 4 h before using. Wastewater fed to activated sludge system was obtained from domestic wastewater contained with glucose and various kinds of nutrient salts to keep the proportion of biochemical oxygen demand (BOD$_5$), ammonia nitrogen(NH$_4^+$-N) and total phosphorus (TP) in a ratio of 100:5:1.

2.2. Experimental conditions

A combined complete-mixed activated sludge SBR reactor with a membrane biofilm reactor was adopted for the purpose of sludge reduction in the continuous tests. The SBR reactor was with a volume of 15 L for the aeration tank and the volume of membrane biofilm reactor was 5 L, inoculated with two composite loading fillers for the biofilm and aerated for creating a suitable habitat for the growth of $T. tubifex$ in the experimental group. The temperature of the reactor was maintained at 20-25°C and the pH of the sludge suspensions was in the range of 6.5-7.5. Sludge samples were then collected from the biofilm reactor for characterization and analysis of EPS. Results represent the average of two experiments for $T. tubifex$ predation and sludge reduction, in which experimental variations did not exceed 5%.

2.3. Analytical

In the continuous tests, chemical oxygen demand (COD), sludge volume index(SVI), mixed liquor suspended solids(MLSS) and mixed liquor suspended solids(MLVSS) concentrations in the biofilm reactor were measured according to the Chinese SEPA Standard Methods\[^8\]. The sludge yield coefficient was calculated by the following equation \((1)\) .

$$Y = \frac{\Delta MLVSS}{\Delta COD} = \frac{MLVSS_{n+1} - MLVSS_n}{\sum COD_n - \sum COD_{out}}$$

where \(Y\) is the sludge yield coefficient, \(MLVSS_n\) and \(MLVSS_{n+1}\) are mixed liquor volatile suspended solids (mg/L) on one day and the next day, \(COD_n\) and \(COD_{out}\) are the influent and effluent concentrations of COD (mg/L), respectively.

The specific resistance of sludge to filtration by pressure filtration was calculated by Eq. (2).

$$r = \frac{2PA^2}{\mu \omega b}$$

where \(r\) is the sludge specific resistance (S$^2$/g), \(P\) is the pressure of pump (Pa), \(A\) is the filter area within the funnel (cm$^2$) and \(\mu\) is the filtrate viscosity (g/cm·s), \(b\) represents the straight slope while \(\omega\) is the weight of filter cake per unit volume (g), respectively.

Sludge sample was collected from the reactor before daily feeding and was then resuspended into 50mL of 0.9% NaCl solution, followed by alkaline treatment with 1 mol/L of NaOH at pH 11. The sludge mixture was stirred slowly for 10 min, followed by centrifugation at 7300 r/min for 10 min. The organic matter in the supernatant was filtered through 0.45 um of membrane filter and readily for extraction of EPS. Both the LB-EPS and TB-EPS extractions were analyzed for proteins and polysaccharides, and they were determined with the Coomassie blue staining method and with the phenol-sulphuric acid method\[^9\].

3. Results and Discussion

3.1. EPS contents at different hydraulic retention times (HRT)

The variation of EPS contents in the activated sludge conditions at different hydraulic retention times (HRT) from 3 to 10 days was listed in Table 1.
It was found that the amount of polysaccharides in EPS in presence of T. tubifex in activated sludge treatment was noticeably decreased from about 27.01 μg/mg to 12.31 μg/mg with a HRT lengthened from 3 to 10 days, whereas in the conditional control group the sludge underwent a slight change average from 21.80 μg/mg to 22.03 μg/mg. Meanwhile, the protein concentration in EPS was obviously increased with a longer HRT in the reactors. However, the amount of LB-EPS in presence of T. tubifex was increased with a longer HRT from about 10.15 μg/mg to 28.51 μg/mg, but TB-EPS content underwent a decline average from 25.63 μg/mg to 10.97 μg/mg, whereas the ratio of LB-EPS to TB-EPS was from 0.39:1 to 2.60:1 along with the HRT from 3 to 10 days. The results demonstrate that HRT is likely to have an effect on composition of EPS from the microbial cells in general.

3.2. Influence of EPS contents on sludge settleability

The growth of T. tubifex in activated sludge with different adding amount EPS was investigated with a view to observe the influence of EPS on sludge settleability (Fig.1).

It was found that different amount of EPS with the growth of T. tubifex lengthen for days of incubation period show a slight change on the SVI of treatment performance. Given the density of EPS varied from 10 μg/mg to 30 μg/mg with the same incubation period, the average SVI value showed a decreasing trend from 84.9 ml/g to 64.2 ml/g respectively, which implied that the sludge samples with a certain amount of EPS had more chances with sedimentation. But when further feeding EPS to 50 μg/mg, the SVI value was up to 72.2 ml/g, resulted in poor performance of sludge sedimentation and compression. Continuous test were conducted to investigate the corresponding results of polysaccharide and protein contents with the increasing the density of EPS by the growth of T. tubifex with an incubation period of 15 days for the activated sludge process and the results were illustrated in Fig 2 and 3.

![Fig. (1)](image1)

**Fig. (1)** Influence of T. tubifex predation on EPS contents and SVI; **Fig. (2)** Influence of T. tubifex predation on EPS contents and polysaccharides content; **Fig. (3)** Influence of T. tubifex predation on EPS contents and protein content

It was found that polysaccharides content increased with elevated EPS concentration in sludge while protein amount in the sludge showed a tendency of decreasing first and a little higher later. Meanwhile, it can be certain that investigated EPS contents varied from 10 to 30μg/mg the relative ratios of polysaccharides to protein in the sludge process with T. tubifex predation was more notable, from 0.7:1, 1.41:1 to 5.11:1. As shown that in the case of 30μg/mg EPS feeding the sludge had a good performance of settleability, it was suggested that a higher abundance of polysaccharides is more likely expected in the biomass feed with EPS than that of protein. The underlying cause for this
phenomenon may be due to the presence of readily biodegradable substrates at the initial in the reactor gives rise to a high level of organic loading for the growth of *T. tubifex*, resulting less of polysaccharides in EPS in sludge be consumed. As for protein, however, when the treatment was increasing the density of EPS from 10 to 30 μg/mg change, the occurrence of ammoniation within the biofilm reactor would lead to decrease the protein content easily, even though proteins are predominance proportion found in the microbial EPS [10].

3.3. **Influence of EPS contents on sludge dewaterability**

The dewaterability of a sludge suspension was determined using the filterability test and the relationship between specific resistance of sludge and EPS content was shown in Fig.4.

![Fig. (4)](image)

**Influence of T. tubifex predation on EPS contents and sludge dewaterability:**

It was found that if EPS contents varied from 10 to 50μg/mg, the SSR of a sludge suspension was decreasing gradually, meaning that the filterability of the sludge was better. However, if the quantity of EPS feeding was above 70μg/mg, the mixed liquors in a sludge suspension had a higher viscosity than in its effluent and the difficulty in sludge dewatering increased. Therefore, it is reasonable to suppose that an increased amount of LB-EPS is mainly for the increase in the viscosity of a sludge suspension, which might lead to a slight poor performance of sludge sedimentation and compression in *T. tubifex* predation compared with the control reactor.

3.4. **Influence of EPS contents on sludge reduction**

The sludge reduction effects of increasing EPS contents on the sludge reduction with *T. tubifex* predation was measured by batch tests and shown in Fig. 5.

It is shown that when investigated EPS contents varied from 10 to 50μg/mg, a total sludge reduction was with trend of decreasing gradually. As for the different role for LB-EPS and TB-EPS, we found that with a high quantity of LB-EPS would worsen the attachment between cells and weaken the structure of the sludge flocs, causing poor performance in bioflocculation and retarded sludge water separation. However, in the turbulent fluid of the activated sludge process, the TB-EPS was noticeably decreased from 25.63μg/mg to 10.97μg/mg with a longer HRT, yet the sludge reduction was not obviously decreased. Thus, it can be argued that an increase in TB-EPS may not affect the biomass separation and no correlation could be established with the TB-EPS content on the sludge-water separation performance in the activated sludge process.

4. **Conclusions**

Sludge characteristics that affect the growth of *T. tubifex* are described as polysaccharides and protein contents with the increasing the amount of EPS feeding into the activated sludge process. Results demonstrate that the sludge yield was to have a negative correlation with the amount of EPS in activated sludge reactors whereas the relative sludge reduction by *T. tubifex* was estimated to be about 40.1% to 31.6% when EPS contents varied from 10 to 50μg/mg. It can be argued that an increase in LB-EPS not TB-EPS may affect the sludge-water separation performance in the activated sludge process.
References
[1] Ratsak CH, Verkuijlen J. 2006. *Hydrobiologia*, 564, 197
[2] Sukhyun Na, Hokyung Shon, Jongho Kim. 2010. *Korean J Chem Eng*, 28, 164
[3] Liu M, Duan YF, Li HF. 2013. *Powder Technology*, 243, 18
[4] Anjum M, Almakishan NH, Barakat MA. 2016. *Process Saf Environ*, 102, 615
[5] Zhang XQ, Tian Y, Wang Q, Lin HL. 2013, *Ecological Engineering*, 58, 406
[6] Bendov E, Shapiro OH, Gruber R, et al. 2008. *FEMS Microbiology Ecology*, 66, 437
[7] Hendrickx TLG., Elissen HJH., Temmink H, et al. 2011. *Water Res*, 45, 4923
[8] Chinese SEPA. 1997. *Water and Wastewater Monitoring Methods, 3rd ed*, Beijing, China,
[9] Liang ZW, Li WH, Yang SH, et al. 2010. *Chemosphere*, 81, 626
[10] Fazal S, Zhang BP, Zhong ZX, et al. 2015. *Journal of Environmental Protection*, 6, 584