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Role of extracellular exopolymers on biological phosphorus removal

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Abstract: Three sequencing batch reactors supplied with different carbon sources were investigated. The system supplied with glucose gained the best enhanced biological phosphorus removal although all of the three reactors were seeded from the same sludge. With the measurement of poly-β-hydroxyalkanoate (PHA) concentration, phosphorus content in sludge and extracellular exopolymers (EPS) with scanning electron microscopy (SEM) combined with energy dispersive spectrometry (EDS), it was found that the biosorption effect of EPS played an important role in phosphorus removal and that the amount of PHA at the end of anaerobic phase was not the only key factor to determine the following phosphorus removal efficiency.

Keywords: biological phosphorus removal; extracellular exopolymers; biosorption

Introduction

With accelerating environmental pollution and water resource shortage, more attention on water pollution has been paid to the nutrient removal. The so-called nutrient, nitrogen and phosphorus, has caused serious global water eutrophication problems. Enhanced biological phosphorus removal (EBPR) can be realized by polyphosphate-accumulation organisms (PAOs) in the activated sludge, which can take up phosphate far more than they need under aerobic condition. The common accepted mechanism of EBPR is that under the anaerobic condition, PAOs use the energy from polyphosphate to turn biodegradable organisms to polyhydroxyalkanoates (PHAs), a kind of storage material. During this course, the polyphosphate is converted to phosphate; during the aerobic phase, PAOs use the energy from PHAs to sustain the cell growth and synthesize glycogen and polyphosphate. The amount of synthesized polyphosphate is greater than that of released phosphate, at the same time the EBPR achieves phosphorus removal by wasting phosphate-rich sludge from system.

Extracellular exopolymers (EPS) is important part of activated sludge. It has good adsorptive properties (Loaëc *et al.*, 1997) and is composed of polysaccharide, proteins, humic acids, smaller amounts of DNA and liquids (Frolund *et al.*, 1996, Jorand *et al.*, 1995, Liao *et al.*, 2001, Görner *et al.*, 2003). The amount of these components is important in controlling the sludge bioflocculation and settleability (Liao *et al.*, 2001), for the part of carbohydrates was presumed to be groups charged negatively and bridged by divalent cations (Jorand *et al.*, 1995). But there are few reports on its role in phosphorus removal (Cloete and Oosthuizen, 2001). The purpose of this study was to compare the role of EPS on phosphorus removal in different carbon source EBPR systems with chemical analysis and energy dispersive spectrometry (EDS). Meanwhile, the fractions of different forms of phosphorus in activated sludge were also analysed to testify the biosorption roles of EPS.

1 Materials and methods

1.1 SBR operation

Three laboratory-scale sequencing batch reactors (SBRs), each with 18 L working volume, were operated with good EBPR performance for more than 90 d. All of them were seeded with the sludge from a full-scale SBR, which was used to treat wastewater from a hotel and has achieved EBPR ability when it was used in this experiment (Fig.1). They were operated under sequencing anaerobic-aerobic (AO) conditions with an 8-h cycle (2.5 h anaerobic phase, 3.5 h aerobic phase, 0.5 h settling, 1.5 h filling/drawing and idle). During the anaerobic phase, nitrogen gas was purged into reactors for 15 min to get anaerobic conditions.

Phosphorus removal efficiencies under different pH, temperature and sludge retention time (SRT) conditions were investigated in batch experiments. Data were not shown here. Results showed that different systems reached to optimal phosphorus removal at pH 7.2. It was also found that temperature had no obvious effect on phosphorus removal in the interval of 10 to 25℃. When SRT was 7 and 20 d, all the systems got better phosphorus removal. Thus, the operation temperature was fixed at 22℃; pH values were controlled around 7.0 ± 0.2 by adding 0.5 mol/L HCl or 0.5 mol/L NaOH; extra sludge was withdrawn from system at the end of each cycle to maintain the SRT around 7 d and mixed liquid suspended solid (MLSS) around 3000 mg/L.

1.2 Synthetic wastewater

Synthetic wastewater was used in this study. The synthetic water was prepared by mixing tap water with acetate. To avoid fermentation, components were

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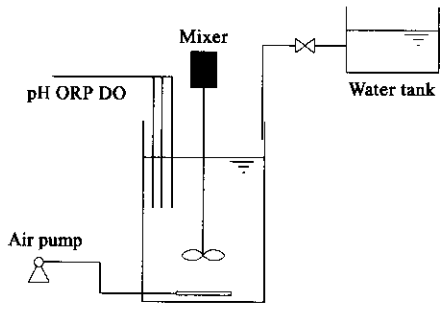

Fig.1 Schematic diagram of the SBR used in this study

added to water tank before the start of each cycle. The compositions of synthetic wastewater are shown in Table 1. The biological treatment units were run for over 3 months under controlled conditions to reach steady phosphorus removal performance. Then the test was carried out, and the performances of whole treatment systems were analyzed.

Table 1 Composition of synthetic wastewater for three reactors

Compound, mg/L	Reactor 1	Reactor 2	Reactor 3
Sodium acetate/Glucose/Skim milk+glucose	741.4	589.5	294.8+197.1
P (KH_2PO_4)	15	15	600
NH_3-N(NH_4Cl, average)	40	40	15+5.4
COD	600	600	40
Mg ($MgCl_2$)	10	10	161.54
Cu ($CuSO_4$)	0.1	0.1	10
Ca ($CaCl_2$)	5	5	0.1
Mn ($MnSO_4$)	0.1	0.1	5
Zn ($ZnCl_2$)	0.1	0.1	0.1
Peptone	10	10	0.1

1.3 Analytical methods

COD, PO_4^{3-}, NH_4-N, NO_3-N, MLSS and mixed liquid volatile suspended solid (MLVSS) were analysed according to standard methods (SEPAC, 1989). Total organic carbon (TOC) was measured by a TOC analyser (TOC-VCPN, Shimadzu). Dissolved oxygen (DO) was measured by a DO meter (JPB-607), and pH was measured by an acidometer (PHB3-PH).

Sodium acetate concentration in mixed liquid was analysed using diethyl ether extraction and gas chromatography (Wang, 2001). The pre-treated samples were analysed by GC (6890 N Agilent), with a capillary column (HP-5, 30 m length, 0.25 mm ID) with a FID detector. The temperature program for the GC was: initial temperature at 70℃ for 3 min, ramping to 120℃ at 10℃/min, then to 180℃ at 35℃ /min, remaining at this final temperature for 1 min. The detector and injector temperatures were set at 250℃ and 210℃, respectively.

Glucose was analysed by the phenol method (Wang, 2001). PHA (poly-β-hydroxyalkanoate) was analysed by the gas chromatogram (Wang, 2001), but some changes were made when compared with what was described by Wang (2001). 5 ml of the mixed suspended solid sample was collected and washed by adding 10 ml of 0.85% NaCl solution. This solution was then centrifuged at 10000 ×g at 4℃ (Anke GL-20G-II) for 15 min. The supernatant was then decanted and replaced with 5 ml of 5.25% sodium hypochlorite. The sample was incubated at 37℃ for 1 h and was then centrifuged again at 10000 ×g at 4℃ for another 15 min. The precipitate at the bottom was added 2 ml acidified methanol (3% H_2SO_4) and 10 ml chloroform. The mixture was transferred into a tube and sealed tightly and heated for 3.5 h at 100℃, cooled down to room temperature, then added 1 ml of distilled water. After sealing, the tube was shaken for 30 min, transferred to a 25 ml extraction funnel and followed by 30 min settling to separate the water phase and the organic phase. The bottom chloroform phase (containing hydrolysed PHA) was used for the GC (6890 N Agilent, the same capillary column as sodium acetate analysis) test. The PHA was calibrated by DL-β-hydroxybutyric (Sigma, Beijing Bailingwei Agent). The temperature program was: initial temperature at 60℃ for 1 min and ramp to 105℃ at 8℃/min and then to 180℃ at 35 ℃/min, hold for 1 min. The standard sample was treated in exactly the same way as above.

Analysis of the phosphorus content of EPS was carried out by means of EDS (Hitachi S-4700). To minimize the error by pre-treatment of scanning electron microscopy (SEM) sample (components were washed away and floc structures were destroyed by fixation with glutaraldehyde or dehydration with different concentrations of ethanol), this test processed the samples as the following: 10 ml sludge was sampled at the end of aerobic phase and settled for 30 min, 1 ml settled sludge was washed with distilled water (1:5 v/v) 3 times, then desiccated for 48 h before coated with platinum (Gatan Model 682). EPS was visualized with SEM and its phosphorus content was also analysed.

Determination of polyphosphate weight percentage in sludge was carried out by the method according to Heymann *et al.* (1989). Phosphorus content in sludge was analysed as the following: 10 ml of mixed liquid was centrifuged at 17960 ×g, 4℃ for 15 min. The precipitate was dried at 105℃ for 6 h, then was weighed and placed in muffle furnace at 500℃ for 4 h. The ash was replaced to polytetrafluoroethylene centrifuge tube. 5 ml of H_2SO_4/$HClO_4$ (v/v: 1/4) was added. The tube was oscillated for 2 h on oscillator, centrifuged again, and made to constant volume. Then phosphorus concentration was measured by the

standard method.

2 Results and discussion

2.1 Phosphorus removal in three different carbon source SBRs

Ortho-phosphate removal efficiencies in the effluent of three SBRs are shown in Fig. 2. It can be seen that the three SBRs reached higher phosphorus removal efficiencies after nearly 100 d operation. The reactor fed with glucose had the best phosphorus removal efficiency, i.e. 96.9%, next was 90.94% of No.1 reactor fed with sodium acetate. While reactor fed with skim milk and glucose had the lowest efficiency of 76.3%.

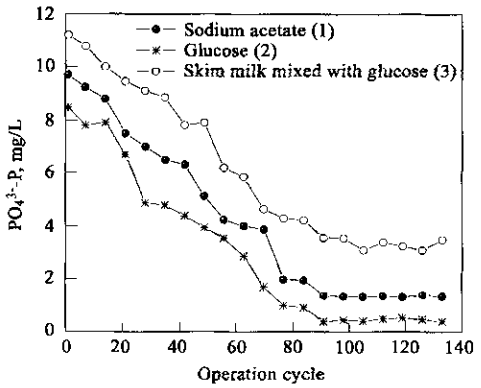

Fig.2 Phosphorus concentrations in effluent of the three SBRs during experiment

2.2 Characteristics of the three SBRs

TOC and phosphate variation profiles in typical cycles are shown in Fig.3a and b. Organic carbon was consumed soon after the beginning of anaerobic phase in all the three reactors. Phosphorus was released during the anaerobic phase in No.1 and No.3 reactors, while it did not happen in No.2 reactor. This phenomenon was different from the previous literature (Lin *et al.*, 2003, Sudiana *et al.*, 1999, Wang *et al.*, 2002, Che and Jone, 2000). To investigate the reason why glucose-fed system did not have phosphorus release in anaerobic phase while its phosphorus removal effect was the best, the polyphosphate variation in acitivated sludge was measured, the results are shown in Fig.3c. As could be seen clearly, there was polyphosphate consumed during anaerobic phase in fact. It was calculated that there was 202.8 mg/L PO_4^{3-}-P released during the anaerobic phase of operation cycle. This amount of released PO_4^{3-}-P was identical to 6.76% of activitated sludge weight percentage. It meant that there was phosphate released in the anaerobic phase, the reason that there was no phosphate released macroscopically might be the relatively stronger absorptive effect of extracellular exopolymer (EPS). During the anaerobic phase, the organic carbon in influent was consumed quickly and the amount of PHA increased at the same

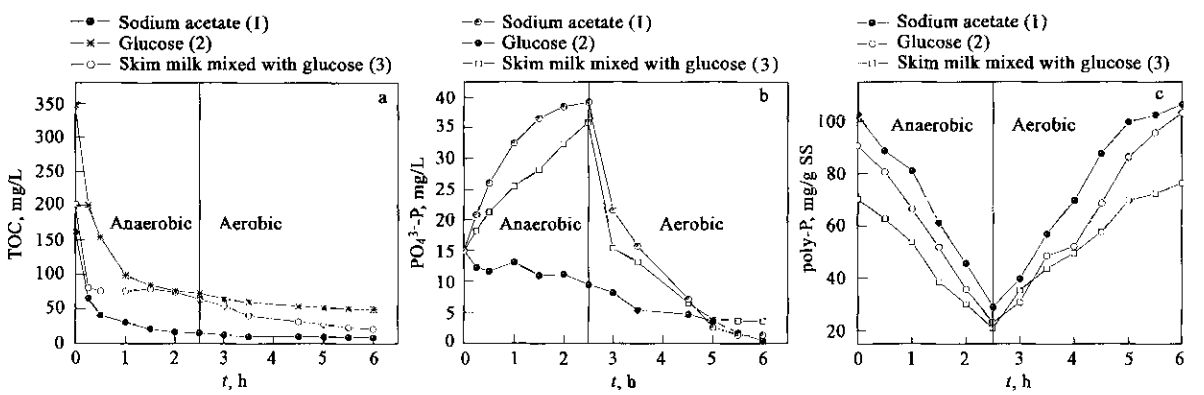

Fig.3 Comparison of TOC (a), phosphate (b) and poly-phosphate (c) change during typical cycles in three reactors

time (Table 2), which demonstrated that the influent organic substrates were converted to intracellular storage compounds PHA completely or partly, because different amounts of PHA was synthesized with the same influent COD. Although No. 2 reactor reached the highest phosphorus removal efficiency, it did not have the most amounts of PHA at the end of anaerobic phase. The result revealed that PHA at the anaerobic phase seemed not to be the key factor of phosphorus uptake in enhanced biological phosphorus removal as described by Randall and Liu (2002).

Table 2 PHA contents in different operation phases

Phase	PHA conc., mg/g MLSS		
	No.1	No.2	No.3
End of anaerobic phase	60.2	43.4	33.5
End of aerobic phase	3.4	3.1	4.9

2.3 Biosorption of EPS

In order to investigate whether the nonlinear relationship between PHA contents and phosphorus removal efficiency was due to the biosorptive effect of EPS, phosphorus content in EPS of aerobic activated sludge was analysed with EDS attached to SEM (Figs. 4 and 5). EPS structure of No.2 SBR is shown in Fig. 5. The elemental percentages of specific field analysed are shown in Table 3. Due to a long-time operation under high phosphorus load, the assimilated phosphorus either combined with metal ions as

extracellular sendimentation or converted to *intracellular* polyphosphate. It could be seen clearly, that the phosphorus content of each reactor was very high. Eliminating the error caused by coating aurum and palladium on samples, phosphorus weight percentages of No.1, 2 and 3 were 7.71%, 9.22% and 8.07%, respectively. It is obvious that the EPS in No. 2 reactor had enough capability to adsorb phosphorus for its phosphorus content 9.22% higher than the phosphorus released by polyphosphate breakdown 6.76%. At the end of aerobic phase, the phosphorus ratios in activated sludge of three SBRs were 11.7%, 14.5% and 13.1% (g P/g VSS%), respectively. Among the three reactors, No.2 had the highest phosphorus percentage in sludge and sludge EPS. The normal phosphorus ratio of dry cell is about 2%, phosphorus ratio in sludge with good EBPR is 8%—12.3% (Liu *et al.*, 2000), while the highest phosphorus ratio in sludge was 14.5% in No.2 reactor. Thus, phosphorus removal could partly be achieved by floc absorption of activated sludge, which was the most evident in No.2 reactor. Polysaccharide is one of the main components of EPS (Frolund *et al.*, 1996, Jorand *et al.*, 1995, Liao *et al.*, 2001, Görner *et al.*, 2001) and the relative amount of polysaccharide plays an important role in bioflocculation and settleability of sludge (Liao *et al.*, 2001). Glucose is the monomer of polysaccharide, so in the glucose-fed system there were the highest amount of polysaccharide and the best biosorption effect. There was better biosorption effect of EPS in No.3 reactor partly fed with glucose, whose phosphorus weight percentage in EPS was 8.07%, while the percentage was 7.71% in No.1 reactor fed with acetate. At the same time, there were high percentage of Mg, Ca, and K in EPS as counter-ions. This phenomenon was consistent with the report by Cloete and Oosthuizen (2001).

Due to variant EPS components in activated sludge, biosorption effects of EPS were different in reactors with different kinds of carbon sources. Analysis of EPS with EDS has its limits, for EDS can only analyse elementary composition of specific point on sample. Thus, further study on pure EPS extracted from activated sludge for elementary analysis is suggested.

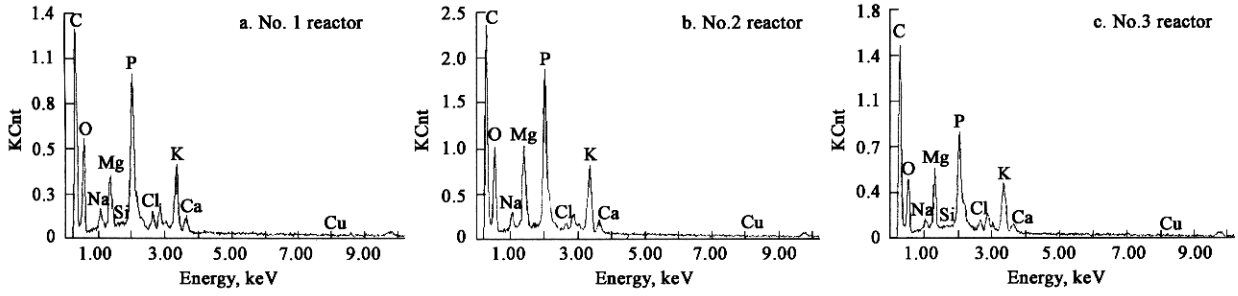

Fig.4 Typical energy dispersive spectrum of EPS of aerobic sludge in three reactors

Table 3 Composition of synthetic wastewater for three reactors

Element	Weight percentage,%		
	No.1	No.2	No.3
Na-K@	0.78	1.04	1.56
Mg-K	1.39	2.16	1.32
Si-K	/	/	/
P-K	7.71	9.22	8.07
K-K	3.77	6.43	6.37
Ca-K	0.16	0.10	0.07
Cu-K	/	/	/
C-K	66.41	58.42	60.05
O-K	19.18	22.28	21.77
Mn-K	/	/	/
Zn-K	/	/	/
Cl-K	0.59	0.34	0.78
S-K	/	/	/

Notes: / No data; @ K indicates the K-shell if specific atom

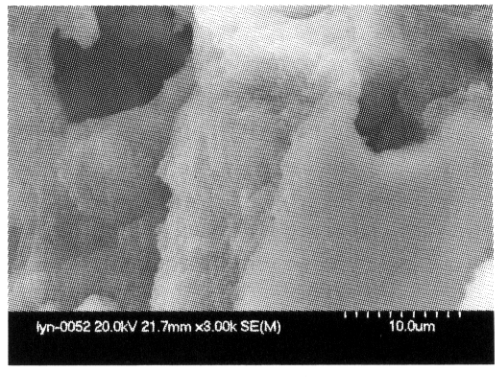

Fig.5 Structure of No.2 EPS cell clusters in reactor at the end of aerobic phase (SEM×3.00k)

3 Conclusions

No.2 SBR fed with glucose had the best phosphorus removal efficiency compared with the other SBRs fed with acetate or glucose combined with skim milk. No.2 SBR had the highest phosphorus weight percentage both in EPS and in sludge, because glucose is the monomer of polysaccharide and a key component influencing bioflocculation and settlea-

bility of sludge. EPS played an important role in phosphorus biosorption removal. Amount of PHA at the end of anaerobic phase was not the unique key factor to affect the following aerobic phosphorus removal efficiency because of the biosorption effect of EPS. The amount change of PHA and polyphosphate in activated sludge of No.2 SBR showed that there should be orthophosphate release during anaerobic phase microscopically and macroscopically no orthophosphate release was caused by the biosorption effect of EPS.

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