DOI:10.11835/j.issn.2096-6717.2020.087 开放科学(资源服务)标识码(OSID):

Effect of free ammonia on nitrogen removal and extracellular polymeric substances in the suspended activated sludge system

CAI Chenjian[1], *WU Changfeng*[2], *YANG Hao*[1], *CHEN Tongsheng*[3], *SUN Hongwei*[1,4]

(1. School of Environmental and Municipal Engineering, LanzhouJiaotong University, Lanzhou 730070, P. R. China; 2. Lanzhou City Water Supply Group Co., Ltd, Lanzhou 730060, P. R. China; 3. South China Institute of Environmental Science, MEE, Guangzhou 510655, P. R. China; 4. School of Environmental and Material Engineering, Yantai University, Yantai 264005, Shandong, P. R. China)

Abstract: To investigate the influences of free ammonia on nitrogen removal, the contents of extracellular polymeric substances and the chemical composition (proteins, polysaccharides, and DNA), four laboratory-scale sequencing batch reactors fed with synthetic wastewater were operated at free ammonia concentrations of 0.5, 5, 10 and 15 mg/L ($R_{0.5}$, R_5, R_{10} and R_{15}, respectively). Results showed that high nitrogen removal efficiencies (97.6%~99.4%) were achieved under four free ammonia concentrations. Free ammonia had a significant impact on the three kinds of extracellular polymeric substance (loosely bound extracellular polymeric substance, tightly bound extracellular polymeric substance and total extracellular polymeric substance, respectively) and their composition. Results showed that with the increased of initial free ammonia concentrations from 0.5 to 10 mg/L, the production of three kinds of extracellular polymeric substance and their components were significantly increased. Further increased of free ammonia concentrations to 15 mg/L caused a decreased trend of them. Moreover, proteins were the main component of loosely bound extracellular polymeric substance, while polysaccharides dominated in tightly bound extracellular polymeric substance and total extracellular polymeric substance under different free ammonia concentrations. It was found that the contents of three kinds of extracellular polymeric substance and their components showed a similar variation tendency to the NO_x^-—N contents during the whole cycle under four free ammonia concentrations.

Keywords: free ammonia; nitrogen removal; extracellular polymeric substance; proteins; polysaccharides

游离氨对活性污泥系统中脱氮性能及胞外聚合物的影响

蔡晨健[1],吴常峰[2],杨浩[1],陈桐生[3],孙洪伟[1,4]

(1. 兰州交通大学 环境与市政工程学院,兰州 730070;2. 兰州城市供水集团有限公司,兰州 730060;3. 生态环境部华南环境科学研究所,广州 510655;4. 烟台大学 环境与材料工程学院,山东 烟台 264005)

摘 要:以人工模拟废水为研究对象,采用4组SBR反应器($R_{0.5}$、R_5、R_{10}和R_{15}),考察了4种游离氨浓度(0.5、5、10和15 mg/L)对生物脱氮效能、胞外聚合物含量及其组分(蛋白质(PN)、多糖(PS)和核酸(DNA))影响。结果表明,4种游离氨浓度条件下都实现了较高的脱氮效果(97.6%~

Received: 2020-05-14

Foundation items: National Natural Science Foundation of China (No. 51668031)

Author brief: CAI Chenjian (1995-), main research interest: sewage biological treatment, E-mail: 2528397863@qq.com.
CHEN Tongsheng (corresponding author), senior engineering, E-mail: chentongsheng@scies.org.

99.4%)。游离氨对3种胞外聚合物(LB-EPS、TB-EPS、总EPS)及其组分有显著影响。当游离氨浓度从0.5 mg/L增加到10 mg/L时,LB-EPS、TB-EPS、总EPS的含量及其组分的含量都显著增加;当游离氨浓度进一步增加到15 mg/L时,LB-EPS、TB-EPS、总EPS的含量及其组分的含量都呈降低的趋势;在4个游离氨浓度下,PN是LB-EPS的主要成分,PS是TB-EPS和总EPS的主要成分;在4个游离氨浓度下的整个反应周期中,3种胞外聚合物及其组分的含量都跟NO_x^-—N有着相同的变化趋势。

关键词:游离氨;脱氮;胞外聚合物;蛋白质;多糖

中图分类号:X703.1　　**文献标志码:**A　　**文章编号:**2096-6717(2021)01-0184-09

1 Introduction

Extracellular polymeric substances (EPS) accumulating on the surface of activated sludge account for 80% of the activated sludge matrix, and play a significant role on wastewater treatment[1-2].

Ammonia nitrogenis the main pollutant in multiple wastewaters. Therefore, there must be a certain concentration of free ammonia (FA) in the presence of NH_4^+—N in any wastewater. The concentration of free ammonia mainly depends on the values of NH_4^+—N, temperature and pH of the wastewater[3]. In recent decades, increasing efforts have been devoted to investigating the effects and mechanisms of free ammonia on nitrification, denitrification, Anammox, phosphorus removal and anaerobic digestion processes[4-7]. But limited literature has reported the effects of free ammonia on extracellular polymeric substance and its components (including proteins, polysaccharides and DNA). Zhang et al.[8] reported that free ammonia pretreatment, with the concentration controlled at 176.5 mg/L, can facilitate the breakdown of extracellular polymeric substances and kill the living cells. But the effects of low concentrations of free ammonia on the production of extracellular polymeric substances and their components are still unknown.

In addition, with respect to the main components of extracellular polymeric substances, there are some different findings. Some studies have reported that polysaccharides (PS) were the predominant components of extracellular polymeric substances[9-10], while other studies have found that proteins (PN) were the main components[11-12]. Furthermore, it was reported that extracellular polymeric substances have some different vital functions as the main components in activated sludge differed. Higher tightly bound extracellular polymeric substance (TB-EPS) contents have been reported to noticeably enable the extracellular polymeric substance to form flocs in activated sludge[13]. Moreover, protein enrichment in the tightly bound extracellular polymeric substance has positive effects on the sedimentation and dehydration of the activated sludge[9,14]. However, it was reported that excessive extracellular polymeric substance contents, especially polysaccharide enriched loosely bound extracellular polymeric substance (LB-EPS), can induce poor dewaterability and bioflocculation[9,14]. The effects of the concentration of free ammonia on the predominant components of extracellular polymeric substance are still unknown. Hence, it is necessary to research the main components of extracellular polymeric substances under different concentratiosns of free ammonia.

Therefore, the objectives of this study were to elucidate the effects of free ammonia on the production of three kinds of extracellular polymeric substance (loosely bound extracellular polymeric substance, tightly bound extracellular polymeric substance and total extracellular polymeric substance) and their components and to build a correlation between nitrogen variation and the production of the three kinds of extracellular polymeric substances and their components under four different concentrations of free ammonia.

2 Material and methods

2.1 Experimental set-up and operational procedure

Four bench-scale sequencing batch reactors (SBR) made of plexiglass15 cm in diameter with 5 L working volume and 40 cm height were used in the present study. The sequencing batch reactors were operated at four different concentrations of free ammonia (0.5, 5, 10 and 15 mg/L, respectively). The long-term operations (lasted for 244 days) were carried out in four sequencing batch reactors fed with synthetic wastewater. The operational cycles of the four sequencing batch reactors with varying concentrations of free ammonia ($R_{0.5}$, R_5, R_{10} and R_{15}) consisted of influent, aerobic reaction, anoxic reaction, settling, decanting and idle. Table 1 shows details of the operational conditions of the four sequencing batch reactors during the entire experimental cycle.

2.2 Inoculated sludge and influent contents

Inoculated activated sludge was collected from a plant mainly treating domestic and brewery wastewater (accounted for about 60%~70% and 30%~40%, respectively). The plant was located at Lanzhou, Gansu province in China, where the anaerobic-anoxic-oxic process was employed. The initial concentration of the mixed liquor suspended solids was 3 000 mg/L. The inoculated sludge was domesticated for 20 days and fed with synthetic wastewater with the following composition per liter: 115 mg of NH_4Cl, 385 mg of CH_3COONa, 26 mg of KH_2PO_4 and trace element solution. The trace element solution consisted of $MgSO_4 \cdot 7H_2O$ 5.07 mg/L, $MnSO_4 \cdot 4H_2O$ 0.31 mg/L, $FeSO_4 \cdot 7H_2O$ 2.49 mg/L, $CuSO_4$ 0.25 mg/L, $Na_2MoO_4 \cdot 2H_2O$ 1.26 mg/L, $ZnSO_4 \cdot 7H_2O$ 0.44 mg/L, NaCl 0.25 mg/L, $CaSO_4 \cdot 2H_2O$ 0.43 mg/L, $CoCl_2 \cdot 6H_2O$ 0.41 mg/L, EDTA 1.88 mg/L. Table 1 presents a summary of the characteristics of the influent of the four sequence batch reactors.

Table 1 Operating conditions of four sequence batch reactors

Influent/(mg·L⁻¹)		Phase time of the SBR/min					Operational parameters				
COD	NH_4^+—N	One cycle	Filling	Aeration	Anoxic	Setting and Decantation	MLSS/(mg·L⁻¹)	FA/(mg·L⁻¹)	Temperature/℃	pH	DO/(mg·L⁻¹)
80	40	620	5	270	300	45	3 900	0.5	20±2.0	7.5±0.2	1.0~2.5
80	90	710	5	300	360	45	4 400	5	25±2.0	8.0±0.2	1.0~2.5
80	130	810	5	360	420	25	4 500	10	30±2.0	8.0±0.2	1.0~2.5
80	55	570	5	240	300	25	4 400	15	35±2.0	8.5±0.2	1.0~2.5

2.3 Extracellular polymeric substance extraction

With respect to extracellular polymeric substance extraction, it mainly included loosely bound extracellular polymeric substance and tightly bound extracellular polymeric substance extraction. The modified two-step thermal extraction method was used to extract loosely bound extracellular polymeric substance and tightly bound extracellular polymeric substance[15]. In the present study, the total extracellular polymeric substance content was regarded as the sum of the loosely bound extracellular polymeric substance and the tightly bound extracellular polymeric substance fractions.

2.3.1 Loosely bound extracellular polymeric substance fraction extraction

In brief: well-mixed sludge water was centrifuged at 2 100 g and 4 ℃ for 10 min to separate the supernatant from the solids, then the obtained supernatant was filtered by a 0.45 μm microporous membrane for analysis, with the collected supernatant regarded as the loosely bound extracellular polymeric substance fraction.

2.3.2 Tightly bound extracellular polymeric substance fraction extraction

Then Ringer solution was added to the residual activated sludge and the mixture was heated at 80 ℃ for 60 min in a constant temperature water bath and subsequently centrifuged again at 12 000 g and 4 ℃ for 10 min. Finally, the obtained supernatant was filtered again by a 0.45 μm microporous membrane for analysis, with the collected supernatant regarded as the tightly bound extracellular polymeric substance fraction.

2.4 Analytical methods

2.4.1 Extracellular polymeric substance quantification

The protein and polysaccharide contents of the loosely bound extracellular polymeric substance and the tightly bound extracellular polymeric substance were measured using the Lowry method and the phenol-sulphuric acid method, with bovine serum albumin and glucose, respectively, used as the standards[16-17]. DNA was determined by means of the ultraviolet absorption method[18]. The sums of the protein, polysaccharide and DNA fractions in the loosely bound extracellular polymeric substance and the tightly bound extracellular polymeric substance were regarded as the content of the loosely bound extracellular polymeric substance and the tightly bound extracellular polymeric substance, respectively.

2.4.2 Conventional analytical

COD, NH_4^+—N, NO_2^-—N, NO_3^-—N and mixed liquor suspended solids were analyzed in accordance with the standard methods[19]. Temperature, pH and dissolved oxygen values were monitored by using on-line probes (WTW Multi 3420, Germany).

The ammonia removal rate, the nitrite accumulation rate and the nitrate accumulation rate were calculated according to the following equation (Eq. (1), (2) and (3)).

$$\mathrm{ARE}(\%) = \frac{[NH_4^+—N]_{\mathrm{influent}} - [NH_4^+—N]_{\mathrm{effluent}}}{[NH_4^+—N]_{\mathrm{influent}}} \times 100\% \quad (1)$$

$$\mathrm{NiAR}(\%) = \frac{NO_2^- - N}{[NO_2^- - N] + [NO_3^- - N]} \times 100\% \quad (2)$$

$$\mathrm{NaAR}(\%) = \frac{NO_3^- - N}{[NO_2^-] + [NO_3^- - N]} \quad (3)$$

The nitrogen consumption and free ammonia concentration were calculated according to the following equation (Eq. (4) and (5)).

$$\mathrm{NC}(\mathrm{mg/L}) = [NH_4^+—N]_{\mathrm{influent}} - \{[NH_4^+—N] + [NO_2^- - N] + [NO_3^- - N]\}_{\mathrm{effluent}} \quad (4)$$

$$\mathrm{FA}(\mathrm{mg/L}) = \frac{17}{14} \times \frac{[NH_4^+—N] \times 10^{\mathrm{pH}}}{\exp\left(\frac{6\,334}{273+T}\right)} + 10^{\mathrm{pH}} \quad (5)$$

3 Results and discussion

3.1 Performance of sequencing batch reactors under different concentrations of free ammonia

The NH_4^+—N concentration in the influent and effluent under four different concentrations of free ammonia are shown in Fig. 1(a). Although the influent NH_4^+—N concentration was controlled at 30.7～45.3 mg/L($R_{0.5}$), 76.0～99.2 mg/L(R_5), 112.4～139.4 mg/L(R_{10}) and 42.1～60.9 mg/L (R_{15}), respectively, the effluent NH_4^+—N concentration was lower than 1.2 mg/L with NH_4^+—N removal efficiency maintained at 97.6%～99.4%. Hence, the present results show that the increase of free ammonia and NH_4^+—N concentration had no adverse effect on the nitrogen removal of the systems. Xu et al.[20] reported that free ammonia pretreatment (below 44.5 mg/L) can improve NH_4^+—N release, and thereby enhance NH_4^+—N removal.

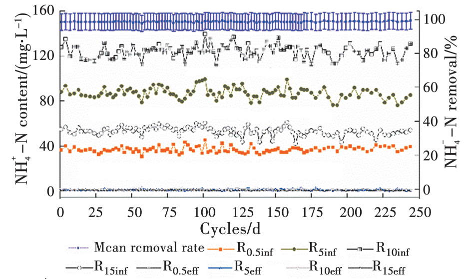
Fig. 1(b) and (c) show the concentration and variation of NO_2^- — N and NO_3^- — N during the whole reaction cycle under different concentrations of free ammonia. The oxidization of NH_4^+—N to NO_2^- — N was mainly achieved at R_{10} and R_{15} with the nitrite accumulation rate stable in 96.6%～99.3% (since 80 cycles), which shows that partial nitrification was achieved at R_{10} and R_{15} (NH_4^+—N →NO_2^- — N→N_2). NH_4^+—N convert to NO_3^- — N was achieved at $R_{0.5}$ and R_5 with the nitrite accumulation lower than 6.3% during the whole reaction, suggesting that $R_{0.5}$ and R_5 were during the full nitrification process (NH_4^+—N→NO_2^- — N

$\rightarrow NO_3^- - N \rightarrow NO_2^- - N \rightarrow N_2$). This shows that high free ammonia concentration was beneficial to the formation of partial nitrification. Liu et al.[21] found that higher free ammonia concentration was conducive to the realization of partial nitrification as the free ammonia exerted a stronger inhibition on nitrite oxidation than on ammonium oxidation.

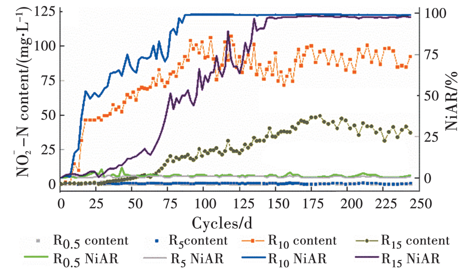
The correlation between free ammonia concentration and nitrogen consumption during the nitrification process was studied (data not shown). Nitrogen consumption increased from 7. 0 mg/L at 0. 5 mg/L free ammonia to 31. 5 mg/L at 10 mg/L free ammonia, and then subsequently decreased to 14. 2 mg/L at 15 mg/L free ammonia, which indicated that high nitrogen removal efficiency was available through added free ammonia concentration. Wang et al.[22] found that the nitrogen removal was significantly improved after free ammonia pretreatment in sequencing batch reactors treating synthetic wastewater.

3. 2 Production of three kinds of extracellular polymeric substance and their components under different concentrations of free ammonia

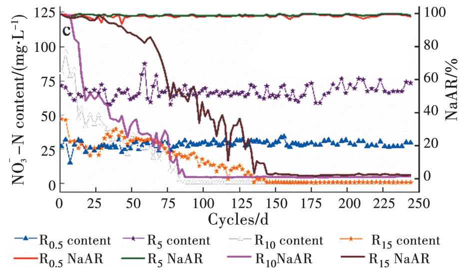
Fig. 2 shows the comparison of the total contents of extracellular polymeric substance, tightly bound extracellular polymeric substance and loosely bound extracellular polymeric substance under different concentrations of free ammonia. Although the contents of total extracellular polymeric substance, tightly bound extracellular polymeric substance and loosely bound extracellular polymeric substance all showed upward trend with the free ammonia concentrations increased from 0. 5 to 10 mg/L, they all showed downward trend when free ammonia concentrations continue increased to 15 mg/L, indicating that the free ammonia concentration was certainly correlated with extracellular polymeric substance production. The mechanism of this phenomenon was that microorganism would produce amount of extracellular polymeric substance to ensure microorganism can survival in toxic environmental condition. However, the contents of total extracellular polymeric substance, tightly bound extracellular polymeric substance and loosely bound extracellular polymeric substance all decreased at 15 mg/L free ammonia by comparison with the 10 mg/L of free ammonia, which showed that the auto-protection ability of the microorganism was limited, so that the higher free ammonia concentrations can cause cell inactivation due to the biocidal impact of free ammonia, which triggers the reduction of metabolites, resulting in extracellular polymeric substance reduction.



(a) NH$_4^+$-N concentration and removal efficiency Plastic strain



(b) NO$_2^-$-N concentration and NiAR values



(c) NO$_3^-$-N concentration and NaAR values

Fig. 1 Long-term performance of SBR system under four different concentrations of free ammonia

Fig. 3 (a), (b) and (c) show the effects of different concentrations of free ammonia on proteins, polysaccharides and DNA in total extracellular polymeric substance, tightly bound extracellular polymeric substance and loosely bound extracellular polymeric substance. The

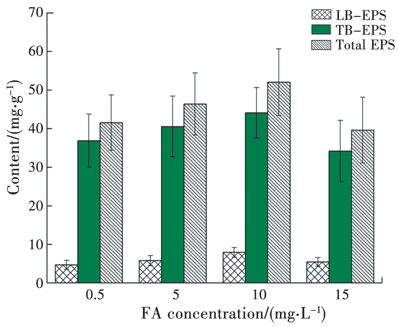


Fig. 2 Effect of the FA concentrations of the influent on the contents of LB-EPS, TB-EPS and EPS

protein and polysaccharide contents in the three fractions of extracellular polymeric substance all showed an upward trend as the free ammonia concentrations was increased from 0.5 to 10 mg/L. However, when the free ammonia concentrations further increased to 15 mg/L, the protein and polysaccharide contents in the three fractions extracellular polymeric substance all showed a downward trend. The DNA content gradually increased as the free ammonia increased, which shows the concentration of free ammonia can cause cell inactivation. Generally, the contents of the three kinds of extracellular polymeric substances and their components increased as the free ammonia concentration increased, triggered by the denser layers of loosely bound extracellular polymeric substance and tightly bound extracellular polymeric substance, which can stop free ammonia from diffusing into the interior of the activated sludge and reduce the free ammonia toxicity to bacteria[23].

Fig. 3(d) shows the percentage of the protein polysaccharide and DNA contents in the three assessed extracellular polymeric substance fractions under four different free ammonia conditions. It can be seen that polysaccharides accounted for between 47.1% and 55.8% of the tightly bound extracellular polymeric substance and total extracellular polymeric substance under four concentrations of free ammonia, followed by proteins (39.8%～46.2%) and DNA (4.2%～7.1%), indicating that polysaccharides were the main component at these four free ammonia conditions. Especially, the protein contents and DNA of the tightly bound extracellular polymeric substance and total extracellular polymeric substance was augmented, while the polysaccharide content decreased as the concentration of free ammonia increased from 0.5 to 15 mg/L.

However, a discrepancy was observed in loosely bound extracellular polymeric substance. Proteins were the dominant component of the loosely bound extracellular polymeric substance (44.3%～64.2%), while polysaccharides was the second largest component (32.8%～53.3%) and DNA was the smallest component (2.4%～3.1%). Furthermore, we found that the percentage of polysaccharides of the loosely bound extracellular polymeric substance was augmented with the increase in the concentration of free ammonia from 0.5 to 10 mg/L, while the percentage of polysaccharides decreased when the concentration of free ammonia was increased to 15 mg/L. An opposite trend was observed in the percentage of proteins of the loosely bound extracellular polymeric substances.

3.3 Variation of extracellular polymeric substance and nitrogen in a typical sequencing batch reactor cycle under different concentrations of free ammonia

To better evaluate the reactor performance, a typical sequencing batch reactors cycle (201 cycle) was examined during nitrification and dentrification under four different concentrations of free ammonia (Fig. 4). Three kinds of extracellular polymeric substance and their components showed an upward trend during the nitrification process, then a downward trend during the denitrification process under the four different concentrations of free ammonia, except for the DNA content. The trend of the total extracellular polymeric substance and its components was negatively correlated with the NH_4^+—N value ($R = -0.787$ to -0.856, $p<0.05$) in the four kinds of free ammonia

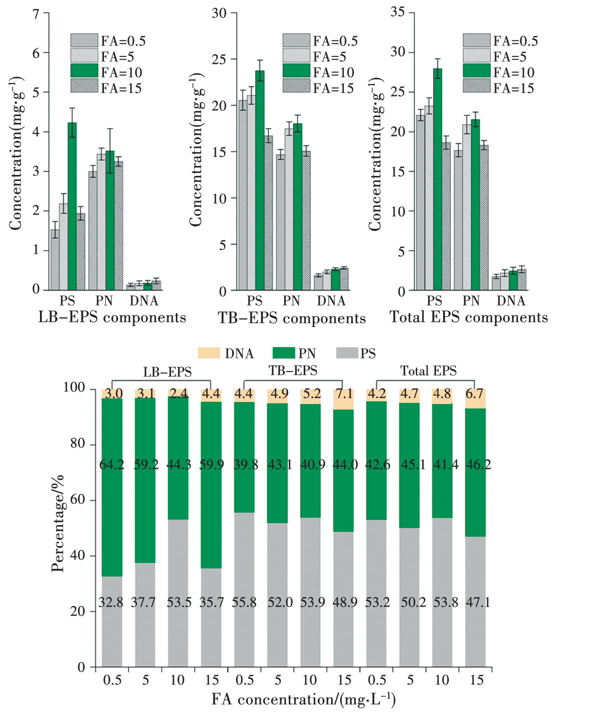


Fig. 3 Effect of the FA concentrations of the influent on the contents of DNA, PS and PN ina) LB-EPS b) TB-EPS c) total EPS d) Effect of the FA concentrations on the percentage of DAN, PS and PN in the three different EPS fractions

conditions during the nitrification process, but was positively correlated with the NO_3^-—N value (R=0.645 to 0.699, $p<0.05$) in $R_{0.5}$ and R_5 and the NO_2^-—N value (R=0.654 to 0.701, $p<0.05$) in R_{10} and R_{15} during the whole reaction cycle, suggesting that certain correlations were stored intracellularly. Sheng et al.[1] believed that the negatively charged proteins are always binding with the positively charged NH_4^+—N through electrostatic interaction. Hence, more proteins were produced to provide sites for the adsorption of NH_4^+—N. Then the NH_4^-—N was further degraded by nitrifying bacteria, resulting in the increase in NO_x^-—N and the protein content during the nitrification process, while the polysaccharide content was reduced during the subsequent denitrification process. The main reason for this was that the extracellular polymeric substance served as an energy source and was utilized by denitrifying bacteria to perform denitrification, which led to the reduction of NO_x^-—N and the total extracellular polymeric substance and its components[24-25].

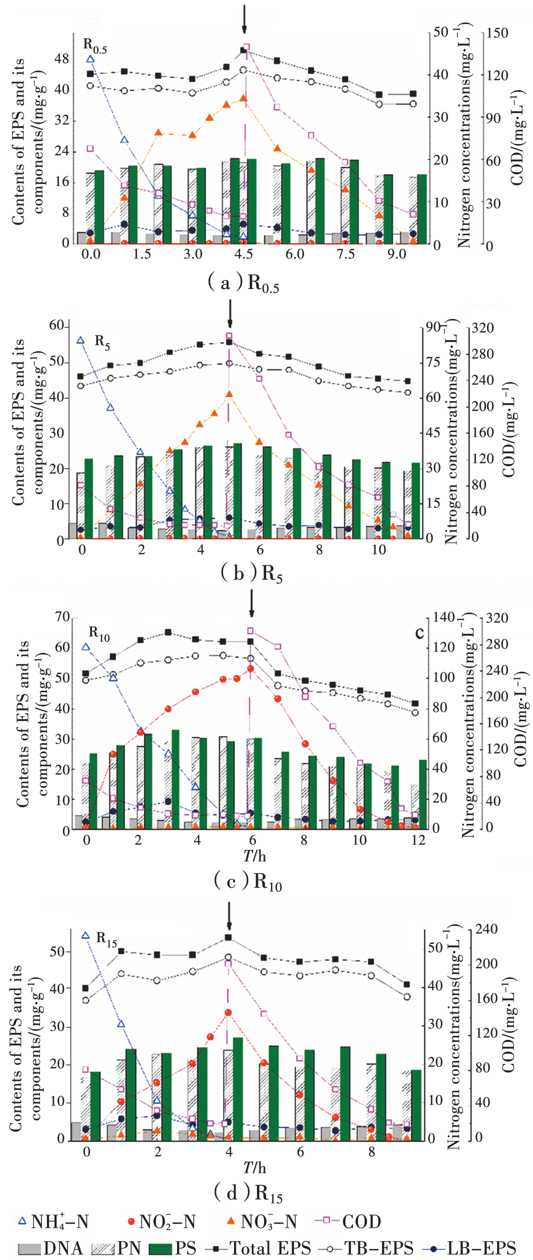


Fig. 4 Variation between nitrogen and EPS and its components during a typical cycle under four different concentrations of FA

4 Conclusions

This paper has presented a comparative study of three kinds of extracellular polymeric substances and their components, as well as the nitrogen removal performance of the influent at different concentrations of free ammonia. The following conclusions may be drawn from this study:

1) Free ammonia concentration at less than 15

mg/L had no obvious effect on the nitrogen removal performance of the sequencing batch reactors.

2) Free ammonia promoted the production of loosely bound extracellular polymeric substance, tightly bound extracellular polymeric substance and their components when its concentration increased from 0.5 to 10 mg/L. However, an opposite result was observed when its concentration was increased to 15 mg/L.

3) Polysaccharides dominated in the tightly bound extracellular polymeric substance and total extracellular polymeric substance fractions, while proteins dominated in the loosely bound extracellular polymeric substance fraction under four different concentrations of free ammonia.

4) The variation of three kinds of extracellular polymeric substance and their components was completely opposite the variation of NH_4^+—N during nitrification, but it was consistent with the variation of NO_x^-—N during the whole reaction cycle under the four different concentrations of free ammonia.

Acknowledgements

The authors would like to acknowledge the financial support from the National Natural Science Foundation of China (Grant No. 51668031).

References:

[1] SHENG G P, YU H Q, LI X Y. Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: A review [J]. Biotechnology Advances, 2010, 28(6): 882-894.

[2] SHI Y H, HUANG J H, ZENG G M, et al. Exploiting extracellular polymeric substances (EPS) controlling strategies for performance enhancement of biological wastewater treatments: An overview [J]. Chemosphere, 2017, 180: 396-411.

[3] ANTHONISEN A C, LOEHR R C, PRAKASAM T B, et al. Inhibition of nitrification by ammonia and nitrous acid [J]. Journal-Water Pollution Control Federation, 1976, 48(5): 835-852.

[4] CHUNG J, SHIM H, LEE Y W, et al. Comparison of influence of free ammonia and Dissolved oxygen on nitrite accumulation between suspended and attached cells [J]. Environmental Technology, 2005, 26(1): 21-33.

[5] YANG G J, XU Q X, WANG D B, et al. Free ammonia-based sludge treatment reduces sludge production in the wastewater treatment process [J]. Chemosphere, 2018, 205: 484-492.

[6] AKTAN C K, YAPSAKLI K, MERTOGLU B. Inhibitory effects of free ammonia on Anammox bacteria [J]. Biodegradation, 2012, 23(5): 751-762.

[7] CALLI B, MERTOGLU B, INANC B, et al. Effects of high free ammonia concentrations on the performances of anaerobic bioreactors [J]. Process Biochemistry, 2005, 40(3/4): 1285-1292.

[8] ZHANG C, QIN Y G, XU Q X, et al. Free ammonia-based pretreatment promotes short-chain fatty acid production from waste activated sludge [J]. ACS Sustainable Chemistry & Engineering, 2018, 6(7): 9120-9129.

[9] BASUVARAJ M, FEIN J, LISS S N. Protein and polysaccharide content of tightly and loosely bound extracellular polymeric substances and the development of a granular activated sludge floc [J]. Water Research, 2015, 82: 104-117.

[10] SHAO Y X, ZHANG H X, BUCHANAN I, et al. Comparison of extracellular polymeric substance (EPS) in nitrification and nitritation bioreactors [J]. International Biodeterioration & Biodegradation, 2019, 143: 104713.

[11] HAN X M, ZHOU Z, MEI X J, et al. Influence of fermentation liquid from waste activated sludge on anoxic/oxic- membrane bioreactor performance: Nitrogen removal, membrane fouling and microbial community [J]. Bioresource Technology, 2018, 250: 699-707.

[12] DASGUPTA S, DE CLIPPELEIR H, GOEL R. Short operational differences support granulation in a lab scale reactor in comparison to another conventional activated sludge reactor [J]. Bioresource Technology, 2019, 271: 417-426.

[13] ZENG T T, RENE E R, ZHANG S Q, et al. Removal of selenate and cadmium by anaerobic granular sludge: EPS characterization and microbial community analysis [J]. Process Safety and Environmental Protection, 2019, 126: 150-159.

[14] LI X Y, YANG S F. Influence of loosely bound extracellular polymeric substances (EPS) on the flocculation, sedimentation and dewaterability of

activated sludge [J]. Water Research, 2007, 41(5): 1022-1030.

[15] CAO Z P, ZHANG H W, ZHANG J L. Effects of sludge retention time on the sludge characteristics and membrane fouling in membrane bioreactor [J]. China Environmental Science, 2009, 29(4): 386-390.

[16] FRØLUND B, PALMGREN R, KEIDING K, et al. Extraction of extracellular polymers from activated sludge using a cation exchange resin [J]. Water Research, 1996, 30(8): 1749-1758.

[17] MASUKO T, MINAMI A, IWASAKI N, et al. Carbohydrate analysis by a phenol-sulfuric acid method in microplate format [J]. Analytical Biochemistry, 2005, 339(1): 69-72.

[18] MENGISTU Y, EDWARDS C, SAUNDERS J R. Continuous culture studies on the synthesis of capsular polysaccharide by Klebsiella pneumoniae K1 [J]. The Journal of Applied Bacteriology, 1994, 76(5): 424-430.

[19] APHA. Standard methods for the examination of water and wastewater [S]. Twentieth ed. American Public Health Association, Washington, DC, USA, 1998.

[20] XU Q X, LIU X R, WANG D B, et al. Free ammonia-based pretreatment enhances phosphorus release and recovery from waste activated sludge [J]. Chemosphere, 2018, 213: 276-284.

[21] LIU Y W, NGO HH, GUO W S, et al. The roles of free ammonia (FA) in biological wastewater treatment processes: A review [J]. Environment International, 2019, 123: 10-19.

[22] WANG Q L, DUAN H R, WEI W, et al. Achieving stable mainstream nitrogen removal via the nitrite pathway by sludge treatment using free ammonia [J]. Environmental Science & Technology, 2017, 51(17): 9800-9807.

[23] MA B R, LI Z W, WANG S, et al. Insights into the effect of nickel (Ni(II)) on the performance, microbial enzymatic activity and extracellular polymeric substances of activated sludge [J]. Environmental Pollution, 2019, 251: 81-89.

[24] MORGAN J W, FORSTER C F, EVISON L. A comparative study of the nature of biopolymers extracted from anaerobic and activated sludges [J]. Water Research, 1990, 24(6): 743-750.

[25] HOA P T, NAIR L, VISVANATHAN C. The effect of nutrients on extracellular polymeric substance production and its influence on sludge properties [J]. Water SA, 2004, 29(4): 437-442.

(编辑　胡英奎)