Characteristic on pollutant removal and extracellular polymeric substances distribution in different periods of an up-flow biological aerated filter

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Abstract. The up-flow biological aerated filter (UBAF) reactor was established to explore the pollutant removal and the extracellular polymeric substances (EPS) distribution at the initial stage of biofilm, maturity of biofilm, and stable operation periods. The parallel factor (PARAFAC) model was applied to analyze the three-dimensional fluorescence characteristics of EPS. Canonical correlation analysis (CCA) was used to study the correlation between the distribution of EPS and pollutant removal. The results showed that the removal efficiency of chemical oxygen demand (COD), ammonia nitrogen (NH₄⁺-N), and total phosphorus (TP) at the maturity of biofilm were higher than other periods, and reached to 95.68%±0.87%, 73.48%±4.32%, and 63.80%±14.27%, respectively. The operation period had a significant correlation with EPS distribution. All of biofilm samples detected four kinds of fluorescent substance of tryptophan-like (C1), protein-like (C2), humic acid (C3), and humic acids-like (C4). The CCA result showed that NH₄⁺-N removal had a very significant positive correlation with EPS concentration and composition (R≥0.9261).

1. Introduction

The BAF process was an effective biological treatment technology and widely applied in municipal wastewater secondary treatment and industrial wastewater treatment [1]. It had the advantages of compact structure, simple operation, low investment, simultaneous nitrogen and phosphorus removal, easy management, lack of sludge bulking issues, etc. [2]. The BAF process mainly used the growth and metabolism of the biofilm on the filter media surface to achieve the pollutant removal. Thus, the biofilm properties were the main factors in the exchange of materials and information, which directly determined the processing efficiency and operating conditions of the BAF process.

EPS was a complex polymer of macromolecules secreted by microorganisms, which was produced in the process of cell lysis and hydrolysis [3]. The main components included polysaccharide (PS), protein (PN) and DNA. It had an important influence on flocculation, structural morphology, dehydration, adsorption capacity, biodegradation and membrane fouling of microbial flocs in wastewater treatment [4]. In biofilm process, EPS could help to immobilize the cells on the filter media surface and to facilitate the spatial arrangement of different species within a biofilm [5]. Therefore, the study of EPS was of great value in mastering biofilm properties.

EPS could affect the wastewater treatment efficiency and microbial community [6]. These studies mainly focused on the composition and distribution of EPS [4], three-dimensional fluorescence
characteristics and the influence of environmental factors on EPS concentration. However, few researchers explored the relationship between EPS characteristics and pollutant removal in wastewater treatment. In addition, the previous studies mainly focused on the pollutant removal efficiency, structural optimization [7], and microbial community structure of biofilm [8]. Few studies reported the characteristics of EPS and pollutant removal at different period in the BAF reactor. These researches could help to further understand the mechanism of pollutant removal.

Therefore, in this study, we aimed to analyze the pollutant removal efficiency, the component distribution EPS, and three-dimensional fluorescence characteristics of EPS in the UBAF reactor. The PARAFAC model was applied to analyze of three-dimensional fluorescence data. Furthermore, the statistical analysis was used to explore the correlation between EPS distribution characteristics and pollutant removal efficiency in different periods of the UBAF reactor.

2. Materials and method

2.1. Experimental setup

The schematic of UBAF reactor was shown in figure 1. The reactor mainly consisted of the reactor body, air compressor, water tank, pump, filter media, air (water) plate, support layer. The reactor body was made of plexiglass with a diameter of 0.350 m, a wall thickness of 0.01 m and a height of 2 m. The reaction zone and the water distribution zone were separated by a uniform water (gas) plate. The plate thickness was 0.02 m. Its surface evenly distributed 25 holes (diameter of 0.006 m). The support layer with a thickness of 0.2 m was consisted of pebbles with a diameter of 0.03 m–0.05 m. The filter media layer was composed of ceramsite with a diameter of 0.003 m–0.005 m.

Figure 1. Schematic diagram of up-flow BAF reactor.

The wastewater and air entered from the bottom of the reactor, which were evenly distributed to the filter media layer through the uniform water distribution plate. The biofilm on the ceramsite surface utilized the pollutant of wastewater and achieved the pollutant removal under aerobic conditions. The wastewater was introduced in the reactor using mechanical diaphragm pump (GM0090PR9(6)MNN). Silent oil-free air compressor (LJ-1530E) was applied to blow the air into the reactor. The flow rate of aeration, wastewater, and backwash water were adjusted by glass rotor flowmeter.

2.2. Reactor start-up and operation

The inoculated sludge was taken from the remaining sludge return well of the third wastewater treatment plant in Xi’an, China. Next, the inoculated sludge was domesticated using the synthetic wastewater of C: N: P=100:5:1. The rapid method of activated sludge was applied the biological inoculation of reactor start-up. The UBAF operation was consisted of the reactor start-up, biofilm culture, and simulation experiment. The parameters of synthetic wastewater (Table 1) were consistent with the previous research [9]. The experiment used glucose as the carbon source, ammonium chloride.
(NH₄Cl) and potassium nitrate (KNO₃) as the nitrogen source, tripotassium phosphate trihydrate (K₃PO₄·3H₂O) as the phosphorus source, calcium chloride (CaCl₂, 20 mg·L⁻¹), magnesium sulfate pentahydrate (MgSO₄·5H₂O, 25 mg·L⁻¹), sodium bicarbonate (NaHCO₃, 110 mg·L⁻¹), ferric chloride (FeCl₃, 1.5 mg·L⁻¹) as a trace element. The frequency of BAF backwashing was a week to restore the treatment efficiency of the reactor.

Table 1. Characteristics of the synthetic wastewater.

| Parameters   | Biofilm culture phases | Simulation experiment phases |
|--------------|------------------------|------------------------------|
| COD (mg/L)   | 300                    | 200-300                      |
| NH₄⁺-N (mg/L)| 13                     | 30-40                        |
| NO₃⁻-N (mg/L)| 2                      | 4-8                          |
| TP (mg/L)    | 3                      | 4-6                          |
| Ca²⁺ (mg/L)  | 20                     | 20                           |
| Mg²⁺ (mg/L)  | 25                     | 25                           |
| Fe³⁺ (mg/L)  | 1.5                    | 1.5                          |
| Na⁺ (mg/L)   | 110                    | 110                          |
| pH           | 7-9                    | 7-9                          |

2.3. Samples collection

Wastewater samples were collected from the outlet of the UBAF reactor. Pollutant removal efficiency from the reactor startup to the stable operation (60 days) was analyzed. All of samples were detected within 1-2 days. In addition, biofilm samples in the reactor operation of 4d, 30d, and 60d were collected, representing the sample of the beginning of startup (RS-4), the biofilm maturity (RS-30), and the stable operation period (RS-60). Next, the EPS in biofilm samples was extracted using heat extraction method.

2.4. Chemical parameters analysis

COD was analyzed using the standard potassium dichromate method (HJ/T 399-2007) with a Speed Digester (DRB200, HACH, New York, NY, USA). NH₄⁺-N was analyzed using the Nessler reagent spectrophotometry (HJ 535-2009). Ammonium molybdate spectrophotometric method (GB 11893-89) was applied to analyze the TP concentration. Total suspended solids (TSS) and volatile organic compounds (VSS) in biofilm were determined by gravimetric analysis.

2.5. EPS extraction and analysis

Xu et al [10] indicated that heat extraction had high efficiency, did not need to introduce foreign substances, and had no effect on the subsequent analysis of EPS. Thus, in this study, heat extraction was applied and optimized in our previous studies [11]. The result showed that the optimum loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) extraction contents were obtained by 0.5% and 0.05% sodium chloride solution respectively at the extraction temperature of 70°C and heat extraction time of 40 min. Detailed extraction steps were shown in our previous studies.

The EPS contents was analyzed according to the previous [11] studies. PN, PS, and DNA were determined by the modified Lowry method, Anthrone method, and diphenylamine colorimetric method, respectively. The total suspended solids (TSSs) and volatile suspended solids (VSS) in biofilm were measured following the standard methods [11]. Its concentrations (mg·g⁻¹·VSS⁻¹) were expressed in amount of substance per gram VSS of PN, PS, and DNA.

2.6. Fluorescence EEM measurement

The FluoroMax-4 was used for analyzing the fluorescent substance of EPS samples. Sheng and Yu [3] indicated that the fluorescence substance mainly included protein-like, humic acid, and fulvic acid. In this study, EEM spectra were gathered with scanning emission (Em) spectra from 290 nm to 550 nm at 2 nm increments. The excitation (Ex) wavelength was from 240 nm to 450 nm at 2 nm increments. In
addition, slit width is 3 nm for all samples.

2.7. **PARAFAC modeling analyse**

PARAFAC modeling was applied to analyze the EEM fluorescence data based on the DOMFluor/PARAFAC toolbox using the Matlab R2014a (Mathworks, Natick, MA) [12]. Analysis step referred the Stedmon and Bro [12] studies. The results of residual analysis and visual inspection found that the four-component model is suitable for further analysis of the EPS samples [13].

2.8. **Statistical analyses**

The Pearson correlation analysis in SPSS 22.0 software (SPSS Inc., USA) was applied to study the correlation between EPS content, fluorescence intensity and operating period. p<0.05(*) and p<0.01(**) indicate the level of correlation, where p<0.01 indicated a very significant correlation between the data and p<0.05 indicated a significant correlation between the data.

The CANOCO 4.5 software was applied to canonical correlation analysis (CCA) between the EPS contents, EPS fluorescence intensity, and pollutant removal. The analytical method was used by Li et al [14] and Licursi et al [15]. Firstly, the date was analyzed using the detrended correspondence analysis (DCA). Each sorting axis had a Lengths of gradient greater than 3. The analysis selected a single peak model for CCA. The arrows indicated environmental factors such as COD, NH\textsubscript{4}\textsuperscript+-N, and TP. The longer the length of the arrow, the greater the influence of the EPS content or the EPS fluorescence intensity [16]. The cosine of the angle between the arrow and the sorting axis indicated the correlation between the environmental factor and the sorting axis.

3. **Results and discussion**

3.1. **Analysis of pollutant removal in different period**

![Graphs showing pollutant removal efficiency over time](image-url)
Figure 2 showed the COD, NH$_4^+$-N and TP removal efficiency and the influent and effluent concentration in different operation periods of the BAF reactor. The COD, NH$_4^+$-N, and TP removal efficiency showed an increasing trend and then decreasing with the increase of operation period. However, COD removal efficiency had not a significant change in different period. The removal efficiency was above 80% at different conditions. Moreover, the highest removal reached 95.68%±0.87% at RS-30. The COD concentration in effluent was basically lower than the first-class A standard requirement (COD: 50 mg·L$^{-1}$) of the Pollutant Discharge Standard for Urban Wastewater Treatment Plants in China (GB18918-2002). The results showed that the BAF reactor had a higher removal efficiency of organic matter, which was consistent with previous studies [1].

The NH$_4^+$-N and TP removal efficiency at different periods was significantly different from the COD removal in the BAF reactor. NH$_4^+$-N removal showed an increasing trend and then a decreasing trend. The removal efficiency in RS-30 reached to 73.48%±4.32%. The distribution could be related to the growth conditions of biofilm. The decreasing removal of nitrogen in RS-60 was related to the lower carbon sources. TP removal presented similarity with the NH$_4^+$-N removal. The removal efficiency in RS-30 was 63.80%±14.27%. However, the removal in RS-60 showed a decreasing trend. In addition, TP removal showed a significant downward trend from the 18~27 day of operation and gradually increased after 27 d. The results were related to the BAF reactor backwash. The TP removal gradually recovered after the reactor was backwashed on 27 d.

3.2. EPS composition and distribution

![EPS composition and distribution graphs](image-url)
**Figure 3.** Distribution of PS, PN, and DNA in EPS.

Figure 3 showed the distribution of EPS component in different period of the BAF reactor. T-EPS indicated the total EPS content of each component. PS, PN, and DNA concentrations showed similar distribution in different EPS layers. The PN concentration of EPS was highest than PS and DNA concentrations. When the operation period was RS-60, the highest concentration of PN was 152.55 mg·g⁻¹±1.38 mg·g⁻¹. In addition, the EPS concentration gradually increased from the outermost layer to the inner layer of the cell. The TB-EPS concentration was significantly higher than others. These results were consistent with previous research [4,15].

As for soluble EPS (SL-EPS) fraction, PS, PN, and DNA concentrations reached the highest in the operation period of RS-30. The distribution was similar to the change of pollutant removal. The result indicated that the EPS distribution was attributed to the difference of pollutant removal. For LB-EPS fraction, EPS concentration had a very significant and positive correlation (p<0.01) with the operation period. The PS, PN, and DNA concentrations in RS-60 were higher than other operation period, and were 20.34 mg·g⁻¹±0.07 mg·g⁻¹, 65.08 mg·g⁻¹±0.59 mg·g⁻¹ and 4.02 mg·g⁻¹±0.03 mg·g⁻¹, respectively. As the operation period of reactor increased, biofilm growth gradually matured. Extracellular cell secretion (such as EPS) was relatively high. For TB-EPS fraction, EPS content showed a decreasing trend and then increasing. The PS concentration in RS-60 was higher than others period. However, PN and DNA concentration in RS-4 were higher, and reached to 97.47 mg·g⁻¹±0.13 mg·g⁻¹ and 9.66 mg·g⁻¹±0.07 mg·g⁻¹, respectively. The result could be related to the structure of microbes in different periods and the surrounding environment. For T-EPS fraction, the PS and PN had a very significant positive correlation (p<0.01) with the operation period.

### 3.3. EEM fluorescence component determination

In the UBAF reactor, the PARAFAC model analysis result on the EEM date was shown in figure 4. The analysis showed that 4-component model was contributed to achieve half-dimension verification of all data. The four fluorescent component peaks in different operation period were C1 (Ex/Em=278/334), C2 (Ex/Em=290/346), C3 (Ex/Em=316/394), and C4 (Ex/Em=360/442(522)), respectively.

**Figure 4.** Contour plots, excitation, and emission loadings of the four components using the PARAFAC model.

Ziegmann et al [17] found that the fluorescence peak with excitation wavelength of 275 nm and emission wavelength of 340 nm was the tryptophan peak, which was closely related to microbial activity and produce. Sheng et al [3] and Murphy et al [18] found that the fluorescence peak
(Ex/Em=280 nm–285 nm/340 nm–350 nm) was protein-like peak. When the excitation wavelength and emission wavelength were 290 nm–310 nm and 370 nm–410 nm, respectively, the fluorescence peak represented the humilic acid peak [9]. The fluorescence peak of humic acid was an excitation wavelength of 339 nm–420 nm and an emission wavelength of 434 nm–520 nm [19]. Thus, the four fluorescent components substance were tryptophan-like (C1), protein-like (C2), humic acid (C3), and humic acids-like (C4).

3.4. Fluorescence intensity analysis of EPS components
The maximum fluorescence intensity distribution of the four components of SL-EPS, LB-EPS and TB-EPS was shown in figure 5. Tryptophan-like (C1) and protein-like (C2) were classified to the protein substance. The maximum fluorescence intensity of C1 and C2 in different EPS layers was significantly higher than others fluorescence components. The maximum fluorescence intensity of C1 in SL-EPS and TB-EPS was highest. The highest maximum fluorescence intensity in LB-EPS was C2. The result was similar with the distribution of EPS contents. Humilic acid (C3) and humic acids-like (C4) belonged to humic acid substance. The maximum fluorescence intensity of C3 and C4 in SL-EPS, LB-EPS, and TB-EPS was significantly lower than the maximum fluorescence intensity of C1 and C2. The result could be contributed to the growth and metabolism of biofilm. In addition, the maximum fluorescence intensity of C3 and C4 showed an increasing trend from SL-EPS to TB-EPS. TB-EPS was linked to the microbial cells. Some dead microorganisms or cell secretions were easily accumulated in TB-EPS layer.

![Figure 5. Maximum fluorescence intensities of EPS components.](image)

As for same EPS fraction, the maximum fluorescence intensity of four components showed the different distribution in different operation period. For SL-EPS fraction, the maximum fluorescence intensity of C1 and C2 in RS-30 was higher than in others operation period. The result in section of pollutant removal found that pollutant achieved a higher pollutant removal in RS-30. These results showed that C1 and C2 might be related to the pollutant removal. For LB-EPS, the maximum fluorescence intensity of four components in RS-4 was lower than others operation period. Because biofilm in the operation period of RS-4 was not formed. The characteristics of sludge flocs and activated sludge were basically similar. When the operation period gradually increased, the characteristics of sludge flocs was significantly different with RS-4 operation period. For TB-EPS fraction, the maximum fluorescence intensity of four components fluorescence substance was significantly higher than others fraction. In addition, C1 substance was not detected in RS-60. The result might be related to the operation condition of the UBAF reactor.

3.5. The CCA analysis between pollutant removal and EPS distribution
Figure 6 showed the CCA analysis results between pollutant removal, EPS contents, and maximum
fluorescence component intensity of EPS in the UBAF reactor. In figure 6(a), COD and TP had a significant negative correlation with the first sorting axis. The correlation coefficient (R) was -0.8854 and -0.8912, respectively. However, there was a significant positive correlation (R=0.9261) between NH$_4^+$-N and second sorting axis. COD, NH$_4^+$-N, and TP had a significant positive correlation (0<R<0.9988) with the second sorting axis in figure 6(b). Moreover, the correlation coefficient (R=0.9988) between NH$_4^+$-N removal and second sorting axis was highest than others. There was basically no correlation (R=-0.0492) between NH$_4^+$-N removal and the first sorting axis. The results showed that NH$_4^+$-N had a great effect on the EPS contents and fluorescence component intensity.

![Figure 6. CCA sequence between pollutants removal, concentrations and fluorescent component of EPS: SL, LB, TB, and T represented the SL-EPS, LB-EPS, TB-EPS, and T-EPS. (a) Pollutant removal and EPS concentration and (b) Pollutant removal and fluorescence component intensity.](image)

As for EPS contents and fluorescence component intensity, COD had a significant positive correlation with TP (R=0.9999) and NH$_4^+$-N (R=0.7756). The result might be mainly related to the removal mechanism of nitrogen and phosphorus. According to different fluorescent component, the absolute abundance between C1, C2, and pollutant in SL-EPS and LB-EPS was significantly higher than other components. The distribution further confirmed that PN might play an important role in pollutant removal. In addition, the absolute abundance of the three operation periods of UBAF reactor and the pollutant factor was RS-30>RS-60>RS-4. The result verified the conclusions obtained previously.

4. Conclusion
Our study demonstrated the characteristic on pollutant removal and EPS distribution in different periods of the UBAF reactor. The operation period significantly affected the of COD, NH$_4^+$-N, and TP removal efficiency and EPS concentration. In addition, the fluorescence substance of protein-like (C1 and C2) and humic acids-like (C3 and C4) were detected in all of biofilm samples. CCA analysis indicated that NH$_4^+$-N showed a very significant positive correlation (R>0.9261) between EPS content and fluorescence intensity. However, in this study, the correlation of pollutant removal, EPS contents and fluorescence substance remained unclear. The specific role of EPS components in wastewater treatment was still not determined. Therefore, future studies should further explore the relationship between EPS and wastewater treatment to reveal the mechanism of pollutant removal in the UBAF reactor.

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References

[1] Yu Q, Huang H, Ren H, Ding L and Geng J 2016 In situ activity recovery of aging biofilm in biological aerated filter: Surfactants treatment and mechanisms study Bioresource Technol. 219 403-10

[2] Pramanik B K, Fatihah S, Shahrom Z and Ahmed E 2012 Biological aerated filters (BAFs) for carbon and nitrogen removal: A review J. Eng. Sci. Technol. 7 428-46

[3] Sheng G and Yu H 2006 Characterization of extracellular polymeric substances of aerobic and anaerobic sludge using three-dimensional excitation and emission matrix fluorescence spectroscopy Water Res. 40 1233-9

[4] Wang B, Liu X, Chen J, Peng D and He F 2018 Composition and functional group characterization of extracellular polymeric substances (EPS) in activated sludge: The impacts of polymerization degree of proteinaceous substrates Water Res. 129 133-42

[5] Abu Bakar S N H, Hasan H A, Mohammad A W, Abdullah S R S, Haan T Y, Ngteni R and Mohamed Yusof K M 2018 A review of moving-bed biofilm reactor technology for palm oil mill effluent treatment J. Clean. Prod. 171 1532-45

[6] Xu H, Cai H, Yu G and Jiang H 2013 Insights into extracellular polymeric substances of cyanobacterium Microcystis aeruginosa using fractionation procedure and parallel factor analysis Water Res. 47 2005-14

[7] Chen T, Peng T, Feng C, Chen N, Hu Q and Hao C 2016 The feasibility of an up-flow partially aerated biological filter (U-PABF) for nitrogen and COD removal from domestic wastewater Bioresource Technol. 218 307-17

[8] Yang K, Yue Q, Kong J, Zhao P, Gao Y, Fu K and Gao B 2016 Microbial diversity in combined UAF-UBAF system with novel sludge and coal cinder ceramic fillers for tetracycline wastewater treatment Chem. Eng. J. 285 319-30

[9] Yu H, Qu F, Sun L, Liang H, Han Z, Chang H, Shao S and Li G 2015 Relationship between soluble microbial products (SMP) and effluent organic matter (EfOM): Characterized by fluorescence excitation emission matrix coupled with parallel factor analysis Chemosphere 121 101-9

[10] Ren J, Cheng W, Wan T, Wang M and Zhang X 2018 Effect of buffer salinity on heat extraction of extracellular polymeric substances from activated sludge Acta Scientiae Cirumstantiae 38 3054-60 (In Chinese)

[11] Yu G, He P and Shao L 2009 Characteristics of extracellular polymeric substances (EPS) fractions from excess sludges and their effects on bioflocculability Bioresource Technol. 100 3193-8

[12] Stedmon C A and Bro R 2008 Characterizing dissolved organic matter fluorescence with parallel factor analysis: A tutorial Limno. Oceanogr. Meth. 6 572-9

[13] Liu L, Huang Q, Zhang Y, Qin B and Zhu G 2017 Excitation-emission matrix fluorescence and parallel factor analyses of the effects of N and P nutrients on the extracellular polymeric substances of Microcystis aeruginosa Limnologica 63 18-26

[14] Li X, He T and Gou M 2018 CCA analysis of phytoplankton community characteristics and environmental factors in Wuliangsuhai Lake during ice-season J. Northeast Agr. Univ. 49 67-78 (In Chinese)

[15] Licursi M, Sierra M V and Gómez N 2006 Diatom assemblages from a turbid coastal plain estuary: Río de la Plata (South America) J. Marine Syst. 62 35-45

[16] Yuan D and Wang Y 2012 Study on the stratification components of extracellular polymeric substances (EPS) in activated sludge and their variation characteristics in physicochemical properties Environ. Sci. 33 3522-8 (In Chinese)

[17] Ziegmann M, Abert M, Muller M and Frimmel F H 2010 Use of fluorescence fingerprints for the estimation of bloom formation and toxin production of Microcystis aeruginosa Water Res. 44 195-204

[18] Murphy K R, Hambly A, Singh S, Henderson R K, Baker Andy, Stuetz R and Khan S J 2011
Organic matter fluorescence in municipal water recycling schemes: Toward a unified PARAFAC model *Environ. Sci. Technol.* **45** 2909-16

[19] Ishii S K L and Boyer T H 2012 Behavior of reoccurring parafac components in fluorescent dissolved organic matter in natural and engineered systems: A critical review *Environ. Sci. Technol.* **46** 2006-17