Analysis of Three-dimensional Fluorescence Characteristics of Extracellular Polymeric Substances at Internal Points in MPR at Low Temperature

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Abstract. Through three-dimension excitation emission matrix (EEM) fluorescence spectroscopy and fluorescence regional integration (FRI), the content and composition changes of extracellular polymeric substances (EPS) in different regions in the Micro-Pressure Inner-Loop Bioreactor (MPR) were analyzed, and the law of EPS in spatial points was found. The analysis shows that the high excitation wavelength tryptophan-like Peak T and the low excitation wavelength tyrosine-like Peak B appear in the spectra of tightly-bounded EPS (TB-EPS) and loosely-bounded EPS (LB-EPS) at different points. The content of protein substances in EPS is relatively high. In addition, the unique flow pattern in the reactor forms a multi-oxygen zone, which causes the total amount of EPS and the content of various organic substances, especially protein substances, to increase from the center of the reactor to the outside.

1. Introduction

The micro-pressure internal circulation multi-biological phase reactor (MPR) is a new type of reactor with a multi-oxygen area inside. The activated sludge forms a circulation inside the reactor under the aeration, which can realize the simultaneous removal of pollutants[1]. Extracellular polymeric substances (EPS) is a macromolecular polymer mainly secreted by microbial cells. According to the different distribution positions of EPS outside the cell, it can be divided into dissolved EPS (SEPS) and loosely bound EPS (LB-EPS) And tightly bound EPS (TB-EPS) [2], which are composed of protein, polysaccharide, humus, nucleic acid, etc., have an important influence on the characteristics of activated sludge. Three-dimensional excitation emission matrix (EEM) fluorescence is a technology with high sensitivity, high selectivity, and high information content. It can be applied to the field of sewage treatment[3]. It can detect dissolved organic matter (DOM) in water without changing the structure of the sample. The composition and content analysis can also be applied to the composition analysis of EPS. Previous research on MPR has rarely involved internal spatial points and EPS. Under the low temperature (9°C) condition of this experiment, temperature has a significant effect on EPS content, and the flow of sludge mixture inside the reactor has uneven characteristics[4]. Therefore, this paper analyzes the EPS at different points in the reactor through EEM and fluorescence region integral (FRI), and finds the rules and characteristics of the EPS at the internal points.
2. Materials and Methods

2.1. Experimental Conditions

The sample was taken from the MPR reactor, and the water temperature was 9°C during operation. The reactor is made of plexiglass plate, the effective volume is 54L, and it is divided into upper and lower parts. The upper part is open, which raises the water level. The size is 500×80×60mm. The lower part of the main reaction zone is 800×600×110mm. The lower left part of the main reaction zone is connected with a perforated aeration pipe, and an air compressor is used for aeration. It adopts intermittent operation mode, and the specific operation process is: instantaneous water intake, aeration 7h, precipitation 4h, instant drainage, and idle 1h. The pH value is controlled between 8.0~8.5, and the dissolved oxygen is controlled at 3.5mg/L. The sludge samples taken in the experiment were sludge samples taken from eight different areas at 9°C and one hour after the start of aeration.

2.2. EPS Extraction

Take 30ml of sludge, centrifuge at 4000r for 5min, and discard the supernatant. Add 9ml of 0.05% NaCL solution and stir evenly, then take the 0.05% NaCL solution heated to 70℃ to make the volume to 30ml, and stir immediately with a glass rod. After centrifugation at 4000r for 10 min, the supernatant is LB-EPS. Use 0.05% NaCL solution to rest ore the remaining sludge to the initial volume of 30ml, stir well and heat it in a water bath at 60℃ for 30min, stir well, centrifuge at 4000r for 15min, and the supernatant is TB-EPS.

2.3. Sample Measurement

The three-dimensional fluorescence spectrum was measured by Hitachi F-7000 fluorescence spectrophotometer, the PMT voltage was 700V, the excitation wavelength scanning range of the spectrum was 200~460nm, the emission wavelength scanning range was 260~550nm, slit width Ex=5nm, Em=5nm, The scanning speed is 2400nm/min, and the blank in the experiment uses Milli-Q pure water. The extracted EPS samples were filtered through a 0.45μm filter membrane, and then subjected to three-dimensional fluorescence detection. The three-dimensional fluorescence spectrum is divided into five regions: Ex/Em=(200~250)nm/(280~330)nm is the fluorescence region of aromatic protein substance I, Ex/Em=(200~250)nm/(330~380)nm is the fluorescence area of aromatic protein substances II, Ex/Em=(200~250)nm/(380~550)nm is the fluorescence area of fulvic acid substances III, Ex/Em=(250~450)nm/(280~380)nm is the fluorescence zone of soluble microbial metabolites IV, and Ex/Em=(250~450)nm/(380~550)nm is the fluorescence zone of humic acid substance V. The area integration method (FRI) is used to analyze the three-dimensional fluorescence spectrum to obtain the integrated volume (Φi) of the five fluorescence regions, and then normalize to obtain the integrated standard volume (Φi,n).

3. Results and Discussion

3.1. Fluorescence Peak Analysis

According to the characteristics of the sludge inside the reactor, as shown in Figure 1, points 1-5, 7-5, 4-2, 4-8 are considered to be the aerobic zone points of the reactor, and points 3-5, 5-5, 4-4, 4-6 is the hypoxic zone. It can be seen from the spectrogram that there are protein substances, fulvic acid substances, soluble microbial metabolites, and humic acid substances in EPS, but the content of protein substances and soluble microbial metabolites is relatively high. There are two peaks in the TB-EPS spectrum, which are near Ex/Em=275~276nm/341~345nm and Ex/Em=222~223nm/331~335nm, which are high excitation wavelength tryptophan-like Peak T1 and low excitation The wavelengths of tyrosine-like Peak B are all protein-like fluorescence peaks. There are also two peaks in the LB-EPS spectrum, which are located near Ex/Em=276~277nm/335nm and Ex/Em=224~225nm/333~335nm, which are also high excitation wavelength tryptophan-like Peak T1 and low excitation Wavelength tyrosine Peak B. It shows that the ingredients in TB-EPS and LB-EPS are basically the same. By analyzing the intensity of the fluorescence peak at each point, it is found that the fluorescence intensity of the protein-like fluorescence peaks (Peak T1 and Peak B) in the outer aerobic zone of the reactor is
higher than that of the inner hypoxic zone of TB-EPS, while the fluorescence intensity of LB-EPS is not displayed. Out of the law.

Figure 1. Sampling point in MPR.

Figure 2. Three-dimensional fluorescence spectrum of LB-EPS.
3.2. Regional Integral Analysis

Analyze the contour map of LB-EPS of each point by area integration method, calculate the integrated standard volume $\Phi_{\text{in}}$ and find $\Phi_{\text{out}}$. From the data in the table 1, it can be seen that the integrated standard volume of points in the outer region is generally significantly higher than that in the inner region, and this feature is more obvious in the regions where there are protein-like fluorescence peaks (I, II, and IV). Related studies believe that dissolved oxygen has an effect on the secretion of extracellular polymer within a certain range, the higher the dissolved oxygen concentration, the higher the EPS content\[^7\]. Zhou J et al. compared EPS under different dissolved oxygen through experiments and found that as the dissolved oxygen increases, microorganisms produce more EPS through secretion and autolysis\[^8\]. This shows that a multi-oxygen zone is really formed inside the reactor, and the dissolved oxygen continues to rise from the center to the outside. Sufficient dissolved oxygen makes the microorganisms in the outer zone have stronger metabolic capacity than the microorganisms in the inner zone, thereby secreting more EPS. Especially protein substances.

Figure 3. Three-dimensional fluorescence spectrum of TB-EPS.

Figure 4. Fluorescence intensity of the fluorescence peaks of TB-EPS and LB-EPS.
Table 1. 3D-EEMs standard integral volume of LB-EPS.

| Point | \( \Phi_{i,n}(\text{au.nm}^2) \times 10^{-5} \) |
|-------|----------------------------------|
|       | I      | II     | III    | IV     | V     | \( \Phi_{i,n}(\text{au.nm}^2) \times 10^{-5} \) |
| Aerobic zone |       |        |        |        |       |        |
| 1-5   | 629.6  | 935.9  | 300.3  | 528.8  | 271.6  | 2666.2 |
| 7-5   | 729.7  | 938.9  | 406.0  | 746.3  | 382.2  | 3203.1 |
| 4-2   | 627.4  | 794.1  | 331.4  | 546.7  | 282.2  | 2626.8 |
| 4-8   | 659.1  | 806.4  | 358.3  | 573.6  | 288.8  | 2686.2 |
| Anoxic zone |       |        |        |        |       |        |
| 3-5   | 703.4  | 793.4  | 295.0  | 566.3  | 276.5  | 2634.6 |
| 5-5   | 590.0  | 698.1  | 270.4  | 478.2  | 252.9  | 2289.6 |
| 4-4   | 648.4  | 771.8  | 319.1  | 525.0  | 271.0  | 2535.3 |
| 4-6   | 607.1  | 700.7  | 347.0  | 474.0  | 244.6  | 2317.8 |

4. Conclusion

The analysis of EPS in different regions in MPR by three-dimensional fluorescence and area integration method reveals that aromatic protein substances, fulvic acids, soluble microbial metabolites and humic acids are present, among which tryptophan-like substances Two types of protein and tyrosine-like substances are important components of EPS, and the content of humic acid and fulvic acid is relatively low. Inside the reactor, an annular flow state of the multi-oxygen zone is formed, and the dissolved oxygen concentration increases from the inner layer to the outer layer, so that the total EPS content and the content of substances in each integral area increase from the inner layer to the outer layer.

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