Extraction method of EPS from the bio-enhanced PAC mixed liquor for municipal wastewater treatment

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Abstract
The optimum Extracellular polymeric substances (EPS) extraction method from bio-enhanced powder activated carbon (PAC) mixed liquor was investigated systematically in wastewater treatment process. Experimental results found that heat temperature influence the quantity of PS, PN and NA obviously, and PS, PN and NA, were all increased as the heated water temperature elevated; the ultrasonic and centrifugation could not extract the EPS from the bio-enhanced PAC mixed liquor completely. Different chemical extraction methods including the acid method, alkaline extraction, formaldehyde - NaOH extraction and EDTA extraction influenced the EPS extraction or test results strongly. The bio-enhanced PAC mixed liquor was fractionated through the optimum thermal and centrifugation extraction into five fractions: (1) supernatant; (2) slime; (3) loosely bound extracellular polymeric substances (LB-EPS); (4) tightly bound EPS (TB-EPS); and (5) pellets (PAC particle attached by microorganism cell). The method and parameters proposed in this research could be useful for understanding the characteristics of sludge EPS from the bio-enhanced PAC dynamic membrane reactor and other sludge EPS analysis.

Keywords
Extracellular polymeric substances; thermal extraction; centrifugation; fractioning protocol; the bio-enhanced PAC mixed liquor

INTRODUCTION
A dynamic membrane bioreactor has been developed in wastewater treatment in recent years. Dynamic membrane is formed on the underlying support mesh when filtering a solution containing fine particles (Chu, 2014). The dynamic membrane formed on the big pore mesh could increase the intrinsic membrane retention capacity, and reduce the membrane module cost and energy consumption. PAC was used as carriers of the microorganisms forming the bio-enhanced PAC mixed liquor to improve pollutants removal, and the mixed liquor deposited on the surface of stainless steel support module to form dynamic membrane to act as the mixed liquor separation. The EPS characteristics of the bio-enhanced PAC mixed liquor has not been investigated yet. This work aimed at determining the optimum EPS extraction methods of the bio-enhanced PAC mixed liquor in municipal wastewater treatment.

MATERIALS AND METHODS
EPS extraction methods of the bio-enhanced PAC mixed liquor
The optimum EPS extraction method of the bio-enhanced PAC mixed liquor was investigated. Firstly, 50 ml bio-enhanced PAC mixed liquor from the aerobic tank was sampled and settled for 2 h; the sediment was collected and re-suspended with distilled water to its original volume. Then, the following different processes were applied subsequently to extract EPS from bio-enhanced PAC mixed liquor, as shown in Fig. 1.

![Figure 1. Schematic diagram of EPS extraction methods](image-url)
RESULTS AND DISCUSSION

Stratification protocol of bio-enhanced PAC mixed liquor

There is no report about the EPS extraction protocol and stratification structure analysis for the bio-enhanced PAC mixed liquor, which is facilitate to indentify the factors that influence the dynamic membrane fouling. Based on the above mentioned extraction methods of EPS, the stratification of the bio-enhanced PAC sludge flocs was well-defined, as well as the extraction methods for each layer, as shown in Fig. 2.

In brief, the bio-enhanced PAC mixed liquor was allowed to settle for 2 h at 4 °C, after which the bulk solution, comprising the supernatant, was collected carefully. The sediments were resuspended with the buffer (0.9% NaCl solution) to the original volume, and then the mixed liquor was centrifuged at 3000 rpm for 15 min, whose bulk solution was collected as the slime (soluble/dissolved EPS). The bottom sediments were also re-suspended with the buffer (0.9% NaCl solution) to the original volumes, and centrifuged at 15000g for 15 min. The bulk solution was collected as LB-EPS. The bottom sediments were again re-suspended with the buffer (0.9% NaCl solution) to the original volumes, and then thermal extracted 15 min at 80 °C and centrifuged again (3000 rpm, 15 min). The centrifuged supernatant was TB-EPS, and the pellet (solid phase) were the PAC particle attached by microorganism cell.

![Supposed stratification structure and optimum EPS extraction protocol](image)

**Figure 2.** Supposed stratification structure and optimum EPS extraction protocol used in analyzing the bio-enhanced PAC mixed liquor

CONCLUSIONS

(1) The main components of EPS from the bio-enhanced PAC mixed liquor, i.e., PS, PN and NA, were all increased as the heated water temperature elevated, and the heat time did not influence the quantity of extracted EPS obviously. The heated water temperature 80 °C is the optimum thermal extraction condition for EPS from the bio-enhanced PAC mixed liquor. The ultrasonic and centrifugation could not extract the EPS from the bio-enhanced PAC mixed liquor completely.

(2) Alkaline had solubilization function to the microorganism’s cell; formaldehyde could consolidate the microorganisms’ cell membrane to prevent cell autolysis, but the color of extracted supernatant was yellow; the NA could not be used to indicate the degree of cell broken under the EDTA extraction. The adopted chemical EPS extraction methods from the bio-enhanced PAC mixed liquor were not optimum.

(3) Five fractions structure of the bio-enhanced PAC mixed liquor was fractionated through the thermal and centrifugation extraction: (1) supernatant; (2) slime; (3) LB-EPS; (4) TB-EPS; and (5) pellets.

REFERENCES