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## Characterization of soluble microbial products in a partial nitrification sequencing batch biofilm reactor treating high ammonia nitrogen wastewater

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#### ABSTRACT

In present study, the characterization of soluble microbial products (SMP) was evaluated in a partial nitrification sequencing batch biofilm reactor (SBBR). During the stable operation of SBBR, the NH<sub>4</sub><sup>+</sup>-N removal efficiency and nitrite accumulation ratio were 96.70  $\pm$  0.41% and 93.77  $\pm$  1.04%, respectively. According to excitation-emission matrix (EEM), the intensities of protein-like substances were reduced under anoxic and aerobic phases, whereas humic-like substances had little change during the whole cycle. Parallel factor analysis (PARAFAC) further indentified two components and their fluorescence intensity scores were both reduced. Synchronous fluorescence spectra revealed that the fluorescence intensity of protein-like fraction decreased with reaction time. Two-dimensional correlation spectroscopy (2D-COS) further demonstrated that protein-like fraction might decrease earlier than the other fractions. The information obtained in present study is of fundamental significance for understanding the key components in SMP and their changes in partial nitrification system by using a spectral approach.

#### 1. Introduction

Biological nitrogen removal is commonly applied for the treatment of both domestic and industrial wastewater (Ma et al., 2016). In traditional N-removal process, ammonia is oxidized to nitrate in aerobic nitrification process and nitrate is reduced to molecular nitrogen in anoxic denitrification process (Zhou et al., 2017). As a cost-effective Nremoval technology, partial nitrification has been widely developed in recent years based on the fact that nitrite is an intermediate compound in both nitrification and denitrification steps (Ciudad et al., 2005). It is generally accepted that partial nitrification via nitrite could save approximately 25% oxygen in nitrification stage and 40% carbon source in denitrification stage (Guo et al., 2009). The key control strategy to achieve partial nitrification is the enrichment of ammonia oxidizing bacteria (AOB) and limitation-inhibition-washout of nitrite oxidizing bacteria (NOB). Till now, it has been successfully achieved by appropriate regulating operational parameters, such as dissolved oxygen (DO), sludge retention time, pH value, temperature and free ammonia (FA) etc. (Ciudad et al., 2007; Peng and Zhu, 2006).

Soluble microbial products (SMP) are defined as a pool of organic compounds that are derived from the substrate metabolism (usually with biomass growth) and biomass decay during complete mineralization of supplying nutrients (Jarusutthirak and Amy, 2007). Indeed, SMP are one kind of heterogeneous mixtures containing various complex organic materials, such as proteins, polysaccharides, humic acids, fulvic acids and organic acids etc. SMP have been drawn intensive attention in the field of wastewater treatment process due to its adverse impact on the effluent quality and treatment efficiency in wastewater treatment plant (WWTP). Many published literatures have confirmed that SMP constitute a major part of organic component in effluent from biological wastewater treatment (Wu et al., 2016; Xie et al., 2012). Additionally, some SMP may cause further environmental hazard to the receiving water after the sewage treatment systems, such as toxicity and metal chelating properties (Kunacheva et al., 2017; Liang et al., 2007). The presence of SMP may also affect the viscosity, flocculating and other physical characters of sludge (Kim et al., 2016; Zhou et al., 2009). Moreover, SMP have a certain effect on the composition changes of microbial community in bioreactor (Kunacheva and Stuckey, 2014).

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Hence, it is essential to clearly identify the key components of SMP for better understanding the fundamental mechanisms of biological activities. However, till now, most researches are related to the full nitrification process in activated sludge reactor, and little information is available on the production of SMP in a partial nitrification system.

To date, a series of advanced analytical methods have been applied to explore the specific components in SMP produced in biological treatment process (Guo et al., 2013). Three-dimensional excitationemission matrix (3D-EEM) has been widely utilized for determining SMP owing to its better selectivity, higher sensitivity, and more simple and convenient operation. Combined with 3D-EEM, parallel factor analysis (PARAFAC) is commonly used to interpret the fluorescence spectra by decomposing the complete map into independent components (Zhang et al., 2016). Although PARAFAC is always applied to explore the further information of fluorescence spectra, it is not able to identify the mutual relationships between different components (Xu and Jiang, 2013). Two-dimensional correlation spectroscopy (2D-COS) could be applied as a versatile tool to express the specific variation order of any slight changes. It has a great advantage in solving the problem of overlapping peaks occurred in the original spectra by extending the overlapped bands in second dimension (Noda, 2006). Therefore, it is of great significant to provide a comprehensive analytical method for explaining the SMP formation in partial nitrification system. However, there is little information available regarding to this point in previous literature.

Based on the above discussion, the objective of present study was to evaluate SMP production in a stable partial nitrification sequencing batch biofilm reactor (SBBR) treating high ammonia nitrogen wastewater. A spectroscopic analysis based on the combination of 3D-EEM, PARAFAC, synchronous fluorescence and 2D-COS was used to characterize SMP samples in various reaction times. The results could provide insightful information on understanding the formation of SMP in a partial nitrification system.

#### 2. Methods and materials

#### 2.1. Experimental set-up

The experiment was carried out in a cylindrical SBBR with a working volume of 3.4 L. The height and inner diameter were 30 and 12 cm, respectively. Cylindrical carriers (K3, plastic media) were applied as biomass support with a packing rate of 40% (v/v). The diameter and height of each carrier were 25 and 15 mm, respectively. The specific gravity and the specific surface area of each carrier were 110 kg/m<sup>3</sup> and 500 m<sup>2</sup>/m<sup>3</sup>, respectively.

The SBBR was operated sequentially in 8 h for each cycle, consisting of 5 min for influent filling, 85 min for anoxic stage, 360 min for aeration, 15 min for settling and 15 min for effluent and idle. Aeration was provided by using an air pump and controlled through a gas flow meter. The exchange volume of SBBR was 50% for each cycle. Influent wastewater was prepared in a water tank and pumped into the bottom of biofilm system.

#### 2.2. Synthetic wastewater and seed sludge

The influent high-strength nitrogen wastewater was shown as follows: COD (as  $C_6H_{12}O_6$ ), 600 mg/L;  $NH_4^+$ -N (as  $NH_4$ Cl), 200 mg/L; P (as  $K_2$ HPO<sub>4</sub>), 15 mg/L; MgSO<sub>4</sub>·2H<sub>2</sub>O, 20 mg/L; CaCl<sub>2</sub>, 40 mg/L; FeSO<sub>4</sub>·2H<sub>2</sub>O, 20 mg/L and trace element solution 1.0 ml/L. The compositions of trace element could be found from previous literature (Tay et al., 2002). The influent pH value was adjusted to 8.0 by using NaHCO<sub>3</sub> and HCl.

Seed sludge for SBBR was collected from a lab-scale SBR of 17 L and mixed liquid suspended solids (MLSS) was controlled at about 3.0 g/L. In present study, high pH value (> 8.0) and ammonia (200 mg/L) was controlled in the influent wastewater, which may provide a feasible

 Table 1

 The contaminant removal performance of SBBR under stable operation.

Parameters	Influent (mg/L)	Effluent (mg/L)	Removal efficiency (%)
COD NH <sub>4</sub> <sup>+</sup> -N NO <sub>2</sub> <sup>-</sup> -N NO <sub>3</sub> <sup>-</sup> -N TN NAR (%)	$594.2 \pm 2.26$ $190.84 \pm 2.52$ $4.94 \pm 0.24$ $195.78 \pm 2.71$ $93.77 \pm 1.04$	$\begin{array}{rrrr} 43.3 \ \pm \ 5.22 \\ 6.29 \ \pm \ 0.71 \\ 80.97 \ \pm \ 2.53 \\ 5.38 \ \pm \ 0.94 \\ 92.64 \ \pm \ 2.75 \end{array}$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$

influent FA inhibition (high of 70 mg/L) on the activity of NOB, as similarly reported in our previous literature (Wei et al., 2017). After approximately 60 days operation, partial nitrification biofilm was successfully achieved, and the biomass concentration increased to 6.0 g/L.

#### 2.3. Fluorescence analysis

SMP samples were obtained from partial nitrification system at various reaction time from 0 to 420 min. Each sample was centrifuged at 8000 rpm for 5 min to separate out the solids. The supernatant was regarded as SMP. Fluorescence spectra were measured by using a Luminescence spectrometer (LS-55, Perkin-Elmer Co., USA). 3D-EEM was obtained by subsequently scanning emission from 220 to 400 nm at 10 nm increments by varying the excitation wavelength from 280 to 550 nm at 0.5 nm increments. PARAFAC analysis was obtained by using MATLAB 7.6 (Mathworks, Natick, MA, USA) with the N-way toolbox for



Fig. 1. Variations of nitrogen compound, DO and pH values in partial nitrification SBBR during one typical cycle.

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