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Qualitative and quantitative analysis of extracellular polymeric substances in partial nitrification and full nitrification reactors

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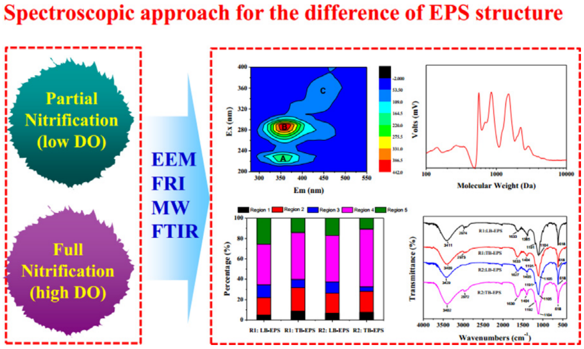
HIGHLIGHTS

- Partial nitrification reactor was achieved by using low DO strategy.
- Higher TN removal efficiency was observed in partial nitrification reactor.
- EEM showed fluorescence property of LB-EPS and TB-EPS from two reactors.
- TB-EPS exhibited a greater number of molecular weight fractions.

GRAPHICAL ABSTRACT



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ABSTRACT

In present study, two column-type sequencing batch reactors with alternative anoxic/aerobic phases were operated and compared under partial nitrification and full nitrification modes by controlling different dissolved oxygen (DO) conditions. During steady state, the characterizations of extracellular polymeric substances (EPS) from two reactors were qualitatively and quantitatively analyzed through chemical and spectroscopic approaches. Data implied that partial nitrification reactor had relatively higher total nitrogen (TN) removal efficiency and loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) contents. According to excitation emission matrix (EEM) spectra, LB-EPS and TB-EPS from two kinds of reactors expressed similar fluorescence peak locations but different intensities. Fluorescence regional integration (FRI) further suggested that Region IV was the main fraction in both types of EPS fractions. Moreover, TB-EPS exhibited a greater number of molecular weight fractions than those of LB-EPS. Both EPS fractions had similar functional groups, which represented the complex nature of EPS compositions.

1. Introduction

Biological nitrogen removal is commonly applied in the field of wastewater treatment, which generally requires a combined two-step process: the conversion of ammonia to nitrate in aerobic nitrification process and subsequent nitrate to nitrogen gas in anoxic denitrification process (Münch et al., 1996). As a promising nitrogen removal technology, partial nitrification via nitrite (100% NH_4^+-N to NO_2^--N) is based on the fact that nitrite is an intermediate compound in both nitrification and denitrification stages (Peng and Zhu, 2006). It is regarded as one of cost-effective and sustainable nitrogen removal processes for treating nitrogen-rich wastewater due to the advantages of saving oxygen consumption in aerobic

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nitrification stage and reducing carbon source requirement in anoxic denitrification stage (Wei et al., 2015).

The critical operational strategy to achieve partial nitrification is the enrichment of ammonia oxidizing bacteria (AOB) and limitation-inhibition-washout of nitrite oxidizing bacteria (NOB) (Wei et al., 2014a). To date, partial nitrification has been successfully achieved at laboratory-scale through controlling different influencing factors, such as dissolved oxygen (DO), solid retention time (SRT), temperature, free ammonia (FA) and free nitrous acid (FNA) etc (Pollice et al., 2002; Wang and Yang, 2004). Guo et al. (2010) evaluated the effect of temperature on partial nitrification in a sequencing batch reactor (SBR) treating domestic wastewater, implying that low temperature did not deteriorate stable partial nitrification performance and nitrite accumulation. Wei et al. (2014b) researched the influent ammonium concentration on the shift of full nitritation to partial nitrification in a SBR, suggesting that FA and FNA were the potential compounds for inhibiting the activity of NOB.

Extracellular polymeric substances (EPS) are regarded as one kind of microbial productions that exhibit a three-dimensional, gel-like, highly hydrated matrix. EPS are mainly consisted of proteins (PN), polysaccharides (PS), humic acid, nucleic acid, and phospholipid etc. The presence of EPS has a significant influence on the physicochemical properties of sludge, such as flocculation, settling, dewatering, and sorption ability etc (Sheng et al., 2010). It is commonly accepted that EPS are generally depicted by a two-layer model, inner and outer, the former layer is composed of tightly bound EPS (TB-EPS), while the latter layer is composed of loosely bound EPS (LB-EPS) (Li and Yang, 2007). Moreover, the structure and key component of two kinds of EPS are closely related to the changes of external parameters of system. Therefore, LB-EPS and TB-EPS fractions may express different characterizations when the reactors operated under partial nitrification and full nitrification modes. However, till now, relatively little attention has been paid to this point.

Hence, this study presented an initial attempt to evaluate the characterizations of LB-EPS and TB-EPS fractions from partial nitrification and full nitrification reactors by using chemical and spectroscopic approaches. To achieve this purpose, two column-type SBRs with alternative anoxic/aerobic phases were operated and compared during their steady state. Low DO supply in aerobic phase was selected as a key parameter for the achievement and maintenance of partial nitrification reactor. A combination of three-dimensional excitation emission matrix (3D-EEM) spectra, fluorescence regional integration (FRI), molecular weight (MW) distribution and Fourier transform infrared spectroscopy (FTIR) was used to assess the structural differences of EPS between the two reactors. The obtained result could provide a better insight for understanding the EPS property of partial nitrification treatment process.

2. Materials and methods

2.1. Experimental set-up

The experiment was carried out in two column-type SBRs (R1 and R2), and each reactor had an internal diameter and working height of 12 and 30 cm, respectively. The volumetric exchange ratio and hydraulic retention time (HRT) of reactors were 50% and 16 h, respectively. Both reactors were operated sequentially by alternating anoxic and aerobic reaction time in an 8-h cycle, including 5 min for influent, 85 min for anoxic process, 300 min for aeration reaction, 20 min for settling, 10 min for effluent and the rest of 60 min for idle.

Mixed liquor suspended solids (MLSS) concentration was controlled approximate 3000 mg/L in both reactors. The main difference between the two reactors was DO concentration during aeration phase. Detailed, for partial nitrification reactor, low DO concentrations between 0.4 and 0.6 mg/L were maintained by turning an on/off air pump, which was adjusted according to an on-line DO probe. In contrast, high DO concentrations between 2 and 8 mg/L were maintained in the full nitrification reactor. After operated for two months, the effluents of two reactors achieved steady state. Then, the nitrogen removal performances of two SBRs were measured and compared. At the same time, LB-EPS and TB-EPS samples were collected from two reactors and qualitatively and quantitatively analyzed in order to evaluate their characteristic difference.

2.2. Nitrogen -rich wastewater

The compositions of synthetic high-strength nitrogen wastewater were used as follows (mg/L): chemical oxygen demand (COD, as sodium acetate), 600 mg/L; NH_4^+-N (as ammonium chloride), 300 mg/L; K_2HPO_4, 112 mg/L; $CaCl_2$, 40 mg/L; $MgSO_4{\cdot}2H_2O$, 20 mg/L; $FeSO_4{\cdot}2H_2O$, 20 mg/L and trace element solution 1.0 ml/L. The influent pH values of wastewater were adjusted to 7.5–7.8 by using $NaHCO_3$ and HCl.

2.3. EPS extraction and fluorescence spectra

LB-EPS and TB-EPS samples were extracted by using a heat method, and the detailed procedure could be found elsewhere (Li and Yang, 2007). 3D-EEM spectra were measured by using a fluorescence luminescence spectrometer (LS-55, Perkin-Elmer Co., USA). A fluorescent EEM for EPS sample was generated with subsequent scanning emission spectra from 280 to 550 nm at 0.5 nm increments by varying excitation wavelength from 200 to 400 nm at 10 nm increments. The width of excitation/emission slit and scanning speed were set to 10.0 nm and 1200 nm/min, respectively.

FRI of EEM was analyzed according to the method reported of Chen et al. (2003). Detailed, EEM spectra could be divided into five regions, including tyrosine and tryptophan (Regions I and II), fulvic acid-like substances (Region III), soluble microbial by-product-like materials (Region IV) and humic acid-like organics (Region V). The excitation/emission (Ex/Em) wavelength range of regions I, II, III, IV and V were 200–250/280–330 nm, 200–250/330–380 nm, 200–250/380–550 nm, 250–400/280–380 nm, and 250–400/380–550 nm, respectively.

2.4. Analytical methods

NH_4^+-N, NO_2^--N and NO_3^--N were analyzed by using the respective standard methods (AWWA, 1998). Total nitrogen (TN) was based on the sum of NH_4^+-N + NO_2^--N + NO_3^--N rather than an independent TN test, as similarly reported by Fu et al. (2009). PS content was measured by means of phenol-sulphuric acid method by using glucose as the standard, while the PN content was measured by means of modified Lowry method by using bovine serum albumin (BSA) as the standard. DO and pH values were measured using their on-line probes (WTW Multi 3420, Germany). Molecular weight (MW) distribution of EPS samples was determined by using high performance size exclusion chromatography (HPSEC) that consisted of a Waters 2487 dual λ absorbance detector, Waters 1525 pump system. FTIR were recorded by using a Perkin-Elmer FTIR spectrometer (United States). Before FTIR spectra analysis, two kinds of EPS samples were freeze-dried at −60 °C.

Nitrite accumulation ratio (NAR) of the reactor was calculated according to the following equation (Eq. (1)):