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Long-term effects of salinity on extracellular polymeric substances, microbial activity and microbial community from biofilm and suspended sludge in an anoxic-aerobic sequencing batch biofilm reactor

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ABSTRACT

The long-term effects of salinity on extracellular polymeric substances (EPS), microbial activity and microbial community from biofilm and suspended sludge (S-sludge) in an anoxic-aerobic sequencing batch biofilm reactor (SBBR) were investigated. The increase of influent salinity from 0% to 8% caused the decrease of specific ammonium oxidation rate (SAOR), specific nitrite oxidation rate (SNOR) and specific nitrate reduction rate (SNRR) in biofilm from 3.89, 4.60 and 52 mg N/(g MLSS h) to 1.01, 0.83 and 18 mg N/(g MLSS h), respectively, and the decrease of SAOR, SNOR and SNRR in S-sludge from 3.57, 3.95 and 29 mg N/(g MLSS h) to 0.71, 0.61 and 9 mg N/(g MLSS h), respectively. As the salinity increased from 0% to 8%, the protein (PN) content in EPS from biofilm and S-sludge increased from 8.35 and 8.77 mg/g VSS to 90.88 and 58.63 mg/g VSS, respectively, and the polysaccharide (PS) content in EPS from biofilm and S-sludge increased from 3.05 and 4.03 mg/g VSS to 57.55 and 62.63 mg/g VSS, respectively. *Nitratireductor lucknowense*, *Micropruina glycogenica*, and *Thiobacillus thioparus* could grow more favorably in biofilm than in S-sludge at 0–8% salinity.

1. Introduction

Compared to traditional suspended activated sludge system, sequencing batch biofilm reactor (SBBR) has higher biomass retention, more abundant microbial species, better process stability, and greater ability to resist inhibitory substances, so it has been widely applied in the treatment of domestic and industrial wastewaters, such as saline wastewaters, heavy metal wastewaters, high strength organic wastewaters, etc. [1–3]. In generally, the main types of microbial aggregates in a SBBR are biofilm and suspended sludge (S-sludge). Biofilm is a tight aggregate which adheres on the carrier, and S-sludge freely disperses in solution. Although biofilm and S-sludge are present in the same reactor, they usually show different physicochemical and biological characteristics. Chen et al. [4] found that the biomass activities of biofilm were significantly higher than those of S-sludge, whether in the anaerobic ammonium oxidation, nitrification, or completely autotrophic nitrogen removal over nitrite processes. Zhang et al. [5] reported that compared to nitrifying bacteria, denitrifying bacteria were more in S-sludge, whereas denitrifying bacteria were fewer in biofilm. Lo et al. [6] found that the main contributions of S-sludge were ammonia oxidation and nitrite oxidation, and denitrification was the major contribution of biofilm. Zhang et al. [7] illustrated that the flocculating capacity of extracellular polymeric substances (EPS) in S-sludge shows extraordinary activity, comparing its counterpart in biofilm. Previous researches showed that biofilm and S-sludge usually had different roles in the removal of organic matters and nutrients in a SBBR.

The roles of biofilm and S-sludge in the removal of organic matters and nutrients could be affected by many factors, such as salinity [8], heavy metals [9], antibiotics [10], and nutrient content [3], etc. Among the above-mentioned influencing factors, salinity is regarded as one of the key factors, due to the inhibition of many

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enzymes, the loss of cellular activities and eventually causing plasmolysis, dehydration, disintegration under salt stress. She et al. [8] found that the increase of salinity from 0.42% to 0.98% inhibited the rates of nitrification and denitrification of biofilm and S-sludge. At 0.98% salinity, nitrification was the main contribution of S-sludge, while the major role of biofilm was denitrification. Zhang et al. [11] reported that as the increase of salinity from 0.2% to 1.0%, the growth of nitrite oxidation bacteria in biofilm was inhibited, whereas anaerobic ammonium oxidation bacteria were enriched. Zhang et al. [12] illustrated that the polysaccharide (PS) content in the loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) from biofilm significantly increased as the increase of salinity from 0.2% to 1.0%, whereas no clear changes of protein (PN) in the LB-EPS and TB-EPS fractions were monitored in an intermittent aerobic SBBR. Although some researchers have reported the long-term effect of salinity on biofilm, little information has been conducted to investigate the differences between biofilm and S-sludge in EPS, microbial activity and microbial community in an anoxic-aerobic SBBR at different salinities.

The major objectives of the present study is (a) to investigate the effect of the increase of influent salinity on the removal of COD and NH_4^+-N in an anoxic-aerobic SBBR at 0–8% salinity, (b) to analyze the variations of the specific ammonium oxidation rate (SAOR), specific nitrite oxidation rate (SNOR), specific nitrate reduction rate (SNRR) from biofilm and S-sludge at different salinities, (c) to evaluate the effect of salinity on the composition of the LB-EPS and TB-EPS from biofilm and S-sludge, and (d) to investigate the variations of microbial community in biofilm and S-sludge as the increase of salinity.

2. Materials and methods

2.1. Reactor set-up and wastewater composition

A lab-scale plexiglass anoxic-aerobic SBBR (an internal diameter of 14 cm, a total height of 55 cm, and a working volume of 7.7 L) was used in the study. Four fibrous carriers with a diameter of 12 cm were placed in the SBBR, and the space between two units was 8 cm. The anoxic-aerobic SBBR was sequentially operated in a 12 h cycle, and one cycle was consisted of 0.25 h influent addition, 3.3 h anoxic reaction, 7.7 h aerobic reaction, 0.5 h settling and 0.25 h effluent withdrawal. The dissolved oxygen (DO) concentration at the anoxic stage was below 0.5 mg/L, and that at the aerobic stage was more than 2.0 mg/L. The seed sludge was taken from a secondary clarifier of municipal wastewater treatment plant in Qingdao (China), and the initial mixed liquor suspended sludge (MLSS) was 3890 mg/L. The composition of synthetic saline wastewater was as follows (mg/L): glucose, 1536; NH_4Cl, 240; KH_2PO_4, 79; $MgSO_4$, 100; KCl, 20; $CaCl_2$, 50; seawater crystal from 0 to 8×10^4 (corresponding to the salinity from 0% to 8%). The composition of seawater crystal was described as Wang et al. [13].

2.2. Analytical methods

The measurements of COD, NH_4^+-N, NO_2^--N, NO_3^--N, MLSS, and mixed liquor volatile suspended solids (MLVSS) were performed according to standard methods [14]. The DO concentration was determined with a dissolved oxygen meter (Oxi 3310, WTW, Germany). The EPS extractions were carried out in accordance with a thermal extraction method [15]. The PN and PS contents in EPS extractions were measured according to the Lowry method [16] and the anthrone-sulfuric acid method [17], respectively. The SAOR, SNOR and SNRR were analyzed according to Wang et al. [18]. The microbial community was evaluated by denaturing gradient gel electrophoresis (DGGE) and sequencing according to Wang et al. [19]. The sequences obtained in this study were submitted to the DDBJ database under accession numbers AB924454-AB924481.

3. Results and discussion

3.1. SBBR performance

Fig. 1 shows the variations of influent and effluent COD, NH_4^+-N, NO_2^--N and NO_3^--N concentrations in an anoxic-aerobic SBBR at different salinities. The biofilm formation was achieved at 0% salinity (0–81 days), and the average removal efficiencies of COD and NH_4^+-N were 96% and 92% during the operation period, respectively. After the formation of biofilm, the influent salinity was gradually increased from 1% to 8%. During the initial period of every salinity changes, the removal efficiencies of COD and NH_4^+-N

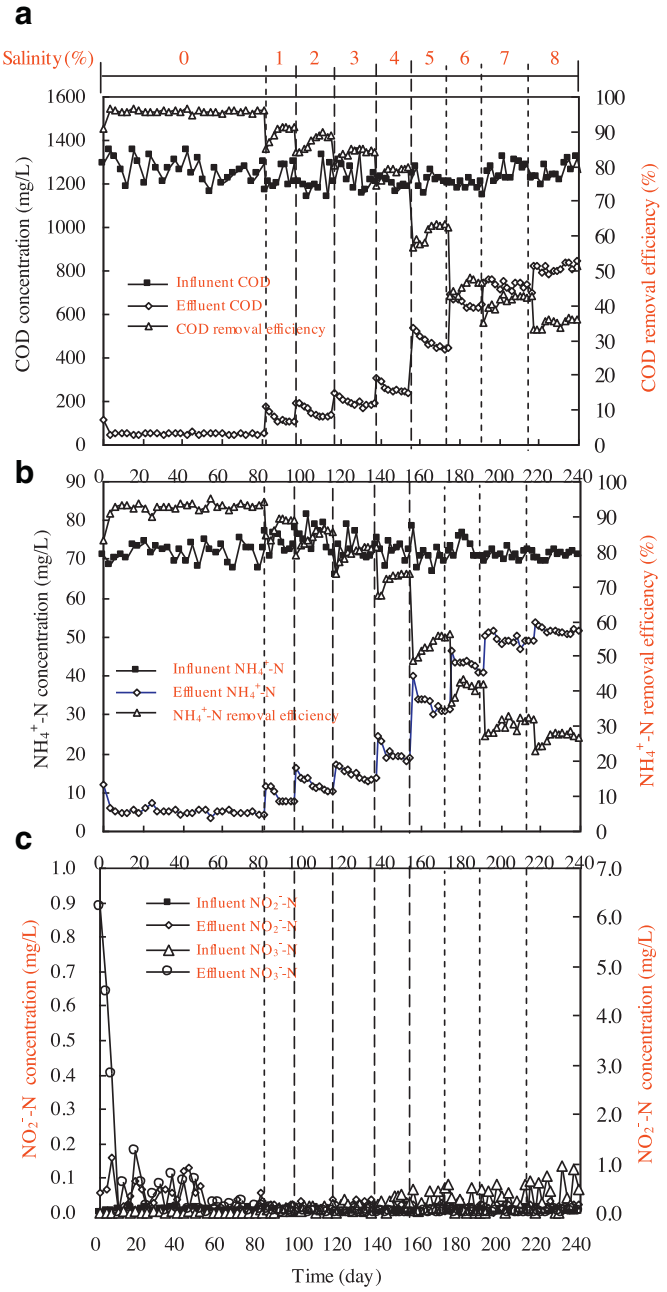

Fig. 1. The variations of COD (a), NH_4^+-N (b), NO_2^--N and NO_3^--N (c) concentrations in the influent and effluent at different salinities.