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Effect of anaerobic/aerobic duration on nitrogen removal and microbial community in a simultaneous partial nitrification and denitrification system under low salinity

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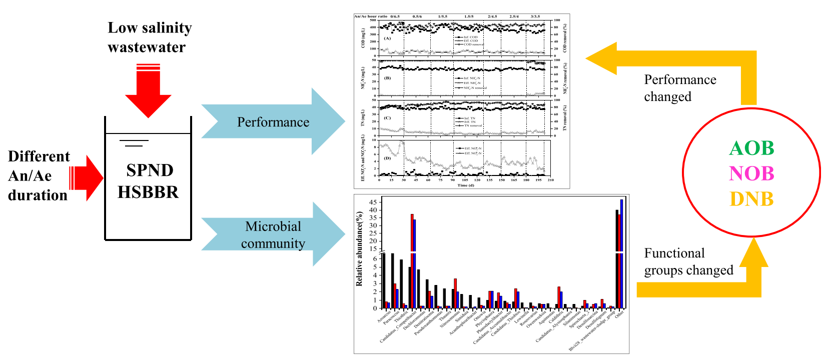
HIGHLIGHTS

- High nitrogen removal was achieved at An/Ae hour ratios of 1/5.5, 1.5/5 and 2/4.5.
- Increasing anaerobic time affected bacterial community structure and composition.
- Similar bacterial composition was observed in S-sludge and biofilm samples.
- *Candidatus_Competibacter* played major role in denitrifying in An/Ae mode.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, the simultaneous partial nitrification and denitrification (SPND) process was investigated in a hybrid sequencing batch biofilm reactor (HSBBR) fed with synthetic wastewater with 1.2% salinity. Different anaerobic/aerobic (An/Ae) durations were selected for evaluating the removal performance of contaminants and the succession of the microbial community in the reactor. The highest organic matter removal efficiency was obtained at An/Ae hour ratio of 0/6.5, with an average chemical oxygen demand (COD) removal of 89.6% at the steady state. Similarly high nitrogen removal efficiencies were achieved at An/Ae hour ratios of 1/5.5, 1.5/5 and 2/4.5,with over 92% of average total nitrogen removed. This represents an increase of more than 10% compared to the mode with An/Ae hour ratio of 0/6.5. High-throughput sequencing analysis revealed that the increase of the An/Ae hour ratio changed the characteristics of the community structures in the HSBBR. *Azoarcus* was the most dominant genus when the An/Ae hour ratio was 0/6.5 in both suspended sludge (S-sludge) and biofilm, while *Candidatus_Competibacter* was the most abundant genus at An/Ae hour ratios of 2/4.5 and 3/3.5. *Nitrosomonas* was the only ammonia oxidizing bacteria (AOB) detected in this study. *Nitrospira*, a kind of nitrite oxidizing bacteria (NOB), was sensitive to salinity and altering the An/Ae mode; this was detected only in S-sludge samples in a fully aerobic mode with a low percentage of 0.1%. S-sludge and biofilm samples shared a similar bacterial composition. This research demonstrated that efficient nitrogen and carbon removal could be achieved via the SPND process by the symbiotic functional groups in a hybrid S-sludge and biofilm reactor.

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1. Introduction

Nitrogen removal from wastewater through simultaneous nitrification and denitrification (SND) is regarded as an efficient and cost effective alternative to conventional nitrogen removal process. During SND, nitrification and denitrification occur concurrently in one reactor under identical operating conditions. The SND process could simplify operating procedures and reduce oxygen requirements and energy consumption (Zhang et al., 2015). SND via nitrite, or simultaneous partial (or shortcut) nitrification and denitrification (SPND or SSND), requires less oxygen, and consumes less energy and carbon resources than SND via nitrate. In an SPND system, the first step of nitrification (oxidizing ammonia to nitrite) was induced but the second step (oxidizing nitrite to nitrate) was suppressed effectively, and denitrification carried out using nitrite (Yoo et al., 1999). The SPND process has already been achieved in a sequencing batch reactor (SBR) (She et al., 2016; Yoo et al., 1999; Zheng et al., 2010), sequencing batch biofilm reactor (Zhang et al., 2009) and membrane bioreactor (Hong et al., 2013) thus far. Some studies have been conducted to determine control parameters for nitrogen removal by SPND. These parameters include temperature (Zhang et al., 2009), dissolved oxygen (DO) level (Yoo et al., 1999), salinity (Wang et al., 2017a) and carbon source (Wang et al., 2017b).

Salinity is one of the crucial factors for achieving SPND as nitrite oxidizing bacteria (NOB) were more sensitive to salt than ammonia oxidizing bacteria (AOB) (Liu et al., 2008; Mosquera-Corral et al., 2005; Wang et al., 2017c). It was reported that a low salinity of 9.0 g NaCl L^{-1} could inhibit NOB activity while enhancing AOB activity (She et al., 2016; Zhang et al., 2010). Stable partial nitrification was achieved in a sequencing batch biofilm reactor (SBBR) when the salt concentration gradually increased to 6.5 g NaCl L^{-1} (She et al., 2016; Zhang et al., 2010). Few studies relating the SPND process to salinity have been performed. Wang et al. (2017a) established a simultaneous nitrification, denitrification and organic matter removal process in an SBBR to treat saline mustard tuber wastewater. Results showed that as salinity increasing, NOB were significantly inhibited, partial nitrification and denitrification (PND) process gradually contributed to nitrogen removal. It was observed that different carbon sources had great impact on microbial communities, and led to different nitrogen removal mechanism in the SPND process treating saline wastewater (Wang et al., 2017b). So far, most studies have focused on establishing the SPND process under saline conditions, instead of on how the operational conditions influence the treatment of saline wastewater using the SPND process. No studies have been performed regarding the effect of different operational modes, especially anaerobic/aerobic duration, on nitrogen removal and the microbial community in the SPND process under low salinity.

The common operational strategies employed in SND systems include fully aerobic mode and anaerobic-aerobic (or anoxic-aerobic) mode. The former is achieved by applying only aeration in the reaction stage, and the latter is realized by introducing a pre-anaerobic (or pre-anoxic) phase prior to the aeration stage (Zhang et al., 2015). In the pre-anaerobic phase, organic matter in influent can be degraded and the amount of oxygen demand in the following aerobic phase can be reduced. Moreover, the alkaline condition produced by denitrification during the pre-anoxic phase is beneficial for subsequent aerobic nitrification (Hu et al., 2011). Some studies have demonstrated that the pre-anoxic mode had a higher denitrification capacity than the fully aerobic and aerobic/anoxic modes (Ersan and Erguder, 2013; Wan et al., 2009). In addition, it is reported that total nitrogen removal and SND efficiencies in the anoxic/aerobic mode increased by 17.8% and 10.1% compared to the fully aerobic mode (Zhang et al., 2015). In an alternating anoxic/aerobic SBR, the highest total nitrogen removal was achieved at the anoxic/aerobic hour ratio of 2/4, although the ammonia removal exhibited a decreasing trend as the anoxic/aerobic time ratio increased (Hu et al., 2011).

The nitrification and denitrification efficiency in a biological nitrogen removal reactor is closely related to the diversity and distribution of the microbial community. High-throughput sequencing technologies have been successfully used as molecular techniques with which to assess the diversity, structure and function of microbial communities in activated sludge and biofilm, and to determine the dynamics of microbial responses to variations in the environment (Tang et al., 2018; Zhang et al., 2017). Zhang et al. (2018) utilized Illumina MiSeq sequencing to analyze the microbial community in a continuous flow reactor operating in an anaerobic/aerobic/anoxic process. They revealed that the predominant bacteria were *Rhodocyclaceae*, *Saprospiraceae* and *Comamonadaceae* on the family level, and that these contributed jointly to nitrogen removal in the system. In another study, high-throughput sequencing was applied to analyze the dynamics of the community of functional microbes (AOB, NOB and anammox bacteria) under conditions of elevated salinity in a CANON system (Wang et al., 2017c). However, high-throughput sequencing technology has not yet been applied to exploring alternating anaerobic/aerobic habitats in an SPND process for treating wastewater with a low salinity.

Interstitial aeration is a typical characteristic for a reactor operated in a sequencing batch mode that could lead to alternating aerobic/anaerobic conditions. Alternating aerobic/anaerobic conditions are beneficial for SND or SPND processes. The SPND process has been achieved in an intermittently aerated moving bed membrane bioreactor, in which the characteristic of nitrification was investigated (Yang and Yang, 2011). In another study, intermittent aeration was used to realize the SPND process in constructed wetlands (Hou et al., 2017). SBR and SBBR have been used as typical SND or SPND situations because of the advantage of interstitial aeration. In recent years, the hybrid sequencing batch biofilm reactor (HSBBR) has attracted interest because it can effectively remove nitrogen via SND based on the interaction of S-sludge and biofilm in a reactor (Lo et al., 2010). In this study, an HSBBR that had been developed by an SPND process under conditions of low salinity was operated with different An/Ae hour ratios. 16S rRNA gene high-throughput sequencing technology was used to characterize the dynamic evolution of the population and structure of the microbial community as the anaerobic duration in the SPND system increased. The main goals of this study were to: (1) determine the appropriate An/Ae hour ratio for nitrogen removal in an SPND process, (2) reveal the changes in the microbial community of both S-sludge and biofilm in the HSBBR as the anaerobic duration increased under low salinity, (3) identify the sensitivities of functional microorganisms needed for nitrogen removal to increasing anaerobic duration in the reactor, and (4) explore the feasibility of using the SPND system to treat saline wastewater and control strategies from the view of microbial ecology.

2. Materials and methods

2.1. Reactor and experimental methodology

This experiment was carried out in an HSBBR, which had been previously operated with increasing salinity from 0.0% to 1.2% for 155 days to establish a steady SPND process. The reactor had a diameter of 19 cm, a height of 33 cm and a working volume of 7.0 L (Fig. 1). Two pieces of soft combination carriers (made of polyvinyl formal fiber and polypropylene hoop) were attached to a rope and hung in the reactor. The diameter of each carrier was 14 cm and the specific surface area was 1236 m^2 m^{-3}. The HSBBR was run in three 8 h cycles a day. Each cycle contained six phases: feeding (15 min), anaerobic, aerobic, settling (60 min), decanting (10 min), and idle (5 min). The reaction time, including anaerobic and aerobic phases, was 6.5 h (390 min) in a cycle. An experiment to determine appropriate reaction time was conducted before this study. The result of the prior study indicated that complete oxidization of ammonium and high COD removal efficiency could be achieved within 6.5 h aeration, so the reaction time in Period 1 was fixed as 6.5 h in the present study. To compare the performance of the