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Response of nitrite accumulation and microbial characteristics to low-intensity static magnetic field during partial nitrification

Wenlin Jia[a,*], Jian Zhang[b], Yumiao Lu[a], Guangchao Li[a], Weihua Yang[a], Qian Wang[a]

[a] School of Chemistry and Materials Science, Jiangsu Normal University, Xuzhou 221116, China
[b] Shandong Provincial Key Laboratory of Water Pollution Control and Resource Reuse, School of Environmental Science and Engineering, Shandong University, Jinan 250100, China

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ABSTRACT

Static magnetic field (SMF) with the intensity of 15 mT was applied during partial nitrification (PN) process to evaluate the impacts on nitrogen transformation and microbial characteristics. Results showed that the startup period of PN process at ambient temperature was markedly shortened by SMF, and the nitrite accumulation increased by 18% due to SMF exposure. The ammonia oxidizing bacteria (AOB) *amoA* gene copy numbers in the reactor with SMF exposure were 40% higher than that without SMF exposure, indicating the AOB abundance was enriched by SMF exposure. The characteristics of extracellular polymeric substances (EPS) changed accordingly. The extracellular protein increased by 30% due to SMF exposure, and it favored the aggregation of sludge flocs. The activated sludge with SMF exposure had a more compact structure, which was in favor of partial nitrification.

1. Introduction

Nowadays, biological nitrogen removal (BNR) technologies are widely used to eliminate the eutrophication occurring in lakes and rivers. The conventional BNR generally involves two processes, i.e. nitrification and denitrification. Recently, partial nitrification (PN) has been achieved and widely explored by controlling the oxidation of nitrite to nitrate during nitrification process (Zhang et al., 2016). Compared with full nitrification, PN can lower the oxygen consumption during aeration period and reduce the carbon source requirement during the following denitrification process. Partial nitrification in combination with anammox process realizes the autotrophic nitrogen removal, which is a cost-effective biotechnology (Desloover et al., 2011). Moreover, PN can potentially combine with bioelectrochemical denitrification to gain an innovative and more efficient BNR process (Kondaveeti et al., 2014; Sevda et al., 2018).

The critical procedure for the success of PN process is obtaining stable nitrite accumulation and controlling the further oxidation to nitrate. It can be achieved by enriching ammonia oxidizing bacteria (AOB) and suppressing nitrite oxidizing bacteria (NOB) in reactors, based on the characteristic differences between the two kinds of bacteria, such as different sludge ages, dissolved oxygen half-saturation coefficients, and anti-toxic capacities (Peng and Zhu, 2006). In order to achieve PN process successfully, various strategies have been performed by changing the operating factors, including temperature, dissolved oxygen (DO), pH value, sludge retention time (SRT), and free ammonia concentration (Guven et al., 2009; Rongsayamanont et al., 2014; Tao et al., 2012). High temperature can expand the differences of specific growth rates between AOB and NOB, and favors the enrichment of AOB and accumulation of nitrite (Tao et al., 2012). It was reported that optimum temperature for PN process was above 30 °C (Do et al., 2008). However, the high running temperature increases the consumption of energy, which limits the development of PN process.

Previous research showed that static magnetic field (SMF) could affect the metabolism of microorganisms, by changing the enzyme activity and cell membrane permeability (Liu et al., 2008). Filipic et al. (2012) reported that SMF with a moderate-intensity (17 mT) can enhance the activities of microbial enzymes and the growth of microorganisms. Although the physiology is still under discussion, the application of SMF to improve the performance of biological wastewater treatment has been widely explored to remove colors, heavy metal, organic compounds and nutrients (Zaidi et al., 2014). Moreover, many previous literatures investigated the impacts of magnetic field on nitrogen transformation. Tomska and Wolny (2008) investigated the performance of nitrification process in an aeration tank with SMF exposure and found nitrification rate was enhanced by periodical exposure to SMF of 40 mT. Liu et al. (2008) studied the impacts of SMF on anammox activity and found the activity was enhanced at week SMF

* Corresponding author.
E-mail address: jiawenlin@jsnu.edu.cn (W. Jia).

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intensity but declined when the intensity was above 75 mT. A recent research demonstrated that SMF of 8.1 mT could shorten nitrification time greatly and favored the growth of AOB (Zielinski et al., 2017). In addition, the growth rate and metabolic activity of *Nitrosomonas europaea*, a key kind of AOB, were found to be enhanced by exposing to SMF of 17 mT (Filipic et al., 2015). It was reported low-intensity SMF could induce the modifications of cell shape, cell surface, and cytoskeleton, which improved the resistant capacity of microorganisms to low temperature (Dini and Abbro, 2005; Niu et al., 2014). Therefore, it is potential to enhance the PN process at ambient temperature by SMF exposure. However, to date, most studies focus on nitrification or other novel BNR processes (such as anammox), and few literature is available regarding the impacts of SMF on nitrogen transformation and microbial characteristics during partial nitrification process.

In this study, sequencing batch reactors (SBRs) were established and exposed to low-intensity SMF, to evaluate the impacts on nitrogen transformation and nitrite accumulation during partial nitrification process. To further explore the response of biomass to SMF, the surface morphology of biomass was characterized by a scanning electron microscope (SEM), and the characteristics of EPS, which surrounded the bacteria cells and were important to the structure of activated sludge, were also analyzed. Moreover, the abundance of AOB and NOB were quantified by quantitative PCR (qPCR), to reveal the impacts of SMF on nitrifying bacteria abundance during partial nitrification. The obtained results could provide a novel strategy to control partial nitrification at ambient temperature.

2. Materials and methods

2.1. Reactor setup and operation

The experiments were carried out in two parallel running SBRs (named R1 and R2) with an effective volume of 2 L. The SBRs were seeded with activated sludge taken from the aeration tank of a local municipal wastewater treatment plant, and the initial concentration of mixed liquor suspended solids (MLSS) was 3000 mg/L. Both SBRs were operated at 25 °C with a cycle time of 6 h, which consisted of 60 min for anoxic process, 180 min for oxic process, 60 min for settling, 10 min for decanting and 50 min for idling. In the first 5 min of anoxic period, one liter synthetic wastewater was pumped into each reactor. An electric agitator with a rectangular paddle was used to keep the sludge suspended during anoxic and oxic periods. An air pump was used to supply oxygen during the oxic period and the aeration rate was controlled by gas flowmeters at 200 mL/min. At the end of oxic period, 100 mL mixed liquor was discharged to keep the SRT at approximately 5 days. After settling, one liter of supernatant was drained, resulting in a hydraulic retention time of 12 h. The pH values in the reactors fluctuated between 7.5 and 8.0, which were recorded but not controlled. In order to investigate the impacts of SMF on PN process, two heteropolar magnetic plates were placed in parallel beside R2 with a spacing of 10 cm, to generate the static magnetic field and the intensity in the middle of the reactor was 15 mT. R1 was set as the control variable without SMF exposure. In order to avoid the influence of SMF on R1, the two reactors were placed on different benches with a distance of 2 m. The experimental system is illustrated in Fig. 1.

During the whole experimental period, the influent and effluent of each reactor were sampled every three days and the nitrogen concentrations were analyzed to evaluate the performance of each SBR. In order to avoid the influence of water quality fluctuation, synthetic feed was used as influent in this study. The composition of synthetic feed can be found in the supplementary material. The complete influent contained 400 mg/L COD, 120 mg/L NH_4-N and 5 mg/L total phosphorus (TP). The experiments were performed for three months.

2.2. Extraction and analysis of EPS

The seeding sludge and acclimated sludge sampled on day 80, 85 and 90 were used to extract the EPS using the heat extraction method described by Li and Yang (2007). Briefly, 50 mL sludge suspension was sampled from each reactor and centrifuged at 4000*g* for 5 min. Then the supernatant was removed and sludge pellet left in the centrifuge tube was resuspended in 0.05% NaCl solution to its original volume of 50 mL. After that, the sludge suspension was heated to 60 °C in a water bath for 30 min and then centrifuged at 4000*g* for 15 min. The collected supernatant was regarded as EPS extraction of sludge. The content of extracellular protein and polysaccharides in the EPS was measured using the modified Lowry method (Frølund et al., 1995) and anthrone-sulfuric method (Wu et al., 2009), respectively. Moreover, the three-dimensional excitation-emission matrix (3D-EEM) fluorescence spectra of the EPS were tested using a fluorescence spectrophotometer (F-4600, HITACHI, Japan). The scanning emission wavelength was set from 300 to 550 nm and the excitation wavelength was set from 220 to 420 nm, both in 5 nm increments. The scanning speed was set as 1200 nm/min.

2.3. Preparation of the specimen for SEM

At the end of the experiment, 10 mL of mixed liquor was collected from each reactor and centrifuged at 4000*g* for 5 min. Then the sludge pellets left in the centrifuge tubes were immobilized by 2.5% glutaraldehyde for 12 h. After washing with phosphate buffer for 3 times, the samples were dehydrated in a graded ethanol series (30%, 50%, 70%, 85%, 95% each for one time and 100% twice, once for 15 min) and then were immersed by isoamyl acetate to replace the ethanol. At last, the samples were dried using a critical point drier. After plating with a thin Au/Pd layer, the samples were observed by SEM (S-8010, HITACHI, Japan).

2.4. DNA extraction and qPCR

The sludge samples of two SBRs were collected at the end of the experiment and centrifuged at 4000*g* for 5 min. Then the total genomic DNA was extracted using the PowerSoil™ DNA Isolation Kit (MO BIO Laboratories, USA).

Quantitative detections of the *amoA* and *nxrA* genes, which represented AOB and NOB, respectively, were carried out by qPCR using Roche LightCycler®480 Real-time PCR System (Roche, USA) on SYBR Green I method. The 20 μl reaction mixtures contained 10 μl SYBR Green Mix (Roche), 0.4 μl each forward and reverse primers (10 μM), 8.2 μl RNAase-free water and 1 μl standard plasmid or DNA template. The primer pairs used for *amoA* and *nxrA* were amoA-1F/amoA-2R and F1norA/R1norA, respectively (Hussain et al., 2011; Poly et al., 2008). The qPCR standard curves were established using the standard plasmids, which were diluted to yield a series of tenfold concentration. The specificity was assured by the melting curves and gel electrophoresis. Each gene was quantified in triplicate with a standard curve and negative control. The final data were generated using Abs Quant/2nd Derivative Max by Roche LC-480 software installed.

2.5. Analytical methods

The analysis of NH_4^+-N, NO_3^--N, NO_2^--N, MLSS was carried out in accordance with the standard methods (APHA-AWWA-WEF, 2012). DO and pH were monitored using a DO meter (HQ30d53LDO™, HACH, USA) and a pH meter (SG2, Mettler Toledo, Switzerland), respectively.

Nitrite accumulation ratio (NAR) was calculated as the percentage of nitrite accumulated to the amount of total nitrogen in the effluent. Specific oxygen uptake rate (SOUR) of the sludge was measured at the end of the experiment by batch experiments with or without inhibitors addition according to the methods of Zhang et al. (2016). The detailed methods were described in supplementary material.