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Denitrification behavior and microbial community spatial distribution inside woodchip-based solid-phase denitrification (W-SPD) bioreactor for nitrate-contaminated water treatment

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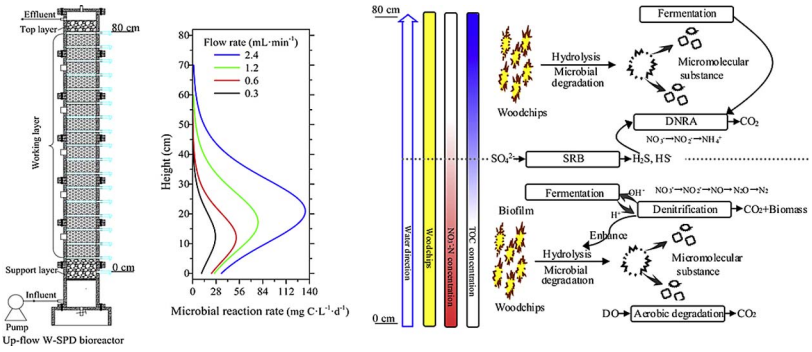
GRAPHICAL ABSTRACT



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ABSTRACT

The NO_3^- removal pathway and microorganisms change along with the height of an up-flow W-SPD bioreactor was investigated in this study. Modeling and microbial community analysis were used to analyze the denitrification behavior in W-SPD bioreactor. The results showed that NO_3^- removal rate matched for zero-order ($R^2 > 0.97$) and first-order ($R^2 > 0.94$) combination Michaelis-Menten kinetics, whereas microbial reaction rate suited for modified logistic model ($R^2 > 0.99$). The excellent denitrification performance (92.5%–96.4%) and microorganisms' quantity occurred in the middle of W-SPD bioreactor. Moreover, high-throughput sequencing analysis revealed that dominant denitrifiers, carbonaceous compound degrading bacteria and fermentative bacteria co-existed in W-SPD system, which was vital for efficiently sustainable NO_3^- removal. Hence, aerobic degradation, heterotrophic denitrification and dissimilatory nitrate reduction to ammonium (DNRA) occurred successively along the water direction in the bioreactor, offering reasonable references for W-SPD bioreactor study and application.

1. Introduction

Nowadays, NO_3^- has become one of the intractable contaminants in surface water and groundwater owing to human activities, including the long-term intensive application of nitrogen fertilizer and wastewater direct discharge and so on (Jafari et al., 2015). Excessive NO_3^- released into the environment has caused serious problems, such as eutrophication, water quality deterioration and potential hazard to

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human (Ghafari et al., 2008). For NO_3^- removal, a number of technologies have been developed that are one or a combination of physical (Bhatnagar and Sillanpää, 2011), chemical (Liu et al., 2015) and biological (Ghafari et al., 2008) processes. Among these competing technologies used for nitrate-contaminated water treatment, biological denitrification has attracted the great attention as its promising, environmentally-friendly and efficient technology (Guo et al., 2017). The heterotrophic denitrifiers reduce NO_3^- to innocuous nitrogen in the presence of organic electron donors under the anoxic conditions (Wang and Chu, 2016). However, challenges still exist regarding the lack of carbon source in nitrate-contaminated water such as surface water (Beutel et al., 2016) and groundwater (Zhang et al., 2012).

In denitrification systems, solid carbon source with high-denitrification efficiency and high-quality slow-release ability captures people's attention (Gibert et al., 2008). Over the past decades, many researches have focused on substrate type (Zhang et al., 2012), longevity denitrification ability (Robertson et al., 2000) and controlling factors (Schipper et al., 2010) in solid-phase denitrification (SPD) bioreactors entirely based on input-output data, without reflecting details of biological process inside the bioreactors along the water direction. Few attempts have been made about removal process inside the bioreactors. Challenges in bioreactor design based on intermediate reaction process inside bioreactor still remain.

Moreover, the duration of effectiveness in SPD bioreactors is determined by the duration of carbon supply to the denitrifiers. In SPD system, solid substrates were further converted into dissolved organic carbon (DOC) by microbial degradation or hydrolysis before it can be used in denitrification (Feng et al., 2017). Previous studies have shown that effluent with high DOC was observed at initial operation period in the bioreactor, while DOC of effluent decreased with passing time (Christianson et al., 2016). Due to the complexity of this process, the accurate amount of solid substrates fed in SPD bioreactors has been little noticed and still difficult to achieve. Especially, natural materials were used as solid carbon source, which resulted in excessive DOC release even NH_4^+ and NO_2^- accumulation (Zhang et al., 2012). As a result, the construction and commercialization of SPD bioreactor was suffering restriction for the further development.

Despite many researches about SPD bioreactor based on input-output data, the NO_3^- removal process inside the bioreactor along the water direction is not clear. Furthermore, the dosage of solid carbon source was hardly determined appropriately due to the complexity of the natural material degradation process. So the second pollution problem occurred frequently in effluent of the SPD bioreactor. Hence, the overall aim of this study was to evaluate the NO_3^- removal performance along the water direction and the optimum position of attainable complete denitrification under different conditions through a pilot-scale up-flow W-SPD bioreactor. The characteristics of NO_3^- removal, woodchip utilization, pH variation, and microbial community structures as well as activity along the height were analyzed to give insight into remediation process and mechanism inside the SPD bioreactor.

2. Material and methods

2.1. Woodchip preparation and characterization

Wood by-products (sawdust/woodchips) have shown an ability to provide sustained NO_3^- removal rates, while requiring minimum maintenance (Robertson, 2010). In this study, woodchips were selected as carbon source to stimulate SPD process. They were coarse with irregular pieces and obtained from fresh clean poplar (grew in Shanxi, China). Before experiment, woodchips were washed by DI water for 3 times and then air dried for 72 h, and then sieved to 1.0–5.0 mm size. The C, H, N, and S contents in the woodchips were 48.04% ± 0.04%, 5.87% ± 0.16%, 0.28% ± 0.05%, and 0.33% ± 0.08% as measured by an elemental analyzer (Flash 2000, Thermo Fisher, Italy).

2.2. Nitrate-contaminated composition

Synthetic wastewater was prepared with a target concentration of 50 mg N·L^{-1} ($NaNO_3$) by tap water. Phosphorus was added in the form of K_2HPO_4 at an N/P ratio of 20 (Zhang et al., 2012) to meet the requirement for microbial growth. All chemical reagents used in the experiment were analytical grade.

2.3. Microorganisms acclimation

Mixed liquor suspended solids (MLSS) for the acclimation of denitrifiers was obtained from a pre-anoxic zone in the Qinghe Sewage Treatment Plant (Beijing, China), then cultured in a 2 L beaker fed with nutrient medium at room temperature (20 ± 2 °C) in the dark. The nutrient medium contained 1.10 g $C_6H_{12}O_6$, 0.85 g $NaNO_3$, 0.10 g KH_2PO_4, 0.10 g K_2HPO_4, 0.10 g $MgSO_4{\cdot}7H_2O$, 0.006 g $FeSO_4{\cdot}7H_2O$ per liter tap water with pH approximately 7.00. The microorganisms were cultivated for one month before inoculation, and the medium was replaced every 3 days. Then, microorganisms in the bioreactor filled with synthetic wastewater were further acclimated for 7 days statically.

2.4. Experimental apparatus

A 7.265 L acrylic glass column (height = 92.5 cm; inside diameter = 10.0 cm) was set up as the up-flow W-SPD bioreactor at room temperature. 17 water-sampling ports (W0–W16) were placed evenly at 5 cm intervals along the height of bioreactor. Meanwhile, the bioreactor was composed of five treatment chambers (from bottom to top: 0–15 cm, 15–30 cm, 30–45 cm, 45–60 cm, 60–75 cm), in which middle was designed with five mixture-sampling ports (M1–M5) spaced evenly.

Along the column height from bottom, the first 7.5 cm height was 2.0–4.0 mm quart sands support layer to make water distribution evenly, and then 80 cm height was working layer containing woodchips and 1.0–2.0 mm quartz sands (dosage ratio (v/v) = 1:1) to assess its denitrification capacity, followed by 5.0 cm top layer of 1.0–2.0 mm quart sands to ensure enough water level. The media porosity (n_e) of the whole bioreactor was 48.0%.

The amounts of woodchips and quartz sands were 360 g and 5448 g, respectively. Before packing into the bioreactor, working layer materials were immersed with 1 L acclimated MLSS for 24 h, stirring hourly through a shovel for homogeneous mixing.

2.5. Experimental procedure

To investigate the influence of NO_3^- loading on the denitrification performance, the column study was divided into four phases (P1–P4). The flow rate was adjusted from 0.3 mL·min^{-1} (day 0) to 0.6 mL·min^{-1} (day 50), 1.2 mL·min^{-1} (day 70), 2.4 mL·min^{-1} (day 90), successively. The steady-state was assumed when changes of the NO_3^- removal efficiency remained below 3% at least during 10-day operation at four periods.

2.6. Microbial reaction rate tests

2.6.1. Counting of microorganisms on media

Solid matrix samples were collected from five mixture-sampling ports at the end of experiment (110^{th} day). 10.0 g solid matrix sample was placed into a 50 mL centrifuge tube containing 30 mL of saline (0.9% NaCl, v/v), and then centrifuged via a high-speed centrifuge (5430R, Eppendorf, Germany) at 20 °C and 2000 rpm for 10 min, then continuously shaking by hands and sampling immediately to analyze adenosine triphosphate (ATP) content via ATP analyzer (AF-100, DKK-TOA, Japan) and total microorganisms count (TMC) via 3 M Petrifilm™ aerobic count plates (6406-PAC, Shanghai Forthright Biological Technology Co., Ltd., China). Another 10.0 g sample was dried at 105 °C for 2 h in an electric thermostatic drying oven (101-2AB, Tianjin Taisite