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Nitrate and COD removal in an upflow biofilter under an aerobic atmosphere

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HIGHLIGHTS

- An aerobic biofilter packed with ceramsite was constructed for nitrate removal.
- *Pseudomonas stutzeri* X31, an aerobic denitrifier isolate, was used as an inoculum.
- The top section of the bioreactor possesses higher COD and NO_3^-—N removal rates.
- *Pseudomonas stutzeri* and *Paracoccus versutus* were the most dominant bacteria.

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ABSTRACT

A continuous-upflow submerged biofilter packed with ceramsite was constructed for nitrate removal under an aerobic atmosphere. *Pseudomonas stutzeri* X31, an aerobic denitrifier isolate, was added to the bioreactor as an inoculum. The influent NO_3^-—N concentrations were 63.0–73.8 mg L^{-1}. The best results were achieved when dissolved oxygen level was 4.6 mg L^{-1} and C/N ratio was 4.5. The maximum removal efficiencies of carbon oxygen demand (COD) and NO_3^-—N were 94.04% and 98.48%, respectively at 30 °C, when the hydraulic load was 0.75 m h^{-1}. The top section of the bioreactor possessed less biofilm but higher COD and NO_3^-—N removal rates than the bottom section. Polymerase chain reaction (PCR)–denaturing gradient gel electrophoresis (DGGE) technique combined with electron microscopic examination indicated *P. stutzeri* X31 and *Paracoccus versutus* were the most dominant bacteria. *Amoeba* sp., *Vorticella* sp., *Philodina* sp., and *Stephanodiscus* sp. were also found in the bioreactor.

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1. Introduction

Groundwater is an important source of municipal water supply for domestic and industrial use (Showers et al., 2008). The removal of nitrate from surface and underground waters has received increased attention on account of the wastewater discharge, deforestation of riparian zones, and the extensive use of fertilizers, etc. Increased nitrate concentrations in drinking water may be responsible for methemoglobinemia and diverse kinds of cancers in humans (Bhatnagar and Sillanpää, 2011). The nitrate removal technologies mainly include ion exchange, reverse osmosis, adsorption, electrodialysis, and biological denitrification. And the biological nitrogen removal process is one of the most commonly used technologies due to its effectiveness and relatively low cost (Haugen et al., 2002). Biological denitrification is considered to be the most economical strategy to use because it does not require post-treatment or produce by-products (Liu et al., 2012).

Denitrification is commonly considered to be achieved under anaerobic or anoxic conditions. However, there have been sporadic reports on aerobic denitrifiers (Kim et al., 2008a; Wang et al., 2013), which are able to conduct an aerobic respiratory process in which nitrate is gradually converted to N_2. It is commonly accepted that dissolved oxygen concentration, C/N ratio, pH and temperature are the rate-limiting parameters, and the first two of them are suggested to be the major factors affecting aerobic denitrification (Bernat and Wojnowska-Baryła, 2007).

As one of the popular biomembrance processes, biofilter process has gradually been adopted in the small communities, since it is simple to manage and able to remove organic materials and suspended solids simultaneously (Kim et al., 2008b). The upflow bioilter is a plug-flow reactor which follows first-level reaction kinetics. In recent years, the submerged filter has been applied to treat nitrate polluted groundwater under an anoxic atmosphere (Gomez et al., 2009; Moreno et al., 2005). Inoculation of a submerged biofilters inoculated with *Hydrogenophaga pseudoflava* showed better results in terms of system stability, higher superficial hydraulic loading and superficial nitrate loading rates than

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activated sludge (Moreno et al., 2005). Optimum C/N ratio was found to range from 1.08 to 2 in the filter (Cortez et al., 2009, 2011; Moreno et al., 2005) for nitrate removal. Although a promising nitrate removal efficiency of over 95% in some cases has been achieved, residual nitrite seemed to be inevitable in the treated water.

The main objective of this study was to utilise aerobic denitrification to remove nitrate in an upflow submerged biofilter. This was carried out by using pure cultures of *Pseudomonas stutzeri* X31, which showed tolerance to high oxygen concentrations (Ji et al., 2013).

2. Methods

2.1. Reactor operation

As shown in Fig. 1, the reactor was a 1800 mm high organic glass column, with 100 mm inner diameter, below which was a long handle filter nozzle in the middle of retainer plate with dozens of holes with a diameter of 0.9 mm, whose function mainly was aeration and backwashing. The bioreactor was filled with a depth of 1400 mm ceramsite filter media (3–5 mm diameter, 1730 kg m^{-3} density, 650 m^2 m^{-3} specific surface, 52% porosity and non-uniform coefficient $K_{80} = d_{80}/d_{10} < 2.0$).

The system was continuously supplied with groundwater of Wuhan, China, which was pumped from the underground zone and supplemented with KNO_3 and NaAc. The influent characteristics were as follows: nitrate–N, 63.0–73.8 mg L^{-1}; nitrite–N, 0.53–3.07 mg L^{-1}; phosphate, 0.4–0.7 mg L^{-1}; sulphate, 39–91 mg L^{-1}; chloride, 53–135 mg L^{-1}; total dissolved solids, 452–546 mg L^{-1}; turbidity, 0.92–2.65 NTU; and pH 7.0–7.5. In order to get the biofilm formation, the precultured *P. stutzeri* X31 isolate (7.9×10^7, 0.5% v/v) were inoculated into the reactor, amended with KNO_3 (2 g L^{-1}), NaAc (3 g L^{-1}) and trace element solution as described (Zhang et al., 2011). Aeration was applied consistently from the bottom of the reactor for 3 days. After the inoculation phase, the reactor was operated at a low hydraulic load of 0.15 m h^{-1} for 7 days, with influent continuously injected into the reactor. Then the hydraulic load was raised gradually to 0.75 m h^{-1} in one week.

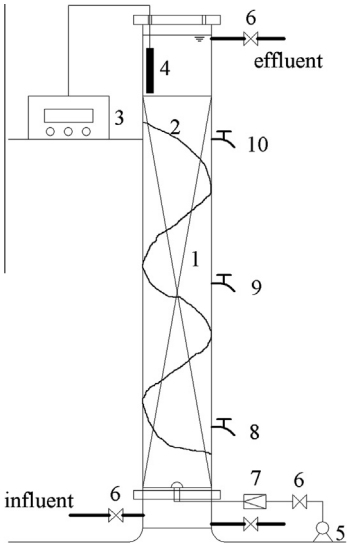

Fig. 1. Schematic diagram of the biofilter: (1) ceramsite filter media; (2) electrical heating belts; (3) temperature controller; (4) temperature sensor; (5) air compressor; (6) valve; (7) gas flowmeter; (8) sampling port A; (9) sampling port B; (10) sampling port C.

Different DO values in the reactor were acquired by controlling the aeration intensity, which were 2.7, 4.2, 5.1, 6.0 and 7.4 mg L^{-1}, when the C/N ratio was controlled at about 5. While the C/N ratios of the influent were adjusted to 1.8, 2.5, 3.5, 4.2, 4.5, and 5 by changing the amount of the carbon source and by maintaining a constant nitrogen concentration. Each operating condition was maintained for 10 days and average variations of nitrogen and COD of seven samples were determined.

2.2. Analysis methods

COD, nitrate nitrogen and nitrite nitrogen of the samples were determined according to the Standard Methods (APHA, 2005). The pH of the influent was measured by an 828 Orion pH meter. Dissolved oxygen (DO) and temperature of the solution were measured with a 52 YSI DO meter. To measure the attached biomass, about 1% of ceramsite were collected from the bioreactor and dried for 2 h at 105 °C. The total weight of the dried ceramsite was measured and the weight of the attached biomass was obtained by subtracting the original weight of the ceramsite. Physiological and biochemical identification (More et al., 2012; Zhang et al., 2011) along with scanning electron microscope examination (VEGA 3 LMU, TESCAN) was used to identify the dominant bacteria preliminarily. For electron microscope examination, biofilm samples were pretreated by fixing with 2.5% pentanediol in a 0.1 M phosphate buffer, then soaked in 1% osmic acid. Afterwards, the samples were washed and dehydrated in a graded series of ethanol solutions (50%, 70%, 80%, 90% and 100%). The samples were dried by the critical point method and coated with gold.

2.3. Assessment of contaminants removal at different heights of the reactor

Biomass and biofilm activity were analysed from sampling port A, B and C (Fig. 1), which were at the height of 25 cm, 75 cm and 125 cm, respectively, from the inlet of the bottom. A certain amount of packing was carefully rinsed with double-distilled water three times. Three equal parts of the biofilm were respectively added to three 500 mL flasks containing sterile oxygen-rich medium, which had the same components as the influent. Then the flask was placed on a magnetic stirrer with a medium stirring speed at 30 °C. After cultured for 1 h, the supernatant was sampled and measured in triplicate. COD and nitrate consumption rates were determined accordingly.

2.4. PCR – denaturing gradient gel electrophoresis (DGGE)

Total bacterial DNA was extracted with a genomic DNA extraction kit (TianGen, China) following the manufacturer's instructions. PCR – DGGE of the community was performed on the partial 16S rRNA gene. The primers used for DGGE were BSF338-GC (5′-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GAC TCC TAC GGG AGG CAG CAG-3′) and BSR518 (5′- ATT ACC GCG GCT GCT GG -3′). The DGGE was performed using the D-Code Universal Detection Mutation System (Bio-Rad). 8% polyacrylamide gels having gradients of 35–70% denaturant were used to run the gel. The 100% denaturing solution strength was defined as 5.6 M urea and 32% (v/v) deionized formamide. The gel was run using TAE buffer for 9 h at 60 °C with 110 V and visualised by modified silver staining. The DGGE band was cut and the DNA was amplified. The amplification products were linked with pMD 18-T Vector and added into competent cells of *Escherichia coli* TOP10. Positive clones were picked and sequenced, and the 16S rRNA partial sequences were aligned with the same region of the closest relative strains available in the GenBank database by BLAST search.

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