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Effects of operational parameters on the treatment of nitrate-rich wastewater by autohydrogenotrophic denitrifying bacteria

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Introduction

The release of nitrate through anthropogenic point (e.g. industrial, domestic wastewater and landfill leachate) and non-point (e.g. agricultural drainage) sources in the environment has augmented the nitrate concentration in surface waters and groundwater, thus posing a worldwide environment challenge and health risk (Feleke & Sakakibara 2002; Ghafari *et al*. 2009a). Uninterrupted consumption of water that contains high concentration of nitrate, which is above the maximum contaminant level in potable water, that is, 45 mg/L NO_3^--N (World Health Organization 2006), can cause several diseases, such as methemoglobinemia, gastric cancer and non-Hodgkin's lymphoma (Sunger & Bose 2009). Different abiotic and biotic methods have been developed to reduce nitrate from water and wastewater. Abiotic techniques have been applied widely, but they pose certain disadvantages, such as waste brine disposal requirements, low efficiency, high capital and operating costs, and thus, biotic methods are favourable and cost-effective methods for removing nitrite and nitrate (Mousavi *et al*. 2012).

Autotrophs and heterotrophs, which are ubiquitous in nature, are used for denitrification and are generally found in ground or surface water and in the sludge of wastewater treatment plants (Chong & Lin 2007). The use of heterotrophic denitrifying bacteria, which consumes organic carbon, is associated with several restrictions, such as reactor clogging, residual organic carbon and by-products that typically incur further costly post-treatment (Mousavi

et al. 2011). Therefore, researchers have focused on the application of autotrophic denitrifying bacteria, which have shown promising results in nitrate elimination. Autotrophs derived energy from inorganic sources (e.g. ferrous iron, manganese and hydrogen sulphide) and utilized carbon dioxide and bicarbonates as carbon sources (Ghafari *et al*. 2008).

A new and comprehensive review by the authors showed that among the inorganic energy sources, H_2 is the perfect alternative for the denitrification process because it is inherently clean and is the best electron donor. In addition, the methods that use H_2 and autohydrogenotrophic bacteria (e.g. *Paracoccus denitrificans, Alcaligenes eutrophs* and *Pseudomonas pseudotiara*) for denitrification are free from the weaknesses of other heterotrophic and autotrophic techniques (Mousavi *et al*. 2011, 2012). Therefore, based on the advantages of this method, as discussed by the authors in previously published reviews, many researchers applied such promising method to treat water and wastewater. Rezania *et al*. (2005) achieved a nitrate specific degradation rate of 740 mg NO_3^-–N/g volatile suspended solids (VSS)·d for synthetic contaminated water containing 20 mg/L NO_3^--N (Rezania *et al*. 2005). Gross *et al*. (1988) reported the commercial scale of this method for water containing 17 mg/L NO_3^--N, and their results show the ability of the plant to treat 50 m^3/h water at the rate of 250 mg/L d NO_3^--N (Gross *et al*. 1988). In 2006, Vasiliadou *et al*. (2006) obtained a nitrogen consumption rate of 2.6 g NO_3^--N/g VSS·d for contaminated water with 30 to 200 mg NO_3^--N/L (Vasiliadou *et al*. 2006). More recently, Ghafari *et al*. (2009b) obtained a nitrate specific degradation rate of 29.6 mg NO_2^-–N/g VSS/h for synthetic contaminated water containing 20 mg/L NO_3^--N (Ghafari *et al*. 2010).

The studies above confirmed the ability of the autohydrogenotrophic bacteria to remove the lowconcentration of nitrate. However, information on the adaptation and the paths to improve the ability of such bacteria to remove the high concentration of nitrate is insufficient Ghafari *et al*. (2009a) obtained the acclimation of autohydrogenotrophic bacteria by using inorganic carbon sources, namely, carbon dioxide (CO_2) and bicarbonate. Their results demonstrated an improved denitrification rate by using an adequate acclimatization process at a low concentration of nitrate of 20 mg/L

(Ghafari *et al*. 2009a).

In the present study, we investigated the growth of a mixed culture of autohydrogenotrophic denitrifying bacteria and the effect of some operational parameters on process in a batch reactor. Among the several factors that affect the rate of nitrate elimination and autohydrogenotrophic bacteria activity, the effects of C/N ratio, hydrogen concentration and initial concentration of nitrate were investigated

Material and methods

Biomass and synthetic wastewater

The activated sludge, which was obtained from an urban wastewater treatment plant in Pantai Dalam, Kuala Lumpur, Malaysia, contained a mixed culture of denitrifying bacteria. The total suspended solids, VSS and the initial sludge volume index of the original activated sludge were 7800, 5200 and 123 mg/L, respectively. The activated sludge was filtered to remove wastes and washed repeatedly to remove the internal nitrogen components (NH_{4+}, NO_{2-} and NO_{3-}). The sludge was then dewatered and kept in a growth medium in a cold room (4°C) for future use. The synthetic wastewater was prepared with varying C/N ratios of 1, 2, 4 and 8 by different concentrations of KNO_3 (200, 800, 1400 and 2000 mg L_{-1} as NO_3-N, respectively) and was fresh for every cycle of the experiments. Sodium bicarbonate ($NaHCO_3$) was used as the carbon source according to the ratios mentioned above, and the phosphorus sources were KH_2PO_4 (1.74 g/L) and K_2HPO_4 (2.14) per 0.1 g/L NO_3-N/, similar to the buffering of solution. Then, 1 ml/L of a stock solution containing trace elements [EDTA (10 mg/L), $ZnSO_4.7H_2O$ (2.2 mg/L), MgSO4 (60 mg/L), $CoCL_2.6H_2O$ (3.2 mg/L), $MnCl_2.4H_2O$ (1.02 mg/L), $CuSO_4.5H_2O$ (0.22 mg/L), $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (2.2 mg/L), $CaCl_2.2H_2O$ (1.1 mg/L), $FeSO_4.7H_2O$ (1.02 mg/L), H_3BO_3 (0.3 mg/L) and $NiSO_4.6H_2O$10] was added into the feeding solution.

Experimental setup

This study was accomplished in five sequencing batch reactors (SBRs) (R1 to R5) with aworking volume of 5 L for a period

of 170 days (Fig. 1). Multiple cycles were set with sequencing stages of reaction (complete removal of nitrate ≥ 22 h), settlement (110 min), decanting (5 min) and filling (5 min), and cycles duration will be 24 h if the complete removal of nitrogen component happen. The length of the total cycle for the SBRs was maintained at complete denitrification within each cycle because of the high concentration of nitrate, slow growth of autohydrogenotrophic bacteria and limited interference in the loss of biomass during decanting time. The controlling factors such as pH (7.8 • } 0.2), temperature (30°C • } 0.5°C) and dissolved oxygen (DO) (≤ 0.2 mg/L) were kept constant under optimal conditions to limit their effects on the process during the experiments (Ghafari *et al*. 2009b; Karanasios *et al*. 2010). The reactors were equipped with a thermostatic jacket, and temperature was maintained at 30 • } 0.5°C by using a thermostatic bath. The SBRs were equipped with two probes to determine the DO and pH value online, and the biomass was suspended via mechanically stirring during the reaction time. The pipes used for gas sparging (H_2 and N_2) were connected to bubble stones at the end. Hydrogen gas was used at different sparging times, with 6 time/d at 3 min for each time, 11 time/d at 30 s for each time and 11 time/d 1 min for each time for the growth and adaptation of the bacteria as electron donor. Nitrogen gas was sparged inside the reactors through the bubble stones to keep the DO concentration under 0.2 mg/L and maintain a suitable anoxic condition. At the end of each settlement phase, 50% of the contents of the reactors were decanted from each reactor and then the reactors were fed by synthetic wastewater for keeping the initial concentration of nitrate constant inside reactor at 100, 400, 700 and 1000 mg NO_3^--N/L. Furthermore, to maintain the value of the mixed liquor suspended solid (MLSS) at 3000 mg/L (SBR = 30), the MLSS of effluent was returned back to the reactors or withdraw from the reactor. Table 1 summarizes the experimental procedure.

Analytical methods

Advanced Compact IC 861 (Metrohm Ltd, Herisau, Switzerland) ion chromatograph with guard column was used to

measure the nitrate and nitrite concentrations. Ultra pure water (18.2 μ s) with dissolved $NaCO_3$ (0.3392 g/L), $NaHCO_3$ (0.084 mg/L) and H_2SO_4 (0.1 M) was used as eluent to determine the anion concentration. Temperature, pH (METTLER

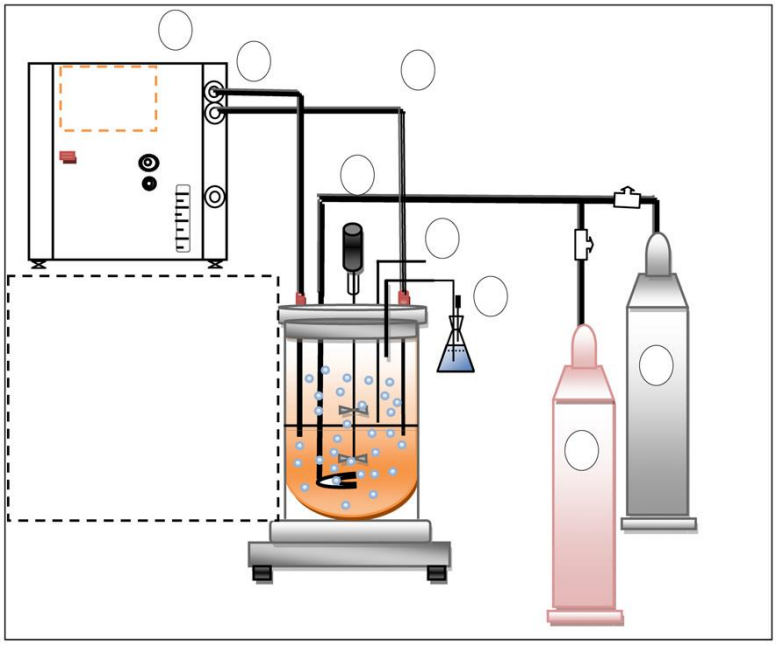

Fig. 1. Schematic diagram of the experimental set-up.

TOLEDO, pH-sensor, Switzerland) and DO (METTLER TOLEDO, O_2-sensor, Switzerland), were continually monitored using a digital controller. The samples were centrifuged and filtered (using 0.2 μ m) prior to analysis. The MLSS, mixed liquor volatile suspended solid (MLVSS) and most probable number (MPN) were also determined using standard methods (American Public Health Association *et al*. 2005). A 5% coefficient of variation was set for all samples. A scanning electron microscope [AURIGA the new CrossBeam Workstation (FIB-SEM) from Carl Zeiss NTS] was used to determine the morphology of the biomass, and it was controlled by a computer system at the magnification range of 5 kV–to 30 kV-fold.

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