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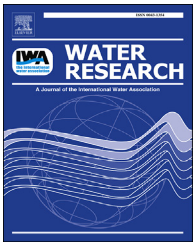

Autotrophic denitrification of nitrate and nitrite using thiosulfate as an electron donor

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ABSTRACT

This study was carried out to determine the possibility of autotrophic denitritation using thiosulfate as an electron donor, compare the kinetics of autotrophic denitrification and denitritation, and to study the effects of pH and sulfur/nitrogen (S/N) ratio on the denitrification rate of nitrite. Both nitrate and nitrite were removed by autotrophic denitrification using thiosulfate as an electron donor at concentrations up to 800 mg-N/L. Denitrification required a S/N ratio of 5.1 for complete denitrification, but denitritation was complete at a S/N ratio of 2.5, which indicated an electron donor cost savings of 50%. Also, pH during denitrification decreased but increased with nitrite, implying additional alkalinity savings. Finally, the highest specific substrate utilization rate of nitrite was slightly higher than that of nitrate reduction, and biomass yield for denitrification was relatively higher than that of denitritation, showing less sludge production and resulting in lower sludge handling costs.

1. Introduction

Denitrification can occur either heterotrophically or autotrophically. Heterotrophic bacteria use an organic carbon source as an electron donor to convert nitrite and nitrate to free nitrogen gas under anoxic conditions. This process is very efficient in wastewater when a sufficient carbon source is available. Depending upon the organic compound used, a carbon:nitrogen (C/N) ratio of 2.7–3 is required for complete nitrate reduction. When a sufficient carbon source is not available in wastewater, inclusion of an additional carbon source can be expensive. In the case of most wastewaters, such as those of old landfill leachate, fertilizers and the leather processing industry, the C/N ratio is very low (Bai et al., 2009; Manconi et al., 2007). With time, the chemical oxygen demand in landfill leachates decreases and the ammonia concentration increases (Price et al., 2003); thus, nitrogen removal from an aged landfill leachate requires an external carbon source during the denitrification process. Also, the additional carbon source needed to address fluctuation in influent nitrogen loading becomes a secondary pollutant if it is not completely oxidized in the denitrification process.

Autotrophic denitrification is an alternative to heterotrophic denitrification of wastewater and landfill leachate that has high nitrogen content and low carbon content. The

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process is carried out by the autotrophic bacteria such as *Thiobacillus denitrificans* or *Thiomicrospira denitrificans* (Hashimoto et al., 1987). Energy required by these microorganisms is derived from the oxidation of certain reduced inorganic compounds (H_2S, $S_2O_3^{-2}$, S, $S_4O_6^{-2}$, SO_3^{-2}) coupled with the reduction of nitrate or nitrite. Theses microorganisms can also grow mixotrophically (i.e. use an inorganic carbon (CO_2, HCO_3^-) and organic carbon for biosynthesis). Autotrophic denitrification has two clear advantages: 1) no need for an external carbon source, i.e., ethanol or methanol, which can reduce the cost of the process and 2) reduced biomass production, which minimizes the handling of sludge (Claus and Kutzner, 1985; Zhang and Lampe, 1999). However, autotrophic denitrification with sulfur increases the sulfate concentration and decreases pH in the effluent (equation (1)).

$$\begin{aligned} &NO_3^- + 0.887S_2O_3^{2-} + 0.456CO_2 + 0.709H_2O \rightarrow 0.454N_2 \\ &+ 1.733\ SO_4^{-2} + 0.773H^+ + 0.0915\ C_5H_7O_2N \end{aligned} \tag{1}$$

When a wastewater with high nitrate concentration and low alkalinity is used, the additional cost to maintain pH in the neutral range required for denitrification is substantial (Liu and Koeing, 2002). Elemental sulfur-limestone autotrophic denitrification has been studied extensively (Liu and Koeing, 2002; Moon et al., 2004; Zhang and Lampe, 1999), and its efficiency was similar to that of heterotrophic denitrification. Although this process seems to be more economical, the use of limestone has a disadvantage in that it increases the hardness and dissolved solids content of water, thus limiting its applicability (Wang and Qu, 2003; Driscoll and Biscogni, 1978).

A stoichiometric equation indicates that 1 g of NO_3^-–N reduced to nitrogen gas produces 12.15 g of sulfate when thiosulfate is used as the electron donor (Matsui and Yamamoto, 1986). This large amount of sulfate production (and accompanying alkalinity consumption) is a disadvantage to autotrophic denitrification of nitrate. In order to overcome the problem of high sulfate production and alkalinity reduction, various researchers (Kim et al., 2004; Liu et al., 2009) have carried out research on combined heterotrophic and autotrophic denitrification of wastewater and drinking water. A shortcut biological nitrification and denitrification was carried out successfully using heterotrophic bacteria (Wang et al., 2008; Ruiz et al., 2006), and 65–95% nitrite accumulation was achieved (Ruiz et al., 2003; Ciudad et al., 2005). Especially, The operational factors of nitrite accumulation have been reported to be the free ammonia and free nitrous acid concentrations, depending on the pH (Anthonisen et al., 1976; Turk and Mavinic, 1987; Abeling and Seyfried, 1992), dissolved oxygen level (Garrido et al., 1997; Pollice et al., 2002; Ruiz et al., 2003; Jubany et al., 2009), temperature (Yang et al., 2007), and sludge retention time (Hellinga et al., 1998; Groeneweg et al., 1994; Pollice et al., 2002). Also, the advantages of heterotrophic denitrification, including a 40% reduction in electron donor use, high denitrification rate and reduced biomass production (Chung et al., 2007; Wang et al., 2008), have been well established. Also, it has been indicated by various researchers that sludge can be acclimated to nitrite reduction and high levels of nitrite can be denitrified smoothly to nitrogen gas (Chung and Bae, 2002; Adav et al., 2010).

Therefore, it is important to study the kinetics of nitrite reduction using autotrophic denitrification in order to overcome the disadvantages of high alkalinity needs and sulfate production during autotrophic denitrification and to also take advantage of shortcut biological nitrification in the autotrophic process. The objective of this study was to investigate the possibility of autotrophic denitritation using thiosulfate as an electron donor and to determine the effects of the S/N ratio and initial pH on the denitritation. Finally, we compared the kinetic parameters of denitrification and denitritation using thiosulfate.

2. Materials and methods

2.1. Preparation of biomass and stock solution

A stock solution of 1000 mg-N/L for nitrate or nitrite was prepared. The S/N ratio was maintained at 5.1 and 2.5 for nitrate and nitrite, respectively. At this ratio, the amount of sulfur was sufficient for complete denitrification. Sufficient amounts of nutrients and minerals were provided for cell synthesis. Phosphorus was provided at a level just sufficient for cell synthesis. The amount of ammonium nitrogen provided was less than that required for cell synthesis so that the cells used nitrate or nitrite as a nitrogen source for growth. Distilled water was used to prepare the stock solution and its dilution. The composition of the medium was (in g/L): KNO_3 7.22, $NaS_2O_2 \cdot 5H_2O$ 19.92 (S/N = 5.1) (for nitrate), KNO_2 6.07, $NaS_2O_2 \cdot 5H_2O$ 10.0 (S/N = 2.5) (for nitrite), $NaHCO_3$ 15, K_2HPO_4 0.057, NH_4Cl 0.056, $CaCl_2$ 0.01, $FeCl_2$ 0.01, $MgSO_4 \cdot 7H_2O$ 0.01 and $MnSO_4 \cdot H_2O$ 0.01.

An autotrophic denitrifying biomass was enriched by inoculating activated sludge collected from a municipal wastewater treatment plant (Ansan, Korea). The sludge was separately acclimated for nitrite and nitrate under anoxic conditions by steadily increasing the NO_2^-–N or NO_3^-–N concentration, respectively, from 10 to 500 mg/L at a temperature of 30 °C. The acclimated sludge was maintained in a 1-L master cell reactor (MCR). The MCR was operated at a temperature of 30 °C and stirred continuously with a magnetic stirrer. The feed solution was changed when the concentration of NO_2^-–N or NO_3^-–N fell below 20 m/L. Every time the medium solution was changed, the MCR reactor was purged with nitrogen gas until the DO concentration fell below 0.3 mg/L. Samples from the MCR were collected every 12–24 h. At every feed change, the initial pH was maintained at 8.0 ± 0.1 by adding HCl solution (0.75 N).

2.2. Experimental setup

In order to test autotrophic denitrification, batch tests were carried out using 500-mL flask bottles under anoxic conditions. The sludge for each batch test was collected from an MCR that had been operated for longer than nine months. For the kinetic study, different initial concentrations of nitrate and nitrite were provided with the same amount of sulfur source, of 1000 mg-S/L of $S_2O_3^{2-}$, in order to avoid inhibition by thiosulfate.