Nitrogen removal in a fluidized bed bioreactor by using mixed culture under oxygen-limited conditions

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Abstract Nitrogen removal involving nitrification and denitrification was investigated in a fluidized bed bioreactor by using mixed culture sludge under oxygen-limited conditions. Methane was used as a sole carbon source for denitrification. In this study, optimal nitrification and denitrification rates were examined by varying methane and oxygen gas dissolution flow rates, 90 ml/min, 400 ml/min and 650 ml/min, in each. Simultaneously nitrification and denitrification was achieved. The total nitrogen removal rate was 15-mg N/g VSS. d, 21-mg N/g VSS. d and 26.4-mg N/g VSS. d at gas dissolution flow rate 90 ml/min, 400 ml/min and 650 ml/min, respectively. No significant accumulation of nitrite was found in this experiment. Nitrogen removal rates depend on gas dissolution flow rates. DO concentration was at 0.5–2 mg/L.

Keywords Denitrification; Fluidizing Bed BioReactor (FBBR); Gas Dissolution Flow Rate (GDFR); methane oxidation; nitrification; Simultaneous Nitrification and Denitrification (SND)

Introduction
Biological nitrogen removal is generally used for elimination of nitrogen compounds from wastewater and is based on nitrification and denitrification. Nitrification is the oxidation of reduced inorganic nitrogen compounds by autotrophic nitrifiers and denitrification is a respiration process in which oxidized nitrogen compounds are reduced to gaseous nitrogen compounds by heterotrophic denitrifying bacteria. Nitrification and denitrification are carried out under different conditions and by different microorganisms. Experience shows that the processes have to be separated in time or space. However, under oxygen-limited conditions or anoxic conditions, autotrophic nitrifiers were able to reduce nitrate and/or nitrite to nitric oxide, nitrous oxide or N₂, depending on the presence of a suitable electron donor (Poth, 1986; Remde and Conrad, 1990; Bock et al., 1995). Kuai and Verstraete (1998) reported that the Oxygen Limited Autotrophic Nitrification and Denitrification (OLAND) process was carried out under oxygen-limited autotrophic conditions. Recently, Strous (2000) reported a new process; called the Completely Autotrophic Nitrogen Removal Over Nitrite (CANON) process, in which ammonium is partially oxidized to nitrite by aerobic ammonium oxidizers and the remaining ammonium oxidized with the nitrite to yield dinitrogen by anaerobic ammonium oxidizer under oxygen-limited conditions. This process is completely autotrophic, therefore, avoiding COD addition, which is often required for the heterotrophic denitrification step in traditional systems.

For the denitrification system, denitrification occurs under an anoxic or anaerobic system and a supplemental organic carbon source, which may serve as electron donor, must frequently be added due to the heterotrophic denitrifying bacteria. A variety of electron donors, such as methanol, acetate, ethanol, lactate and glucose (McCarty et al., 1969; Blaszczyk and Przytocka-Jusiak, 1981; Grabinska-Loniewska, 1991; Tam et al., 1992; Akunna et al., 1993) has been used for this purpose. Later, methane serving as a possible sole electron donor for denitrification under oxygen-limited conditions was demonstrated (Davies, 1973; Sollo et al., 1976; Werner and Kayser, 1991; Thalasso et al., 1997; Costa et al., 2000; Lee et al., 2001).
Denitrification with methane is carried out by methanotrophic/methylotrophic association. Methanotrophs are strict aerobes and are capable of growing only on methane. An association of methanotrophs oxidized methane to carbon dioxide and water (Mechsner and Hamer, 1985), in which process they are unable to denitrify, but are able to excrete some organic intermediate compounds such as citrate (Rhee and Fuhs, 1978), methanol (Mechsner and Hamer, 1985), polysaccharides and proteins (Linton and Buckee, 1977; Ivanova and Nesterov, 1988; Mshenskii et al., 1988; Nesterov et al., 1988) and acetate (Costa et al., 2000) under certain environmental conditions (Megraw and Knowles, 1989; Roy and Knowles, 1994; Amaral and Knowles, 1995). Several authors have demonstrated the ability of organic intermediate compounds to serve as a carbon source for aerobic or anoxic denitrifying.

The ammonia oxidizer appeared to be common in the methane-oxidizing environment. Methanotrophs are able to oxidize ammonia, although they apparently cannot grow lithotrophically by using ammonia as sole electron donor (Thomas and Michael, 1988). In both ammonia oxidizers and methanotrophs, methanol is metabolized to CO₂ and cell C (Jones and Morita, 1983). Their metabolic correlation has been reinforced with five supporting observations: (i) Similarities in the structure of their genetic coding (i.e., DNA composition); (ii) Similarities in their substrate oxidizing monooxygenase enzymes; (iii) Similarities in the structure of their cell membranes each being extensively intruded or corrugated; (iv) Apparent similarities in their required substrate forms (e.g. both consuming unionized forms having molecular weights either 16 or 17); and (v) The fact that nitritifiers can co-metabolize the methanotroph’s substrate, methane (Bedrard and Knowles, 1989). There is no doubt that the competition for dissolved oxygen (DO) exists between methanotrophs and ammonia oxidizers.

The above researches imply that the complete conversion of ammonium into nitrogen gas and nitrate into nitrogen gas by using the mixed culture sludge in a single reactor under oxygen-limited conditions is possible. Based on this implication, a new process, simultaneous nitrification and denitrification (SND) under oxygen-limited conditions can be proposed. SND implies that nitrification and denitrification occur concurrently in the same reactor under identical overall operating conditions (Wartchow, 1990; Watanabe et al., 1992; Munch et al., 1996). SND may have a significant advantage over the elimination of multi-reactor systems or single reactors with intermittent aeration for complete nitrogen removal.

In this study, a possibility of SND was examined in a fluidized bed reactor containing mixed culture sludge under oxygen-limited conditions. Therefore, the study was performed to evaluate the optimum rate of nitrogen removal at various methane and oxygen gas dissolution rates.

**Materials and methods**

**Preparation of seed sludge**

The culturing of seed sludge was carried out as follows. The mixed methanotrophic culture was taken from a landfill cover soil. The cover soil was mixed with a nutrient solution to obtain the bacteria. After the soil was settled, the supernatant was transferred to a new nutrient solution and fed with oxygen and methane. This procedure was repeated for 2 weeks with culturing methanotrophs. The mixed culture activated sludge was taken from a domestic wastewater treatment plant. The sludge was fed with NH₄Cl, NaHCO₃ and nutrient solution under oxygen-limited conditions. The sludge was separately fed with NaNO₃, methanol and nutrient solution under anoxic conditions. Culturing of autotrophic nitrifiers and denitrifiers was carried out for 2 weeks. Before the start of the experiments, all cultured microorganisms were mixed together and pretreated for 2 weeks. Pretreatment was carried out by passing the dissolved oxygen and methane in the inoculum, which contains NH₄Cl, NaNO₃, NaHCO₃ and nutrient solution. The pretreatment was carried out to eliminate any residual carbon source associated with the sludge inoculum.
Synthetic wastewater

Synthetic wastewater was prepared by addition of ammonia and nitrate to a mineral medium in the form of NH$_4$Cl and NaNO$_3$. The composition of nutrient solution in all experiments was (in mg/L): NaHCO$_3$ (300), MgSO$_4$.7H$_2$O (200), NaHPO$_4$ (200), KCl (40), CaCl$_2$ (15), FeSO$_4$.7H$_2$O (1), ZnSO$_4$.7H$_2$O (0.07), MnSO$_4$.5H$_2$O (0.01), H$_3$BO$_3$ (0.01), CuSO$_4$.5H$_2$O (0.005). pH was adjusted to 7.5.

Experimental setup

A laboratory scale up-flow fluidized bed bioreactor (FBBR) was designed as shown in Figure 1. The unit consisted of three columns. Column I was the biological fluidized bed reactor (21 L), Column II (2.5 L) was the methane gas dissolution reactor and Column III (2.5 L) was the oxygen gas dissolution reactor. Re-circulation rate was used for completely mixed conditions. Peristaltic pumps were used for influent as well as re-circulation.

Experimental procedure

About 3,000 mg/L MLVSS of sludge inoculum was filled into the fluidized bed bioreactor. The influent flow rate was 0.75 L/hr and recycling flow rate was 38 times higher than the influent flow rate. Hydraulic retention time was maintained at 20 hr. 95% Methane and 80% Oxygen were supplied into Column II and Column III at a rate of 30 ml/min in each column. The gas dissolution flow rates (GDFR) of Column II and Column III were varied 90 ml/min, 400 ml/min and 650 ml/min each. Experiments were carried out in three steps. Bulk DO concentration was around 0.5–2 mg/L. These experiments were operated at ambient temperature (32–34°C).

Analytical methods

Samples from influent and effluent streams of FBBR were analyzed daily. NH$_4$N, NO$_2$N and MLVSS were analyzed as per Standard methods (APHA, AWWA, WEF 20th ed., 1998). NO$_3$N was measured spectrophotometrically at 420 nm (Schwoerbel, 1980; Frevert,

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**Figure 1** Schematic illustration of the fluidized bed reactor

(A) Feed Tank, (B) Fluidized Bed Bioreactor, (C) Methane Gas Dissolution Reactor, (D) Oxygen Gas Dissolution Reactor, (E) Pump, (F) DO probe, (G) Gas collector, (H) Effluent, (I) Gas flow meter, (J) Gas sampling port, (K) FBR sampling port
Gas composition was analyzed by 14A Shimadzu Gas Chromatograph. Total and soluble organic carbon were quantified with a Shimadzu TOC Vcsn. pH was measured by Ecoscan pH 6 pH meter, Eutech. DO was measured by Cyberscan DO 100 Meter, Eutech.

Results and discussion

Optimal ammonium nitrogen removal at different gas dissolution flow rates

The experiment was carried out in 3 steps with different GDFR (90, 400, 650 ml/min). Steady NO\textsubscript{3}N loading rate was maintained by controlling influent NO\textsubscript{3}N concentration (=20 mg/L) at all steps. In each step, NH\textsubscript{4}N loading rate was gradually increased by increasing influent NH\textsubscript{4}N concentration. This was also done to determine the optimum NH\textsubscript{4}N removal rate at each GDFR. For each condition, the experiments were run for a number of days to achieve steady NH\textsubscript{4}N, NO\textsubscript{2}N and NO\textsubscript{3}N removal rate. Influent, effluent and % NH\textsubscript{4}N removal of each GDFR are presented in Figure 2. The optimum removal NH\textsubscript{4}N removal rates were 6.6 mgN/gVSS.d (16.4 ± 1 mg/L), 10.0 mgN/gVSS.d (25 ± 1 mg/L), and 12.5 mgN/gVSS.d (31.2 ± 1 mg/L) at GDFR 90, 400 and 650 ml/min respectively. Influent and effluent NO\textsubscript{2}N concentrations were lower than 1 mg/L at all GDFR (Data not shown).

Figure 3 shows that NO\textsubscript{3}N removal efficiency was obtained 90 ± 8% at an effluent NH\textsubscript{4}N concentration of less than 7 mg/L. NO\textsubscript{3}N removal efficiency was reduced to 65 ± 5% when effluent NH\textsubscript{4}N concentration was 20 ± 2 mg/L. It was observed that the maximum NO\textsubscript{3}N removal efficiency decreases with increasing bulk NH\textsubscript{4}N concentration. The bulk NH\textsubscript{4}N concentration inhibited denitrification as evidenced by increasing effluent NH\textsubscript{4}N concentration and decreasing denitrification efficiency. Decreasing of denitrification efficiency may have possibly resulted from a carbon limitation because NH\textsubscript{4}+ can reduce the growth rate of many methanotrophs and inhibition of methane oxidation (Hyman and Wood, 1983; Whittenbury, 1970).

Optimal nitrate nitrogen removal at different gas dissolution rates

The experiments were carried out in 3 steps with different GDFR (90, 400, 650 ml/min). At each step, influent NH\textsubscript{4}N loading rates were fixed by optimal NH\textsubscript{4}N removal concentration obtained from previous experiments. Influent NO\textsubscript{3}N loading rates were gradually increased by influent NO\textsubscript{3}N concentrations. This was done to determine the optimum NO\textsubscript{3}N removal rates at each GDFR. For each condition, the experiments were run for a number of days to achieve steady state effluent concentrations of NH\textsubscript{4}N, NO\textsubscript{2}N and NO\textsubscript{3}N values. Influent, effluent and % NH\textsubscript{4}N removal of each GDFR were presented in Figure 4.

![Figure 2](https://iwaponline.com/wst/article-pdf/50/6/313/420076/313.pdf)
Figure 4 shows that average NH$_4$N removal efficiency was between 80–95% at all steps. For all GDFR, effluent NH$_4$N concentrations were below 5 mg/L. Influent, effluent and % NO$_3$N removal of each GDFR are presented in Figure 5. The optimum removal NO$_3$N removal rates were 8.9 mgN/gVSS.d (22.1 ± 2 mg/L), 11.8 mgN/gVSS.d (29.6 ± 2 mg/L), and 16.1 mgN/gVSS.d (40.2 ± 2 mg/L) at GDR 90, 400 and 650 ml/min respectively.

Simultaneous ammonium and nitrate nitrogen removal at different GDFR

This experiment was performed under oxygen-limited conditions (0.5–2 mg/L). External carbon source for denitrification was not added in the synthetic wastewater. Only methane was used as carbon source for denitrification under oxygen-limited conditions. Influent and effluent pH and N$_2$ production from the reactor were measured. The experiments were carried out in 3 steps with different methane and oxygen GDFR (90, 400, 650 ml/min) each. In each step, the experiments were run for a number of days to achieve steady removal rate of NH$_4$N, NO$_2$N and NO$_3$N. Influent, effluent and % NH$_4$N removal of each GDFR are presented in Figure 6 and influent, effluent and % NO$_3$N removal of each GDFR are presented in Figure 7.

From the experimental results, NH$_4$N and NO$_3$N removal efficiencies were obtained 80–95% and 85–95%, respectively from all steps. Effluent NO$_2$N was less than 0.1 mg/L for all steps. It is evident from the experimental results that there was no appreciable build up of NO$_2$N in the system at any stage. Both ammonia and nitrate were removed simultaneously under oxygen-limited conditions. Influent and effluent pH was 7.8–7.3. pH was
depleted when nitrification occurred. However, pH was increased by alkalinity production from denitrification. pH returned to close to the initial value. Therefore, SND has an advantage over a separate nitrification/denitrification system in that intensive pH control is not necessary as both consumption (denitrification) and production (nitrification) of proton occurs in one reactor.

Figure 5 NO$_3$N removal at fixed influent NH$_4$N concentration

Figure 6 Optimal NH$_4$N removal at different GDFR

Figure 7 Optimal NO$_3$N removal at different GDFR
The gas analysis also showed a steady state generation of N₂ and CO₂ gas with the decrease in ammonium and nitrate nitrogen concentration (data not shown).

Table 1 summarizes the removal rates of ammonium and nitrate nitrogen during SND at different GDFR. The highest ammonium and nitrate nitrogen removal were 11.5 and 14.9 mg N/g VSS.d respectively at GDFR 650 ml/min (DO concentration of 1.6–2.0 mg/L). The lowest ammonium and nitrate nitrogen removal were 6.9 and 8.1 mg N/g VSS.d respectively at GDFR 90 ml/min (DO concentration of 0.5–0.9 mg/L).

There are some assumptions supporting the occurrence of simultaneous nitrification and denitrification under oxygen-limited conditions.

First, ammonium nitrogen partially oxidized to nitrite or nitrate, which is a general metabolism of nitrifiers with insufficient DO and in the subsequent denitrification step, nitrite or nitrate is converted to dinitrogen gas with ammonium as electron donor by anaerobic ammonia oxidizers, which is called the Oxygen Limited Autotrophic Nitrification and Denitrification (OLAND) and Complete Autotrophic Nitrogen removal Over Nitrite (CANON) process. Denitrification, removal of nitrate under oxygen-limited conditions by using methane as a sole carbon source, is effectively possible. Because no other carbon source than methane was present, it can be concluded that this carbonaceous compound was produced directly or indirectly by methanotrophic active biomass. Several observations favour the hypothesis of an association of methanotrophic and heterotrophic denitrifying active biomass.

Second, SND occurs as a consequence of DO concentration gradients within microbial flocs or biofilm due to diffusional limitations. That is, the outer layer of flocs will become an aerobic region and the inner layer of flocs will become an anoxic region. Nitrifiers and Methanotrophs exist in regions with high dissolved oxygen concentrations, whereas the anaerobic ammonia oxidizer and denitrifiers will preferentially be active in regions with very low dissolved oxygen concentration. Ammonium nitrogen oxidized to nitrite and nitrate nitrogen by nitrifiers and dissolved methane gas oxidized to organic carbon by methanotrophs. Then, anaerobic ammonia oxidation and denitrification occurred by anaerobic ammonia oxidizer and denitrifiers.

**Conclusion**

The simultaneous nitrification and denitrification was evaluated in the fluidized bed bioreactor by using mixed culture sludge under oxygen-limited conditions. Nitrite and nitrate were not produced significantly during the ammonium nitrogen removal and influent nitrate nitrogen was removed significantly under aerobic conditions. Experimental results also substantiated that dissolved methane gas can be used as a carbon source for denitrification. The DO and dissolved methane concentration were the main parameters for nitrate nitrogen removal.

**References**


