Bioresource Technology 130 (2013) 592–598

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Effect of electron donors on anammox coupling with nitrate reduction for removing nitrogen from nitrate and ammonium

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HIGHLIGHTS

- Anammox coupled with NO_3^- reduction was stimulated by addition of swine wastewater, acetate, hydrogen and iron.
- No inhibitory effect on anammox in a medium with NO_2^-/NH_4^+ was induced by these electron donors, except for iron.
- Swine wastewater carried an exponential increase in denitrification under high concentrations.
- Hydrogen did not carry an increase in denitrification even when its addition was increased.

ARTICLE INFO

Article history:
Received 6 October 2012
Received in revised form 11 December 2012
Accepted 13 December 2012
Available online 21 December 2012

Keywords:
Anammox
Denitrification
Simultaneous removal of NO_3^- and NH_4^+
Swine wastewater
Hydrogen

ABSTRACT

Anammox coupling with nitrate reduction under various electron donors was studied using sludge acclimatized to have anammox and denitrification activities. Due to a deficiency in electron donors for NO_3^- reduction, anammox activity in an inorganic medium containing NO_3^- and NH_4^+ was lower than that in NO_2^- and NH_4^+. Anammox could use NO_2^- competitively against denitrifiers under a very limited NO_2^- concentration, and additions of swine wastewater or acetate stimulated anammox activity in an inorganic medium containing NO_3^- and NH_4^+ with no inhibition effects. However, a high concentration of swine wastewater caused an exponential increase in denitrification activity. The addition of hydrogen and iron stimulated anammox activity in an inorganic medium containing NO_3^- and NH_4^+, but iron showed an inhibitory effect on anammox in a medium containing NO_2^- and NH_4^+. Hydrogen was shown to be advantageous since it did not increase denitrification even when its addition was increased.

1. Introduction

Biological nitrogen removal has been conventionally achieved by nitrification followed by denitrification. However, some kinds of wastewater characterized by a low BOD/N (Biological Oxygen Demand/Nitrogen) ratio (e.g. livestock wastewaters) make it difficult to remove nitrogen using such a removal process because they have an insufficient supply of electron donors for denitrification (Boursier et al., 2005; Waki et al., 2010a). In recent years, anaerobic ammonium oxidation (anammox), which oxidizes NH_4^+ to N_2 using NO_2^- as an electron acceptor under anoxic conditions, has been introduced (Mulder et al., 1995; Strous et al., 1997). Since it can remove nitrogen without an additional electron donor for denitrification, the process is expected to be applicable for removing nitrogen from low BOD/N ratio wastewater.

Most anammox treatment studies were carried out using an inorganic medium containing NO_2^- and NH_4^+, which is most suitable for producing an anammox reaction. Actual wastewater was purified to remove BOD, followed by partial nitrification to contain NO_2^- and NH_4^+, and was then used for anammox treatment (Tokutomi et al., 2011; van der Star et al., 2007). Process manipulation methods with dissolved oxygen concentration, inorganic carbon concentration, temperature, inhibitors, and heat shock of sludge, have been proposed for attaining partial nitrification (i.e., maintaining dominance of the aerobic ammonium oxidizer and suppressing the nitrite oxidizer) (Isaka et al., 2008; Peng and Zhu, 2006; Tokutomi et al., 2010). However, such manipulations require an advanced and extensive process. Without a manipulation for partial nitrification, wastewater treated by an aerobic process is inclined to contain NO_3^-. For example, swine wastewater treated by an activated sludge treatment frequently contains NO_3^-, as well as residual NH_4^+ and BOD (Waki et al., 2010a). Therefore, a nitrogen

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http://dx.doi.org/10.1016/j.biortech.2012.12.101

removal method from a NO_3^- and NH_4^+ mixture would generally be applicable as a post treatment for an already existing aerobic process.

Recently, nitrogen removal from the NO_3^- and NH_4^+ mixture using electron donors such as acetate, VFA, and sulfide has been proposed (Kalyuzhnyi et al., 2008; Kalyuzhnyi et al., 2006; Sumino et al., 2006). In such processes, the coordination of anammox and denitrifiers which reduce NO_3^- to NO_2^- is thought to contribute to nitrogen removal (Fig. 1). A stoichiometric reaction shows that a NO_3^- reduction to N_2 gas requires 10 mol of electrons for the production of 1 mol of N_2 gas (Eq. (1)) (Tchobanoglous et al., 2003). NO_3^- reduction to NO_2^- would require 2 mol of electrons at a production rate of 1 mol of NO_2^- (Eq. (2)) (Richardson and Watmough, 1999). Therefore, upon coupling with anammox, only 2 mol of electrons would be consumed in the production of 1 mol of N_2 gas (Eqs. (3) and (4)). Anammox coupling with NO_3^- reduction could thus save electron donor consumption compared to traditional denitrification. However, there is a shortage of detailed information regarding nitrogen removal. Anammox poses some disadvantages in practical use compared to denitrification. For instance, the anammox process is inhibited by some popular electron donors such as methanol (Guven et al., 2005). Also, anammox bacteria grow very slow with a doubling time of 9–14 days (Strous et al., 1998; Yasuda et al., 2011) and tend to be washed out from a continuously operating reactor by the predominance of denitrifiers. Therefore, the absence of inhibitory effects of electron donors on anammox and excess growth of denitrifiers are the key to nitrogen removal by nitrate reduction and anammox.

$$2NO_3^- + 12H^+ + 10e^- \rightarrow N_2 + 6H_2O \text{ (denitrification)} \quad (1)$$

$$NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O \text{ (nitrate reduction to nitrite)} \quad (2)$$

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O \text{ (anammox)} \quad (3)$$

$$NO_3^- + NH_4^+ + 2H^+ + 2e^- \rightarrow N_2 + 3H_2O \text{ (anammox coupling with nitrate reduction to nitrite)} \quad (4)$$

The objective of this work is to clarify the most suitable conditions for anammox activity coupled with nitrate reduction. Sludge which was acclimatized to contain both anammox and denitrification activities was evaluated, in batch incubation with various electron donors such as swine wastewater, acetate, sulfide, hydrogen, and iron, under varying pH and temperature conditions.

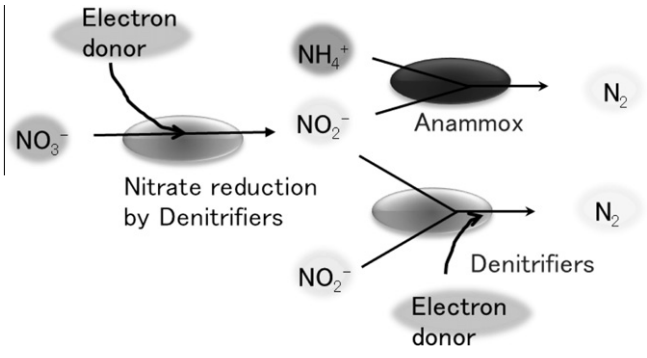

Fig. 1. Expected nitrogen flow of simultaneous nitrate reduction, denitrification and anammox.

2. Methods

2.1. Test sludge acclimatization

Sludge for the batch incubation experiment was collected from the lab-scale sequencing batch reactor. Activated sludge collected from a swine wastewater treatment plant was added to the reactor as seed sludge. The reactor had a working volume of 500 mL, was anaerobically operated at 20 °C, and was fed with the effluent from activated sludge treatment of livestock wastewater containing an average 16 mg/L of BOD, 200 mgN/L of NO_3^- and 200 mgN/L NH_4^+. Reactor operating conditions were an HRT of 2.6 days, MLVSS of an average 3100 mg/L, and a mean pH of 7.4. The removal of NO_3^- and NH_4^+ was observed using an average nitrogen removal rate of 0.071 gN/L/day and a removal ratio of NO_3^-/NH_4^+ that averaged 2.3 at the sampling period.

2.2. Measurement of anammox and denitrification activity in the presence of various electron donors, pH and temperature conditions

Anammox activity and denitrification activity of the test sludge were measured using the tracer method (Yoshinaga et al., 2011). The test sludge was washed to remove attached inorganic nitrogen compounds by centrifuging and suspension to an inorganic medium (Waki et al., 2010b), and then the centrifuged sludge was resuspended to be 100 mg/L MLVSS in the medium. Ten mL of the suspended mixture was anaerobically batch-incubated in 25 mL glass bottles with gas-tight butyl rubber stoppers. Inorganic nitrogen compounds were added to the suspended mixture to be 2.5 mM each of $^{15}NO_2^-/^{14}NH_4^+$, $^{14}NO_2^-/^{15}NH_4^+$, $^{15}NO_3^-/^{14}NH_4^+$, $^{14}NO_3^-/^{15}NH_4^+$, $^{15}NO_3^-$, or $^{15}NH_4^+$. ^{15}N atom% of the chemicals was 99.71% for $^{15}NO_2^-$, 99.92% for $^{15}NH_4^+$, and 99.92% for $^{15}NO_3^-$. The effect of adding raw swine wastewater on ^{15}N atom% of $^{15}NO_3^-$ was negligibly small, since ^{15}N atom% for $^{15}NO_3^-$ was more than 99.75%, and the effect of anammox activity of the addition was less than 2.0%. To investigate the effect of organic carbon compounds, raw swine wastewater, which included 2200 mg/L of BOD, 5200 mg/L of COD, 1600 mg/L of TOC, 500 mg/L of TN, 1.0 mg/L of $N\text{-}NO_3^-$, 0.3 mg/L of $N\text{-}NO_2^-$, and 380 mg/L of $N\text{-}NH_4^+$, 7.6 of pH, was added for a final concentration of 10–130 mg/L of BOD (26–310 mg/L of COD, 8–93 mg/L of TOC) in a vial. Sodium acetate was added for a final amount of 6 mg/L of CH_3COO^- (6.4 mg/L of COD) in a vial. To investigate the effect of inorganic electron donors, sulfur powder of 30 μmol (1 mg), hydrogen gas of 13 μmol or 130 μmol, steel wool of 50–90 μmol (3–5 mg), and iron powder (particle size pass of 150 μm) of 40 μmol (2 mg) were added in a vial. Anammox activity and denitrification activity were estimated from $^{29}N_2$ and $^{30}N_2$ production in incubation, respectively. The incubation time was set at 30 h for the investigation of anammox competitiveness against denitrifiers, to measure a slight difference, and at 24 h in other experiments since anammox activity linearly increased during this incubation period. The effect of the electron donors was evaluated by the relative activity, comparing it to anammox activity in the control condition in incubation with $^{15}NO_2^-/^{14}NH_4^+$ without an electron donor addition at the same day assay, because the fresh sludge sample was obtained from the reactor frequently and the activity varied among experimental days at 0.98–2.2 μmol/vial/day.

The original pH of the inorganic medium was set at 7.6 as a neutral condition, but for investigation of the pH effect, the inorganic medium was controlled to reach a pH from 5.8 to 8.8 by adding HCl or NaOH, and raw swine wastewater at a final amount of 20 mg/L was then added. The original temperature condition was set at 20 °C regarding water temperature in livestock wastewater treatment facility without heating in Japan, but upon investigation