

The effects of different electron donors on anaerobic nitrogen transformations and denitrification processes in Lake Taihu sediments

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Abstract Nitrogen transformations in anaerobic sediments and leachate in Lake Taihu were simulated in the laboratory. Ammonium, nitrate and nitrite were analyzed after incubation under anaerobic conditions. Different reductive states and pH values were obtained by using different electron donors, such as glucose, sucrose, potato starch and sodium acetate. Chemical nitrogen transformation mechanisms were discussed relative to physico-chemical properties of lake sediment. Results demonstrated that nitrogen transformations in anaerobic conditions supplemented with different electron donors varied, and supplementation with certain electron donors may enhance nitrogen removal from anaerobic sediments. Among the four electron donors studied, higher nitrogen removal efficiencies were observed with acetate and starch. Saccharides, such as glucose, sucrose and starch, stimulate nitrate reduction to nitrite, while acetate stimulates nitrate reduction to ammonium.

Keywords Electron donor · Anaerobic · Nitrogen · Transformation · Sediment · Lake Taihu

Introduction

Denitrification is a microbial process that converts nitrate to nitrogen gas via several intermediates, including nitrite and nitrous oxide (Tiedje, 1982). It is recognized as the most important process removing fixed nitrogen in most natural environments (Boicourt et al., 1996). Transformations involve nitrogen reduction and therefore, require electron donors (Devlin et al., 2000). Denitrification is a respiratory process which also requires an electron donor for energy (Islas-Limaa et al., 2004). Denitrifying bacteria can use nitrate and/or nitrite as an alternative electron acceptor for metabolic activities when molecular oxygen is unavailable (Bae et al., 2004). However, in natural anaerobic systems including sediments, denitrification often is limited by readily biodegradable electron donors.

Studies have demonstrated enhancement of nitro-reduction rates following addition of organic electron donors, such as glucose, pyruvate, formate, starch, acetate, hydrogen gas, elemental sulphur, thiosulphate, aqueous ferrous iron and pyrite (Preuss et al., 1993; Boopathy, 1994; Roberts et al., 1995; Devlin et al., 2000).

Guest editors: B. Qin, Z. Liu & K. Havens
Eutrophication of shallow lakes with special reference to
Lake Taihu, China

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Saccharide is an abundant biomolecule in the natural environment. Glucose is a monosaccharide, and sucrose is a disaccharide, which yields two monosaccharides upon hydrolysis. Starch contains two polysaccharides: amylose and amylopectin (Yu et al., 2004). Acetate is an organic acid and has been used in waste water treatment for nitrogen removal (Graaf et al., 1996; Cervantes et al., 1999).

Addition of electron donors to enhance reduction processes is, therefore, a potential method for in-place treatment of contaminated sediments (Gerlach et al., 1999). This may be an important treatment for eutrophic lakes, such as Lake Taihu, which is the third largest freshwater lake in China (Qin et al., 2007). Water quality in Lake Taihu has deteriorated due to rapid economic development and intensive use of water resources in recent years. As a result, cyanobacteria blooms occur over large areas in summer, and these blooms impact the aquatic ecosystem detrimentally (Pu et al., 1998a, b).

Most studies have focused on nitrate and ammonium removal in waste water systems (Cervantes et al., 2001; Schmidt et al., 2003), and comparative studies on denitrification and electron donor supplements in fresh water sediments are scarce.

Objectives of the present study were to compare total nitrogen (TN) reduction effects in Lake Taihu sediments stimulated by saccharide electron donors, such as glucose, sucrose, potato starch and organic acid acetate; and to evaluate effects of nitrogen species transformation under different electron donor additions.

Materials and methods

Sediment samples collection

Six surface sediment samples were collected using a Peterson grab bucket on 13–15 July 2003 from Lake Taihu. Locations of sampling sites are shown in Fig. 1. Samples were stored in a cooler until return to the laboratory, freeze-dried, sieved to ~ 0.28 mm aggregate size, mixed together, homogenized by gentle shaking, and stored at 4°C in a sealed polyethylene bag.

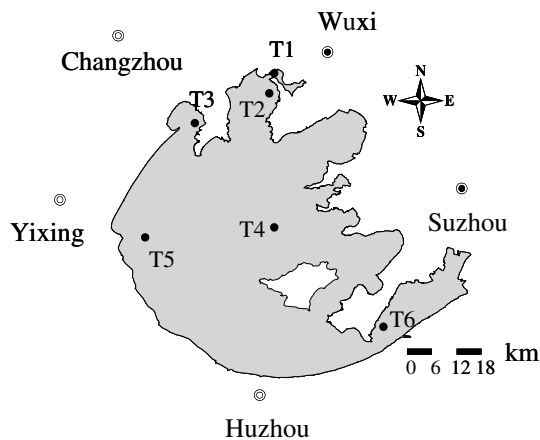


Fig. 1 Geographic locations of sampling sites

Anaerobic incubation experiment

Microcosms were constructed using 20 g of sediment and 150 ml of artificial freshwater (1 mg l⁻¹ ammonium, 1 mg l⁻¹ nitrate and 0.1 mg l⁻¹ nitrite), which was purged with high purity nitrogen (99.99% N₂) for 10 min to remove oxygen before use in 250-ml serum bottles. Microcosms were prepared in triplicate, and electron donors glucose, sucrose, potato starch (Sigma Co.) and sodium acetate added 2 g in stoichiometric excess. Two electron donor-free control groups, one sterilized with 0.1 g HgCl₂, were established. Table 1 lists the treatment conditions and microcosm abbreviations. Test bottles were aerated with high purity nitrogen for 3–5 additional minutes, sealed with butyl rubber stoppers and continuously mixed with a planar shaker rotating at 200 rpm at 25°C.

Table 1 Treatment conditions and abbreviations of microcosms

Abbreviation	Treatment
A	Sterilized control
B	Electron donor free control
C	Microcosm of glucose as electron donor
D	Microcosm of sucrose as electron donor
E	Microcosm of potato starch as electron donor
F	Microcosm of sodium acetate as electron donor

Sampling was done in a glove bag (I²R Glove BagTM, Model X-27-27, USA) purged with nitrogen before use. Bottles were vigorously shaken; pH and Eh were determined using a portable meter (Thermo Orion Model 250, USA). Ten milliliter of sediment/water slurry was poured into a 10 ml centrifuge tube, and the same quantity was added (10 ml artificial freshwater contained 1.33 g sediment). After sampling, sediment samples were centrifuged at 4000 rpm for 20 min and filtered through a 0.45 μm pore-size cellulose acetate filter to separate sediment and leachate at once.

Chemical analysis

For leachate samples, ammonium, nitrate and nitrite were determined within 24 h after sampling. Nitrate was determined by ion chromatography (Dionex 4500i using an AS-14 4-mm anion column), nitrite with *N*-(1-naphthyl) ethylene diamine dihydrochloride (APHA, 1998a), and ammonium with salicylic-hypochlorite colorimetry (Standard method of People's Republic of China, 1987).

Total nitrogen in sediments was analyzed using persulfate according to standard methods for examination of water and wastewater (APHA, 1998b). Weights of sediments reported are on an oven-dry basis.

Results and discussion

Formal potentials and pH conditions in the microcosms

Formal potential consists of measurement of the electromotive force of an electrochemical cell in which, under the specified conditions, the analytical concentration of the two oxidation states is varied. The formal potential includes correction factors for activity coefficients, acid-base phenomena, complex formation, and the liquid junction potential used between the reference electrode and the half-cell in question. It may often leads to better predictions than standard potential because it represents quantities subject to direct experimental measurement. In this

paper, it is expressed by p_e , which is calculated from the determined Eh values using Nernst Equation. The p_e can be defined as the negative logarithm of electron activity in a solution. It is a parameter for the redox intensity gives the electron activity at equilibrium and measures the relative tendency of a solution to accept or transfer electrons (Stumm & Morgan, 1981).

Values of pH and p_e are shown in Fig. 2. In all microcosms, pH declined with time. Trends are divided into three groups. Microcosms treated with glucose and sucrose had similar trends. pH declined sharply in the first 96 h and dropped to 4.3 by completion. pH in starch additions also declined, but more slowly than glucose and sucrose, dropping to 4.8 at the end of the incubation. pH in the sterilized control, electron donor-free control and sodium acetate addition declined in the first 48 h, then remained around 7.4 to 7.5.

Formal potentials in electron donor systems reached a strong reductive environment after 2 or 3 days. p_e dropped in the first 48 h in glucose and sucrose microcosms and in the first 96 h in starch and sodium acetate microcosms. Afterwards, the

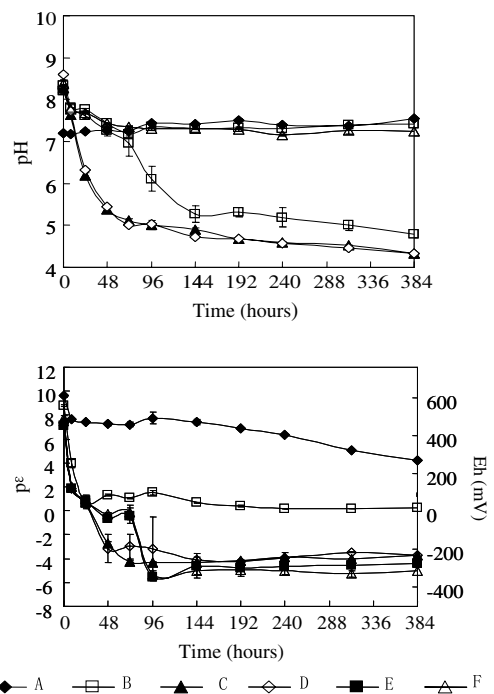


Fig. 2 pH and p_e values of microcosms

reductive environment in these microcosms reached steady-state and remained constant at -4 to -6 . The sterilized control did not attain reductive conditions, and the electron donor-free control barely reached a mild reductive environment.

Systems supplemented with electron donors achieved more reduction potential than controls. It is believed that Eh values is featured by reduction when they below 200 mV. (Song et al., 1990) Sediments from Lake Taihu in our experiment were strongly anaerobic except two control groups, and additional electronic donors could accelerate reducing conditions in microcosms.

pH and reductive potential are important factors controlling the fate of pollutants, especially those at the soil-water interface (Cao et al., 2001). pH and reductive potential also affect nitrogen composition (Stumm & Morgan, 1981). pH in all microcosms treated with electron donors declined, except the system treated with sodium acetate, likely due to acetate hydrolysis. In our experiment, the acetate microcosm may have maintained pH of 7.2–7.4 via its strong buffering capacity (Terra & Regel, 1995). However, in other three electron donor microcosms, pH values declined and remained below 5. At this pH, acid producing microorganisms may not survive, leading to stable pH.

Transformation of nitrogen species

Nitrogen transformations in control and electron donor microcosms are shown in Figs. 3 and 4. Results show that, in all microcosms, nitrate in leachate increased early in the incubation, then decreased. Nitrate, which is formed during organic matter degradation under oxic conditions (ammonification/nitrification), may be transported (mixing, currents, upwelling etc.) to anoxic environments where it can undergo denitrification (Boyd, 2001). In the present study, early nitrate increases may be caused by degradation of organic matter using residual oxygen in the sediment. For in the first 24 h, formal potential in all microcosms was positive, and pH was about 7, indicating characteristics of sediments were weak oxic or weak reduction in this condition.

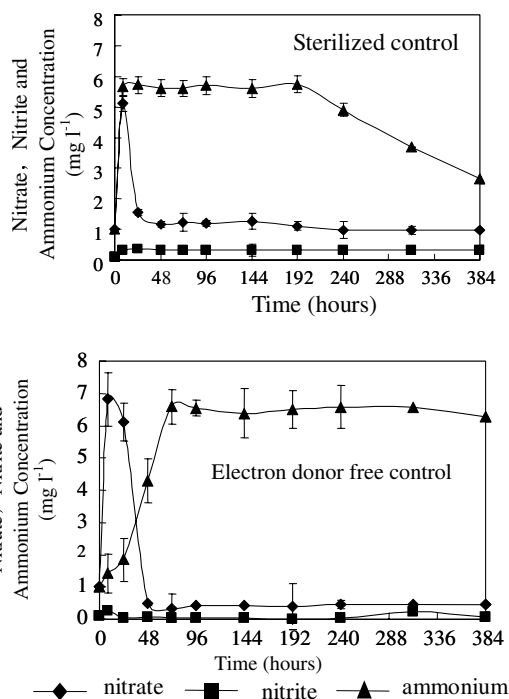


Fig. 3 Transformation of nitrogen species in two control microcosms

Nitrogen transformations in glucose and sucrose microcosms were similar. Nitrate in glucose and sucrose systems were lower than controls. Highest nitrate concentrations in glucose and sucrose microcosms were 5.2 and 4.7 mg l⁻¹, respectively, in the first 8 h and then decreased sharply. Highest nitrite concentrations in leachate occurred simultaneously with nitrate maxima. Ammonium decreased to below detection limits in 48 h, which indicates that monosaccharide and disaccharide electron donors may stimulate anaerobic nitrogen transformations.

Nitrate concentration in the starch addition reached 7.4 mg l⁻¹ in 24 h, and ammonium concentration increased to a similar value as the controls, then decreased to below detection limits after 96 h.

In anoxic, nitrate-containing environments, two pathways of dissimilatory nitrate reduction have been identified: denitrification, by which nitrogen oxides (mainly NO₃⁻) are reduced to nitrite (NO₂⁻) and then to dinitrogen gases (N₂O and N₂), and dissimilatory nitrate reduction to ammonia (DNRA), producing ammonia by

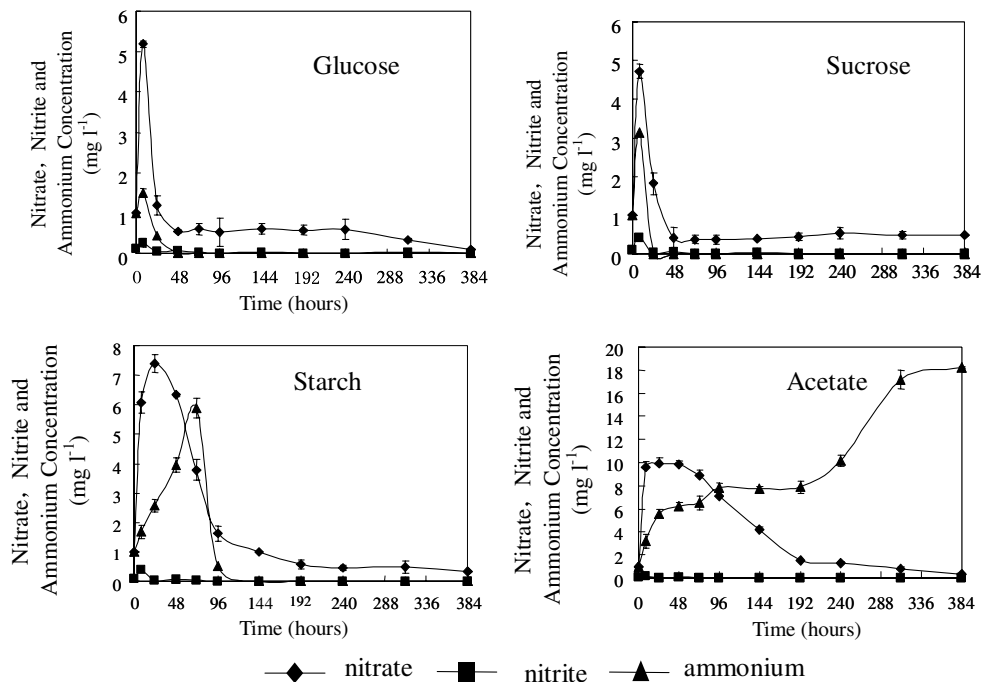


Fig. 4 Transformation of nitrogen species in different electron donor treated microcosms

reduction of nitrate and/or nitrite (Brunet & Garcia-Gil, 1996).

Apparently, nitrate reduced in microcosms supplemented with glucose, sucrose and starch as electronic donors were denitrified. Ammonium could not be detected after 48 h in glucose and sucrose systems and 96 h in the starch system. This indicates that saccharide, including monosaccharide (glucose), disaccharide (sucrose) and polysaccharides (starch), may stimulate nitrate reduction to nitrogen gas, although the dinitrogen gases (N_2O and N_2) were not determined in our experiment.

Nitrate and nitrite in the sodium acetate system was different from other electron donor systems. Nitrate in leachate reached 10 mg l^{-1} and maintained this value for 96 h. Nitrite concentration was half that of glucose and sucrose microcosms. However, ammonium increased to about 8 mg l^{-1} within 96 h and remained steady to 240 h. Afterwards, ammonium concentration increased to 18.3 mg l^{-1} by the end of incubation. Ammonium increased early maybe caused by ammonification of organic matter, for nitrate did not decrease at the first 96 h. But latter the high

ammonium value indicated that nitrate was reduced to ammonium in this microcosm. The removal of eight electrons during NO_3^- reduction to NH_4^+ makes this process potentially advantageous in reduced environments (Tiedje et al., 1982). DNRA may be quantitatively important nitrogen cycling of marine sediments (Sorensen, 1978).

Ammonium concentrations in the control systems were higher than electron donor addition systems except for acetate. Ammonium in the sterilized control increased for 8 h, then remained constant at approximately 6 mg l^{-1} from 8 h to 192 h. In the electron donor-free control system, ammonium increased slowly for 192 h. These systems may be controlled by pH and redox potential. According to p_e -pH diagrams (Wang, 2001), at pH about 7.5 and p_e about 0–8, ammonium is the predominant nitrogen species. This may explain why ammonium in the starch microcosm increased for 96 h and then decreased to detection limits. pH and p_e conditions in the starch microcosm up to 96 h were within the ammonium predominance area. And, nitrate reduction seems follow denitrification for 96 h in

the starch system since pH was lower than 6 and p_e decreased sharply to about -6 . This condition overstepped the ammonium predominance area. So, nitrate reduction to ammonium stimulated by sodium acetate may be due to comparatively high pH caused by acetate hydrolysis. In soil, similar results were reported that DNRA was favored at high pH (Stevens et al., 1998).

TN removal by denitrification

The ability to enhance sediment denitrification via addition of various electron donors was tested in sediment–water microcosms using TN reduction as a gross indicator of denitrification activity. TN removal from sediments in microcosms over 384 h are shown in Fig. 5. TN removal was observed in all systems but was not significant in the sterilized control, which increased for 48 h, then stayed steady for 384 h. Denitrification in the electron donor-free control increased for 144 h, and TN reduction percentage was 21.8%. Compared to control microcosms, potato starch and sodium acetate systems obtained higher reductive efficiency (45.5% and 42.5%, respectively). At the beginning of the incubation, denitrification in glucose and sucrose additions was lower than controls. However, reductive efficiency increased sharply at the end of the incubation (31.4% and 34.7%, respectively).

These results demonstrate that additional electronic donors may accelerate. Among the four electron donors, starch and acetate have largest effects. TN reduction ratios of starch and acetate

were 10% higher than glucose and sucrose. Since the starch used had low solubility, starch may have been more associated with sediments than the other electron donors (Gerlach et al., 1999). However, TN reduction in the sterilized control increased slightly throughout the experiment, indicating that $HgCl_2$ addition may not have been sufficient to eliminate microbial activity; but the increase in TN reduction was significantly less than the non-inhibited microcosms. However, this TN reduction was not observed in the electronic donor-free controls, indicating that either the sterilization agent affected abiotic redox activity, or microorganisms could reduce TN without electron donor addition.

Conclusions

The results presented in this paper show certain electron donors may be used to enhance TN removal in anaerobic sediments. Higher nitrogen removal efficiencies were observed when acetate and starch were added. However, nitrogen transformations in anaerobic conditions supplemented with different electron donors varied. Saccharides, such as glucose, sucrose and starch, stimulated nitrate reduction to nitrite, and acetate stimulated nitrate reduction to ammonium. Nitrogen transformations in these microcosms were controlled by pH and formal potential.

Acknowledgements This work was supported by the Key Project of Chinese Academy of Sciences, (Grant No.KZCX1-SW-12-II-32) and National Key Basic Research Support Foundation of China (Grant No.2002CB412304).

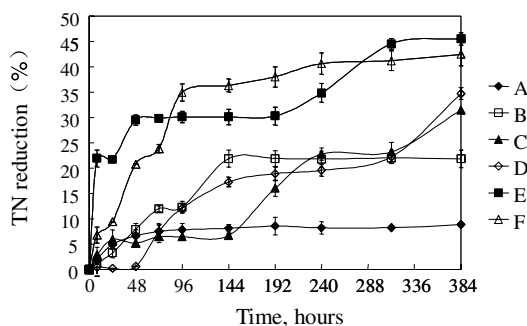


Fig. 5 TN removal ratio of sediments in microcosms

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