Bioresource Technology 201 (2016) 195–207

Contents lists available at ScienceDirect

Bioresource Technology

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

Nitrogen removal characteristics of enhanced in situ indigenous aerobic denitrification bacteria for micro-polluted reservoir source water

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HIGHLIGHTS

- The indigenous aerobic denitrifiers were enhanced in situ reservoir system.
- The enhanced system performed very well in terms of nitrogen and carbon removal.
- The carbon utilization diversity of the microbial communities varied significantly over the spatial and temporal variation.

ARTICLE INFO

Article history:
Received 4 October 2015
Received in revised form 16 November 2015
Accepted 17 November 2015
Available online 30 November 2015

Keywords:
Aerobic denitrification
Indigenous
Oligotrophic
In situ
Nitrogen removal

ABSTRACT

Indigenous oligotrophic aerobic denitrifiers nitrogen removal characteristics, community metabolic activity and functional genes were analyzed in a micro-polluted reservoir. The results showed that the nitrate in the enhanced system decreased from 1.71 ± 0.01 to 0.80 ± 0.06 mg/L, while the control system did little to remove and there was no nitrite accumulation. The total nitrogen (TN) removal rate of the enhanced system reached 38.33 ± 1.50% and the TN removal rate of surface sediment in the enhanced system reached 23.85 ± 2.52%. TN removal in the control system experienced an 85.48 ± 2.37% increase. The densities of aerobic denitrifiers in the enhanced system ranged from 2.24×10^5 to 8.13×10^7 cfu/mL. The abundance of *nir*S and *nir*K genes in the enhanced system were higher than those of in the control system. These results suggest that the enhanced in situ indigenous aerobic denitrifiers have potential applications for the bioremediation of micro-polluted reservoir system.

1. Introduction

Over the last century, extensive researches have been conducted on denitrification for several reasons. First, denitrification constitutes the main branch of the biogeochemical nitrogen cycle, which maintains the balance of the global nitrogen budget (Jetten, 2008). Second, denitrification is an important process for achieving biological nutrient removal, and a process that has been widely applied in engineered wastewater treatment systems for targeted water quality improvement (Grady et al., 2012). Third, biological denitrification can contribute to the global greenhouse effect through nitrous oxide emissions, which area approximately 300 times more potent than carbon dioxide (Brown et al., 2000). Although denitrification potential is widely found in bacteria, archaea, and some eukaryotes, nitrate reduction in natural and engineered ecosystems is primarily accomplished by functional bacterial species.

Bioremediation refers to the use of microorganisms to eliminate or reduce the concentrations of hazardous wastes at a contaminated site. One important characteristic of bioremediation is that it is carried out in non-sterile open environments comprised of a variety of microorganisms. An increasing number of researches have focused on bioremediation using N-functional bacteria for the effective and economic characteristics in engineered treatment systems for targeted water quality improvement. For example, Patureau et al. (2001) have achieved a combined phosphate and nitrogen removal in a sequencing batch reactor (SBR) using the aerobic denitrifier *Microvirgula aerodenitrificans*. The study conducted by Naeem et al. (2008), the bioaugmented reactor showed better performance regarding nitrogen and chemical oxygen demand (COD) removal with a novel heterotrophic nitrifier *Pseudonocardia ammonioxydans* H9T. Chen et al. (2015) concluded that PCN bacteria (the mixture of three heterotrophic nitrification–aerobic denitrification bacteria, named *Agrobacterium tumefaciens* LAD9,

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http://dx.doi.org/10.1016/j.biortech.2015.11.041
0960-8524/

Comamonas testosteroni GAD3 and *Achromobacter xylosoxidans* GAD4) capable of heterotrophic and aerobic nitrogen remove was successfully applied for bioaugmented treatment with municipal wastewater in a pilot-scale SBR, which could satisfy the first class requirement of the National Municipal Wastewater Discharge Standards of China.

However, there are many reasons that bioremediation fails. For example, the selected strains have often failed to show the abilities under natural environmental conditions that were evident in the laboratory as pure cultures (Boon et al., 2000). Assays in test tubes or in laboratory scale bioreactors have been unable to mimic the complexity innate to natural ecosystems, and the interaction of the inoculated microorganisms with their new biological and non-biological environments in terms of activity, survival, and migration can be critical to the success of any bioaugmentation strategy (El Fantroussi and Agathos, 2005). Bioaugmentation can change the composition of the indigenous microbial community due to competition or inhibition (van Veen et al., 1997), which could result in environmental safety problems that must be addressed (Wu et al., 2014). Therefore, environmentally benign and sustainable bio-measures have become attractive options for the in situ remediation of polluted surface waters. It had rarely been reported that N-functional bacteria were used to purify a surface water ecosystem, and there has been limited research on the use of this bacteria to remediate a reservoir ecosystem.

Traditional denitrification occurs in anaerobic or anoxic conditions with the reduction of nitrate to nitrogen gas. Oxygen inhibits the reaction steps, which makes the reactions impractical in natural waters, especially reservoir ecosystems. However, since the isolation of the first aerobic denitrification bacteria (*Paracoccus pantotrophus*) by Robertson and Kuenen (1983), the bacteria has been studied to a great extent. Compared to conventional anaerobic denitrification, there are attractive advantages; nitrification and denitrification can occur in the same system (Joo et al., 2006) and denitrification can cause sufficient alkalinity to partially balance the acidity of nitrification (Carter et al., 1995). There have been recent reports of aerobic denitrifiers isolated from canals, ponds, rivers (Huang et al., 2013), lakes (Guo et al., 2013), and reservoirs (Huang et al., 2015). Moreover, aerobic denitrification has also occurred in a natural system. For example, Gao et al. (2010) demonstrated that aerobic denitrification existed in permeable Wadden Sea sediments, and Coban et al. (2015) quantified the rates of aerobic denitrification in a horizontal subsurface-flow constructed wetland. According to our previous studies, Huang et al. (2012) carried out pilot research on micro-pollutant removal from source water by a combined process of water-lifting aeration (WLA) and oligotrophic biofilm, and the nitrogen removal performances can meet the requirements of class3, based on the Chinese Surface Water Environmental Quality Standard (GB3838-2002). Huang et al. (2015) explored the nitrogen removal characteristics of Zhoucun reservoir source water by adding indigenous aerobic denitrifiers, and found that the nitrate and TN declined by at least 70% and 50%, while that in the control system declined only 20% and 20–30%, respectively. The density of aerobic denitrifiers in an experimental system was higher by a 1–2 magnitude than that in the control system. Therefore, an investigation was conducted into the nitrogen removal characteristics of enhanced indigenous aerobic denitrification bacteria for micro-polluted reservoir source water by enclosure in situ, which attempted to simulate the environments of WLA operation.

In order to explore the nitrogen removal characteristics of indigenous aerobic denitrification bacteria, the nitrate, nitrite, ammonia, TN, total dissolved nitrogen (TDN) concentrations of the water system, and the TN of the surface sediment system were measured. The densities of aerobic denitrification (AD) bacteria were determined by plate count method and the abundances of denitrification functional genes (*nir*S and *nir*K) quantified using real-time polymerase chain reaction (PCR) in the enhanced system. Moreover, the removal characteristics of carbon (C) and inhibition performances of phosphorus (P) in the enhanced system in situ were explored.

2. Methods

2.1. Experimental system

2.1.1. Enclosure system

The enhanced enclosure system was used to simulate the operational environment of WLA (see Supporting information Fig. S1). The enhanced system was installed in Zhoucun reservoir with a steel pipe (inner diameter of 1.0 m) that was used to fix the experimental system to the reservoir bottom and a polyethylene body (height of 14.0 m). The enclosure system was filled with ~11,000 L raw water to simulate natural reservoir conditions. Compressed air was released into the bottom of the enhanced experimental system in the form of small bubbles, which maintained dissolved oxygen (DO) concentrations at lower water levels (4–5 mg/L) through direct mixing and oxygenation. The low DO conditions of the enhanced system lasted 1 month, and the in situ indigenous aerobic denitrification bacteria completed the enhanced operation. Next, nitrate was added to the enclosure system to maintain the nitrogen concentration at 2–2.5 mg/L. After 2 days, the enclosure system reached a stable state. The nitrogen and carbon removal performances of water and the surface sediment in the enclosure system were investigated.

2.1.2. Control system

All reservoir water except for the enclosure system was considered the control system.

2.2. Physical and chemical analysis

Water temperature, DO, pH, chlorophyll-a (Chl-a), SpCond (electrical conductivity (EC)), and oxidation–reduction potential (ORP) were measured in situ at 0.5-m increments using a multi parameter water quality analyzer (Hydrolab DS5, Hach Company, USA). The water column was defined as thermally stratified if the temperature difference exceeded 1 °C within a 1 m depth at the metalimnion.

2.3. Nitrogen removal performance of the enclosure system

In order to explore nitrogen removal performances, the nitrate, nitrite, ammonia, TN, and TDN were examined in the enhanced enclosure and control systems. The TN of surface sediment was also determined in order to reflect the inhibition performance in the enhanced enclosure and control systems.

2.4. Changes of oligotrophic aerobic denitrifiers in the enclosure system

In order to investigate whether the densities of indigenous bacteria could be increased in the enhanced enclosure experiment, the numbers of oligotrophic aerobic denitrifiers were measured in the enclosure and reservoir systems. The water samples (from 0.5 m, 5 m, 7.5 m, 10 m, 13 m water layers) were sampled via gradient dilution in triplicate. The gradient dilutions were as follows: 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}, respectively. The diluents were streaked on a solid screening medium (SM) of pH 7.2 (in g L^{-1}: 0.1 CH_3COONa, 0.02 $NaNO_3$, 0.02 $K_2HPO_4{\cdot}3H_2O$, 0.01 $CaCl_2$, 0.01, $MgCl_2{\cdot}6H_2O$, 20.00 agar) and incubated at 30 °C for 5 days. Prominent single colonies were harvested and calculated.