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Research article

Impact of aerobic acclimation on the nitrification performance and microbial community of landfill leachate sludge

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ABSTRACT

Nitrogenous pollution of water is regarded as a global environmental problem, and nitrogen removal has become an important issue in wastewater treatment processes. Landfill leachate is a typical large source of nitrogenous wastewater. Although the characteristics of leachate vary according to the age of the landfill, leachates of mature landfill have high concentrations of nitrogenous compounds. Most nitrogen in these leachates is in the form of ammonium nitrogen. In this study, we investigated the bacterial community of sludge from a landfill leachate lagoon by pyrosequencing of the bacterial 16S rRNA gene. The sludge was acclimated in a laboratory-scale reactor with aeration using a mechanical stirrer to promote nitrification. On 149 days, nitrification was achieved and then the bacterial community was also analyzed. The bacterial community was also analyzed after nitrification was achieved. Pyrosequencing analyses revealed that the abundances of ammonia-oxidizing and nitrite-oxidizing bacteria were increased by acclimation and their total proportions increased to >15% of total biomass. Changes in the sulfate-reducing and sulfur-oxidizing bacteria were also observed during the acclimation process. The aerobic acclimation process enriched a nitrifying microbial community from the landfill leachate sludge. These results suggested that the aerobic acclimation is a processing method for the nitrification ammonium oxidizing throw the enrichment of nitrifiers. Improvement of this acclimation method would allow nitrogen removal from leachate by nitrification and sulfur denitrification.

1. Introduction

The nitrogenous pollution of water is regarded as a global environmental problem and nitrogen (N) removal has become an important issue in wastewater treatment processes. Landfill leachate is a typical large source of nitrogenous wastewater. Leachates are generated at landfill sites as a result of precipitation, infiltration, compression and waste degradation. The quality and quantity of landfill leachates are highly variable (Gong et al., 2014; Kulikowska and Klimiuk, 2008; Statom et al., 2004; Cheung et al., 1993, 1997). According to a previous study, during a 2–6 year period of landfill operation, ammonia (NH_3) concentration increased from 98 to 364 mg L^{-1}, total N (TN) concentration increased from 95 to 401 mg L^{-1}, chemical oxygen demand (COD) decreased from 1800 mg L^{-1} to 610 mg L^{-1} and the biochemical oxygen demand (BOD_5)/COD ratio decreased from 0.4 to 0.13 (Kulikowska and Klimiuk, 2008). In another study, for ten years after landfill closure, NH_3 concentration decreased gradually from 500 to 400 mg L^{-1}, TN concentration decreased gradually from 600 to 400 mg L^{-1} and COD decreased from 1200 to 500 mg L^{-1} (Statom et al., 2004). Although the characteristics of leachate vary according to the age of the landfill, leachates of a mature landfill have high concentrations of nitrogenous compounds and almost all N in the leachates is in the form of ammonium N (NH_4^+-N). A cost effective process for N removal from landfill leachate is required for the stabilization and maintenance of landfill.

For N removal from wastewater, the nitrification–denitrification process has traditionally been used (Riaño and García-González, 2014; Munch et al., 1996). Aerobic nitrification and anoxic denitrification are costly treatment processes. Recently, new biological approaches for N removal, including partial nitrification (PN: nitritation without nitratation) and anaerobic ammonia oxidation (A: Anammox) processes, have been extensively studied (Cema et al., 2006; Strous et al., 1997); it is estimated that full-scale

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plants using the PN/A process for nitrogen removal exceed 100 in the world (Lackner et al., 2014).

Nitrification is a two-step process carried out by two groups of chemolithoautotrophic nitrifying bacteria: the oxidation of ammonium (NH_4^+) to nitrite (NO_2^-) by ammonia-oxidizing bacteria (AOB) and the oxidation of NO_2^- to nitrate (NO_3^-) by nitrite-oxidizing bacteria (NOB). Based on phylogenetic analysis of the 16S rRNA gene sequence, AOB are divided into two monophyletic groups, β-proteobacteria including the genera *Nitrosospira* and *Nitrosomonas* and γ-proteobacteria including the genera *Nitrosospira* and *Nitrosococcus* (Junier et al., 2008; Aakra et al., 2001; Head et al., 1993). NOB are comprised of at least five different genera: *Nitrobacter*, *Nitrospina*, *Nitrococcus*, *Nitrospira* and the recently discovered *Candidatus Nitrotoga* (Kruse et al., 2013; Alawi et al., 2007). In general, AOB and NOB have slow growth rates and they are also believed to be sensitive to environmental changes such as toxic shocks, pH, salinity, temperature changes, free NH_3, free nitrous acid (HNO_2) concentrations, hydraulic retention time (HRT) and dissolved oxygen (DO) concentrations (Li et al., 2012; Park et al., 2010; Coskuner and Curtis, 2002). Because of these characteristics, it is difficult to obtain and maintain sufficient nitrifying bacteria in wastewater treatment plants (Zhang et al., 2011). In previous studies, the relative abundance of β-proteobacterial AOB and NOB (*Nitrobacter* and *Nitrospira*) of raw landfill leachate treatment sludge was investigated (Fudala-Ksiazek et al., 2014; Yapsakli et al., 2011). However, there is relatively little known about whole bacterial communities of raw landfill leachate treatment sludge.

Landfill leachate from an industrial waste landfill in Osaka, Japan was sent into a lagoon for about 1 month prior to treatment in a nitrification–denitrification plant. In this study, we analyzed the characteristics of the landfill leachate and sewage of the landfill leachate lagoon, and investigated the bacterial communities of sludge from the landfill leachate lagoon. Additionally, the sludge was operated in the laboratory-scale reactor with aeration using a mechanical stirrer to promote nitrification, and the bacterial community was analyzed after nitrification was achieved. Pyrosequencing of the bacterial 16S rRNA gene was used to characterize the bacterial community structures.

2. Materials and methods

2.1. Materials

The industrial waste landfill, located in Izumiotsu, Osaka, Japan, received industrial waste from 1992 to 2002. Leachates from this landfill are collected at the bottom of the landfill by a drainage system and are then pumped to a lagoon. The biodegradation lagoon treats an average landfill leachate volume of 200,000 m^3 per month. The treated landfill leachates are discharged into the wastewater treatment plant.

The raw landfill leachates and the leachate lagoon water and sludge were obtained from the Izumiotsu industrial waste landfill leachate lagoon in 2012.

2.2. Analytical methods

The characteristics of the raw landfill leachates and leachate lagoon water were determined. NH_4^+-N was measured by an improved method of the Berthelot reaction using phenol and salicylate (Rhine et al., 1998). NO_2^--N and NO_3^--N were analyzed by the colorimetric method (AWWA, APHA, WPCF, 1995) using an ion chromatography with anion-exchange column (ICS 1100, Ion Pac AS23 Column, Dionex, California, USA). Total dissolved N (TDN) and dissolved organic carbon (DOC) were analyzed using a Shimadzu TOC-VCSN total organic carbon analyzer equipped with a TNM-1 total nitrogen measuring unit (Shimadzu, Kyoto, Japan). DO and pH were measured using a DO meter (D-55, HORIBA, Kyoto, Japan) and a pH meter (B-211, HORIBA, Kyoto, Japan), respectively.

Free NH_3 and free HNO_2 concentrations were calculated by equilibrium as follows (Anthonisen et al., 1976):

$$NH_3\ (mg\ L^{-1}) = 17/14 \times \text{Total ammonia as N}\ (mg\ L^{-1}) \times 10^{pH} / [\exp\{6344 / (273 + T)\} + 10^{pH}]$$

$$HNO_2\ (mg\ L^{-1}) = 47/14 \times NO_2\text{–}N\ (mg\ L^{-1}) / [\exp\{-2300 / (273 + T)\} \times 10^{pH}]$$

2.3. Aerobic acclimation of lagoon sludge

A reactor with an effective volume of 5.0 L, an internal diameter of 135 mm and a height/diameter ratio of 3.3 was used for the aerobic acclimation of lagoon sludge (Fig. 1). Sludge (1.0 L) collected from the landfill leachate lagoon was charged into the reactor. A mechanical stirrer was installed from the top of the reactor, ~130 mm from the surface of the sludge. Aerobic acclimation was carried out for 209 days using aerobic synthetic leachate continuously introduced by a peristaltic pump. The temperature was controlled at 25 °C by a heater. The aerobic synthetic leachate had the following composition: NH_4^+-N 25 mg L^{-1}, NO_2^--N 25 mg L^{-1}, KH_2PO_4 43.4 mg L^{-1}, $NaHCO_3$ mg L^{-1}, $MgSO_4 \cdot 7H_2O$ 328 mg L^{-1}, $CaCl_2 \cdot 2H_2O$ 235.2 mg L^{-1}, $FeSO_4 \cdot 7H_2O$ 16 mg L^{-1}, $Na_2 \cdot$EDTA 16 mg $L^{-1}$and NaCl 3.0%. pH of the synthetic leachate was kept at 9.0 by addition of $NaHCO_3$. The sludge washed out from the reactor during operating was collected in the effluent tank and returned to the reactor once daily.

To evaluate the activity of nitrifiers, nitrification rates were measured at batch scale outside of the reactor. Ammonia uptake rate (AUR) was determined by measuring the consumption of NH_4^+-N (Dytczak et al., 2008; Yu et al., 2011). The AUR was calculated based on changes in measured NH_4^+ concentration versus time as described previously (Dytczak et al., 2008, 2007). Nitrate production rate (NAPR) was determined by measuring the concentration of NO_3^--N and was calculated based on changes in measured NO_3^- concentration versus time (Fudala-Ksiazek et al., 2014).

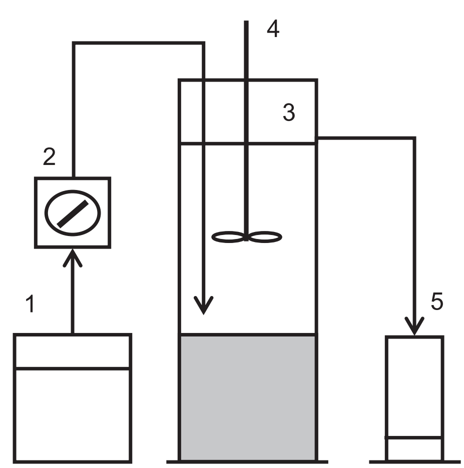

Fig. 1. Schematic diagram of the reactor used in this study (1, Influent tank; 2, Pump; 3, Reactor; 4, Stirrer; 5, Effluent tank). Synthetic leachate flows from the influent tank to the reactor using the pump.