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Monitoring influential environmental conditions affecting communities of denitrifying and nitrifying bacteria in a combined anoxic–oxic activated sludge system

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ABSTRACT

The goal of this study was to improve biological nitrogen removal (BNR) efficiency by investigating links between environmental factors and the dynamics of denitrifying and nitrifying bacteria in a combined anoxic–oxic activated sludge system. Principal component analysis (PCA) supported a positive correlation of *narG*, *nirS* and *nosZ* genes of denitrifying bacteria with SCN^- and CN^-, constitute one part of total nitrogen (TN). Supplemental ammonia produced from the biodegradation of those cyanide compounds increased NO_2^- and NO_3^- by nitrification, increasing concentrations of the denitrifying bacteria by increasing their N substrates. On the other hand, concentrations of both influent COD and phenol adversely affected the dominant nitrite oxidizing bacteria (NOB), *Nitrobacter* spp., exhibiting an inverse relationship both with the complete nitrification performance (end products, NO_3^-) as well as with an accumulation of NO_2^-. In this BNR system, therefore, creation of favorable conditions by pretreatment of influent phenol and organic matter may allow achievement of complete nitrification and an increase in quantities of nitrifying bacteria.

Introduction

An ecosystem's response to increased levels of the two most prominent nutrients in aquatic systems, nitrogen and phosphorus, is eutrophication. Thus, in an attempt to control eutrophication, these two major nutrients have been targeted for removal via wastewater treatment plants (WWTPs). For nitrogen removal, biological nitrogen removal (BNR) employing autotrophic nitrification followed by heterotrophic denitrification represents standard practice: (1) autotrophic ammonia oxidation to nitrite by ammonia oxidizing bacteria (AOB); (2) autotrophic nitrite oxidation to nitrate by nitrite oxidizing bacteria (NOB) and (3) heterotrophic nitrate or nitrite reduction to nitrogen gas by denitrifiers (Sun et al., 2014).

In engineered BNR systems, nitrification is regarded as the rate-limiting step reflecting low growth rates of nitrifying bacteria and susceptibility to environmental conditions such as pH, temperature and toxic contaminants (Do et al., 2008). Since wastewater targeted in this study was generated during the manufacture and processing of iron, levels of various toxic compounds have presented impediments to maintaining stable nitrification performance (Vázquez et al., 2006; Cho et al., 2014). In comparison to nitrification, relatively less concern has been paid to biological denitrification in engineered BNR systems due to its being less sensitive to environmental factors excepting fluctuations in dissolved oxygen (DO) (Kim et al., 2008, 2011a; Sun et al., 2014). However, denitrification can be adversely affected by environmental conditions such as a substrate shortage via shifts in the nitrification performance, reflecting the characteristics of a sequential anoxic–oxic system employing a single-sludge along with the recycling of nitrified effluent. Therefore, information about denitrification is just as

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important as for nitrification since complete BNR is achieved via a combination of denitrification and nitrification. Notably, understanding and diagnosing what environmental conditions determine both the dynamics and functions of denitrifying and nitrifying bacteria can lead to successful achievement of BNR in the WWTP. Although the causes for many failures arising during BNR have been reported (Figuerola and Erijman, 2010; Wells et al., 2011), further information is still required due to the unique differences in the selective pressure of each different WWTP. Even more, the causes for BNR failures in full-scale WWTPs are not always obvious and research targeting full-scale WWTPs has rarely been attempted due to either sampling problems or difficulties in proper WWTP selection. This study was, therefore, undertaken to: (1) monitor seasonal fluctuations in communities of both denitrifying and nitrifying bacteria as well as in the characteristics of the influent (2) explore links between environmental factors and bacterial communities both to identify significant influential factors and to diagnose the targeted BNR system with the goal of improving BNR efficiency and functional stability of the system.

Materials and methods

Description of WWTP

Targeted full-scale WWTP employed a pre-denitrification activated sludge system composed of sequential anoxic and oxic tanks (Fig. 1). Nitrified effluent was recycled from the oxic tank to the anoxic tank. This system receiving wastewater generated from coke manufacturing plants in a steel company performed N-removal via sequential denitrificaiton-nitrification as well as removal of phenol, thiocyanate (SCN^-) and total cyanide (TCN). During this study, the mixed-liquor suspended solids (MLSS) of the system were controlled at 2900 ± 360 mg l^{-1}; the average hydraulic retention time (HRT) was 4.7 days; the average solid retention time (SRT) was 29 days. DO level of the oxic tanks was maintained over 3.0 mg l^{-1}; that of the anoxic tanks below 0.2 mg l^{-1}.

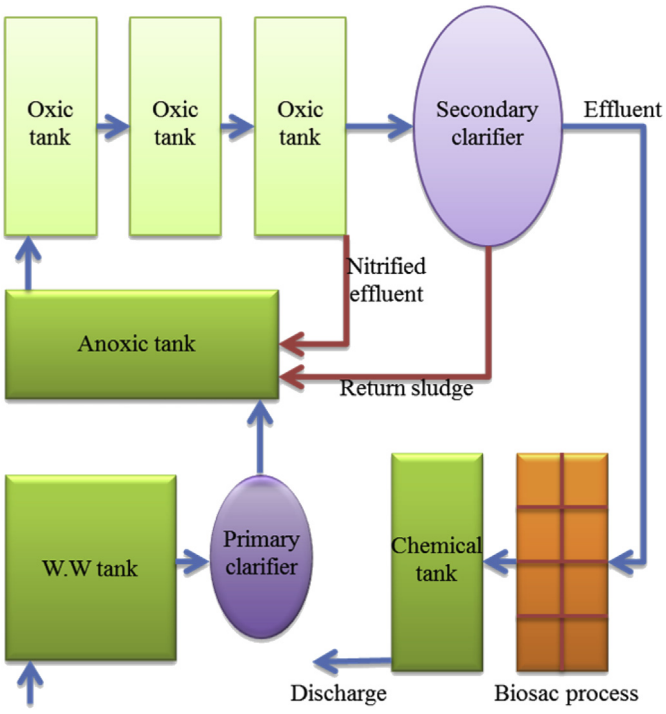

Fig. 1. Schematic diagram of a combined anoxic–oxic activated sludge system.

Chemical analysis

Collected samples were analyzed as follows: relying on standard methods, ammonia, phenol, chemical demand oxygen (COD) and SCN^- were analyzed via the colorimetric method using a spectrophotometer (Genesys TM-5, Spectronic Inc., USA). TCN concentration was determined by the pyridien-pyrazolone method after distillation. Nitrite and nitrate concentrations were analyzed with an ion chromatograph (ICS-1000, DIONEX Co.). Total nitrogen (TN) were measured with a TOC/TN analyzer (TOC-V csu, TNM-1, Shimadzu CO., Japan).

qPCR analysis

Biomass samples were periodically collected and stored at −80 °C for subsequent processing. All DNA in the pellets was extracted using an automated nucleic acid extractor (Magtration System 6GC, PSS, Chiba, Japan). Quantities of total bacteria (TB), nitrifying bacteria (AOB, NOB: *Nitrospira* spp. and *Nitrobacter* spp.) and denitrifying functional genes (*narG*, *nirS*, *nirK* and *nosZ*) were determined in triplicate via quantitative PCR (qPCR) via previously documented protocols (Kim et al., 2011b). Standard curves for qPCR were generated using serial 10-fold dilutions of plasmid DNA containing specific target gene inserts. Primer specificity and the absence of primer-dimers were confirmed via melt curve analysis.

Principal component analysis

Principal component analysis (PCA) was applied to reveal any statistically significant links between environmental factors (influent/effluent water qualities) and bacterial communities. PCA has been widely used to reduce the dimension of a large dataset; thereby it can be used to extract any significantly correlated groups, retaining the variation of a dataset in the original data set (Jolliffe, 2002). Prior to applying PCA, a non-parametric Kolmogorov–Smirnov test was applied to identify the normality of the dataset. The logarithmic transformation is applied if the data does not follow a normal distribution. By applying PCA, principal components (PCs) are estimated to identify the inter-correlations among variables in the original dataset.

Results and discussion

Performance and operating conditions of a WWTP

During the study, the influent temperature was maintained at an average of 32 ± 1.5 °C via cool water, with the pH of the influent being controlled at 9.0 ± 0.3 by adding H_3PO_4 solution to the influent storage tank (Fig. 2a). Wastewater flowed into the system in the range of 41–58 m^3 h^{-1} and the internal recycling ratio varied in the range of 3–5 (Fig. 2b). Phenol and SCN^-, representative organic and nitrogen pollutants in this wastewater, flowed into the full-scale WWTP in the dynamic range of 287–502 mg l^{-1} and of 346–545 mg l^{-1}, respectively (Fig. 2c, d). Most phenol was first degraded in the anoxic tanks with any remaining phenol almost completely degraded in the oxic tanks with its concentration remaining less than 0.06 mg l^{-1}, whereas almost all SCN^- was completely removed by various autotrophic bacteria such as *Thiobacilllus* sp. only in the oxic tanks (Kim et al., 2011c). Ammonia and TN removal fluctuated widely in the full-scale system (Fig. 2e), as did the ratios of the end products (NO_2^- and NO_3^-) of nitrification, producing NO_2^- in the range of 0.6–31.9 mg–N l^{-1} and NO_3^- concentrations in the range of 1.9–28.5 mg–N l^{-1}, respectively (Fig. 2f). Perhaps adjusting variations in the environmental conditions in the WWTP affects either partial or full nitrification