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Short Communication

Acclimatization of communities of ammonia oxidizing bacteria to seasonal changes in optimal conditions in a coke wastewater treatment plant

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HIGHLIGHTS

- The optimal conditions of AORs were investigated according to seasonal changes.
- The optimum temperature and pH of specific AORs showed seasonal variations.
- There were no seasonal changes in AOB communities.
- Variations in optimal conditions relied on the acclimation of a stable AOB community.

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ABSTRACT

The goal of this study was to investigate the correlation between optimal conditions of ammonia oxidation rates (AORs) and communities of ammonia oxidizing bacteria (AOB) adapting to seasonal changes in a full-scale wastewater treatment plant (WWTP). The optimal temperature and pH of specific AORs reflected seasonal variation patterns, showing the lowest values during the cold season, while the highest values in the warm season. Throughout the study period, *Nitrosomonas europaea/eutropha* and *Nitrosomonas nitrosa* remained the dominant AOB, indicating resistance to the influences of a changing environment. These results show that the optimal conditions for AOR can be adjusted to accommodate changing environmental conditions, relying on the acclimatization of a stable AOB community to given conditions, without any visible shift in the AOB community.

1. Introduction

Steel industries generate various wastewaters during the manufacture and processing of iron. Of these, coke wastewater is mostly generated either in the cooling step after coking coals at high temperatures (900–1100 °C) or in the liquid stripping step of the coke oven gas produced during the process (Kim et al., 2008). Coke wastewater contains different nitrogen compounds such as ammonia, thiocyanate and cyanides in a high concentration range (Vázquez et al., 2006).

A biological nitrogen removal (BNR) system employing autotrophic nitrification followed by heterotrophic denitrification forms an integral component in the overall management of coke wastewater in achieving the highly regulated effluent discharge levels in many nations (Kelly et al., 2004; Wyffels et al., 2004). In an engineered BNR system, overall treatment efficiency depends upon the execution of two consecutive steps of nitrification: (1) autotrophic ammonia oxidation to nitrite by ammonia oxidizing bacteria (AOB) and (2) autotrophic nitrite oxidation to nitrate by nitrite oxidizing bacteria (NOB). Ammonia oxidation, the first step in nitrification, is regarded as the rate-limiting step both because of low growth rates of AOB and their susceptibility to changes in growth conditions (Wagner et al., 1995; Kim et al., 2011).

Temperature and pH are some of the most significant conditions influencing the growth of AOB and the rate of ammonia oxidation. Although much information exists in the literature about the effects of temperature and pH on nitrification, the reported optimal conditions for nitrification vary considerably (Jones and Hood, 1980; Painter and Loveless, 1983; Shammas, 1986; Antoniou et al., 1990). Variations in diversity and amount of AOB in WWTP can create differences in the optimal conditions for AOB. However, the relationship between the optimal conditions to oxidize ammonia and the communities of AOB is not well understood. This study was, therefore, initiated to: (1) investigate the optimal conditions for the AOB activities in a full-scale coke

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wastewater treatment plant using a response surface analysis (RSA) and (2) use culture-independent molecular tools to investigate the effect of seasonal fluctuations on the ecology of AOB variations. To accomplish these objectives, full-scale sampling was conducted over a period of one year. These samples were analyzed via 16S rRNA gene-based Terminal Restriction Fragment Length (T-RFLP) to identify the AOB lineages, and quantitative PCR (qPCR) to measure the quantities of the AOB.

2. Methods

2.1. Wastewater treatment plant operation and samples

Activated sludge used in this study was sampled over a one year period from the aerobic tank of a full-scale WWTP of coke manufacturing plant in a steel company located in Korea. Nitrification activity was directly measured from fresh samples. During this study, the mixed-liquor suspended solids (MLSS) of the system were controlled in the range of 2100–4750 mg/L; the average hydraulic retention time (HRT) was 4.0 days; the average solid retention time (SRT) was 25 days. Analyzed parameters for the system are shown in Table 1. Biomass samples were periodically collected and stored at −80 °C for subsequent processing.

2.2. Chemical analysis

The collected samples were centrifuged at 3500 rpm for 3 min (MF550, Hanil Sci. Ind., Korea), and the supernatants were analyzed as follows: according to standard methods, ammonia was analyzed with the phenate method using a spectrophotometer (Genesys TM-5, Spectronic Inc., USA). Nitrite and nitrate concentrations were analyzed with an ion chromatograph (ICS-1000, DIONEX Co.).

2.3. Experimental design and batch RSA tests

A RSA experiment was employed to optimize the most significant factors (temperature and pH) for AOB. The ammonia oxidation reactions for two independent factors were conducted at three different levels (temperature: 25, 35, 45 °C; pH: 7.0, 8.0, 9.0 corresponding to −1, 0, +1) in batch tests. The experimental design used for this study was followed as previously described Lee et al. (2011), resulting in a total number of 11 experiments on every sampling date. Batch experiments for ammonia oxidation were carried out in 500 mL Erlenmeyer flasks filled with 100 mL of test solution containing 50 mg-N/L of ammonium, 1.0 g/L $NaHCO_3$, buffer solution and trace metals. Without any pretreatment each flask was inoculated with freshly activated sludge obtained directly from the full-scale aerobic tank and then agitated with excess oxygen on a thermostatic shaker at 200 rpm, maintaining the pH by adding 1 M HCl and NaOH solution at the desired temperature.

Table 1
Influent and effluent characteristics and operational parameters of the full-scale coke WWTP.

Parameters	Value	Parameters	Value
Inf pH	8.5–9.6	Eff NO_2^--N	1–23 mg/L
Inf Tem	24–41 °C	Eff NO_3^--N	17–35 mg/L
Inflow rate	43–61 m^3/h	Eff NH_4^+-N	16–90 mg/L
Inf COD	2860–3800 mg/L	Eff TN	42–100 mg/L
Inf CN^-	12–24 mg/L	Eff COD	78–114 mg/L
Inf phenol	384–534 mg/L	Eff CN^-	0.6–1.1 mg/L
Inf SCN^-	367–642 mg/L	Eff phenol	<1.0 mg/L
Inf NH_4^+-N	85–121 mg/L	Eff SCN^-	<1.0 mg/L
Inf TN	215–270 mg/L		

Abbreviations: Inf, Influent; Eff, Effluent; Temp, Temperature; TN, Total nitrogen; CN^-, Cyanide; SCN^-, Thiocyanate.

2.4. DNA extraction, T-RFLP analysis, cloning and sequencing

All DNA in the pellet was extracted using an automated nucleic acid extractor (Magtration System 6GC, PSS, Chiba, Japan). Temporal dynamics of AOB lineages were monitored via T-RFLP using the protocol of a previous study based on the known 16S rRNA gene of AOB (Siripong and Rittmann, 2007). The relative amounts of each terminal fragment (TF) were calculated as the ratio of the peak height for that TF to the sum of the peak height for all TFs in a given profile and expressed as a percentage. Cloning and sequencing were performed as previously described Kim et al. (2011). The sequencing results were compared with the reference sequences in the GenBank database. Sequence alignment and construction of phylogenetic tree were performed using MEGA ver. 4.0.

2.5. qPCR analysis

To determine the quantities of the AOB, qPCR assays were conducted in triplicate using TaqMan probes targeting the AOB 16S rRNA gene (Hermansson and Lindgren, 2001). Total bacterial abundance was also quantified using universal 16S rRNA targeted primers (Ferris et al., 1996; Harms et al., 2003). Serial 10-fold dilutions of plasmid DNA in which the target genes had been inserted were used to generate standard curves. The mixture for each reaction was prepared as previously described Kim et al. (2011).

3. Results and discussion

3.1. Variations in conditions to achieve optimal ammonia oxidation rates

Among various environmental conditions, temperature and pH are some of the most important factors influencing the ammonia oxidation rate (AOR). A full-scale WWTP was operated in a temperature range from 24 °C (in winter) to 38 °C (in summer; highest temp: 41 °C) according to the seasonal changes. Also, the pH value fluctuated in a range from 8.5 (in winter) to 9.5 (in summer). Optimal conditions for AOB were determined by two-and three-dimensional contour plots of the model for specific AORs reflecting the variations in these parameters (temperature and pH) (Fig. 1a). Through RSA experiments which had been performed according to seasonal changes (Fig. 1a), variations in the optimal temperature and pH conditions for the maximum specific AORs are enumerated in Fig. 1b.

Generally, the optimal temperature of the ammonia oxidizer was reported in the range of 25–30 °C (Jones and Hood, 1980). As shown in Fig. 1b, the optimal temperature of specific AORs (between 30 and 35 °C) were slightly higher than the values reported in a previous study (Jones and Hood, 1980; Grunditz and Dalhammar, 2001). The optimal temperature during the cold season was the lowest, while the optimal temperature in the warm season showed higher values. Previous studies indicated that the optimal pH for nitrification ranges between 8 and 8.5 (Jones and Hood, 1980; Shammas, 1986). Fig. 1b shows that the optimal pH of specific AORs fluctuated in the range of 7.5–8.0. Interestingly, the changing pattern in optimal pH was similar to that of temperature. The optimal pH was lower in the cold season and higher in the warm season. Despite insignificant variations, overall, the optimal temperature and pH values for specific AORs varied according to the season. This suggests that the optimal conditions for AOR can be adjusted responding to variations in environmental conditions, relying on the ability of AOB to acclimate under given conditions. Therefore, the reported