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Influence of phenol on ammonia removal in an intermittent aeration bioreactor treating biologically pretreated coal gasification wastewater

Chunyan Xu, Hongjun Han, Shengyong Jia, Qian Zhao*

State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, Harbin 150090, China

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ABSTRACT

A laboratory-scale intermittent aeration bioreactor was investigated to treat biologically pretreated coal gasification wastewater that was mainly composed of NH_3-N and phenol. The results showed that increasing phenol loading had an adverse effect on NH_3-N removal; the concentration in effluent at phenol loading of 40 mg phenol/(L·day) was 7.3 mg/L, 36.3% of that at 200 mg phenol/(L·day). The enzyme ammonia monooxygenase showed more sensitivity than hydroxylamine oxidoreductase to the inhibitory effect of phenol, with 32.2% and 10.5% activity inhibition, respectively at 200 mg phenol/(L·day). Owing to intermittent aeration conditions, nitritation-type nitrification and simultaneous nitrification and denitrification (SND) were observed, giving a maximum SND efficiency of 30.5%. Additionally, ammonia oxidizing bacteria (AOB) and denitrifying bacteria were the main group identified by fluorescent *in situ* hybridization. However, their relative abundance represented opposite variations as phenol loading increased, ranging from 30.1% to 17.5% and 7.6% to 18.2% for AOB and denitrifying bacteria, respectively.

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Introduction

Coal gasification wastewater (CGW) is a typical high strength industrial wastewater, characterized by high concentrations of phenol and NH_3-N, which causes serious deterioration to the environment if disposed of without adequate treatment (Jia et al., 2014). Therefore, efficient treatment for CGW is indispensable for the sustained rapid and sound development of the coal gasification industry. To date, anaerobic and aerobic hybrid processes have been developed to treat this wastewater (Li et al., 2011; Wang et al., 2011) and much attention has been paid on shortening the hydraulic retention time (HRT) and reducing the footprint. However, under conditions of short HRT, residual phenol and NH_3-N in biologically pretreated CGW (BPCGW) require further removal.

According to previous reports, phenol can cause serious damage to the cell membranes of nitrifying and denitrifying bacteria (Van Schie and Young, 2000). Neufeld et al. (1986) found that phenol had an acutely adverse effect on the rate of ammonia biooxidation to nitrite. In contrast, other researchers reported that ammonia and phenol could be simultaneously removed in a single-stage activated sludge process with cross-flow filtration, probably owing to the use of activated sludge biomass that had been acclimated with

* *Corresponding author*. E-mail: han13946003379@163.com (Hongjun Han).

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phenol for years (Yamagishi et al., 2001). Fang et al. (2013) evaluated the performance of a biological contact oxidation reactor treating CGW after it was augmented with phenol-degrading bacteria, resulting in increased total phenol and NH_3-N removal efficiencies from 66% and 5% to 80% and 25%, respectively. Therefore, it was speculated that the use of activated sludge with high activity was crucial and indispensible for simultaneous phenol and NH_3-N removal.

The main difficulty of NH_3-N oxidation lies in maintaining adequate levels of nitrifiers and enhancing their low growth rates. In particular, nitrification is generally a rate-limiting step in biological nitrogen removal processes. What's more, nitritation–denitritation, avoiding the oxidation of nitrite to nitrate by repressing nitrite oxidizing bacteria (NOB) and allowing for the reduction of nitrite to N_2 by heterotrophic denitrification, could decrease the organic carbon demand for total nitrogen removal by 40% and save 25% of the aeration costs (Turk and Mavinic, 1986). In previous reports, ammonia oxidizing bacteria (AOB) have been suggested to outcompete NOB at low dissolved oxygen (DO) concentrations due to their higher oxygen affinity than that of NOB, thereby resulting in nitritation (Ma et al., 2009; Zeng et al., 2010).

It has been suggested that with the same DO level during continuous aeration and intermittent aeration, nitrogen removal efficiencies were similar, which revealed that intermittent aeration could shorten the duration of aeration without any loss of process efficiency (Yang et al., 2015). In addition, efficient nitrification and stable microbial communities were achieved using a novel fluidized bed reactor-membrane bioreactor under the condition of an intermittent aeration cycle (Guadie et al., 2014). Therefore, intermittent aeration was expected to be a feasible method to efficiently remove NH_3-N with less energy consumption.

This study aimed to evaluate the influence of phenol loading on NH_3-N removal in BPCGW by a nitritation process in an intermittent aeration bioreactor. The inhibitory effect of phenol on the enzyme activity of bacteria with respect to NH_3-N oxidation was also estimated. In addition, fluorescent *in situ* hybridization (FISH) technology was used to reveal the main components of microbial community structures. A better understanding of the adverse effect of phenol on NH_3-N biodegradation was also anticipated.

1. Material and methods

1.1. Experimental setup, inoculums and CGW characteristics

Six reactors were made of Plexiglas with a working volume of 5 L and operated around 28–32°C. The activated sludge was taken from the full-scale aerobic tank treating CGW from a wastewater treatment plant, and the amount of suspended solids inoculated in reactors was 3000 mg/L. The BPCGW used in this study mainly contained NH_3-N at 55–60 mg/L and phenol at 10–50 mg/L.

1.2. Experimental procedures

The HRT applied in this study was set at 6 hr. The influent was supplied by peristaltic pumps (BT100 2J, Longer pump, China). The operation process was divided into 2 phases using a time controller, I (aerobic, 4 hr) and II (anoxic, 2 hr). The continuous experiments were operated for 100 days at phenol loadings of 40, 80, 120, 160 and 200 mg phenol/(L·day), respectively. A bioreactor without phenol addition was used as a background control.

The concentrations of NH_3-N, NO_2^--N and phenol were analyzed daily. Six activated sludge samples were collected from each bioreactor on the last day (day 100) for the FISH analysis. Batch experiments were carried out to evaluate the variations of DO, phenol, NH_3-N and NO_2^--N. These parameters were determined every 15 min.

It was reported that ammonia monooxygenase (AMO), a key enzyme responsible for ammonia oxidation, catalyzes the oxidation of NH_3-N to hydroxylamine (You et al., 2009), and hydroxylamine oxidoreductase (HAO) catalyzes the oxidation of hydroxylamine to nitrite (Stein and Arp, 1998). In order to contrast the enzyme activity at different phenol loadings, aliquots of ammonium (NH_4NO_3), hydroxylamine ($NH_2OH{\cdot}H_2SO_4$) and nitrite ($NaNO_2$), each at a final concentration of 10 mg/L N (0.7 mmol/L), were added to the individual respirometric bottles at around 300 sec after initiation of data acquisition (Choi et al., 2009). Owing to nitrification, a decrease in the DO in the closed respirometric vessels was continuously monitored.

The inhibition was calculated by the relative oxygen uptake rate (OUR) involved in reduced nitrogen oxidation for AMO and HAO, as described explicitly in Eq. (1). Each batch of

Table 1 – Oligonucleotide probe names, sequences and target microbial groups used in this study.

Probe name	Target organism	Probe sequence (5′-3′)	Reference
EUB338 I	Most Bacteria	GCTGCCTCCCGTAGGAGT	(Amann et al., 1990)
EUB338 II	*Planctomycetales*	GCAGCCACCCGTAGGTGT	(Daims et al., 1999)
EUB338 III	*Verrucomicrobiales*	GCTGCCACCCGTAGGTGT	(Daims et al., 1999)
NEU	Most halophilic and halotolerant *Nitrosomonas* spp.	CCCCTCTGCTGCACTCTA	(Wagner et al., 1995)
	Competitor for NEU	TTCCATCCCCCTCTGCCG	(Wagner et al., 1995)
Nso1225	Betaproteobacterial ammonia-oxidizing bacteria	CGCCATTGTATTACGTGTGA	(Mobarry et al., 1996)
Cluster 6a 192	*Nitrosomonas oligotropha* lineage (Cluster 6a)	CTTTCGATCCCCTACTTTCC	(Adamczyk et al., 2003)
	Competitor for Cluster 6a 192	CTTTCGATCCCCTGCTTTCC	(Adamczyk et al., 2003)
Ntspa662	Genus *Nitrospira*	GGAATTCCGCGCTCCTCT	(Daims et al., 2001)
	Competitor for Ntspa662	GGAATTCCGCTCTCCTCT	(Daims et al., 2001)
Ntspa712	Phylum *Nitrospirae*	CGCCTTCGCCACCGGCCTTCC	(Daims et al., 2001)
	Competitor for Ntspa 712	CGCCTTCGCCACCGGTGTTCC	(Daims et al., 2001)
Den650	Denitromonas	AGTTTCCTCTCCGAACAA	(Xiao et al., 2010)

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