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

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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.

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

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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 NH₃-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 NH₃-N removal.

The main difficulty of NH₃-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 NH₃-N with less energy consumption.

This study aimed to evaluate the influence of phenol loading on NH₃-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 NH₃-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 NH₃-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 NH₃-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 NH₃-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, NH₃-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 NH₃-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 ($NH_2OH·H_2SO_4$) 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)
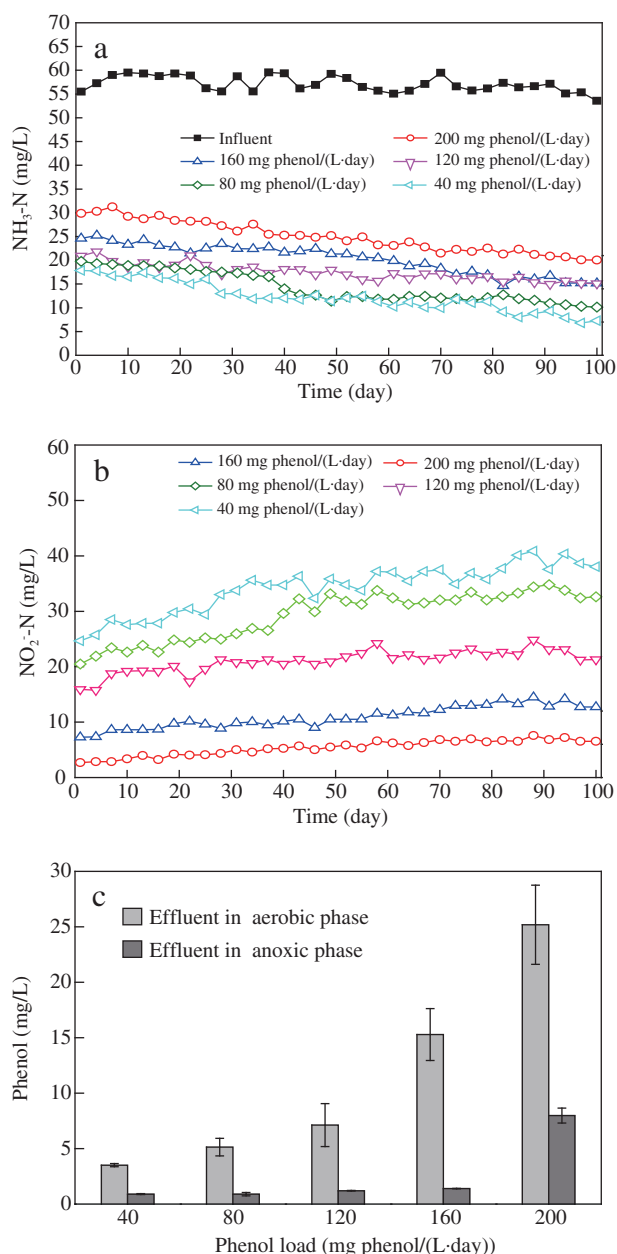
Fig. 1 – NH$_3$-N (a), NO$_2^-$-N (b) and phenol (c) variations in effluent with increasing phenol loading (c, values represent the mean values, error bars represent variance at each phenol loading).

respirometric tests was accompanied by a positive control (*e.g.*, untreated nitrifying biomass only) and experiments were performed in triplicate (Choi et al., 2009).

$$\text{Inhibition} = \frac{\text{OUR}_{\text{control}} - \text{OUR}_{\text{treated}}}{\text{OUR}_{\text{control}}} \times 100\% \tag{1}$$

Simultaneous nitrification and denitrification (SND) efficiency is defined as the loss of nitrogen (Lo et al., 2010) (Eq. (2)).

$$\text{SND} = \frac{\text{NH}_{3,e} + \text{NO}_{\overline{2},e}}{\text{NH}_{3,i} - \text{NH}_{3,e}} \times 100\% \tag{2}$$

where NH$_{3,i}$ (mg/L) is the NH$_3$-N concentration in influent; NH$_{3,e}$ (mg/L) is the NH$_3$-N concentration in effluent; and NO$^-_{2,e}$ (mg/L) is the NO$_2^-$-N concentration in effluent.

The NH$_3$-N consumption specific rate (CSR) and NO$_2^-$-N production specific rate (PSR) were determined every 5 days.

1.3. Analytical methods

Phenol, NH$_3$-N and NO$_2^-$-N were measured according to Standard Methods (AHPA, 1998). DO values were determined daily with a hybrid meter (30d, HACH, USA). FISH on fixed activated sludge samples was performed as previously described (Braguglia et al., 2012) and microscopy and fluorescence signal quantification were carried out by methods described in (Gagliano et al., 2015). The probes used in this study are listed in Table 1.

2. Results and discussion

2.1. NH$_3$-N, NO$_2^-$-N and phenol variations

As shown in Fig. 1, for each phenol loading, the effluent NH$_3$-N showed a declining trend as the operation time was extended, implying a gradually enhanced nitrification process; in addition, the phenol loading showed a significant influence on NH$_3$-N removal. On the start day, slight NH$_3$-N decreases were observed at phenol of 200 and 160 mg phenol/(L·day), reaching concentrations of 29.9 and 24.6 mg/L in effluent, respectively; while a remarkable NH$_3$-N decrease was found, reaching 17.9 mg/L, with the lowest phenol loading of 40 mg phenol/(L·day). In this study, NH$_3$-N removal on the start day was probably owing to the high activities of NH$_3$-N degrading bacteria, while the differences among different phenol loadings can be explained by the inhibitory effect of phenol. In previous reports, the ammonia oxidation process was found to be extremely sensitive to phenolic compounds (Amor et al., 2005), while with decreasing phenol loading, this inhibition was weakened, which favored NH$_3$-N biodegradation. After 100 days' operation, the NH$_3$-N reached 7.3 mg/L with 40 mg phenol/(L·day), which was 12.8 mg/L lower than that with 200 mg phenol/(L·day). However, in the previous research, higher consumption efficiencies of phenol and ammonium were observed in continuous culture than in this study (Pérez-González et al., 2012). This significant difference was attributed to two reasons: (1) the loading rate of phenol/NH$_3$-N of the referenced literature was 0.25 compared to 1.35 in this study; and (2) the DO of the referenced literature was 4.0 ± 0.3 mg/L compared to 1.0 ± 0.3 mg/L in this study.

Owing to the intermittent aeration, nitritation-type nitrification was observed, resulting in NO$_2^-$-N accumulation as operation time was extended. Due to its low concentration (<3 mg/L) throughout the experiments, NO$_3^-$-N was not shown in Fig. 1b. As shown in Fig. 1b, on the start day, NO$_2^-$-N reached 24.7 mg/L with 40 mg phenol/L, while at 200 mg phenol/(L·day), NO$_2^-$-N decreased to 2.7 mg/L. In addition, after 100 days of operation, the NO$_2^-$-N increment were 3.8 and 13.3 mg/L at 40 and 200 mg phenol/(L·day), respectively. It was speculated that the adverse effect of phenol on nitritation was gradually weakened at low loading, which was not found at high loading.

In the previous study, phenol was regarded as a readily biodegraded compound at sufficient HRT (Jia et al., 2014). But

this was only observed at low phenol loading at short HRT in this study. As shown in Fig. 1c, significant decrease of phenol was found in the anoxic phase at 160 mg phenol/(L·day), and the effluent concentration reached 1.4 mg/L. In addition, shock phenol loading (200 mg phenol/(L·day)) not only inhibited the NH_3-N biodegradation but also limited its removal.

2.2. NH_3-N oxidation and its inhibition

As shown in Fig. 2a, at 40 mg phenol/(L·day), the NH_3-N CSR was 19.9 mg NH_3-N/(g VSS·day), while the NO_2^--N PSR was 16.3 mg NO_2^--N/(g VSS·day). With regard to the control bioreactor without phenol addition, the NH_3-N consumption efficiency and nitrifying yield maintained at a relatively stable level. However, as phenol loading increased, the NH_3-N CSR gradually decreased, reaching 14.6 mg NH_3-N/(g VSS·day) at 200 mg phenol/(L·day), which was 73.4% of the value for 40 mg phenol/(L·day). A general characteristic of AMO is a broad substrate range, as well over 40 compounds have been shown to be substrates of AMO that can competitively inhibit NH_3-N oxidation, due to the competition for the active site (McCarty, 1999). In particular, when phenol was used as substrate, the major product was hydroquinone (Keener

and Arp, 1994), showing more toxicity to the nitrifiers (Wang et al., 2011).

As shown in Fig. 2b, the enzymes of AMO and HAO involved in nitrification showed different responses to phenol loading, with AMO being more sensitive than HAO. The results showed that low phenol loading did not initially cause a complete inactivation of AMO activity, but higher loading than 120 mg phenol/(L·day) acutely inhibited its activity. As phenol loading increased, the maximum loss of 32.2% of AMO activity was observed at 200 mg phenol/(L·day), which might partially explain why the lowest NH_3-N CSR occurred under this condition.

Compared with NH_3-N CSR, NO_2^--N PSR exhibited an acute decrement with increasing phenol loading. Fig. 2a shows only a slight change in NO_2^--N PSR between no phenol addition and 40 mg phenol/(L·day). However, a dramatic decrease of NO_2^--N PSR was observed when the phenol loading increased from 80 to 200 mg phenol/(L·day), with values ranging from 13.9 to 2.8 mg NO_2^--N/(g VSS·day). Generally, lower and more stable inhibition of HAO at the phenol loading range from 80 to 200 mg phenol/(L·day) would promote NO_2^--N accumulation; by contrast, the concentrations showed a sharp decrease from 32.6 to 6.5 mg/L (Fig. 1b). It was speculated that this observation might be related to the denitrification process, in which phenol as organic carbon acted as an electron donor and energy source, under the conditions of the intermittent aeration environment.

2.3. Denitrification

It has been suggested that SND could achieve efficient nitrogen removal at low DO concentrations of 0.5–1.5 mg/L (Meyer et al., 2005). Therefore, it was possible to achieve SND under the intermittent aeration conditions in this study.

Due to the low COD/NH_3-N ratio, the inherent organic carbon in BPCGW is inadequate to achieve complete nitrogen removal through denitrification, so that supplemental organic carbon was necessary. As shown in Fig. 3a, low SND efficiencies were observed without phenol addition and with less than 120 mg phenol/(L·day), and significant improvement of SND occurred when phenol loading increased to 160 mg phenol/(L·day), achieving a maximum value of 30.5%, probably owing to the supplementary organic carbon provided by phenol. However, a slight decrease of SND efficiency was found with phenol loading increasing from 160 to 200 mg phenol/(L·day). Meanwhile, the DO variations and phenol degradation were determined in the batch experiments, and the result is shown in Fig. 3b. It was found that DO stayed at a high and steady level, ranging from 1.20 to 1.42 mg/L, which dramatically decreased when aeration stopped. In addition, a gradual decrement of phenol was observed as operation time was extended. The phenol degradation rate reached 8.96 mg/L hr in the aerobic period, which increased by 24.8% in the anoxic period, giving a value of 11.18 mg/L hr. In the previous study, it was suggested that phenol was easily biodegraded in an aerobic environment (Jia et al., 2014), while under anaerobic conditions, the phenol concentration was increased due to the transformation of polyhydric phenols; by contrast, significant phenol degradation was observed first in the anoxic environment, probably owing to its use in the denitrification process as an organic carbon source. As shown in Fig. 3b, at
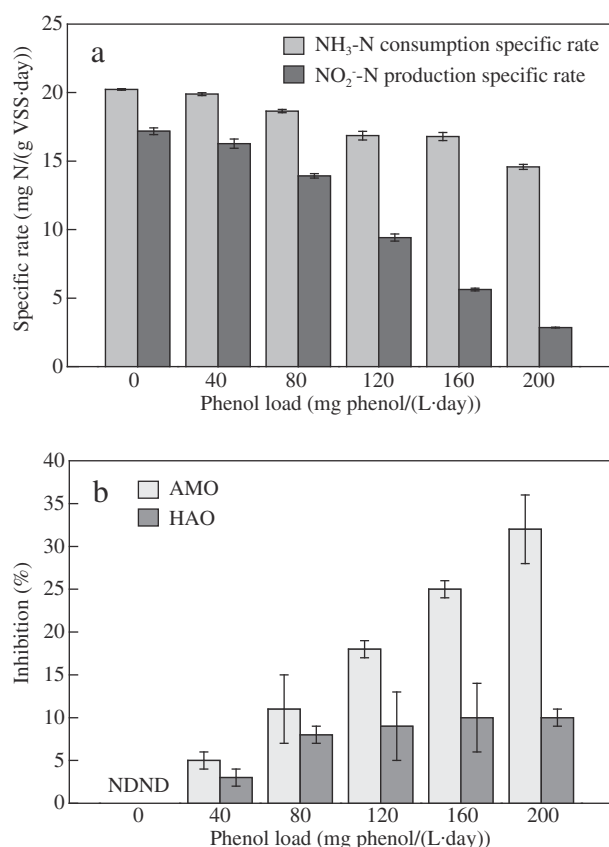
Fig. 2 – NH_3-N consumption specific rate (CSR) and NO_2^--N production specific rate (PSR) (a) variations and ammonia monooxygenase (AMO) and hydroxylamine oxidoreductase (HAO) variations (b) as phenol increased (values represent the mean values, error bars represent variance at each phenol loading).
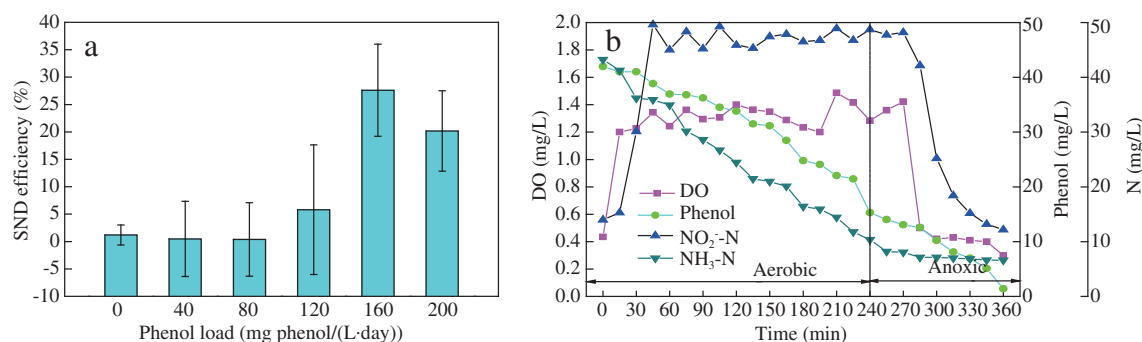
Fig. 3 – Simultaneous nitrification and denitrification (SND) efficiency with phenol loading increase (a) and variations of dissolved oxygen (DO), phenol, NH$_3$-N and NO$_2^-$-N in a operation cycle (b, with 160 mg phenol/L d) (values represent the mean values, error bars represent variance).

the phenol loading of 160 mg phenol/(L·day), after 240 min operation, the phenol concentration decreased to 15.3 mg/L, and it was much lower at loadings of 40 and 80 mg phenol/(L·day) (data not shown) which inhibited the denitrification process due to lack of an organic carbon source. Nonetheless, too high phenol loading (200 mg phenol/(L·day)) had an adverse effect due to damage to cell membranes (Van Schie and Young, 2000). Therefore, it was speculated that the SND process was affected by phenol based on the dynamic balance between its competing roles of inhibition as a toxic compound and denitrification as a supplier of organic carbon.

2.4. FISH analysis

Meng et al. (2010) has suggested that FISH technology represents an approach that can measure the relative abundance of microorganisms in bioreactors. In this study, FISH analysis was further employed to evaluate the abundance of each microbial component and follow the population dynamics at different phenol loadings.

AOB and denitrifying bacteria, related to nitrogen removal in biodegradation (Zhao et al., 2013), were target bacteria of the FISH analysis. However, as shown in Appendix A Figs. S1 and S2, their relative abundance represented opposite variation trends with increasing phenol loading. For instance, Fig. S1 shows that AOB contributed substantially to ammonia oxidation under aerobic conditions, and thrived in all the activated sludge samples at different phenol loadings; however, the fluorescence intensity became weakened as the phenol loading increased, while the denitrifying bacteria gradually accumulated.

As shown in Fig. 4, even though a similar microbial composition was finally observed in reactors, the dynamics during the reactor operation were totally different. Without phenol addition, AOB relative abundance indicated higher density than *Nitrospira* and *Nitrobacter*, which were affiliated to NOB, reaching the value of 30%, which then gradually decreased as the phenol loading increased (Zhu et al., 2013). Decrement of AOB relative abundance indicated an inhibited NH$_3$-N oxidation effect, probably due to the adverse influence by high concentrations of phenol. By contrast, *Denitromonas* relative abundance showed an increasing trend, ranging from 7.6% to 18.2% with phenol loading increasing from 40 to

160 mg Phenol/L d, suggesting promotion of the denitrification effect (Ma et al., 2015), and this result was consistent with SND efficiency variations. In addition, it was noteworthy that *Nitrospira* and *Nitrobacter*, with respect to nitrite oxidation, occupied less and less relative abundance, indicating that phenol presented more serious inhibition to the nitrite oxidation process in this study.

3. Conclusions

Intermittent aeration reactors were developed to treat BPCGW. The results showed that increasing phenol loading had adverse effects on NH$_3$-N removal. The AMO enzyme was found to be more sensitive than HAO to the inhibition effect caused by phenol. Owing to intermittent aeration conditions, nitritation-type nitrification and SND were observed, giving a maximum SND efficiency of 30.5%. Additionally, AOB and denitrifying bacteria were the main group of bacteria identified by FISH. However, their relative abundance showed opposite variation trends with increasing phenol loading, with a decrease for AOB and increase for denitrifying bacteria.
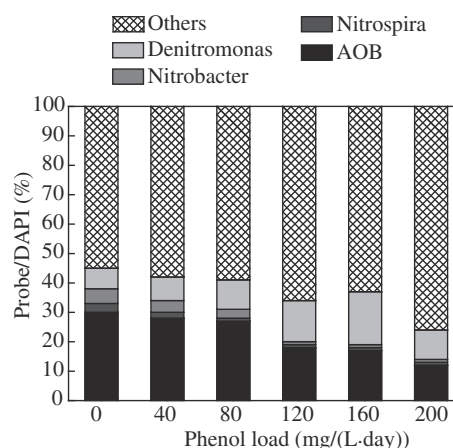

Fig. 4 – Microbial population dynamics estimated by FISH analysis in bioreactors with different phenol loading (DAPI (4′,6-diamidino-2-phenylindole) fluorescent staining was performed for determining total cell numbers).

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jes.2015.08.013.

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