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Simultaneous Removal of Phenol and Ammonium Using *Serratia* sp. LJ-1 Capable of Heterotrophic Nitrification-Aerobic Denitrification

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Abstract The simultaneous removal of phenol and ammonium using heterotrophic nitrifying-denitrifying bacterium *Serratia* sp. LJ-1 was investigated. The maximum removal rates of ammonium nitrogen and phenol were 1.08±0.05 and 2.14±0.08 mg L^{-1} h^{-1}, respectively. The ammonium oxidation had much higher tolerance to phenol toxicity than that of the autotrophic nitrifying bacteria. The increase in phenol concentration led to an increase in ammonium oxidation rate under the phenol concentration of 600 mg L^{-1}. The increase in ammonium concentration caused an increase in phenol biodegradation rate under the ammonium nitrogen concentration of 150 mg L^{-1}. Maximum rates of phenol biodegradation and total nitrogen removal in the treatments with nitrification metabolite (nitrate or nitrite) as the sole nitrogen source were more than 30 % lower than those of the treatment with ammonium as the sole nitrogen source. Ammonium was removed through nitrification and subsequent aerobic denitrification while phenol was biodegraded through the *ortho*-cleavage pathway and subsequently mineralized. Since phenol often coexists with nitrogen pollutants, these findings have significant environmental implications in terms of the simultaneous removal of these contaminants.

Keywords Phenol · Ammonium · Biodegradation · Heterotrophic nitrification

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1 Introduction

Hazardous aromatic organic pollutants often coexist with ammonium in wastewater (Amor et al. 2005). Among these organic toxicants, phenol is the most common (Liang and Ni 2009). Phenol is very toxic, with a fixed low admissible level of 0.5 mg L^{-1} in water (Polat et al. 2006; Girods et al. 2009). Ammonium ion (NH_4^+) is one of the important pollutants in municipal sewage and industrial wastewater because it can cause increased oxygen demand and eutrophication in rivers and lakes (Liang and Ni 2009). Therefore, the wastewater biological treatment requires the simultaneous removal of aromatic organic compounds and ammonium. Nitrification has been proven to be an effective method for ammonium removal (Rittmann and McCarty 2001). However, the nitrification process may be severely inhibited in the presence of phenol because phenol is a common nitrification inhibitor (Neufeld et al. 1980). A previous study showed that ammonium oxidation could be inhibited 75 % by phenol at a concentration of 5.64 mg L^{-1} in sewage sludge (Staford 1974).

Simultaneous removal of phenol and ammonium had been achieved in an activated sludge reactor by two successive steps, relying on the cooperation between autotrophic bacteria and heterotrophic bacteria which may subsequently lead to complicated bioreactor operation (Amor et al. 2005).

Nitrification and denitrification have been traditionally regarded as essentially separate phenomena, carried out by different bacteria in segregated areas of soils, sediments, water, or reactors (Kuenen and Robertson 1994). Recently, bacteria possessing abilities of heterotrophic nitrification and aerobic denitrification have been studied as potential microorganisms that may be used to overcome problems inherent in the conventional methods (Joo et al. 2005). Low molecular weight organic acids, glucose, and methanol are usually used as the carbon sources for these bacteria (Robertson et al. 1988; Arts et al. 1995). Little information is available on the nitrification and subsequent denitrification of bacteria with heterotrophic nitrification abilities in the presence of hazardous organic pollutants. Studies have shown that autotrophic ammonium oxidation bacteria such as *Nitrosomonas europaea* have the ability to simultaneously oxidize ammonium and phenol (Hyman et al. 1985; Shi et al. 2004). However, the phenol could only be partly oxidized (i.e., not mineralized), causing the accumulation of hydroquinone regarded as the dead-end metabolite (Hyman et al. 1985). Additionally, ammonium was only oxidized into nitrite (NO_2^-) or nitrate (NO_3^-) rather than removed as dinitrogen (N_2). There is no report on microorganisms with the ability to simultaneously remove phenol as carbon dioxide (CO_2) and ammonium as N_2.

Our previous research showed that bacteria with heterotrophic nitrification ability can remove the ethoxylate groups of nonylphenol ethoxylates, suggesting that this kind of bacteria might have potential for the biodegradation removal of some hazardous organic pollutants (Lu et al. 2008a). The removal of ammonium as N_2 occurred simultaneously during the biodegradation of nonylphenol ethoxylates. In this study, the simultaneous removal of phenol and ammonium using *Serratia* sp. LJ-1 capable of heterotrophic nitrification-aerobic denitrification was investigated. The tolerance of nitrification on phenol toxicity was evaluated. The effect of ammonium concentration on the simultaneous removal of phenol and ammonium was investigated. The biodegradation pathway of phenol was also elucidated. The objective of this study was to obtain initial information on biodegradation of phenol and the simultaneous metabolism of ammonium by heterotrophic nitrifying-denitrifying bacterium. Phenol was chosen as the model aromatic compound for this study since it was a typical aromatic pollutant of environmental interest. The finial goal was to determine the feasibility of the simultaneous removal of aromatic organic pollutants and nitrogen pollutants by taking advantage of heterotrophic nitrifier.

2 Materials and Methods

2.1 Chemicals and Reagents

High-performance liquid chromatography (HPLC)-grade methanol was purchased from Tedia (Fairfield, OH, USA). Phenol, hydroquinone, catechol, 2-hydroxymuconic semialdehyde (2-HMS), and *cis*, *cis*-muconic acid were purchased from the Sigma-Aldrich Corporation (St. Louis, MO, USA). High-purity Milli-Q water was produced by a Milli-Q Plus system (Millipore, Billerica, MA, USA). All other reagents used were of reagent grade.

2.2 Microorganism and Experimental Setup

Heterotrophic nitrifier, *Serratia* sp. LJ-1 (GenBank access number EU137875) (Lu et al. 2008b) with simultaneous nitrification and denitrification ability, was isolated from a membrane bioreactor following previously reported methods (Lin et al. 2004). The red colonies of *Serratia* sp. LJ-1 grown on Luria-Bertani (LB) agar plate were transferred in LB medium with phenol (100 mg L^{-1}) and cultivated at 30 °C and 120 rpm on an orbital shaker in darkness. After 7 days (168 h), the cells were harvested by centrifugation at 4,000×*g* for 10 min and washed twice with 20 mM potassium phosphate buffer solution (pH=7.0) before being used for wastewater treatment.

The incubations were performed in 500-mL flasks. One-hundred-milliliter synthetic wastewater containing phenol and ammonium was added into each flask. The cells of *Serratia* sp. LJ-1 were suspended in the wastewater at 1.0 g L^{-1} dry weight. Incubations were performed under room temperature (30 °C) on magnetic stirrers in darkness. All the treatments were performed in triplicate. The basic composition of the synthetic wastewater was as follows: $MgCl_2{\cdot}6H_2O$ (0.1 g L^{-1}), $CaCl_2$ (0.1 g L^{-1}), $FeCl_2{\cdot}4H_2O$ (0.01 g L^{-1}), K_2HPO_4 (0.27 g L^{-1}), KH_2PO_4 (0.35 g L^{-1}), and $NaHCO_3$

(2.5 g L^{-1}). Ammonium was used as the nitrogen source while phenol was added as carbon source. Trace salts were also added into the medium according to Somsamak et al. (2001), and the pH was adjusted to 7 using 1 M HCl.

2.3 Experiments on the Simultaneous Removal of Phenol and Ammonium

To investigate the simultaneous removal of phenol and ammonium during wastewater treatment, initial concentrations of ammonium nitrogen (NH_4^+-N) and phenol were 100 and 400 mg L^{-1}, respectively. Evolution of ammonium metabolites during wastewater treatment was monitored. An uninoculated control (prepared with no additional inocula) and a heat-killed control (prepared by sterilizing the wastewater in an autoclave at 120 °C for 20 min) were performed. To investigate the effect of different phenol concentrations on nitrification and phenol biodegradation, the initial concentrations of phenol were 0, 15, 30, 60, 150, 300, 400, 600, 1,000, 1,500, and 2,000 mg L^{-1}. To determine the effect of different ammonium concentration on phenol removal and nitrification, the initial concentrations of NH_4^+-N were 50, 100, 150, 500, and 1,000 mg L^{-1}.

2.4 Phenol Biodegradation Assays with Different Nitrification Metabolites of Ammonium

To evaluate the effect of typical nitrification metabolite of ammonium, the ammonium in the synthetic wastewater was replaced by nitrate or nitrite. Initial concentrations of nitrogen (NH_4^+-N, NO_2^--N, NO_3^--N) and phenol were 100 and 400 mg L^{-1}, respectively. To obtain further evidence on the enhancement of nitrate or nitrite on phenol biodegradation, the control using the NH_4^+-N as the sole nitrogen source was also included.

2.5 Biodegradation Assays of Phenol and Its Metabolites

To monitor the evolution of intermediates of phenol during wastewater treatment, concentrations of potential intermediates including catechol, hydroquinone, 2-HMS, and *cis*, *cis*-muconic acid were monitored. To get more information on the phenol biodegradation, the biodegradation of two detected phenol biodegradation intermediates (catechol and *cis*, *cis*-muconic acid) of phenol was investigated. In these tests, phenol in the synthetic wastewater was replaced by catechol or *cis*, *cis*-muconic acid as the sole carbon source. Initial concentration of NH_4^+-N was 100 mg L^{-1} while those of phenol, catechol, and *cis*, *cis*-muconic acid were 400, 5, and 5 mg L^{-1}, respectively. Phenol biodegradation intermediates were spiked at low level based on their concentration levels in the phenol biodegradation experiment. All treatments had two controls, including an uninoculated control (prepared with no additional inocula) and a heat-killed control (prepared by sterilizing the wastewater in an autoclave at 120 °C for 20 min).

2.6 Experiments on Carbon and Nitrogen Mass Balances

To obtain further evidence on the simultaneous removal of phenol and ammonium by *Serratia* sp. LJ-1, experiments focusing on carbon and nitrogen mass balances were performed, following previous methods (Alva and Peyton 2003; Joo et al. 2005). Incubations were conducted in 1-L serum bottles sealed with rubber septa for 120 h. The wastewater (100 mL) was flushed with high-purity oxygen for about 15 min, and the headspace of the bottles was oxygen. Wastewater was added at low volume to ensure the oxygen was sufficient for the maintenance of aerobic conditions during the experiment. The carbon recovery refers to the percentage of sum of carbon contribution from inorganic carbon, cell carbon, and aqueous organic carbon to the total carbon (initial phenol carbon) (Alva and Peyton 2003). The inorganic carbon refers to the sum of carbon contribution from inorganic carbon in water and carbon dioxide in headspace. The nitrogen recovery refers to the percentage of sum of nitrogen contribution from final NH_4^+-N, nitrification product nitrogen (NO_2^--N, NO_3^--N, and hydroxylamine nitrogen (NH_2OH-N)), cell nitrogen, and denitrification product nitrogen (N_2-N, nitrous oxide nitrogen (N_2O-N), and nitric oxide nitrogen (NO-N)) to the total input nitrogen (initial NH_4^+-N) (Joo et al. 2005). The intracellular carbon and nitrogen contents were calculated from the dry cell weight by assuming biomass composition to be $C_5H_7O_2N$ (Kim et al. 2005). Other forms of carbon and nitrogen were obtained through instrument analysis.

2.7 Analytical Methods

All aqueous samples were centrifuged for 15 min at 21,100×*g* before being subjected to subsequent analysis.

Phenol and its metabolites were analyzed according to Costa et al. (1999) with slight modification. HPLC analysis was performed using a Hewlett-Packard Series 1100 HPLC (Agilent Technologies, Palo Alto, CA, USA) equipped with a diode array detector. The chromatographic separation was carried out in the reversed-phase mode with a Hypersil ODS column (250 mm×4.6 mm i.d. particle packing size of 5 μm). The mobile phase used was a mixture of 5 mM K_3PO_4 (pH 3.4 with phosphoric acid)-methanol (80:20, *v*/*v*), and the flow rate was maintained at 1.0 mL min^{-1}. Phenol, catechol, hydroquinone, *cis*, *cis*-muconic acid, and 2-HMS were determined by the comparison of the retention time and the UV/visible absorption spectra (200–800 nm). HPLC quantification was performed with an injection volume of 20 μL, and the column effluent was monitored at 270 nm. The method limit of detections (LODs) of phenol, catechol, and hydroquinone were 0.1 mg L^{-1}, while those of *cis*, *cis*-muconic acid and 2-HMS were 0.5 mg L^{-1}.

Ammonium was determined using a spectrophotometric method (APHA 1998), and NH_2OH was measured according to Frear and Burrell (1955) using a UV/visible spectrophotometer (UV-2450PC, Shimadzu, Japan). Concentrations of nitrate and nitrite (NO_2^-) were measured using a MIC ion chromatograph (Metohm, Switzerland). Inorganic carbon and total soluble nitrogen of the supernatant were measured simultaneously using a TOC-5050A total carbon analyzer (Shimadzu, Japan). Headspace samples of N_2, N_2O, NO, and CO_2 from the mass balance experiment were measured using a Hewlett-Packard 5890 series gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with two separation columns and a thermal conductivity detector (TCD). Column 1 was a Porapack Q column (hp 19006C) used for the separation of N_2O, CO_2, H_2O, and the mixture of N_2 and O_2. Column 2 was a Molsieve column (hp 19096A-010) used for the separation of N_2 and O_2 in the effluent of column 1. The detector temperature was 200 °C. Dissolved oxygen within the media was monitored with a Thermo Orion Model 830A DO meter (Thermo Orion, Beverly, MA, USA) to confirm maintenance of aerobic conditions. The maximum removal rates of NH_4^+-N, total soluble nitrogen (TN), and phenol were determined following the previously described method (Lu et al. 2008c).

Biomass content in water samples was determined by measuring the optical density at 600 nm (OD_{600}) using a spectrophotometer following previous method (Shim et al. 2005). A linear correlation between cell dry weight and OD_{600} was obtained, and one unit of OD_{600} was found to be equivalent to 0.31 g cell dry weight per liter. The maximum growth rate was determined using logistic growth model (Perni et al. 2005). Biomass production was calculated using difference between the biomass at the end the experiment and the biomass at the beginning the experiment.

3 Results

3.1 Simultaneous Removal of Phenol and Ammonium

Simultaneous removal of ammonium and phenol occurred when phenol was degraded by the heterotrophic nitrifying strain LJ-1 (Fig. 1). At the end of the experiments, more than 90 % of both total soluble organic carbon and nitrogen were removed, indicating that phenol was mineralized while the ammonium was totally removed by strain LJ-1. The maximum removal rates of phenol and NH_4^+-N were 2.14±0.08 and 1.08±0.05 mg L^{-1} h^{-1}, respectively. Total soluble nitrogen was concomitantly removed. The maximal removal was 0.62±0.02 mg L^{-1} h^{-1}, which was nearly 60 % of the ammonium nitrogen removal rate. The nitrogen mass balance study (Table 1) showed that most of ammonium was recovered as N_2 (54 %), indicating that ammonium was removed as N_2.

Changes in the concentration of ammonium, hydroxylamine, nitrite, and nitrate during the biodegradation period of phenol were also monitored to get further information for the effect of phenol on the heterotrophic nitrification pathway. The consumption of ammonium was concomitant with the removal of dissolved nitrogen. Hydroxylamine, nitrite, and nitrate were formed during this heterotrophic nitrogen removal process. Ammonium was firstly oxidized to hydroxylamine, which was rapidly oxidized into nitrite. The concentration of hydroxylamine reached its peak at 96 h, much earlier than that of nitrate. Interestingly, there were two peaks in the concentration of nitrite. The formation of hydroxylamine and nitrite led to their accumulation from 72 h. Nitrite was then oxidized into nitrate which led to the later accumulation of nitrate. The subsequent reduction of nitrate led to the second accumulation of nitrite.

The rapid growth and reproduction of strain LJ-1 confirmed the feasibility of using nitrifying-denitrifying bacterium for the simultaneous removal of phenol and ammonium during wastewater treatment.

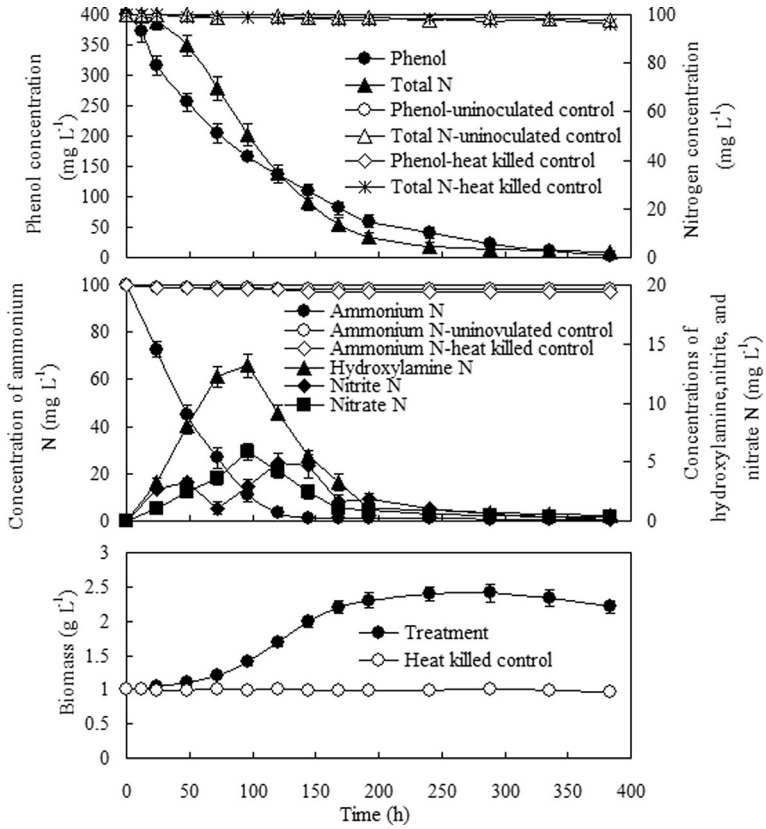

Fig. 1 Simultaneous removal of phenol and ammonium and growth of *Serratia* sp. LJ-1 during incubation. The initial concentration of ammonium nitrogen (N) was 100 mg L^{-1} while that of phenol was 400 mg L^{-1}. Nitrite and nitrate were not detected in controls

The bacterium grew rapidly with a short lag period, and the maximum biomass content (2.42±0.10 g L^{-1}) was observed after 200-h incubation. The maximum growth rate was 12.5±0.2 mg L^{-1} h^{-1}.

3.2 Effect of Phenol Concentration on Ammonium Oxidization and Phenol Biodegradation

The nitrification is usually sensitive to phenol (Staford 1974; Neufeld et al. 1980). To evaluate the tolerance of nitrification in heterotrophic nitrifier *Serratia* sp. LJ-1 to phenol toxicity, ammonium oxidation tests were performed under different phenol concentrations. The results showed that inhibition of ammonium oxidation by phenol occurred only at very high phenol concentrations (Fig. 2). The maximum oxidation rate of ammonium nitrogen (1.38±0.23 mg L^{-1} h^{-1}) was reached when the initial concentration of phenol increased to 600 mg L^{-1} and almost ceased (0.06±0.01 mg L^{-1} h^{-1}) when the phenol concentration increased to 1,500 mg L^{-1}. At low phenol concentrations, the removal rates of ammonium and phenol increased in direct proportion to phenol concentration. The increase in phenol concentration led to a linear increase in the removal rates of both ammonium and phenol under the phenol concentration of 600 mg L^{-1}. The ammonium oxidation rate increased from 0.04±0.00 to 1.35±0.16 mg L^{-1} h^{-1} when the initial ammonium concentration increased from 15 to 600 mg L^{-1} while phenol removal rate increased from 0.12±0.01 to 3.13±0.28 mg L^{-1} h^{-1}.

3.3 Effect of Ammonium Concentration on Ammonium Oxidization and Phenol Biodegradation

The biodegradation rate of phenol and ammonium oxidation rate at different ammonium concentrations were measured to obtain further information on the relationship between heterotrophic nitrification and phenol biodegradation. At low ammonium concentrations, the increase in ammonium concentration caused the increase in both ammonium oxidation rate and phenol removal rate (Fig. 3). The phenol removal rate increased from 1.69±0.10 to 2.16±0.14 mg L^{-1} h^{-1} when the initial ammonium N concentration increased from 50 to 150 mg L^{-1} while the ammonium oxidation rate increased from 0.65±0.04 to 1.05±0.08 mg L^{-1} h^{-1}. The phenol removal rate

Table 1 Nitrogen balance of ammonium removal

		Treatment	Uninoculated control	Heat-killed control
NH_4^+-N (mg)	Initial NH_4^+-N	10.00	10.0	10.00
	Final NH_4^+-N	0.08	10.0	9.94
Nitrification products (mg)	NH_2OH-N	0.30	0	0
	NO_2^--N	0.12	0	0
	NO_3^--N	0.18	0	0
Intracellular N (mg)		2.56	0	0
Dinitrification products (mg)	N_2-N	5.36	0	0
	N_2O-N	0.36	0	0
	NO-N	0	0	0
Nitrogen recovery (%)[a]		90	100	99

The mass balance experiment was performed in sealed bottles containing 100 mL of wastewater. Initial concentrations of ammonium nitrogen (NH_4^+-N) and phenol were 100 and 400 mg L^{-1}, respectively

[a] The nitrogen recovery refers to the percentage of sum of nitrogen contribution from final NH_4^+-N, nitrification product nitrogen (NH_2OH-N, NO_2^-N, and NO_3^--N), cell nitrogen, and denitrification product nitrogen (N_2O-N, NO-N, N_2-N) to the total input nitrogen (initial NH_4^+-N)

began to decrease at relatively high NH_4^+-N concentrations (>150 mg L^{-1}), indicating the occurrence of the inhibition on phenol biodegradation. Ammonium with high concentration could compete with phenol for the same electron acceptor (oxygen), which subsequently led to the substrate inhibition of phenol biodegradation.

3.4 Effects of Nitrification Metabolites on the Phenol Biodegradation and Nitrogen Removal

The effects of nitrification metabolites including nitrite and nitrate on phenol biodegradation were investigated to get further information on the influence of nitrification process on phenol biodegradation (Table 2). The results showed that the phenol removal rate was 1.21± 0.05 mg L^{-1} h^{-1} when nitrite was added as the sole nitrogen source while that of the nitrate treatment was 1.39±0.05 mg L^{-1} h^{-1}. Total nitrogen removal rate was 0.40±0.04 mg L^{-1} h^{-1} when nitrite was added as the sole nitrogen source while that of the nitrate treatment was 0.44±0.03 mg L^{-1} h^{-1}. Compared with the ammonium treatment, both the phenol biodegradation rates and nitrogen removal rates in the presence of nitrite and nitrate were relatively low.

3.5 Phenol Biodegradation Pathway

To determine the biodegradation pathway of phenol by strain LJ-1, the evolution of typical metabolites during phenol biodegradation was monitored (Fig. 4). The results showed that two typical intermediates (catechol and *cis*, *cis*-muconic acid) (Parke et al. 2000; Alva and Peyton 2003) were detected. The rapid biodegradation of phenol led to the accumulation of catechol which reached its concentration peak at 96 h. The catechol appeared transiently and was later degraded. The subsequent removal of catechol was followed with the accumulation of *cis*, *cis*-muconic acid which reached its concentration peak at 168 h. Additionally, both of two

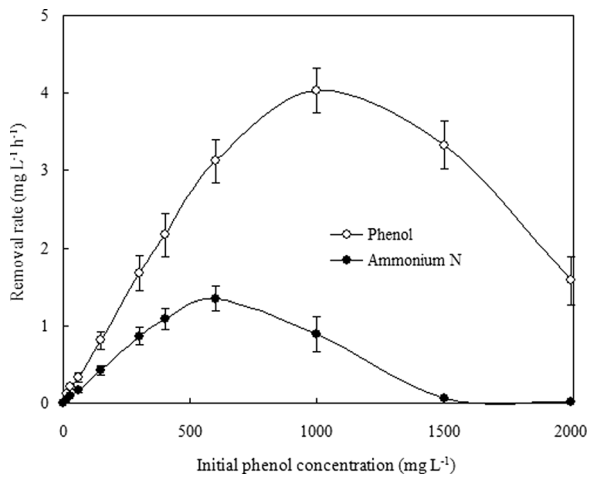

Fig. 2 Effect of phenol concentration on ammonium oxidization and phenol biodegradation. The initial concentration of ammonium nitrogen (N) was 100 mg L^{-1}. Initial concentrations of phenol were 0, 15, 30, 60, 150, 300, 400, 600, 1,000, 1,500, and 2,000 mg L^{-1}

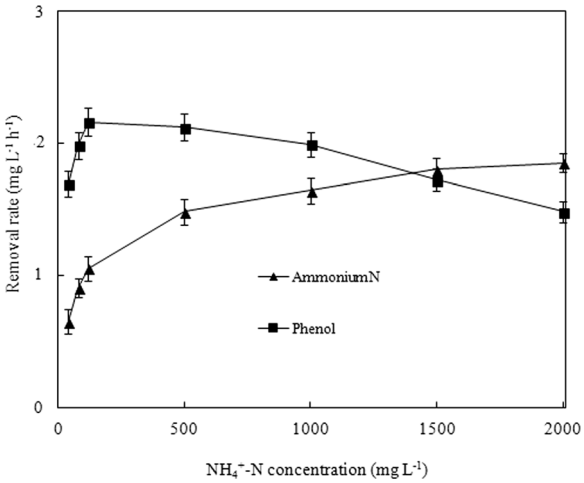

Fig. 3 Effect of ammonium concentration on ammonium oxidization and phenol biodegradation. The initial concentration of phenol was 400 mg L^{-1}. Initial concentrations of ammonium nitrogen (N) were 50, 100, 150, 500, 1,000, 1,500, and 2,000 mg L^{-1}

typical intermediates (catechol and *cis*, *cis*-muconic acid) were removed rapidly when they were used as the sole carbon source. The carbon mass balance experiment showed that the fate of phenol carbon in terms of inorganic carbon, cell carbon, metabolites (catechol and *cis*, *cis*-muconic acid), and residual phenol gave high carbon recovery of 87 % (Table 3). Among all the phenol carbon, inorganic carbon contributed 50 %, indicating the high degree of phenol mineralization during biodegradation.

4 Discussion

The simultaneous removal of phenol and ammonium usually relies on the cooperation between autotrophic bacteria and heterotrophic bacteria which may subsequently lead to complicated bioreactor operation (Amor et al. 2005). To our knowledge, this is the first report of the simultaneous removal of phenol and ammonium by heterotrophic nitrifying-denitrifying bacterium, which can biodegrade phenol and remove ammonium through the heterotrophic nitrification-aerobic denitrification. The maximum removal rates of phenol and NH_4^+-N in this study are similar to the previous report on activated sludge process with nitrification function (Amor et al. 2005), indicating considerable removal ability for phenol and ammonium by strain LJ-1. The nitrogen mass balance study showed that most of ammonium was recovered as N_2 (54 %), indicating that ammonium was removed as N_2. In the presence of heterotrophic nitrifier LJ-1, ammonium was transformed into N_2 and removed from the aqueous phase through nitrification-aerobic-dinitrification process. In the presence of heterotrophic nitrifiers, ammonium can be transformed into N_2 and removed from the aqueous phase through nitrification-aerobic-dinitrification process (Joo et al. 2005). Evolution of ammonium, hydroxylamine, nitrite, and nitrate during the biodegradation period of phenol showed that the nitrification pathway was similar to that in both the heterotrophic nitrifiers (Joo et al. 2005; Lu et al. 2008a) and the autotrophic nitrifier (Rittmann and McCarty 2001), indicating that phenol had no effect on the nitrification pathway.

The ammonium oxidation using heterotrophic nitrification bacterium had good tolerance to phenol toxicity, suggesting the advantage of using heterotrophic nitrifying bacterium for the simultaneous removal of phenol and ammonium. The nitrification process is usually sensitive to organic toxicants such as phenol (Staford 1974; Neufeld et al. 1980), which leads to the challenge for the simultaneous removal of phenol and ammonium. Previous studies showed that ammonium oxidation by autotrophic nitrification bacteria could be easily inhibited at phenol concentrations as low as 5.64 mg L^{-1} (Staford 1974; Neufeld et al. 1980). Phenol removal rate kept increasing with the increase in phenol concentration at relatively high phenol concentrations (>600 mg L^{-1}) when the ammonium oxidation began to be inhibited, indicating relatively high phenol biodegradation potential in the presence of heterotrophic nitrifying bacterium.

Phenol is necessary for ammonium removal since ammonium removal did not occur in the absence of organic carbon (phenol), confirming that the ammonium oxidization was catalyzed by heterotrophic but not autotrophic nitrifier. At low phenol concentrations, the

Table 2 Effects of nitrification metabolite on the removal of phenol and total nitrogen

	Removal rate of phenol (mg L^{-1} h^{-1})	Removal rate of total nitrogen (mg L^{-1} h^{-1})
Nitrate N	1.39±0.05	0.44±0.03
Nitrite N	1.21±0.05	0.40±0.04
Ammonium N (control)	2.13±0.10	0.64±0.03

Initial concentrations of nitrogen (ammonium N, nitrite N, or nitrate N) and phenol were 100 and 400 mg L^{-1}, respectively

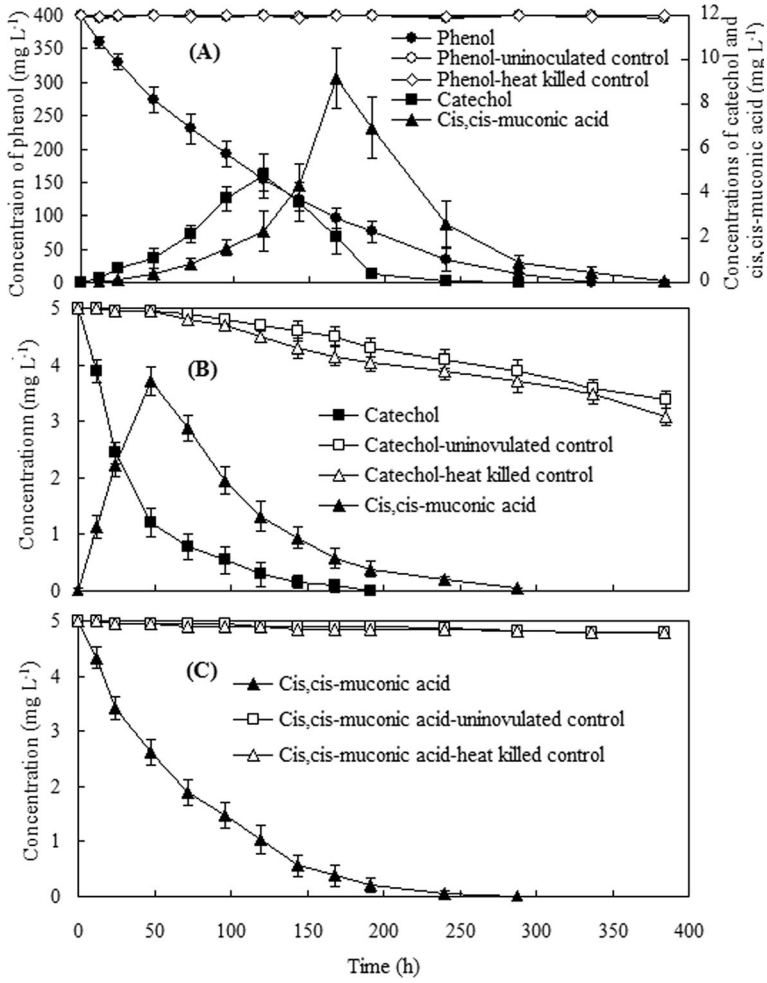

Fig. 4 Changes of the concentrations of phenol, catechol, and *cis*, *cis*-muconic acid profiles in the biodegradation assays of phenol (**a**), catechol (**b**), and *cis*, *cis*-muconic acid (**c**). The initial concentration of ammonium N in all the biodegradation experiments was 100 mg L^{-1}. Initial concentration of phenol was 400 mg L^{-1} in phenol biodegradation experiment. Initial concentrations of catechol and *cis*, *cis*-muconic acid were 5 mg L^{-1} in catechol and *cis*, *cis*-muconic acid biodegradation experiment, respectively

removal rates of ammonium increased in direct proportion to phenol concentration. The reasonable explanation for this phenomenon is that the oxidation of phenol can provide energy and carbon for the heterotrophic nitrifier strain LJ-1, which can subsequently promote the oxidation of ammonium. According to previous studies (Verstraete and Focht 1977; Castignetti and Hollocher 1982), heterotrophic nitrifiers are distinct from autotrophic nitrifiers, which use energy gained solely from the oxidation of inorganic nitrogen compounds. They oxidize reduced nitrogenous compounds and utilize organic carbon as carbon and energy sources.

NH_4^+-N is the preferable N source for strain LJ-1 due to its heterotrophic nitrification ability. Maximum rates of phenol biodegradation and total nitrogen removal in the treatments with nitrate or nitrite as the sole nitrogen source were more than 30 % lower than those of the treatment with NH_4^+-N as the sole nitrogen source. The presence of the ammonium could bring additional energy besides phenol oxidation for strain LJ-1 through nitrification process, which subsequently enhanced the ammonium oxidation and phenol biodegradation. For this reason, the treatment with ammonium as the nitrogen source had the highest removal rates of phenol and nitrogen pollutants. Since heterotrophic nitrifiers could use energy gained from the oxidation of both inorganic nitrogen compounds and organic carbon (Verstraete and Focht 1977; Castignetti and Hollocher 1982), the oxidation of ammonium could also provide energy for the heterotrophic nitrifier strain LJ-1, which could subsequently promote the oxidation of phenol. The increase in ammonium concentration caused the increase in phenol removal rate.

Catechol but not hydroquinone was detected when phenol was rapidly degraded, suggesting that *ortho*-to-hydroxy attack by one oxygen atom occurred and led to

Table 3 Carbon balance of phenol biodegradation

	Treatment	Uninoculated control	Heat-killed control
Phenol carbon (mg)			
Initial phenol carbon	30.64	30.64	30.64
Final phenol carbon	1.15	30.70	30.28
Catechol carbon (mg)	0.77	0	0
cis, *cis*-Muconic acid carbon (mg)	2.53	0	0
Inorganic carbon (mg)[a]	15.40	0	0
Cell carbon (mg)	6.66	0	0
Carbon recovery (%)[b]	87	100	99

The mass balance experiment was performed in sealed bottles containing 100 mL of wastewater. Initial concentrations of ammonium nitrogen and phenol were 100 and 400 mg L^{-1}, respectively

[a] The inorganic carbon refers to the sum of carbon contribution from inorganic carbon in water and carbon dioxide in headspace

[b] The carbon recovery refers to the percentage of sum of carbon contribution from inorganic carbon, cell carbon, final phenol carbon, and metabolite carbon to the total carbon (initial phenol carbon)

the formation of catechol. Catechol is usually formed in the primary step of the phenol oxidation process in heterotrophic bacteria, while hydroquinone is formed in autotrophic nitrifying bacteria (Hyman et al. 1985). Phenol is usually oxidized into catechol, and then the ring of catechol is further cleaved by two known pathways, namely, the *ortho*-cleavage pathway, through which catechol is metabolized into *cis*, *cis*-muconic acid, and *meta*-cleavage pathway, through which catechol is transformed into 2-HMS (Parke et al. 2000; Alva and Peyton 2003). In this experiment, catechol appeared transiently and was later degraded. The removal of catechol was followed with the accumulation of *cis*, *cis*-muconic acid, a metabolite of the *ortho*-cleavage pathway, while the central metabolite in the *meta*-cleavage pathway (2-HMS) was not detected, suggesting that phenol was biodegraded through the *ortho*-cleavage pathway. Additionally, both of two typical intermediates (catechol and *cis*, *cis*-muconic acid) of phenol *ortho*-cleavage pathway could be degraded rapidly when they were used as the sole carbon source, confirming that phenol was biodegraded through the *ortho*-cleavage pathway. In the carbon mass balance experiment, the fate of phenol carbon in terms of inorganic carbon, cell carbon, metabolites, and residual phenol gave high carbon recovery, indicating reasonability for the assumed phenol biodegradation pathway. Moreover, high inorganic carbon recovery (50 %) showed the high degree of phenol mineralization during biodegradation. The ring structure often contributes to the toxicity and persistence of aromatic compounds, which makes ring cleavage the most important step in the biodegradation process of the aromatic compounds (Bouchez et al. 1996; Parke et al. 2000; Alva and Peyton 2003). The ring cleavage ability of heterotrophic nitrifying strain LJ-1 suggests that this heterotrophic nitrification bacterium has the potential advantage of phenol mineralization.

5 Conclusion

In this study, simultaneous removal of phenol and ammonium using heterotrophic nitrifying-denitrifying bacterium *Serratia* sp. LJ-1 was firstly investigated. The removal rates of both contaminants were notable. Phenol was degraded through the *ortho*-cleavage pathway before being mineralized while ammonium was removed as N_2 through nitrification and subsequent aerobic denitrification. Phenol and ammonium could promote their removal efficiencies with each other at relatively low concentrations. The ammonium oxidation had much higher tolerance to phenol toxicity than that of the autotrophic nitrifier. The treatment with ammonium but not nitrification metabolites as the nitrogen source had the highest removal rates of phenol and nitrogen pollutants. Since phenol often coexists with nitrogen contaminants, these findings have significant environmental implications in terms of the simultaneous removal of these contaminants.

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