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SIMULTANEOUS AMMONIUM AND p-HYDROXYBENZALDEHYDE OXIDATION IN A SEQUENCING BATCH REACTOR

OXIDACIÓN SIMULTÁNEA DE AMONIO Y p-HIDROXIBENZALDEHIDO EN UN REACTOR DE LOTES SECUENCIADOS

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Abstract

The simultaneous ammonium and p-hydroxybenzaldehyde (pOHBD) oxidation capacity of a nitrifying sludge was investigated in a sequencing batch reactor (SBR). At all initial pOHBD concentrations tested (25-400 mg C/L), both ammonium (100 mg NH $_4^+$ -N/L) and pOHBD were consumed with efficiencies of 99.2 ± 1.5 % and 100 ± 1 %, respectively. At pOHBD concentrations lower than 100 mg C/L, the main product of ammonium oxidation was nitrate with a yield (Y_{NO_3}) of 0.97 ± 0.03 g NO $_3^-$ -N/g NH $_4^+$ -N consumed. At 200 and 400 mg pOHBD-C/L, Y_{NO_3} decreased to 0.78 ± 0.05 and nitrite was detected (Y_{NO_2} = 0.04 ± 0.01 g NO $_2^-$ -N/g NH $_4^+$ -N consumed). p-Hydroxybenzoate (pOHBT) was detected as product of pOHBD oxidation. pOHBT accumulation was significant in the first operation cycles at 25 mg pOHBD-C/L. Afterward, pOHBT was completely removed and no aromatic intermediates were detected. At low C/N ratio values (0.25-4), a dissimilatory nitrifying respiratory process was maintained (Y_{BM} = 0.03 ± 0.01 g biomass-N/g NH $_4^+$ -N consumed). These results show that nitrifying SBR can be successfully used for the simultaneous removal of ammonium and p-hydroxybenzaldehyde in a unique reactor. This information might be useful for treating industrial wastewaters contaminated with nitrogen and recalcitrant phenolic compounds.

Keywords: ammonium, biological oxidation, p-hydroxybenzaldehyde, nitrification, sequencing batch reactor.

Resumen

La capacidad de un lodo nitrificante para oxidar simultáneamente amonio y p-hidroxibenzaldehido (pOHBO) fue evaluada en un reactor de lotes secuenciados (SBR). A todas las concentraciones ensayadas (25-400 mg C-pOHBO/L), el amonio (100 mg N-NH $_4^+$ /L) y el pOHBO fueron consumidos con eficiencias de 99.2 ± 1.5 % y de 100 ± 1 %, respectivamente. Hasta 100 mg C-CpOHBO/L, el nitrato fue el principal producto de la oxidación del amonio con un rendimiento (Y_{NO_3}) de 0.97 ± 0.03 g N-NO $_3^-$ g/g N-NH $_4^+$ consumido. A 200 y 400 mg C-pOHBO/L, Y_{NO_3} disminuyó a 0.78 ± 0.05 y nitrito fue detectado ($Y_{NO_2} = 0.04 \pm 0.01$ g N-NO $_2^-$ /g N-NH $_4^+$ consumido). El p-hidroxibenzoato (pOHBT)) se detectó como producto de la oxidación del pOHBO. El pOHBT se acumuló significativamente en los primeros ciclos de operación, pero posteriormente fue completamente consumido y no se detectó ningún intermediario aromático. A valores de relación C/N bajos (0.25-4), se mantuvo un proceso respiratorio nitrificante desasimilativo ($Y_{BM} = 0.03 \pm 0.01$ g N-biomasa/g N-NH $_4^+$ consumido). Estos resultados muestran que los reactores SBR nitrificantes pueden ser exitosamente utilizados para la eliminación simultánea de amonio y p-hidroxibenzaldehido en un solo reactor. Esta información puede ser útil para el tratamiento de aguas residuales industriales contaminadas por nitrógeno y compuestos fenólicos recalcitrantes.

Palabras clave: amonio, oxidación biológica, p-hidroxibenzaldehido, nitrificación, reactor de lotes secuenciados.

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1 Introduction

There are several industries that can generate effluents containing high concentrations of ammonium and phenolic compounds (petrochemical, chemical, steel manufacturing, resin producing industries, among others) (Olmos *et al.*, 2004). Phenolic compounds (phenol, cresols, chlorophenols, etc.) are toxic, and might be carcinogenic, mutagenic and teratogenic at high concentrations (Autenrieth *et al.*, 1991). Nitrogen pollution by ammonium is linked to ecological (eutrophication, acidification), toxicological and economical impacts (Arredondo-Figueroa *et al.*, 2007; Cervantes, 2009).

Biological nitrification and denitrification processes are considered economically feasible technologies for nitrogen removal from wastewaters. Nitrification is the biological oxidation of ammonium (NH_4^+) via nitrite (NO_2^-) to nitrate (NO_3^-) , and denitrification is the biological reduction of nitrate to nitrogen gas (N₂). Recently, nitrifying processes have been proposed as novel alternative technologies for the simultaneous removal of ammonium and phenolic pollutants from industrial wastewaters (Beristain-Cardoso et al., 2011; Silva et al., 2011). Several nitrifying consortia have been reported to oxidize simultaneously ammonium and various phenolic compounds, being in some cases nitrate and carbon dioxide the major products (Yamaghisi et al., 2001; Amor et al., 2005; Vázquez et al., 2006; Texier and Gómez, 2007; Silva et al., 2009; Martínez-Hernández et al., 2011). results might be useful for using nitrifying reactors for the simultaneous removal of ammonium and phenolic compounds in one step from wastewaters of chemical complexity. There is no work reporting the simultaneous oxidation of p-hydroxybenzaldehyde (pOHBD) and ammonium in nitrifying reactors. pOHBD is a phenolic compound used in the chemistry, medicine and pharmaceutical industries and one of the intermediates from the p-cresol oxidation p-Cresol oxidation follows the same pathway. initial pathway under different aerobic and anaerobic conditions, consisting of the transient and sequential formation of p-hydroxybenzylalcohol, pOHBD, and p-hydroxybenzoate (pOHBT) (Häggblom et al., 1990). Preliminary results from batch experiments suggested that pOHBD oxidation would be the limiting step in p-cresol mineralization by a nitrifying consortium (Silva et al., 2009). However, more studies in biological reactors are necessary to evaluate the feasibility of using nitrifying sludge to remove both ammonium and pOHBD in a sole bioreactor.

In recent years, conventional suspended-growth activated sludge system has been replaced by costeffective and high-efficiency sequencing batch reactor (SBR), particularly for biological nutrient removal (Singh and Srivastava, 2011). SBR has proven to be a viable alternative to the continuous-flow systems for nitrogen removal through nitrification and denitrification biological processes (Puig et There is a need to improve the al., 2004). overall performance of the SBRs treating wastewaters of chemical complexity. The use of nitrifying SBRs for simultaneous removal of ammonium and carbonaceous compounds could be very attractive for wastewater treatment.

In this study, a nitrifying SBR was used to oxidize simultaneously ammonium and pOHBD. The nitrification performance in the SBR and capability of the microbial sludge to remove pOHBD were evaluated at different initial concentrations of pOHBD throughout the operation cycles. Mass balances of both nitrogen and carbon were established, while efficiencies (ammonium and pOHBD consumption) and yields (nitrite, nitrate, and biomass production) were used as response variables of the respiratory processes.

2 Materials and methods

2.1 Inoculum

The sludge used for inoculating the SBR was obtained from a continuous stirred tank reactor (CSTR) operated at steady-state nitrification. The composition of the medium used for the CSTR was (g/L): (NH₄)₂SO₄ (1.73), NH₄Cl (1.40), KH₂PO₄ (2.73), MgSO₄ (0.60), NaCl (1.0), NaHCO₃ (9.30) and CaCl₂ (0.05). The CSTR was continuously aerated and operated at 200 rpm, 30° C \pm 3, pH of 7.8 \pm 0.3 and a hydraulic retention time of 3.5 d. At a NH₄ loading rate of 116 ± 9 mg N/L.d, the complete oxidation of ammonium (99.0 ± 1.6% of removal efficiency) into nitrate (yield of 0.90 ± 0.03 g NO_3^--N/g NH_4^+-N consumed) was obtained. There was no accumulation of nitrite and ammonium in the continuous reactor. These results confirmed that nitrification in steadystate was achieved in the CSTR and the stabilized nitrifying sludge could be used as inoculum for the SBR.

2.2 Reactor setup and operation

A 2-L SBR was inoculated with 0.7 ± 0.1 g volatile suspended solids (VSS)/L of sludge previously stabilized in the CSTR. The operating conditions used for the SBR are presented in Table 1. Each 12 h cycle consisted of 10 min influent addition, 11 h aerated reaction, 30 min settling and 20 min effluent withdrawal. The hydraulic retention time was 0.55 d and the volumetric exchange ratio of liquid was 90%. The chemical composition of the basal medium was (g/L): (NH₄)₂SO₄ (0.43), NH₄Cl (0.36), KH₂PO₄ (2.70), K₂HPO₄ (1.80), MgSO₄ (0.40), NaCl (0.40), NaHCO₃ (3.40) and CaCl₂ (0.03). At the beginning of each cycle, the initial NH₄ concentration was 101 ± 3 mg N/L. NaHCO₃ was used both as pH buffer and as carbon source. The reactor was operated at ambient temperature. Firstly, the SBR was operated without pOHBD feeding as control test to evaluate the nitrifying respiratory process under the experimental conditions used. After 70 cycles, pOHBD was added into the reactor at initial concentrations ranging from 25 to 400 mg C/L (50 to 800 mg pOHBD-C/L.d). According to the initial NH₄⁺-N and pOHBD-C concentrations, the C/N ratio varied between 0.25 Samples were taken periodically in the influent and effluent of the SBR, filtered (0.45 μ m), and analyzed for ammonium, nitrite, nitrate, pOHBD and pOHBT. Microbial performance was evaluated in terms of consumption efficiency (E, %, (g of N or C consumed/g of N or C fed) × 100) and yield (Y, g of N or C produced/g of N or C consumed). Additionally, mass balance was established for both N and C compounds.

2.3 Analytical methods

Ammonium nitrogen was analyzed by a selective electrode (Phoenix Electrode Co., USA). Nitrite, nitrate, pOHBD, and pOHBT concentrations were determined by HPLC as previously described by Silva *et al.* (2009). The volatile suspended solids (VSS) were determined according to standard methods (APHA, 1998). Lowry's method was employed to measure microbial protein concentration (Lowry *et al.*, 1951). In order to express the biomass concentration as biomass-N and biomass-C in the mass balances, it was considered that 16% of microbial protein is nitrogen and 50% of biomass is carbon (Bailey and Ollis, 1986). pH and dissolved oxygen concentrations were determined by selective electrodes. Analytical methods had a variation coefficient of less than 10%.

Table 1. Operating conditions of the nitrifying sequencing batch reactor.

Conditions	Value
Working volume	2 L
Cycles time	12 h
VSS	$0.7 \pm 0.1 \text{ g/L}$
pН	8.1 ± 0.4
Dissolved oxygen	5.3 ± 0.1 mg/L
Temperature	$25 \pm 5^{\circ}\text{C}$
Agitation	225 rpm
Aeration flow	2 vvm

vvm: volumes of air per volume of liquid per minute

3 Results and discussion

3.1 Nitrifying performance of the SBR fed with p-hydroxybenzaldehyde

Nitrifying performance of the SBR fed at different initial pOHBD concentrations (25-400 mg C/L) is presented in Fig. 1 and Table 2. In the first period (cycles 1 to 70) when the reactor was fed with ammonium alone, the ammonium consumption efficiency was $99.5 \pm 0.7\%$ and the main product was nitrate with a yield of 0.96 ± 0.07 g NO_3^- N/g NH₄⁺-N consumed. These results indicated that the sludge showed a stable nitrifying activity under the experimental conditions used in the SBR. At all initial pOHBD concentrations tested (25-400 mg C/L), ammonium was totally consumed with an average efficiency of 99.2 \pm 1.5%. At pOHBD concentrations ranging from 25 to 100 mg C/L, the main nitrogenous product of ammonium oxidation was nitrate with a Y_{NO_3} of 0.97 ± 0.03 g NO_3^- -N/g NH_4^+ -N consumed. There was no nitrite accumulation in the SBR. At a pOHBD concentration of 200 mg C/L, nitrate formation tended to decrease. At 400 mg pOHBD-C/L, the Y_{NO_3} value dropped to 0.83 \pm 0.10 g NO_3^- N/g NH₄-N consumed while nitrite was detected in the culture $(Y_{NO_2} = 0.04 \pm 0.01 \text{ g NO}_2^-\text{-N/g NH}_4^+\text{-N}$ consumed). In all cases, except at 200 mg pOHBD-C/L, the recovery percentage of nitrogen products was higher than 90% from the ammonium initially added. Considering a variation coefficient of 10% in our results due to variations in analytical methods, this result is satisfactory. However, at 200 mg pOHBD-C/L, the recovery percentage decreased to 76%. In this period, an increase in the pH value was observed in

the SBR, which might provoke nitrogen volatilization as NH₃. As soon as the pH value was newly controlled in the system (at 400 mg pOHBD-C/L), the recovery percentage increased to 90%, showing that the main products of ammonium oxidation were detected as nitrite, nitrate and biomass. These results suggest that pOHBD at higher concentrations than 100 mg C/L might alter the nitrifying performance of the SBR as nitrate production tended to diminish and nitrite to accumulate. As shown in Table 2, biomass formation increased with the initial pOHBD concentrations, indicating an increase in nitrogen assimilation. However, the maximum Y_{BM} value was 0.03 ± 0.01 g biomass-N/g NH₄⁺-N consumed. This shows that only 3% of the ammonium consumed was used for biosynthesis and the nitrifying process kept essentially dissimilatory. The addition of organic matter in bioreactors generally causes an increase in the microbial growth and alters the nitrifying performance due to competition for ammonium and dissolved oxygen between nitrifiers and heterotrophs in the sludge (Hanaki et al., 1990). However, in our study, the pOHBD-C/N ratio was maintained at low values (0.25 - 4.00) and biomass growth was limited.

The experimental results demonstrated that nitrification can successfully occur at an initial pOHBD concentration up to 100 mg C/L, corresponding to a pOHBD loading rate of 200 mg C/L.d. Benzaldehydes are known for having an antibacterial activity and it has been suggested that

they act on the cell surface by reacting with sulfhydryl groups (Ramos-Nino et al., 1998). Results from the study of Silva et al. (2009) performed in batch experiments indicated that pOHBD could be the main intermediate from p-cresol oxidation that would be responsible for nitrification inhibition. The authors observed the inhibitory effect through a decrease in the nitrification specific rates. This suggests that in the present study, the nitrification process might have been slower in the SBR due to the pOHBD inhibitory effect but the 12 h cycles were sufficiently longer to reach high values for E_{NH_4} and Y_{NO_3} . At higher pOHBD concentrations than 100 mg C/L, the inhibitory effect of the aromatic compound might induce a decrease in nitrate formation and a nitrite accumulation in the reactor. However, further research is needed to understand better how pOHBD affects nitrification processes, including studies with axenic cultures and nitrifying consortia, in batch cultures as in biological reactors. Recently, Silva et al. (2011) observed in batch assays that pOHBD would not be inhibitory for nitrification when the sludge was previously fed with p-cresol, suggesting that the previous exposure of the sludge to a phenolic compound might contribute to a better tolerance to pOHBD. In the present work, the use of a SBR as bioreactor where there is a repetitive exposure of the sludge to the phenolic compound might contribute to a higher tolerance of the consortium to the toxic throughout the cycles.

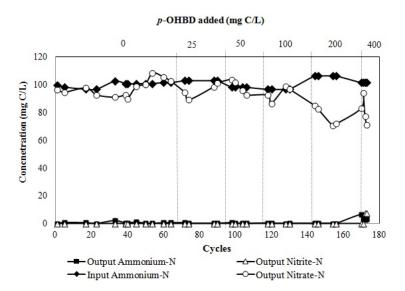


Fig. 1. Nitrifying performance of the SBR fed with p-hydroxybenzaldehyde (p-OHBD) at different initial concentrations (25-400 mg C/L).

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p nydroxyoenzaidenyde.						
	Added	Input-N (mg/L)		Output	-N (mg/L)	
	pOHBD	NH ₄ +N	NH ₄ +N	NO ₂ -N	NO ₃ -N	Biomass-N
	(mg C/L)					
	0	101.1 ± 0.6	0.5 ± 0.7	0	96.7 ± 6.9	0.4 ± 0.1
	25	103.0 ± 0.1	0.2 ± 0.2	0	95.7 ± 5.3	0.4 ± 0.1
	50	98.2 ± 0.1	0.2 ± 0.3	0	98.6 ± 5.2	0.9 ± 0.1
	100	96.7 ± 0.1	0.1 ± 0.1	0	93.6 ± 5.4	1.6 ± 0.1
	200	106.4 ± 0.2	0.0 ± 0.0	0	77.5 ± 7.3	3.1 ± 0.1
	400	101.5 ± 0.1	4.0 ± 1.8	3.7 ± 1.2	81.3 ± 9.9	2.1 ± 0.1

Table 2. Nitrogen mass balance in the nitrifying SBR fed with *p*-hydroxybenzaldehyde.

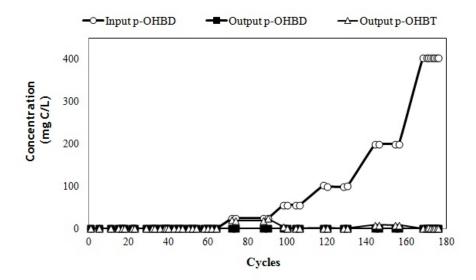


Fig. 2. *p*-Hydroxybenzaldehyde (p-OHBD) consumption and *p*-hydroxybenzoate (p-OHBT) production in a nitrifying sequencing batch reactor.

Bacteria can develop mechanisms to change membrane configuration and acquire a higher resistance to toxics such as benzaldehydes (van Schie and Young, 2000). Nonetheless, further works are required in order to understand this phenomenon.

3.2 p-hydroxybenzaldehyde oxidation in the nitrifying SBR

At all initial concentrations (25-400 mg C/L), pOHBD consumption was complete, resulting in an E_{pOHBD} of 100% (Fig. 2 and Table 3). It has been previously reported that in abiotic assays under conditions of aeration and agitation similar to those used in the

present study, the lost of pOHBD by volatilization was negligible (Silva *et al.*, 2011). *p*-Hydroxybenzoate (pOHBT) was detected as intermediate of pOHBD oxidation by the consortium. In the first cycles operated at 25 mg pOHBD-C/L (cycles 70 to 100), pOHBT was accumulated at 21.1 ± 1.9 mg C/L, corresponding to 85% of the pOHBD initially added. However, in the following SBR cycles, pOHBT was removed at efficiencies higher than 95%. It was also verified by HPLC that no aromatic compounds were accumulated in the culture. It has been previously observed in batch studies that pOHBD is a recalcitrant phenolic compound that shows low specific consumption rates under nitrifying conditions

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	p-nydroxy benzardenyde.						
Added	Input-C (mg/L)	Output-C (mg/L)					
pOHBD	pOHBD	pOHBD	pOHBT	Biomass-C			
(mg C/L)	_		_				
0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0			
25	24.7 ± 1.3	0.0 ± 0.0	21.1 ± 1.9	2.0 ± 0.1			
50	56.1 ± 0.9	0.0 ± 0.0	1.7 ± 1.6	4.1 ± 0.3			
100	100.0 ± 1.9	0.0 ± 0.0	1.0 ± 0.2	7.9 ± 0.5			
200	195.6 ± 2.2	0.0 ± 0.0	8.7 ± 0.2	15.0 ± 0.3			
400	402.5 ± 5.8	0.0 ± 0.0	0.7 ± 2.5	16.3 ± 0.3			

Table 3. Carbon mass balance in the nitrifying SBR fed with *p*-hydroxybenzaldehyde.

(Silva et al., 2009, 2011). In our work, it was shown that under the experimental conditions used in the SBR, the consortium was able to oxidize pOHBD at a maximum concentration of 400 mg C/L, corresponding to a loading rate of 800 mg C/L.d. In spite of the recalcitrance of pOHBD, the SBR could be a good technology for pOHBD treatment. This type of reactor has previously been shown to allow the metabolic adaptation of sludge through the operation cycles, resulting in higher specific consumption rates of recalcitrant pollutants (Zhuang et al., 2005). As it can be seen in Table 3, carbon assimilation for biomass synthesis increased with the initial pOHBD concentration. However, in terms of biomass yield, it was found that the process was dissimilatory as the maximum Y_{BM} was only of 0.08 biomass-C/g pOHBD-C consumed. In the study of Eiroa et al. (2005), the simultaneous removal of formaldehyde and ammonium in a lab-scale activated sludge unit was investigated. High removal efficiencies were obtained for both ammonium and formaldehyde (99.9 and 99.5%, respectively). However, at formaldehyde loading rates higher than 0.48 g COD/L.d, the nitrate concentration in the effluent decreased. According to the authors, this decrease can be basically attributed to denitrification and ammonium assimilation by the heterothophs. However, the authors did not present data of N₂ production or biomass yield. In the present study, the low value for Y_{BM} indicated that growth of the heterotrophic population of the consortium was limited under the experimental conditions used in the SBR (low C/N ratio, lithoautotrophic medium, physiologically stable nitrifying inoculum).

Conclusions

In a SBR fed with ammonium at 100 mg N/L (200 mg N/L.d) and pOHBD at concentrations between 25 and 400 mg C/L (50 to 800 mg C/L.d), the nitrifying sludge achieved total removal of ammonium and pOHBD with efficiencies of $99.2 \pm 1.5\%$ and $100 \pm 1\%$, respectively. At pOHBD concentrations lower than 100 mg C/L, nitrification was not affected and nitrate was the main end product of the nitrifying pathway. Nitrite was not accumulated and the biomass formation kept very low. These results show that nitrifying SBR could be a good alternative for the simultaneous removal of ammonium and recalcitrant phenolic compounds from wastewaters. Additionally, results showed a novel and interesting aspect of using nitrifying consortia physiologically stable in wastewater treatment as they might perform various biological respiratory processes in a unique reactor for oxidizing ammonium and organic pollutants.

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