



Sequential nitrification–denitrification process for nitrogenous, sulfurous and phenolic compounds removal in the same bioreactor



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HIGHLIGHTS

- 4-Methylphenol, sulfide and ammonium oxidation by an aerobic granular sludge.
- Aerobic granular sludge showed metabolic capability to denitrify.
- Elemental sulfur produced as strategy of nitrification–denitrification coupling.
- Removal of 4-methylphenol, sulfide and ammonium in the same bioreactor.
- Ammonium was biotransformed to molecular nitrogen.

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ABSTRACT

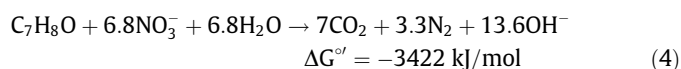
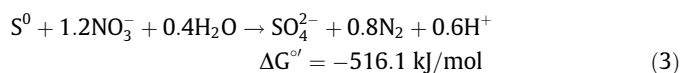
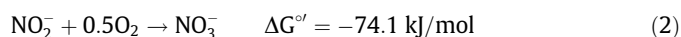
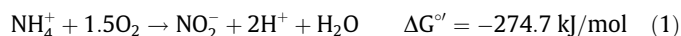
The kinetic and metabolic behavior of an aerobic granular sludge to nitrify, denitrify and nitrify–denitrify was evaluated in batch cultures. In nitrification control, ammonium, 4-methylphenol and sulfide were consumed efficiently (~100%) and recovered as NO_3^- , CO_2 , S^0 and SO_4^{2-} , respectively. In denitrification control, S^0 and nitrate were efficiently consumed and recovered as SO_4^{2-} and N_2 , respectively. Sequential nitrification–denitrification process was evaluated by applying oxic/anoxic conditions. Ammonium, 4-methylphenol and sulfide were oxidized to nitrate, CO_2 and mainly S^0 , respectively, under aerobic conditions. After that, anoxic conditions were established where S^0 reduced all nitrate to N_2 , with molecular nitrogen yield (Y_{N_2}) of 1.03 ± 0.06 mg/mg NH_4^+-N consumed. This is the first study to show the capability of an aerobic granular sludge in simultaneous removal of ammonium, 4-methylphenol and sulfide by sequential nitrification–denitrification process in the same bioreactor.

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1. Introduction

Industrial activities such as oil refining, chemical and paper manufacturing have generated wastewaters containing ammonium, sulfide and phenolic compounds with concentrations ranging from one to several hundred mg/L (Olmos et al., 2004). These compounds have been reported to cause environmental and public health damage (Altas and Buyukgungor, 2008). Phenolic compounds are toxic to humans and lethal to aquatic organisms at concentration of 5 mg/L (Autenrieth et al., 1991; Sublette et al., 1998; Veeresh et al., 2005). Ammonium can contribute to eutrophication, resulting in loss of flora and fauna because of the diminishing of dissolved oxygen (DO) in body waters (Cervantes-Carrillo et al., 2000). On the other hand, sulfide has negative effects on environmental and human health, as it might cause slight troubles at 10 mg/L and a severe respiratory failure at concentrations of 700 mg/L (ATSDR, 1992).

Nowadays, in order to remove these pollutants, usually treatment trains, based on nitrogen cycle are used, where ammonium is first oxidized to nitrate by nitrification, and afterward nitrate is reduce to N_2 by lithotrophic or organotrophic denitrification, according to next equations:



Several research groups have studied the simultaneous removal of sulfurous, carbonaceous and nitrogenous compounds, using either nitrifying or denitrifying conditions, suggesting the participation of different microbial populations. Beristain-Cardoso et al.

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(2010) have shown that a metabolically stable nitrifying sludge removed simultaneously three compounds; ammonium, sulfide and *p*-cresol. Aromatic compounds such as benzene, toluene and xylene have also been removed under nitrifying conditions (Amor et al., 2005; Kim et al., 2008). On the other hand, it is worth pointing out that the presence of phenolic compounds, such as phenol, *p*-cresol or *m*-cresol is not a limiting factor for denitrification, since those compounds were simultaneously removed by using nitrate as oxidizing power (Oh et al., 2001; Sierra-Álvarez et al., 2007; Meza-Escalante et al., 2008; Zhang et al., 2008; Beristain-Cardoso et al., 2009).

Researchers have identified bacteria with the metabolic capability to carry out either the nitrification or denitrification, such as *Thiosphaera pantotropha*, *Alcaligenes faecalis*, *Pseudomonas putida*, among others. Those strains are able to perform denitrification even in presence of dissolved oxygen (DO), concomitantly with the nitrifying process (Robertson et al., 1988; Baumann et al., 1996; Gupta, 1997; Daum et al., 1998; Takaya et al., 2003; Miyahara et al., 2010). These coupling of bioprocesses also have been carried out alternating oxic and anoxic periods, or anaerobic-oxic-anoxic periods (Kishida et al., 2006; Yilmaz et al., 2008; Adav et al., 2009). However, simultaneous removal of 4-methylphenol, ammonium and sulfide by sequential nitrification–denitrification process in the same reactor, and using aerobic granular sludge as inoculum has not been reported.

Thus, the goal of this study was to evaluate the metabolic capability of aerobic granular sludge, in batch cultures, to nitrify and denitrify in a sequential way in order to biotransform 4-methylphenol, sulfide and ammonium into CO_2 , SO_4^{2-} and N_2 , respectively.

2. Methods

2.1. Experimental set-up

The continuous stirred tank reactor (CSTR) had a working volume of 5 L, which was equipped with pH and temperature controllers. Temperature and pH were controlled at 25 ± 0.2 and 7.0 ± 0.3 °C, respectively. The air was supplied through the reactor liquid phase using an air sparger at the bottom. Dissolved oxygen (DO) inside the CSTR was kept at 4.0 ± 0.3 mg/L. Full mixing inside the reactor was achieved with one set of mechanical stirrer, which is at the bottom of the liquid medium, comprising of two blades. The feeding solution of synthetic wastewater was added to the reactor using peristaltic pump (Master Flex, ColeParmer), having a hydraulic retention time of 2 days.

2.2. Biomass and synthetic wastewater

The CSTR was inoculated with 3.5 g VSS/L of a nitrifying sludge coming from a laboratory-scale aerobic bioreactor. The sludge inoculated was of granular type, with average diameter of 4 mm and of yellow–brown color. This was fed with 400 mg $\text{NH}_4^+-\text{N}/\text{L}$, 100 mg 4-methylphenol-C/L, 100 mg *p*-hydroxybenzoate-C/L and 50 mg phenol-C/L. The basal mineral medium was composed of (g/L): K_2HPO_4 (4.5), KH_2PO_4 (3.0), NaHCO_3 (3.5), and trace elements solution supplied at 1 ml/L. The trace components solution contained (g/L): EDTA (0.05), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.015), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (0.07), MnCl_2 (0.03), $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ (0.015), FeCl_3 (0.015), MgCl_2 (0.02). Aerobic sludge from the CSTR was washed with saline solution before using as inoculum in batch experiments.

2.3. Batch experiments

2.3.1. Nitrifying activity

Batch experiments were performed in stirred instrumented experimental units with working volume of 1.0 L. The sludge em-

ployed in these experiments was obtained from the CSTR during the steady state, having an initial sludge concentration of 3.0 g VSS/L. The mineral culture medium used was described in the Section 2.2. The reactors were spiked with 52.5 mg/L of NH_4^+-N , 100 mg/L of 4-methylphenol-C and 100 mg/L of S^{2-} . The air was continually supplied through the reactor liquid phase by using an air sparger at the bottom, reaching a DO concentration of 4.5 ± 0.3 mg/L. The assays were performed by duplicate at 25 ± 2 °C, 200 rpm and pH of 7.0 ± 0.5 . Biotic controls lacking ammonium, 4-methylphenol or sulfide were also performed in order to make corrections due to substrate losses not linked to the nitrification process.

2.3.2. Denitrifying activity

Batch experiments were undertaken in serologic bottles of 60 ml, containing 48 ml of mineral medium, using the same mineral medium for the nitrifying activity cultures. Granular sludge employed in these experiments was collected from the CSTR during the steady state. The initial sludge concentration was of 3.0 g VSS/L. The experimental bottles were spiked with 100 mg S^0/L as electron donor, and 60 mg $\text{NO}_3^--\text{N}/\text{L}$. The pH value was controlled at 7.0 ± 0.2 in all batch assays, which was adjusted by a phosphate buffer (g/L): K_2HPO_4 (4.5), KH_2PO_4 (3.0). The bottles were flushed with helium gas for about 5–10 min and were subsequently put in a shaker at 200 rpm. Batch cultures assays were performed by duplicate at 25 °C. Biotic controls lacking nitrate or elemental sulfur were also performed in order to make corrections due to substrate losses not linked to the denitrification process.

2.3.3. Sequential nitrification–denitrification process

Batch experiments were performed in stirred tank reactors with working volume of 1.0 L. The experimental units were spiked with 60 mg $\text{NH}_4^+-\text{N}/\text{L}$, 100 mg 4-methylphenol-C/L and 100 mg S^{2-}/L , using the same mineral medium for the nitrifying activity experiments. The inoculum used was the granular sludge produced in the CSRT in steady state at initial concentration of 3.0 g VSS/L. Bioassays were carried out by duplicate at 25 ± 2 °C, 200 rpm and a pH value of 7.5 ± 0.5 . The bioreactor was aerated as described in the experimental set-up. The reactors were continuously aerated by 9 h. After this time, aeration flow was stopped and the residual DO from either the headspace or liquid medium was removed by a helium gas flow for 5–10 min in order to establish anoxic conditions.

The metabolic and kinetic aspects were evaluated in terms of consumption efficiencies [E ; (g of N, S or C consumed/g of N, S or C fed) \times 100%], production yields [Y ; g of N, S or C produced/g of N, S or C consumed], specific consumption rates (q_s ; mg of N, S or C consumed/g VSS h) and specific production rates (q_p ; mg of N, S or C produced/g VSS h). The average specific rates were calculated from the kinetic data, using the integrated Gompertz model (González-Blanco et al., 2012). The experimental data were statistically analyzed by Tukey–Kramer test at the $\alpha = 0.05$ level, using a SPSS predictive analytics software.

2.4. Analytical methods

Nitrate, nitrite, thiosulfate and sulfate were determined by capillary electrophoresis (Beckman Coulter, proteomeLab PA 800). The buffer to measure the last ions was prepared with 2.8 mL of Na_2CrO_4 (32.5 mM), H_2SO_4 (0.17 mM) and a commercial solution CIA Pak OFM anion-BT (Waters) plus 7.2 mL of deionizer water. A microcapillar of melted silica (60 cm long and 75 μm internal diameter) was used. The absorbance was measured in the ultraviolet region using a mercury lamp at 254 nm and 25 °C. Ammonium was determined by selective ammonium electrode (pHoenix electrode Co. Mod. NH331501). The molecular nitrogen (N_2) and ni-

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