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Simultaneous nitrification and *p*-cresol oxidation in a nitrifying sequencing batch reactor

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ABSTRACT

The tolerance, kinetic behavior and oxidizing ability of a nitrifying sludge exposed to different initial concentrations of *p*-cresol (25–150 mg/l) were evaluated in a sequencing batch reactor (SBR) fed with 200 mg NH_4^+-N/l d. The nitrifying SBR operated up to 300 mg/l d of *p*-cresol, achieving simultaneously the complete ammonium oxidation to nitrate and the total consumption of *p*-cresol and its transitory intermediates from the culture. *p*-Cresol induced a significant decrease in the values for specific rates of ammonium consumption, showing that the ammonium oxidation pathway was mainly inhibited. After 7 months of operation in SBR, the specific rates of NH_4^+-N oxidation, NO_3^--N formation, and total organic carbon consumption were 0.6 g NH_4^+-N/g microbial protein-N h, 0.3 g NO_3^--N/g microbial protein-N h, and 0.24 g total organic carbon/g microbial protein h, respectively. The microbial growth rate was always low (maximum value of 12.2 ± 0.4 mg protein-N/l d) and settleability of the sludge was good with sludge volume index values lower than 21 ml/g. The oxidation of *p*-cresol and its intermediates was carried out faster throughout the cycles and nitrification inhibition decreased with the number of cycles.

1. Introduction

Biological nitrification, the sequential oxidation of ammonia (NH_3) via nitrite (NO_2^-) to nitrate (NO_3^-), is frequently the initial step in the removal of ammonia from wastewaters. Nitrate is subsequently reduced to molecular nitrogen (N_2) by denitrification process. The inhibitory effect of organic compounds on biological nitrification is well documented and it is a known fact that the stability of nitrification systems in wastewater treatment plants is challenged by incoming toxic or inhibitory chemicals (Schweighofer et al., 1996; Winther-Nielsen and la Cour Jansen, 1996; McCarty, 1999). Phenolic compounds and ammonia can be found at high concentrations in petrochemical and industrial effluents (Mueller et al., 1985; Fang et al., 1993; Olmos et al., 2004). While there has been significant research on the biodegradation of phenolic compounds under denitrifying conditions using the ability of heterotrophic denitrifiers to consume organic compounds as carbon and energy source, less attention has been paid to the inhibitory effect of cresols on nitrification processes and the tolerance and oxidizing ability of nitrifying sludge exposed to cresols. Stafford (1974) observed that nitrifying bacteria in activated sludge were affected by phenols and reported the following concentrations (mg/l) that would give 75% reduction of ammonia oxidation: 5.6 for phenol and *p*-cresol, 4.4 for *o*-cresol, and 4.0 for *m*-cresol. Dyreborg and Arvin (1995) estimated concentrations for a 100% inhibition of nitrification to 1.3 mg/l for *o*-cresol and 3.5 mg/l for phenol.

Earlier batch studies have shown that a nitrifying consortium produced in steady-state nitrification can simultaneously oxidize ammonia to nitrate and aromatic compounds (*p*-cresol, benzene, toluene, and *m*-xylene) to intermediates,

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such as volatile fatty acids (Texier and Gomez, 2002; Zepeda et al., 2003, 2004, 2006). These results suggested that nitrification as the initial step in the removal of ammonia from wastewaters might be used to oxidize simultaneously ammonia and recalcitrant aromatic compounds, allowing the production of organic matter easier to consume by denitrification pathway. Thus, it is of interest to carry out investigations in dynamic systems, such as sequencing batch reactors (SBR), to evaluate the experimental conditions allowing to maintain high ammonia consumption efficiency and nitrifying yield in presence of phenolic compounds.

The aim of this study was to evaluate the tolerance of a nitrifying SBR to *p*-cresol and the ability of the sludge to consume this phenolic compound. The overall performance of the nitrifying SBR was evaluated when *p*-cresol was added or not into the microbial culture by measuring NH_4^+-N consumption efficiency and NO_2^--N and NO_3^--N formation. Kinetic studies were also carried out at different initial concentrations of *p*-cresol (0–150 mg/l) to evaluate the evolution of specific rates of nitrification at different operation cycles. Microbial growth and settleability of the sludge were also determined in the SBR system.

2. Materials and methods

2.1. Inoculum and culture medium composition

The sludge used for inoculating the SBR was obtained from a 6 l continuous reactor which was kept operating for over two years under steady-state nitrification. Steady-state operating conditions and performance of the continuous reactor were previously described by Texier and Gomez (2002). The initial microbial protein concentration in the SBR culture was 250 mg/l.

The medium used for the SBR culture was a mixture of two media (A and B). The chemical composition of medium A was (g/l): $(NH_4)_2SO_4$ (1.45), KH_2PO_4 (1.73), $MgSO_4$ (0.74), NaCl (1.23) and NH_4Cl (1.17). Medium B consisted (g/l) of $CaCl_2$ 0.11 and $NaHCO_3$ 2.88. Media A and B were pumped at 20 and 70 ml/min, respectively. $FeSO_4 \cdot 7H_2O$ was added daily into the reactor at 0.02 g/l. The following ratios were maintained: C/N of 2.3 (without addition of *p*-cresol), N/S of 1.2 and N/P of 1.6. The initial NH_4^+-N concentration in the SBR was 0.1 g/l.

2.2. Nitrifying sequencing batch reactor

A laboratory-scale SBR with a working volume of 5 l was operated with cycles of 12 h. Each cycle consisted of the four following periods: fill (40 min, corresponding to 25 min static and 15 min mixed and aerated feeding); aerated react (10 h including 15 min during the filling phase); settle (40 min) and draw (55 min). All aeration, mixing, and discharge functions were controlled electronically with programmed timers. Air was pumped into the system at a constant flow of two volumes of air per volume of liquid per minute and stirred constantly at 300 rpm. Temperature was measured and controlled automatically at 30 °C. The initial pH value was 7.6. At the end of the filling period, *p*-cresol was added to the reactor at 45 ml/min for 8 min and at initial concentrations ranging from 25 to 150 mg/l according to the following schedule (Fig. 1). For initial concentrations of 25, 50, and 75 mg/l, *p*-cresol was added into the nitrifying SBR culture as a shock load in so far as the sludge previously exposed to *p*-cresol was washed with a solution of NaCl (9 g/l) and newly used in nitrifying SBR culture without *p*-cresol during nearly 60 consecutive cycles before to add again *p*-cresol. For initial concentrations of 100 and 150 mg/l, the SBR was continuously fed with *p*-cresol without break periods. Before adding *p*-cresol at a new initial concentration, biomass was purged from the reactor to obtain a microbial protein concentration of 506 ± 25 mg/l (1.1 ± 0.1 g volatile suspended solids/l).

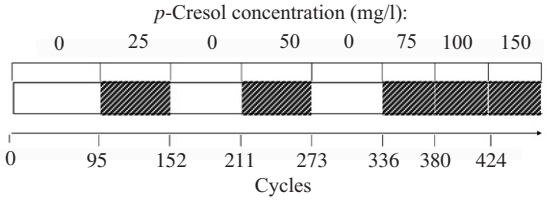

Fig. 1 – Schedule for *p*-cresol addition in the nitrifying SBR.

At the beginning of each cycle, 3.6 l of culture medium and 0.36 l of *p*-cresol solution (0.35–2.07 g/l) were added to 1 l of settled sludge from the previous cycle, and at the end of the settling period 4 l of effluent was withdrawn from the reactor. The hydraulic retention time was 15 h. Samples were withdrawn daily at the end of SBR culture and at different times over 12 h cycles for conducting kinetic studies. All samples were filtered (0.45 μm) and analyzed for NH_4^+-N, NO_2^--N, NO_3^--N, total organic carbon (TOC), and *p*-cresol. NH_4^+-N consumption efficiency (E-NH_4^+, (g NH_4^+-N consumed/g NH_4^+-N fed) × 100), nitrifying yield (Y-NO_3^-, g NO_3^--N/g NH_4^+-N consumed) and specific rates for nitrification were determined to evaluate the physiological response of the nitrifying sludge to the presence of *p*-cresol. The volumetric rates of NH_4^+-N consumption and NO_3^--N production were estimated from the kinetic curves and expressed as mg N/l h. With the microbial protein concentration known and assuming that 16% of total microbial protein is nitrogen, the specific rates of NH_4^+-N consumption and NO_3^--N production were calculated and expressed as g N/g microbial protein-N h. To establish the carbon mass balance, it was assumed that 65% of the microbial biomass is protein and 50% is carbon. Culture samples were analyzed daily for total microbial protein and sludge volume index (SVI) as indicated below.

2.3. Analytical methods

p-Cresol was measured by HPLC (Thermo Separation Products) and TOC by using a TOC-meter (Shimadzu TOC-5000A). Ammonium nitrogen (NH_4^+-N) was analyzed by a selective electrode (Phoenix Electrode Co., USA) and nitrite and nitrate by capillary electrophoresis (Waters Capillary Ion Analyzer). Lowry's method was employed to measure microbial protein concentration. To prevent interference of *p*-cresol on measurement of protein by the Lowry's method, culture samples (5 ml) were centrifuged (9500 *g*, 10 min) and the pellet was