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p-Cresol mineralization and bacterial population dynamics in a nitrifying sequential batch reactor

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ABSTRACT

The ability of a nitrifying sludge to oxidize *p*-cresol was evaluated in a sequential batch reactor (SBR). *p*-Cresol was first transformed to *p*-hydroxybenzaldehyde and *p*-hydroxybenzoate, which were later mineralized. The specific rates of *p*-cresol consumption increased throughout the cycles. The bacterial population dynamics were monitored by using denaturing gradient gel electrophoresis (DGGE) and sequencing of DGGE fragments. The ability of the sludge to consume *p*-cresol and intermediates might be related to the presence of species such as *Variovorax paradoxus* and *Thauera mechernichensis*. *p*-Cresol (25 to 200 mg C/L) did not affect the nitrifying SBR performance (ammonium consumption efficiency and nitrate production yield were close to 100% and 1, respectively). This may be related to the high stability observed in the nitrifying communities. It was shown that a nitrifying SBR may be a good alternative to eliminate simultaneously ammonium and *p*-cresol, maintaining stable the respiratory process as the bacterial community.

Introduction

The use of sequential batch reactors (SBRs) in the biological treatment of wastewaters has been widely extended from lab-scale studies to wastewater treatment plants. The major advantages of SBR systems over traditional continuous flow systems are the design simplicity, the absence of a separate clarifier, the operating flexibility and the low cost for operation. The SBR technology can be used for eliminating ammonium nitrogen from the water through the coupled respiratory processes of nitrification and denitrification (Puig et al., 2004). Nitrification is an aerobic respiratory process carried out by two groups of gram negative lithoautotrophic bacteria that are phylogenetically unrelated, where ammonium is oxidized to nitrate. Nitrate is subsequently reduced to molecular nitrogen by denitrification. Nitrification is the succession of the oxidation of ammonium to nitrite by the ammonia-oxidizing bacteria (AOB) and the subsequent oxidation of nitrite to nitrate by the nitrite-oxidizing bacteria (NOB).

It is well known that a wide variety of organic compounds could be toxic or provoke inhibitory effects on nitrification (McCarty, 1999). Inhibitory effects of phenolic compounds on the nitrification process have been previously reported (Kim et al., 2008). However, knowledge on the inhibitory effects of cresols on the nitrification respiratory process is still limited (Silva et al., 2009). Ammonium and cresols can be found in various industrial effluents (petrochemical, pharmaceutical, chemical, coke wastewater, synthetic resin wastes) (Olmos et al., 2004; Kim et al., 2008). Phenolic compounds including cresols are toxic and can cause persistence and bioaccumulation effects in animal and vegetable

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organisms. Many of these organic chemicals might be also carcinogenic, mutagenic and teratogenic (Davì and Gnudi, 1999).

Several studies have reported the ability of nitrifying consortia to oxidize various organic compounds (Silva et al., 2011). These compounds include recalcitrant aromatic compounds such as benzene, toluene, *m*-xylene and phenol (Amor et al., 2005; Zepeda et al., 2006). Previous results showed that nitrifying SBR might be a good alternative to eliminate simultaneously ammonium and inhibitory aromatic compounds from wastewaters (Texier and Gomez, 2007). Nevertheless, further work is required to kinetically characterize the ability of the nitrifying SBR culture for oxidizing inhibitory compounds such as *p*-cresol throughout the cycles and evaluate the accumulation of intermediates.

Nowadays, attempts are made to relate the physiological response of microbial consortia with their structure and population dynamics (Wittebolle et al., 2008). Several molecular techniques have been developed to evaluate microbial communities in natural samples and bioreactors (Talbot et al., 2008). Denaturing gradient gel electrophoresis (DGGE) is a powerful tool to determine the genetic diversity of microbial communities and to identify the phylogenetic affiliation of community members in bioreactors operated under different experimental conditions (Xia et al., 2005; Martínez-Hernández et al., 2009). Based on a DGGE analysis and sequencing of the microbial community structure of cresol-degrading aerobic granules, Lee et al. (2011) found 15 predominant bacterial species, including the genera *Bacillus*, *Acinetobacter*, *Corynebacterium*, and *Nocardioides*. Other bacteria, including *Pseudomonas*, *Arthrobacter*, *Alcaligenes*, and *Rhodococcus* metabolized *p*-cresol as carbon and energy source (Kang et al., 1998; Gallego et al., 2008; Wang et al., 2009; Ho et al., 2010; Lee et al., 2011).

Therefore, the aim of this study was to evaluate the ability of a nitrifying sludge to consume *p*-cresol and its intermediates as well as to monitor the population dynamics of the bacterial community throughout the operation cycles in a SBR system.

1. Materials and methods

1.1. Nitrifying sequential batch reactors

The sludge used for inoculating the SBRs was obtained from a continuous stirred tank reactor in steady-state nitrification. The system was operated continuously at 300 r/min, 25 ± 2°C, and pH of 8.0 ± 0.5. The dissolved oxygen concentration was maintained at 4.3 ± 0.1 mg/L. The hydraulic retention time was 1.8 days. The reactor was fed with a medium containing the following nutrients (g/L): $(NH_4)_2SO_4$ (1.18), NH_4Cl (0.94), KH_2PO_4 (1.40), $MgSO_4$ (0.60), NaCl (1.0), $NaHCO_3$ (9.33) and $CaCl_2$ (0.05). The loading rate of NH_4^+-N was 141 ± 6 mg/(L·day). Under steady state conditions, the ammonium consumption efficiency was close to 100% while nitrate was the main product from ammonium oxidation.

The initial concentration of volatile suspended solids (VSSs) in the SBRs was of 1 g/L. Two laboratory-scale SBRs (Model p100, Applikon, Dover, NJ, USA) were operated with cycles of 12 hr for more than 5 months. The 2 L SBR_A was fed with culture medium and used as control while the 1.7 L SBR_B was fed with different initial *p*-cresol concentrations. Each cycle consisted of the four following periods: fill (0.25 hr), reaction (11.25 hr), settle (0.25 hr) and draw (0.25 hr). All of the periods of the SBRs were controlled electronically by timers. The volumetric exchange ratio of liquid was 90% and the hydraulic retention time was 0.56 days. The medium used for the SBR cultures was the mixture of two media as nitrogen (A) and carbon (B) sources. The chemical composition of medium A was (g/L): $(NH_4)Cl$ 0.45, $(NH_4)_2SO_4$ 0.54, KH_2PO_4 0.65, $MgSO_4$ 0.5, and NaCl 0.5. Medium B consisted (g/L) of $NaHCO_3$ 4.5 and $CaCl_2$ 0.03. Media A and B were added at 60 and 40 mL/min, respectively. At the beginning of each cycle, the initial concentration of NH_4^+-N was 100 mg/L, corresponding to 200 mg NH_4^+-N/(L·day). $FeSO_4·7H_2O$ was added to the reactors daily at 0.01 g/L. A *p*-cresol solution (536–4288 mg/L) was added to the SBR_B at 17 mL/min for 6 min to obtain initial *p*-cresol concentrations ranging from 25 to 200 mg *p*-cresol-C/L (50 to 400 mg *p*-cresol-C/(L·day)). A constant aeration and agitation of 225 r/min were maintained into the cultures to give a dissolved oxygen concentration of 5.3 ± 0.1 mg/L at the beginning of each cycle. Samples were withdrawn at different times over 12 hr cycles for conducting kinetic studies. Additionally, the possible adsorption of *p*-cresol onto the sludge was evaluated by sterile controls (the sludge was sterilized at 120°C for 20 min) in serological bottles according to the methodology described by Silva et al. (2009). Furthermore, abiotic batch cultures (in the absence of sludge) were conducted to discard the possible loss of *p*-cresol by volatilization or chemical reaction.

1.2. Analytical methods

Ammonium nitrogen was analyzed by a selective electrode (Ammonia gas sensing ISE, Cole-Parmer, Equipar, Mexico), total organic carbon (TOC) by using a TOC-meter (TOC-5000A, Shimadzu, Columbia, Maryland, USA), and VSSs were determined according to standard methods (APHA, 1998). Nitrite, nitrate, *p*-cresol, *p*-hydroxybenzaldehyde (pOHBD), and *p*-hydroxybenzoate (pOHBT) concentrations were determined by HPLC as previously described by Silva et al. (2009). Oxygen concentration was determined by a dissolved oxygen meter (HI 98186, Hanna, Romania) with a polarographic probe (HI76407/4F, Hanna, Romania). Analytical methods had a variation coefficient of less than 10%.

1.3. Culture evaluation

The ammonium consumption efficiency (E_{NH_4} was expressed as (g NH_4^+-N consumed/g NH_4^+-N initial) × 100 and nitrate production yield (Y_{NO_3}) as g NO_3^--N/g NH_4^+-N consumed. To determine the biomass yield (Y_{BM}, mg biomass-C produced/mg consumed-C), it was considered that 50% of the microbial biomass (VSS) is carbon. The Gompertz model was used to analyze the kinetic data of *p*-cresol consumption (Draper and Smith, 1981). In this model, the consumed *p*-cresol concentration (S_c) may be expressed as a function of the time according to the following Eq. (1):

$$S_c = \alpha \exp(-\beta \exp(-kt)) \quad (1)$$

where, $S_c = (S_0 - S)$, S_0 (mg/L) is the initial substrate concentration, S (mg/L) is the substrate concentration, α (mg *p*-cresol/L) is