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Simultaneous oxidation of ammonium and *p*-cresol linked to nitrite reduction by denitrifying sludge

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ABSTRACT

The metabolic capability of denitrifying sludge to oxidize ammonium and *p*-cresol was evaluated in batch cultures. Ammonium oxidation was studied in presence of nitrite and/or *p*-cresol by 55 h. At 50 mg/L NH_4^+-N and 76 mg/L NO_2^--N, the substrates were consumed at 100% and 95%, respectively, being N_2 the product. At 50 mg/L NH_4^+-N and 133 mg/L NO_2^--N, the consumption efficiencies decreased to 96% and 70%, respectively. The increase in nitrite concentration affected the ammonium oxidation rate. Nonetheless, the N_2 production rate did not change. In organotrophic denitrification, the *p*-cresol oxidation rate was slower than ammonium oxidation. In litho-organotrophic cultures, the *p*-cresol and ammonium oxidation rates were affected at 133 mg/L NO_2^--N. Nonetheless, at 76 mg/L NO_2^--N the denitrifying sludge oxidized ammonium and *p*-cresol, but at different rate. Finally, this is the first work reporting the simultaneous oxidation of ammonium and *p*-cresol with the production of N_2 from denitrifying sludge.

1. Introduction

The major pollutants in wastewaters industrials are nitrogen and carbon aromatic compounds, without excluding other pollutants. For example, the three isomeric forms of cresol are used individually or in mixtures as disinfectants, preservatives, and solvents or as intermediates in the production of antioxidants, fragrances, herbicides, insecticides, dyes, and explosives (Sanders et al., 2009). Specifically the phenolic compound, 4-methylphenol (*p*-cresol), is a natural product present in crude oil, foods, and is also detected in animal and human urine (Yan et al., 2005). The *p*-cresol exposition or ingestion can cause: (1) at high concentration, death (Monma-Ohtaki et al., 2002), (2) hepatotoxicity (Kamijo et al., 2003) and (3) the *p*-cresol in the human body is conjugated, with *p*-cresylsulphate as the main metabolite, the last cause toxic effects such as uremia (Schepers et al., 2007). Hence, it is of utmost importance to diminish the phenolic compound level in industrial effluents to tolerant limits prior to being released into the environment (Banerjee and Ghoshal, 2010). On the other hand, nitrogen compounds are usually found as ammonium, nitrite and nitrate. Ecological and toxicological effects caused by these compounds can give rise to major environmental problems to name a few: water acidification, can deplete dissolved oxygen levels in receiving waters, eutrophication and can reach toxic levels for human beings and affect the suitability of wastewater for reuse (Wolfe and Patz, 2002; Smith, 2003; Camargo and Alonso, 2006).

Literature frequently mentions the nitrite inhibitory effect towards bacteria and aquatic life. Usually the presence of nitrite is undesired in wastewater treatment. However, some recent biological processes use mainly nitrite as an intermediate (Philips et al., 2002) as the anaerobic ammonium oxidation (Anammox), single reactor high activity ammonia removal over nitrite (Sharon) and oxygen limited autotrophic nitrification denitrification (Oland) processes (Van de Graaf et al., 1996; Jetten et al., 1998; Hellinga et al., 1998; Kuai and Verstraete, 1998).

Nitrite is an intermediate in different oxidative or reductive biochemical pathways that occur in water or soil: nitrification, denitrification, dissimilatory nitrate reduction to ammonium and Anammox processes (Fig. 1). Nitrite nitrogen has an oxidation state of +3, which allows it acting as donor or electron acceptor. Nonetheless, the thermodynamics of biological process which involves the nitrite could change in function of energy source, as can be seen in next equations:

$$NH_4^+ + NO_2^- \rightarrow N_2 + H_2O \qquad \Delta G^{\circ\prime} = -427.2\text{kJ/mol} \tag{1}$$

$$C_7H_8O + 11.33NO_2^- + 4.33H^+ \rightarrow 7HCO_3^- + 5.66N_2 + 2.66H_2O$$
$$\Delta G^{\circ\prime} = -4413.8\text{kJ/mol} \tag{2}$$

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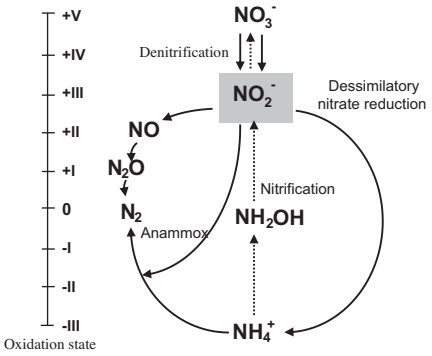

Fig. 1. Oxide-reduction state of nitrogen cycle (modified of Codispoti et al., 2001).

$$CH_3COO^- + 2.66NO_2^- + 1.66H^+ \rightarrow 2HCO_3^- + 1.33N_2 + 1.33H_2O$$
$$\Delta G^{\circ\prime} = -1033.95 kJ/mol \quad (3)$$

According to these reactions, using a chemical structure complex, like *p*-cresol, the reaction is more exergonic in regarding to ammonium or acetate. For instance, the low $\Delta G^{\circ\prime}$ value for the ammonium oxidation with nitrite (Eq. 1), it is possible to predict that the ATP production will be low. Thus, the growth of the ammonium oxidizing bacteria will be also very low, because cellular biosynthesis is limited by the energy availability.

On the other hand, under anoxic conditions, Anammox bacteria oxidize ammonium to molecular nitrogen whereas nitrite is the electron acceptor and carbon dioxide is used for growth (Molinuevo et al., 2009). The metabolic versatility of Anammox bacteria has been showed, since this microflora can also oxidize organic matter like acetic and propionic acid and this metabolic versatility might improve the Anammox process for energy generation (Kartal et al., 2007, 2010). Nonetheless, high concentration of organic matter can negatively affect the Anammox process (Dapena-Mora et al., 2007). Many of the ammonium containing wastewaters also contained organic matter, thus utilization of autotrophic Anammox process would not be suitable in such cases. There are authors that suggest that the coexistence of Anammox and denitrification might allow the simultaneous removal of ammonium in presence of simple organic matter (Kartal et al., 2007). Organotrophic denitrification is able to use a wide variety of organic compounds, such as toluene and phenolic compounds as carbon and energy source (Beristain-Cardoso et al., 2009a). Nitrite is an unusual compound in wastewater, but it can be coming from nitrate partial reduction due to the presence of phenolic compounds, like *p*-cresol or phenol (Meza-Escalante et al., 2008; Beristain-Cardoso et al., 2009b). The transitory nitrite formation from organotrophic denitrification might be a good strategy for ammonium oxidation. There are information in the literature that the denitrifying process can simultaneously remove ammonium and acetate or ammonium, acetate and sulfide in a single reactor without previous adaptation to Anammox conditions (Cervantes et al., 1999, 2001; Beristain-Cardoso et al., 2011).

It is important to note that there is no work reporting the simultaneous oxidation of ammonium and phenolic compounds from denitrifying sludge. Nowadays, the oxidation of ammonium and the mineralization of phenolic compounds require different types of reactors connected in series. The use of one only bioreactor for wastewater treatment will be a feasible process. The purpose of this research was to evaluate the metabolic capability of denitrifying sludge for ammonium oxidation with nitrite either in presence or absence of *p*-cresol. This research might be crucial because the real wastewaters are highly heterogeneous and several of them have high phenolic compound concentrations.

2. Methods

2.1. Inoculum and culture medium composition

The denitrifying sludge used for the batch experiments was produced in a 5.0 L continuous stirrer tank reactor (CSTR) operated at 30 ± 0.2 °C, pH 9.3 ± 0.2 with a hydraulic retention time of 3.2 d. The basal mineral medium was composed of (g/L): K_2HPO_4 (4.5), KH_2PO_4 (3.0), and trace elements solution supplied at 1.5 ml/L. The trace element solution contained (g/L): EDTA (5), $CuSO_4 \cdot 5H_2O$ (1.57), $CaCl_2 \cdot 2H_2O$ (5.54), $MnCl_2$ (5.0), $(NH4)_6Mo_7O_{24} \cdot 4H_2O$ (1.1), $FeCl_3$ (05.0), $CoCl_2 \cdot 6H_2O$ (1.6), $MgCl_2$ (5.0). The CSTR was fed with the following loading rates (mg/L d): 128 of NO_3^--N, 258 of acetate-C and 22 of NH_4^+-N. In denitrifying steady state the consumption efficiencies for nitrate, acetate and ammonium were 100%, 76% and 89%, respectively, being the main products N_2 and

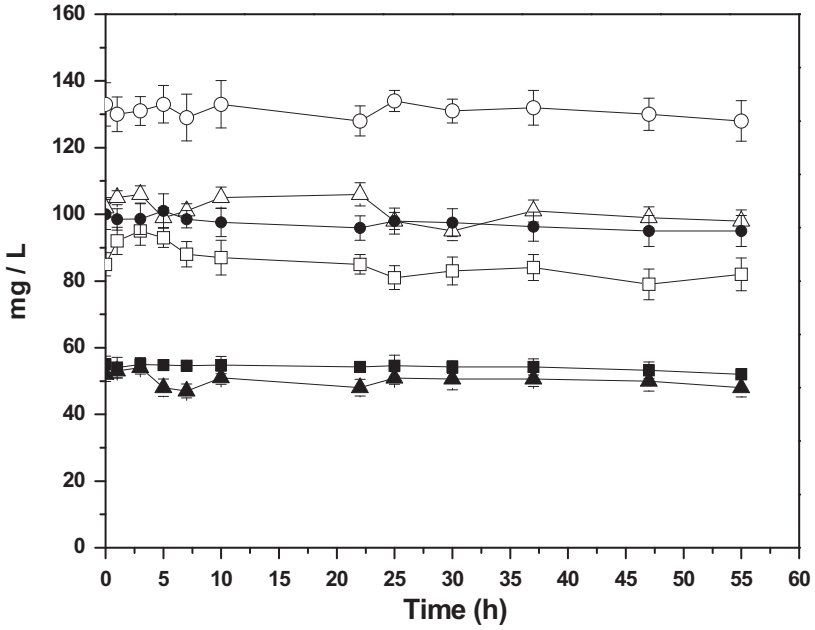

Fig. 2. Control assays. Abiotic: (○) NO_2^--N, (Δ) NH_4^+-N, (□) *p*-cresol-C. Biotic: (●) NO_2^--N, (▲) NH_4^+-N, (■) *p*-cresol-C.