ELSEVIER

Water Research 39 (2005) 2915–2920

WATER RESEARCH

www.elsevier.com/locate/watres

# Phenol biodegradation and its effect on the nitrification process

L. Amor, M. Eiroa, C. Kennes, M.C. Veiga*

*Faculty of Sciences, Chemical Engineering Laboratory, University of A Coruña, Alejandro de la Sota nº 1, 15008-A Coruña, Spain*

Received 17 January 2005; received in revised form 18 May 2005; accepted 18 May 2005
Available online 5 July 2005

**Abstract**

Phenol biodegradation under aerobic conditions and its effect on the nitrification process were studied, first in batch assays and then in an activated sludge reactor. In batch assays, phenol was completely biodegraded at concentrations ranging from 100 to 2500 mg $l^{-1}$. Phenol was inhibitory to the nitrification process, showing more inhibition at higher initial phenol concentrations. At initial phenol concentrations above 1000 mg $l^{-1}$, the level of nitrification decreased. In the activated sludge reactor, the applied ammonium loading rate was maintained at 140 mg $N–NH_4^+$ $l^{-1}$ $d^{-1}$ (350 mg $N–NH_4^+$ $l^{-1}$) during the operation time. However, the applied organic loading rate was increased stepwise from 30 to 2700 mg COD $l^{-1}$ $d^{-1}$ by increasing the phenol concentration from 35 up to 2800 mg $l^{-1}$. High phenol removal efficiencies, above 99.9%, were maintained at all the applied organic loading rates. Ammonium removal was also very high during the operation period, around 99.8%, indicating that there was no inhibition of nitrification by phenol.


*Keywords:* Phenol; Ammonium; Aerobic biodegradation; Nitrification; Toxicity

## 1. Introduction

Phenol is a toxic compound present in wastewaters from many different industries, such as petrochemical industries, chemical industries and resin producing industries. In some cases nitrogen may be present as well. Therefore, the biological treatment of these wastewaters requires the simultaneous removal of phenol and nitrogen, which can be done in an activated sludge reactor in two successive steps. During the nitrification step, ammonium is oxidized to nitrate under aerobic conditions; and during the denitrification step, nitrate is reduced to molecular nitrogen in the presence of a carbon source under anoxic conditions. Nitrification is commonly the rate-limiting step of the overall nitrogen removal. In the presence of toxic compounds as phenol, even at low concentrations, the nitrification process may be inhibited.

Phenol removal has been the subject of numerous investigations. There are several references about phenol biodegradation in both anoxic (Fang and Zhou, 1997; Blaszczyk et al., 1998; Sarfaraz et al., 2004) and aerobic conditions (Buitrón et al., 1998; González et al., 2001; Yamagishi et al., 2001). Buitrón et al. (1998) studied the degradation of phenol by acclimated activated sludge and by isolated bacteria. Activated sludge was acclimated for 70 days to phenol and chlorophenols and then the microorganisms responsible for degradation were isolated. The acclimated sludge degradation rates were higher than those of pure strains. The specific phenol uptake rate for acclimated activated sludge was 407 mg $g^{-1}$ VSS $d^{-1}$ while it was 54 mg $g^{-1}$ VSS $d^{-1}$ for

*Corresponding author. Tel.: 0034 981 167000; fax: 0034 981 167065.
*E-mail address:* veiga@udc.es (M.C. Veiga).

0043-1354/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.watres.2005.05.019

the more active pure strain. González et al. (2001) worked with two different aerobic systems: a stirred tank and a fluidized bed reactor, in order to evaluate phenol biodegradation by a pure culture of *Pseudomonas putida*. Both bioreactors showed high phenol degradation efficiencies, higher than 90%, for phenol loading rates up to 4000 mg $l^{-1}$ $d^{-1}$. Yamagishi et al. (2001) studied the simultaneous removal of phenol and ammonium by activated sludge biomass which had been acclimated with phenol for over 15 years. Phenol was completely removed, and ammonium was simultaneously nitrified to nitrate. The nitrification rate reached 200 mg N–$NH_4^+$ $l^{-1}$ $d^{-1}$ when phenol was removed at a rate up to 714 mg COD $l^{-1}$ $d^{-1}$.

In wastewaters from resin producing factories the organic matter is present mainly as formaldehyde but odd effluents with high concentrations of phenol are also generated when the manufacturing process is changed. Thus, it is necessary to study the treatment of these wastewaters with high levels of phenol and ammonium. The effect of phenol on nitrogen removal at high phenol loading rates and using unacclimated biomass has not been investigated deeply. The goal of this work was to analyze phenol biodegradation under aerobic conditions and its effect on the nitrification process. Therefore, aerobic assays were undertaken first in batch and then in an activated sludge reactor.

## 2. Materials and methods

### 2.1. Aerobic batch assays

Duplicate experiments were undertaken under aerobic conditions in 500 ml screw-capped vials containing 100 ml aqueous medium. The vials were inoculated with 3.5 g VSS $l^{-1}$ using sludge obtained from the aerobic chamber of the industrial wastewater treatment plant of a resin producing factory. The medium was supplemented with 10 ml nutrients solution and 0.05 ml trace elements solution. The composition of these solutions was mentioned elsewhere (Eiroa et al., 2004). The initial pH was adjusted to 7.9 using $NaHCO_3$ buffer.

Finally, phenol (100–2500 mg $l^{-1}$) and 250 mg N–$NH_4^+$ $l^{-1}$ were added in order to study phenol biodegradation and the nitrification process. Phenol, nitrite, nitrate and ammonium concentrations were measured at regular time intervals. Assays were performed in a thermostatic chamber at 20 °C and with constant shaking at 200 rpm.

### 2.2. Activated sludge reactor

A lab-scale activated sludge reactor consisting of an aeration basin coupled to an external settler with a useful volume of 1.8 l was used. The scheme of the continuous experimental unit was shown elsewhere (Eiroa et al., 2005a). In this unit, water to be treated was aerated and mixed with the sludge in the aeration basin. The treated water was separated from the sludge in the settler and the sludge was recycled in a temporized fashion to the aeration basin. Diffusers, located at the bottom of the aeration basin, supplied air of an air pump and maintained complete mixing. The reactor was initially inoculated with sludge obtained from the aerobic chamber of the full-scale wastewater treatment plant of a resin producing industry.

A synthetic wastewater containing nutrients (350 mg N–$NH_4^+$ $l^{-1}$), trace elements (0.05 ml $l^{-1}$), $NaHCO_3$ (4800 mg $l^{-1}$) and phenol was continually fed to the unit by a peristaltic pump. The unit was operated at a hydraulic retention time of 2.5 days. The applied ammonium loading rate was maintained constant during all the operation time (140 mg N–$NH_4^+$ $l^{-1}$ $d^{-1}$). However, the applied organic loading rate was increased stepwise (30–2700 mg COD $l^{-1}$ $d^{-1}$) by increasing the phenol concentration (35–2800 mg $l^{-1}$). Each organic loading rate was maintained in the system until reaching steady-state conditions. Phenol, nitrite, nitrate, ammonium, COD, VSS, dissolved oxygen and pH were measured on a regular basis.

### 2.3. Analytical methods

All samples were filtered through a 0.45 μm nylon membrane before analysis. Phenol was determined using a Hewlett-Packard 1100 liquid chromatograph equipped with a C-18 ODS column (25 cm × 4 mm ID) and a UV diode-array detector. The mobile phase was methanol:-water (60:40) and detection was performed at 280 nm. Nitrite and nitrate were analyzed by capillary electrophoresis using a Hewlett-Packard $^{3D}$CE system with a microcapillary tube of fused silica (40 cm × 50 μm ID). Sodium phosphate solution was employed as the electrolyte. UV detection was undertaken at a wavelength of 214 nm and 450 nm as reference. Ammonium, dissolved oxygen, COD, VSS and pH were evaluated according to *Standard Methods* (APHA, 1998). Ammonium and dissolved oxygen were determined using selective electrodes. COD was analyzed by the closed reflux colorimetric method while VSS were analyzed by the gravimetric method.

## 3. Results and discussion

### 3.1. Aerobic batch assays

#### 3.1.1. Nitrification in the presence of phenol

In the batch assays the pH was between 7.3 and 7.9, which is close to the optimum range for nitrifying bacteria (Antoniou et al., 1990). The dissolved oxygen