



# Simultaneous nitrification and *p*-nitrophenol removal using aerobic granular biomass in a continuous airlift reactor



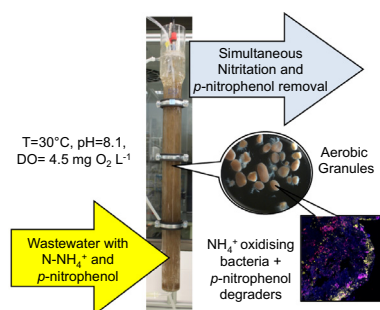
Zulkifly Jemaat<sup>1</sup>, María Eugenia Suárez-Ojeda<sup>\*</sup>, Julio Pérez, Julián Carrera

Department of Chemical Engineering, School of Engineering, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain

## HIGHLIGHTS

- Nitritation and *p*-nitrophenol removal in a continuous granular reactor is feasible.
- Bioaugmentation and a specific feeding strategy were the keys for success.
- Granules were composed of  $\text{NH}_4^+$  oxidising bacteria and *p*-nitrophenol degraders.
- Stable  $\text{NH}_4^+$  oxidation was obtained, with less than 0.3% of nitrate in the effluent.
- Complete *p*-nitrophenol degradation at non-limiting dissolved oxygen concentration.

## GRAPHICAL ABSTRACT



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## ABSTRACT

The chemical and petrochemical industries produce wastewaters containing ammonium and phenolic compounds. Biological treatment of these wastewaters could be problematic due to the possible inhibitory effects exerted by phenolic compounds. The feasibility of performing simultaneous nitrification and *p*-nitrophenol (PNP) biodegradation using a continuous aerobic granular reactor was evaluated. A nitrifying granular sludge was bioaugmented with a PNP-degrading floccular sludge, while PNP was progressively added to the feed containing a high ammonium concentration. Nitritation was sustained throughout the operational period with ca. 85% of ammonium oxidation and less than 0.3% of nitrate in the effluent. PNP biodegradation was unstable and the oxygen limiting condition was found to be the main explanation for this unsteadiness. An increase in dissolved oxygen concentration from 2.0 to 4.5  $\text{mg O}_2 \text{L}^{-1}$  significantly enhanced PNP removal, achieving total elimination. *Acinetobacter* genus and ammonia-oxidising bacteria were the predominant bacteria species in the granular biomass.

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## 1. Introduction

Currently, biological nitrogen removal via nitrite route is considered as the technology with the cheapest costs and the lowest environmental foot-print for treating ammonium-rich wastewa-

ters (Van Hulle et al., 2010). However, several industries such as the chemical, petrochemical, coke plant and refineries produce complex wastewaters containing both, ammonium and phenolic compounds (Morita et al., 2007; Milia et al., 2012). Very often, expensive physico-chemical treatments are advisable for the treatment of these industrial wastewaters, due to the potential inhibitory effect of phenolic compounds over microorganisms in the biological treatment (Oller et al., 2011), specifically the inhibition of the nitrification process by phenolic compounds (Hu et al., 2005; Liu et al., 2005; Morita et al., 2007).

<sup>\*</sup> Corresponding author. Tel.: +34 935868372; fax: +34 935812013.

E-mail address: [mariaeugenia.suarez@uab.es](mailto:mariaeugenia.suarez@uab.es) (M.E. Suárez-Ojeda).

<sup>1</sup> Present address: Department of Chemical Engineering, Faculty of Chemical and Natural Resources Engineering, Universiti Malaysia Pahang, Lebuhraya Tun Razak, 26300 Kuantan, Pahang, Malaysia.

A possible option for achieving this challenge with only biological treatments could be the technology of aerobic granules. The systems based on aerobic granular biomass are known to perform better in front of inhibitory or toxic compounds compared to activated sludge systems because granule architecture causes diffusion gradients contributing to protect sensitive bacteria (Adav et al., 2009; Maszenan et al., 2011). In this sense, some studies have shown the possibility of aerobic granules to perform simultaneous removal of ammonium and phenolic compounds (Liu et al., 2011; Suja et al., 2012).

Besides, the development and maturation of aerobic granules is performed, in general, in sequencing batch reactors (SBR) where high hydrodynamic stress is applied to form and to maintain the granules (Gao et al., 2011). Nevertheless, conventional batch operation is not advisable for treatment of phenolic compounds that usually exhibit inhibition by substrate (Martín-Hernández et al., 2009). To solve this problem, continuous operation could be a suitable option, since the concentration of the recalcitrant compounds in the reactor is expected to be low due to the high removal efficiency, reducing their toxic effect in the reactor. This high removal efficiency could be attained from the beginning of the operation by performing a controlled enrichment of the specific degrading biomass, i.e. by feeding this kind of compounds progressively during the start-up (Martín-Hernández et al., 2009).

In this sense, the development of a biological treatment dealing simultaneously with phenols and ammonium-rich wastewaters is of paramount importance.

In this study, *p*-nitrophenol (PNP) was selected as model compound of the nitrophenols family that are widely used, as reflected by their inclusion in the list of high volume production chemicals (OECD, 2008). So that, the simultaneous nitrification and PNP removal using a continuous aerobic-granular airlift-reactor is investigated and evaluated for the treatment of an industrial wastewater containing PNP and a high concentration of ammonium. Granular biomass was characterized in terms of size, density, sludge volumetric index, settling velocity and contents of exopolymeric substances (EPS). Fluorescence in situ hybridization (FISH) coupled with confocal laser scanning microscopy (CLSM) was also performed for identification and quantification of the predominant bacteria species and to determine their spatial arrangement in the granules.

## 2. Methods

### 2.1. Experimental set-up and reactor conditions

A glass airlift reactor with a working volume of 2.6 L was used. The internal diameter of the down-comer was 62.5 mm. The riser had a height of 750 mm and an internal diameter of 42.5 mm, and it was at 8 mm from the bottom of the down-comer. Fig. S1 in supporting information depicts a schematic diagram of the experimental set-up. Compressed air was supplied through an air diffuser placed at the bottom of the reactor. The reactor was equipped with dissolved oxygen (DO) (Crison DO 6050) and pH probes (Crison pH 5333) that were connected to a data monitoring system (Crison Multimeter 44). The temperature in the reactor was maintained using a temperature controller coupled with a belt-type heating device (Horst, Germany). pH of the reactor was maintained by a regular addition of NaHCO<sub>3</sub> into the reactor. Feeding to the reactor was made with a membrane pump (ProMinent Gamma-L). Air flow-rate in the reactor was regulated by rotameter (Aalborg, USA) with a range of 125–500 mL min<sup>-1</sup>. Samples were regularly withdrawn from the effluent and filtered through 0.20 μm syringe filter driven unit from Milipore® provided with a high-density polyethylene housing and membrane of hydrophilic

Durapore® (PVDF) prior to analysis. The operational conditions in the reactor during the experimental period were: Temperature of 30 ± 1 °C and pH of 8.1 ± 0.4.

### 2.2. Wastewater composition

The airlift reactor was fed with synthetic wastewater containing 3.6 g L<sup>-1</sup> NH<sub>4</sub>Cl (950 ± 25 mg N-NH<sub>4</sub><sup>+</sup> L<sup>-1</sup>) and the following compounds and micronutrients (concentrations are expressed in mg L<sup>-1</sup>): CH<sub>3</sub>COONa, 48.0; glucose, 12.5; sucrose, 11.9; CaCl<sub>2</sub>·2H<sub>2</sub>O, 88.0; KH<sub>2</sub>PO<sub>4</sub>, 41.0; NaCl, 176.0; MgCl<sub>2</sub>·7H<sub>2</sub>O, 198.0; FeSO<sub>4</sub>·7H<sub>2</sub>O, 4.0; MnSO<sub>4</sub>·H<sub>2</sub>O, 3.0; ZnSO<sub>4</sub>·7H<sub>2</sub>O, 4.0; CuSO<sub>4</sub>·5H<sub>2</sub>O, 2.0; and H<sub>3</sub>BO<sub>3</sub>, 0.02; CO(NH<sub>2</sub>)<sub>2</sub>, 12.0 and yeast extract, 2.0. In addition, an increasing amount of PNP was added to the influent, for details see Section 2.4 and Fig. S2 in supporting information.

### 2.3. Inoculum and reactor operation before bioaugmentation

The airlift reactor was inoculated with 1 L of granular biomass from a granular sequencing batch reactor (GSBR) at pilot scale treating low-strength wastewater for simultaneous carbon, nitrogen and phosphorus removal (Isanta et al., 2012). Then, the reactor was operated in continuous using a synthetic high-strength ammonium wastewater (950 ± 25 mg N-NH<sub>4</sub><sup>+</sup> L<sup>-1</sup>) to obtain nitrification maintaining the DO/N-NH<sub>4</sub><sup>+</sup> concentration ratio below 0.02 mg O<sub>2</sub> mg<sup>-1</sup> N (Bartrolí et al., 2010). Prior to bioaugmentation, the reactor was performing partial nitrification, oxidising ca. 48% of ammonium to nitrite at 0.3 ± 0.1 g N L<sup>-1</sup> d<sup>-1</sup> of volumetric nitrogen loading rate (NLR<sub>v</sub>). The average effluent concentrations were: ammonium, 505 ± 40 mg N L<sup>-1</sup>; nitrite, 462 ± 40 mg N L<sup>-1</sup> and nitrate, 2 ± 1 mg N L<sup>-1</sup>. Just before bioaugmentation, the granular biomass characteristics were as follow: mean size (mm) 1.1 ± 0.7, settling velocity (m h<sup>-1</sup>) 66 ± 27, sludge volumetric index (SVI<sub>5</sub>) (mL g<sup>-1</sup> TSS) 8 ± 2, ratio SVI<sub>5</sub>/SVI<sub>30</sub> 1.0 and biomass density (g VSS L<sup>-1</sup><sub>particle</sub>) 370 ± 140.

### 2.4. Bioaugmentation and operational strategy

At day 0, the airlift reactor was bioaugmented with 500 mL (2 g VSS L<sup>-1</sup>) of a floccular sludge from a SBR performing stable PNP degradation (Martín-Hernández et al., 2009). The bioaugmented biomass was accounted to be 15% of the total biomass inside the airlift reactor. Martín-Hernández et al. (2012) suggested using ca. 5% w/w for a successful bioaugmentation and retention of specialised biomass in a SBR. Considering that the airlift reactor was operating in continuous and biomass wash out would be inevitable, 15% of bioaugmented biomass was added instead of the 5% used by Martín-Hernández et al. (2012). The bioaugmentation procedure was repeated twice, on days 7 and 14.

The microbial composition of the PNP-degrading floccular sludge was characterized through fluorescence in situ hybridization (FISH) coupled to confocal laser scanning microscope (CLSM) following the protocol developed by Suárez-Ojeda et al. (2011). The FISH-CLSM results allowed identification and quantification of *Arthrobacter* sp. (26 ± 2%) and genus *Acinetobacter* (31 ± 10%) as the PNP-degraders in the floccular sludge, whereas no hybridization was found for *Burkholderia* sp. and *Pseudomonas* spp.

The PNP concentration was progressively increased during the experimental period to minimise its potential toxic or inhibitory effect over the granular biomass. During the first 14 days, PNP concentration in the influent was of 5 mg PNP L<sup>-1</sup>, then, it was increased to 10 mg PNP L<sup>-1</sup> during the next 115 days, and finally it reached 15 mg PNP L<sup>-1</sup> for the last 111 days of the experiment (the reader is kindly referred to the Fig. S2 in supporting information for a graphical overview of the feeding strategy).

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