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# Long-term impact of salinity on the performance and microbial population of an aerobic granular reactor treating a high-strength aromatic wastewater

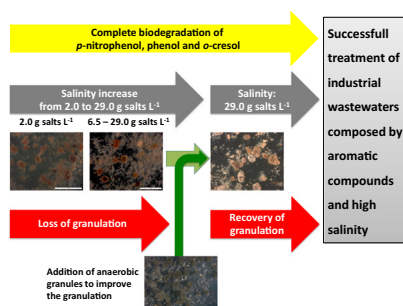
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## HIGHLIGHTS

- A salinity increase ( $29 \text{ g L}^{-1}$ ) caused the loss of aerobic granulation.
- Biodegradation of aromatics was successful in spite of the loss of granulation.
- New aerobic granules were formed after addition of anaerobic granules.
- The microbial population changed with the increase of the salts concentration.

## GRAPHICAL ABSTRACT



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## ABSTRACT

The effect of salinity over granular biomass treating a mixture of aromatic compounds (phenol, *o*-cresol and *p*-nitrophenol) was evaluated in a continuous airlift reactor. To mimic an industrial wastewater, increasing concentrations (from 2.0 to  $29.0 \text{ g salts L}^{-1}$ ) of a mixture of salts ( $\text{MgSO}_4$ , NaCl, KCl,  $\text{CaCl}_2$  and  $\text{NaHCO}_3$ ) were introduced in the influent. The gradual salinity increase led to a good acclimation of the biomass obtaining complete biodegradation of the aromatic compounds and no accumulation of metabolic intermediates. However, a deterioration of the morphology of aerobic granules with a complete loss of granulation after 125 days was produced at  $29.0 \text{ g salts L}^{-1}$ . At that moment, anaerobic granules were added to promote granulation and after 50 days new aerobic granules were formed. These new aerobic granules remained stable for more than 100 days at the highest salinity condition with 100% removal of the mixture of aromatic compounds.

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## 1. Introduction

Industrial wastewaters are composed by numerous pollutants constituting, therefore, a very complex matrix difficult to be treated. The effluents produced by agro-industries, paper-making, petrochemicals, pharmaceuticals, landfill leachates, chemical manufacturing, pesticides and herbicides industries can contain

significant amounts of inorganic dissolved salts (Lefebvre and Moletta, 2006). For instance, carbonates ( $1\text{--}16 \text{ g L}^{-1}$ ) (Olmos et al., 2004), chlorides ( $1\text{--}40 \text{ g L}^{-1}$ ) (Manekar et al., 2013; Olmos et al., 2004) and sulphates ( $10\text{--}25 \text{ g L}^{-1}$ ) (Manekar et al., 2013). Besides, most of these wastewaters also contain organic compounds, particularly toxic and recalcitrant pollutants, such as aromatic compounds: phenol ( $0.3\text{--}31 \text{ g L}^{-1}$ ) (Bai et al., 2010; Kim and Kim, 2003; Olmos et al., 2004) and *o*-cresol ( $0.03\text{--}6 \text{ g L}^{-1}$ ) (Kim and Kim, 2003; Olmos et al., 2004).

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Industrial wastewaters are often treated by physico-chemical processes. However, these technologies have serious drawbacks (Al-Khalid and El-Naas, 2011; Kim and Ihm, 2011): (i) high costs due to the required conditions of temperature and pressure and the use of some chemicals, (ii) incomplete degradation of the recalcitrant/toxic organic compounds and (iii) generation of other hazardous by-products (secondary pollutants). Biological processes can satisfactorily overcome some of the disadvantages of physico-chemical processes. Technologies based on flocculent biomass, such as activated sludge systems, are the main biological processes implemented at full-scale, however its practical application for treating complex industrial wastewaters is rather limited because activated sludge systems are widely known to be inhibited by aromatic compounds (Kim and Ihm, 2011) and also to be affected by high salinity. Inorganic salts can influence negatively over the structure and settling properties of microbial flocs (Lefebvre and Moletta, 2006). This fact is related to the density of salty water, which is higher than that of freshwater, thus creating greater resistance to decantation through higher buoyant forces (Lefebvre and Moletta, 2006).

To overcome the inhibition caused by organic compounds and the detrimental effect of salts, a promising alternative to activated sludge systems is the application of reactors with aerobic granular biomass (Gao et al., 2011). The application of aerobic granules allows retaining slow growing microorganisms and protects them from high concentrations of pollutants due to the diffusion gradient produced through the granule (Gao et al., 2011), favouring gradual adaptation to stressing conditions.

To the best of the authors knowledge, aerobic granules have been usually used for treating a single toxic/recalcitrant compound and a single salt (usually NaCl) (Li and Wang, 2008; Pronk et al., 2013; Taheri et al., 2012; Wan et al., 2014). Moreover, these studies have been carried out in sequencing batch reactors (SBRs). However, conventional batch operation is not the best option for the treatment of toxic/recalcitrant compounds, since the occurrence of high concentrations of these compounds at the beginning of a cycle can generate inhibitory conditions for the microorganisms. In this sense, continuous reactors would be a better option compared to SBRs in order to prevent these inhibitory effects since the bulk liquid concentration of the toxic/recalcitrant compound in a continuous reactor is expected to be low if the removal efficiency is high.

Therefore, taking into account this lack of practical information about the performance of aerobic granular reactors treating complex wastewaters containing mixtures of aromatic compounds and salts in continuous reactors, this study aims to evaluate the long-term effect of salinity on the biodegradation of a mixture of aromatic compounds by aerobic granules in a continuous airlift reactor.

## 2. Methods

### 2.1. Reactor

A glass airlift reactor with a working volume of 2.6 L was utilized in this study. 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. Compressed air was supplied through an air diffuser placed at the bottom of the reactor at an upflow velocity of 0.2–0.3 cm s<sup>-1</sup>. Airflow rate in the reactor was regulated manually between 150 to 250 mL min<sup>-1</sup> by a rotameter (Aalborg, USA) and it was enough to ensure an appropriate flow in the airlift reactor. The reactor was equipped with dissolved oxygen (DO) (Crison DO 6050), temperature (Crison Pt1000) and pH probes (Crison pH 5333) that were

connected to a data monitoring system (Crison Multimeter 44). DO was manually maintained between 4.0 and 6.0 mg O<sub>2</sub> L<sup>-1</sup> along the reactor performance. The DO concentrations were increased stepwise: 4.0, 4.5, 5.0 and 6.0 mg O<sub>2</sub> L<sup>-1</sup>, when the salinity increased: 2.0, 6.5, 13.0 and 29.0 g salts L<sup>-1</sup>, respectively. These concentrations were selected (i) to maintain a concentration over 4 mg O<sub>2</sub> L<sup>-1</sup> to achieve successful aerobic biodegradation of *p*-nitrophenol (Jemaat et al., 2013) and (ii) to avoid a decreased oxygen transfer to the liquid phase due to the decrease in the oxygen maximum solubility at high salinity conditions, which can lead to DO limitations. A Programmable Logic Controller (PLC) coupled to a Supervisory Control And Data Acquisition (SCADA) system regulated temperature, pH and feeding. pH was maintained at 8.0 ± 0.2 by a regular addition of NaHCO<sub>3</sub> whereas temperature in the reactor was maintained at 30 ± 0.5 °C using a temperature controller coupled with a belt-type heating device (Horst, Germany). Feeding to the reactor was made with a membrane pump (ProMinent Gamma/L).

### 2.2. Granular biomasses

Aerobic granular sludge from a continuous airlift reactor performing simultaneous biodegradation of *p*-nitrophenol, phenol and *o*-cresol was used as inoculum. This reactor exhibited complete biodegradation of the aromatic compounds and their metabolic intermediates at an organic loading rate of 0.61 g COD L<sup>-1</sup> d<sup>-1</sup> in presence of a concentration of salts of 2.0 g L<sup>-1</sup>. Stable granules were obtained throughout the long-term operation (more than 250 days). Some of the granular biomass characteristics were as follows: average granule size of 220 ± 20 µm, sludge volumetric index (SVI) at 30 min of 26 ± 1 mL g<sup>-1</sup> of Total Suspended Solids (TSS) and SVI<sub>30</sub>/SVI<sub>5</sub> ratio of 1.0. More information can be found in Ramos et al. (2015).

On day 125 of the reactor operation, 260 mL (10% of the working volume of the reactor) of anaerobic granular biomass was added to the reactor to promote granulation. The anaerobic granular biomass was obtained from a full-scale internal circulation (IC) reactor treating an industrial wastewater and their characteristics were SVI<sub>30</sub> of 8.0 ± 0.5 mL g<sup>-1</sup> TSS, SVI<sub>30</sub>/SVI<sub>5</sub> of 1.0, average granule size of 1100 ± 10 µm and 87% of biomass with a diameter higher than 0.2 mm. The wastewater treated by these anaerobic granules had a salinity of 8.0 g salts L<sup>-1</sup>.

### 2.3. Wastewater composition and operational conditions

The airlift reactor was continuously fed with synthetic wastewater. The organic carbon source was maintained constant along the experiment and it was composed by several aromatic compounds: phenol (563 ± 21 mg L<sup>-1</sup> equivalent to 1340 ± 50 mg COD L<sup>-1</sup>), *p*-nitrophenol (350 ± 17 mg L<sup>-1</sup> equivalent to 565 ± 30 mg COD L<sup>-1</sup>) and *o*-cresol (95 ± 6 mg L<sup>-1</sup> equivalent to 240 ± 10 mg COD L<sup>-1</sup>). Sucrose and glucose were added along the whole operational time as co-substrate in a *p*-nitrophenol:(glucose + sucrose) ratio of 0.4 (as COD). Therefore, the total concentration of organic matter in the influent was 2890 ± 240 mg COD L<sup>-1</sup>, where the aromatic compounds represented around 75% of the COD. The composition of the micronutrients in the synthetic wastewater was maintained constant along the whole experimental period and it was composed as follows (expressed as mg L<sup>-1</sup>): 88 of CaCl<sub>2</sub> × 2H<sub>2</sub>O; 106 of NH<sub>4</sub>Cl; 41.0 of KH<sub>2</sub>PO<sub>4</sub>; 176.0 of NaCl; 198.0 of MgCl<sub>2</sub> × 7H<sub>2</sub>O; 4.0 of FeSO<sub>4</sub> × 7H<sub>2</sub>O; 3.0 of MnSO<sub>4</sub> × H<sub>2</sub>O; 4.0 of ZnSO<sub>4</sub> × 7H<sub>2</sub>O; 2.0 of CuSO<sub>4</sub> × 5H<sub>2</sub>O; 0.02 of H<sub>3</sub>BO<sub>3</sub>; 12.0 of CO(NH<sub>2</sub>)<sub>2</sub> and 1.0 of yeast extract.

Along the reactor operation, the biomass was exposed to increasing salts concentrations, maintaining constant the concentrations of the organic carbon sources. The salinity in the

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