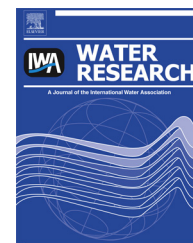


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# Partial nitrification and o-cresol removal with aerobic granular biomass in a continuous airlift reactor

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## ABSTRACT

Several chemical industries produce wastewaters containing both, ammonium and phenolic compounds. As an alternative to treat this kind of complex industrial wastewaters, this study presents the simultaneous partial nitrification and o-cresol biodegradation in a continuous airlift reactor using aerobic granular biomass. An aerobic granular sludge was developed in the airlift reactor for treating a high-strength ammonium wastewater containing  $950 \pm 25 \text{ mg N} - \text{NH}_4^+ \text{ L}^{-1}$ . Then, the airlift reactor was bioaugmented with a p-nitrophenol-degrading activated sludge and o-cresol was added progressively to the ammonium feed to achieve  $100 \text{ mg L}^{-1}$ . The results showed that stable partial nitrification and full biodegradation of o-cresol were simultaneously maintained obtaining a suitable effluent for a subsequent anammox reactor. Moreover, two o-cresol shock-load events with concentrations of 300 and  $1000 \text{ mg L}^{-1}$  were applied to assess the capabilities of the system. Despite these shock load events, the partial nitrification process was kept stable and o-cresol was totally biodegraded. Fluorescence in situ hybridization technique was used to identify the heterotrophic bacteria related to o-cresol biodegradation and the ammonia oxidising bacteria along the granules.

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## 1. Introduction

Several industrial processes such as petroleum refinement, coal tar processing, petrochemicals manufacturing, paints and resins production release wastewaters containing both, ammonium and phenolic compounds (Morita et al., 2007; Milia et al., 2012). In particular, o-cresol is one of the phenols most commonly found in these effluents (Veeresh et al., 2005). The presence of phenolic compounds in these industrial wastewaters could advise for (expensive) physico-chemical treatments due to the potential inhibitory or toxic effects

over a biological treatment (Oller et al., 2011). Nevertheless, there is no doubt that autotrophic biological nitrogen removal (BNR), i.e. partial nitrification plus anammox, could be regarded as the technology with the cheapest costs and the lowest environmental foot-print available nowadays for treating ammonium-rich wastewaters (Ahn, 2006). However, phenolics compounds are recognised as inhibitors of both, partial nitrification and anammox processes. On one hand, the inhibition of nitrifying microorganisms by phenolic compounds is well documented (Liu et al., 2005; Morita et al., 2007; Suárez-Ojeda et al., 2010) and several specific studies reported how

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nitrification was specifically inhibited by *o*-cresol (Dyreborg and Arvin, 1995; Radniecki et al., 2010). On the other hand, the inhibition of anammox microorganisms by phenolic compounds has also been reported; for example, by phenol (Yang and Jin, 2012) or by toluene (Martínez-Hernández et al., 2013). Therefore, the development of an autotrophic BNR process with a high removal capacity for both nitrogen and phenolic compounds from high-strength wastewaters will be a significant improvement in the current state-of-the-art. A possible technological option could be a two-sludge system composed by a first partial nitrification reactor followed by an anammox reactor. The partial nitrification reactor should guarantee an effluent suitable for the subsequent anammox stage, i.e. an effluent with a nitrite/ammonium ratio around one and without phenolic compounds.

Among the wide spectrum of reactors used in wastewater treatment, those based on granular biomass are considered a robust alternative for the treatment of wastewaters containing inhibitory or toxic organic compounds (Maszenan et al., 2011). The diffusion gradients existing in aerobic granules could contribute to reduce the inhibitory effect of these compounds protecting sensitive bacteria (Liu et al., 2005; Morita et al., 2007; Maszenan et al., 2011). The development of aerobic granules is commonly achieved through sequencing batch reactors (SBRs) by applying short settling times and high shear stress (Gao et al., 2011). However, conventional batch operation is not advisable for the treatment of recalcitrant compounds, and alternative strategies like distributed feeding along the SBR cycle have been proposed to minimize substrate inhibition (Martín-Hernández et al., 2009). Continuous reactor operation would avoid this drawback since the bulk liquid concentration of the recalcitrant

compound in the reactor is expected to be low if the removal efficiency is high, therefore, mitigating the toxic effects over the biomass.

In the present study, a continuous reactor with granular biomass performing nitrification of a high-strength ammonium wastewater was bioaugmented with activated sludge specialised in the degradation of phenolic compounds. The main aim was to develop granular sludge with a special architecture, in which an external layer of heterotrophs would degrade *o*-cresol and, at the same time, would protect ammonia-oxidising bacteria (AOB) against the potential inhibition or toxicity caused by this phenolic compound. The results will serve to assess the feasibility of a biological treatment for the simultaneous partial nitrification and *o*-cresol removal to potentially feed an anammox reactor for autotrophic BNR of complex industrial wastewaters.

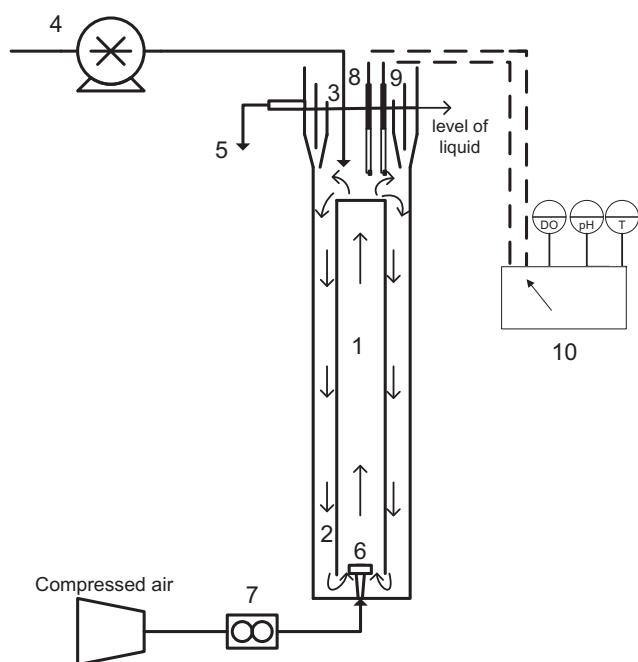
## 2. Materials and methods

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

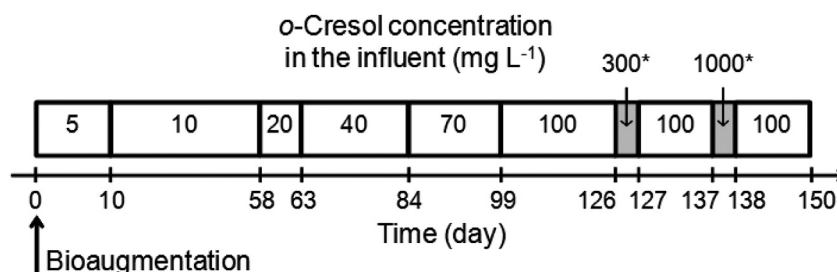
A glass airlift reactor with a working volume of 2.6 L was utilised in this study (Fig. 1). The internal diameter of the down-comer was 62.5 mm. The riser had a height of 750 mm and an internal diameter of 42.5 mm, and it was at 8 mm from the bottom of the down-comer. Compressed air was supplied through an air diffuser placed at the bottom of the reactor at an upflow velocity of  $0.4 \text{ cm s}^{-1}$ . Air flow rate in the reactor was regulated at  $250 \pm 50 \text{ mL min}^{-1}$  by 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) and pH probes (Crison pH 5333) that were connected to a data monitoring system (Crison Multimeter 44). DO was set at  $2.0 \pm 0.3 \text{ mg O}_2 \text{ L}^{-1}$  and pH was maintained at  $8.3 \pm 0.2$  by a regular addition of  $\text{NaHCO}_3$ . The temperature in the reactor was maintained at  $30 \pm 1^\circ \text{C}$  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). Samples were regularly withdrawn from the effluent and filtered through  $0.20 \mu\text{m}$  syringe filter driven unit from Milipore® provided with a high-density polyethylene housing and membrane of hydrophilic Durapore® (PVDF) prior to analysis.

### 2.2. Wastewater composition

The airlift reactor was fed with synthetic wastewater with  $3.63 \text{ g L}^{-1} \text{ NH}_4\text{Cl}$  ( $950 \pm 25 \text{ mg N} - \text{NH}_4^+ \text{ L}^{-1}$ ) and the following compounds and micronutrients (concentrations are expressed in  $\text{mg L}^{-1}$ ):  $\text{CH}_3\text{COONa}$ , 48.0; glucose, 12.5; sucrose, 11.9;  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 88.0;  $\text{KH}_2\text{PO}_4$ , 41.0;  $\text{NaCl}$ , 176.0;  $\text{MgCl}_2 \cdot 7\text{H}_2\text{O}$ , 198.0;  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 4.0;  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ , 3.0;  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 4.0;  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 2.0; and  $\text{H}_3\text{BO}_3$ , 0.02;  $\text{CO}(\text{NH}_2)_2$ , 12.0 and yeast extract, 2.0. In addition, an increasing amount of *o*-cresol was added to the influent after performing bioaugmentation, for details see Section 2.4 and Fig. 2.



**Fig. 1** – Experimental set-up of the continuous granular airlift reactor. (1) Riser; (2) down comer; (3) separator; (4) feed pump; (5) effluent port; (6) air sparger; (7) rotameter; (8) pH probe; (9) DO probe; (10) monitoring panel.



**Fig. 2 – o-Cresol feeding strategy imposed during the continuous operation of the granular airlift reactor. Note how bioaugmentation (thick arrow) shows the beginning of the experimental period here reported. \*Shock load experiment for a period of 24 h.**

### 2.3. Achievement of an aerobic granular biomass with a high nitrification rate before addition of o-cresol in the influent

The airlift reactor was inoculated with 1 L of granular biomass from a granular sequencing batch reactor (GSBR) at pilot scale treating a low-strength wastewater for simultaneous carbon, nitrogen, phosphorus removal (Isanta et al., 2012). The reactor was operated in continuous with the synthetic wastewater described in Section 2.2 aiming to obtain nitrification, following the strategy described by Bartolí et al. (2010). At the time of the study (i.e. prior to bioaugmentation and addition of o-cresol in the influent), the reactor was operating in stable conditions (data not shown), oxidising ca. 66% of ammonium into nitrite at a volumetric nitrogen loading rate ( $NLR_v$ ) of  $0.4 \pm 0.1 \text{ g N L}^{-1} \text{ d}^{-1}$ , low nitrate concentration (average of  $3 \pm 1 \text{ mg N L}^{-1}$ ) and the following biomass characteristics: mean size (mm)  $1.5 \pm 1.2$ , settling velocity ( $\text{m h}^{-1}$ )  $61 \pm 24$ , sludge volumetric index ( $SVI_5$ ) ( $\text{mL g}^{-1} \text{ TSS}$ ) 8.1, ratio  $SVI_{30}/SVI_5$  1.0 and biomass density ( $\text{g VSS L}^{-1}_{\text{particle}}$ )  $290 \pm 120$ .

A batch test was carried out with this aerobic granular biomass to assess its capacity to biodegrade o-cresol (data not shown). The test consisted on the addition of a pulse of ammonium and o-cresol in an aerated batch reactor. The results of this test showed that the aerobic granular biomass was not able to biodegrade o-cresol. Therefore, bioaugmentation was chosen as a good strategy to improve the o-cresol removal capacity of the aerobic granular biomass.

### 2.4. Bioaugmentation

The ability of phenols-acclimated sludge to degrade high-strength cresol wastewater has been previously reported (Lee et al., 2011). Activated sludge specifically enriched to degrade *p*-nitrophenol (PNP) was used to bioaugment the reactor, given its ability to also biodegrade o-cresol (Fernández et al., 2013). The microbial composition of the PNP-degrading activated 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. ( $26 \pm 2\%$ ) and genus *Acinetobacter* ( $31 \pm 10\%$ ) as the PNP-degraders in the activated sludge, whereas no hybridization was found for *Burkholderia* sp. and *Pseudomonas* spp.

A volume of 500 mL with a concentration of  $2 \text{ g VSS L}^{-1}$  of the PNP-degrading activated sludge was bioaugmented in the reactor, meaning 12% w/w of the total volatile suspended solids (VSS) in the reactor. This was used as the starting event of the experimental period here reported (i.e. day 0). Simultaneously, on same day-0, o-cresol was added to the wastewater. Five percent (% w/w) of specialized sludge was required for a successful bioaugmentation with PNP-degraders in SBR operation since the biomass was retained by settling prior effluent discharge (Martín-Hernández et al., 2012). Considering that the reactor used in this study was operated in continuous mode and a strong washout of the bioaugmented activated sludge would (unavoidably) happen, a higher proportion of bioaugmented biomass was added in this study (12% w/w) compared to the work of Martín-Hernández et al. (2012) (5% w/w).

### 2.5. Operational strategy of the bioaugmented aerobic granular airlift reactor

o-Cresol concentration in the influent was progressively increased throughout the operational period to minimise the potential inhibitory/toxic effects (Fig. 2). This operational strategy was selected by two reasons (i) the progressive increase of o-cresol will allow for both, high degradation rate and development of the specific heterotrophic bacteria over the aerobic granules, and (ii) the AOB potential inhibition/toxicity by o-cresol would be minimised. Hydraulic retention time (HRT) was set between 0.85 and 2.3 d depending on the  $NLR_v$  imposed to the reactor.

Additionally, to test the ability of the system against shock load events, two strong disturbances were applied to the o-cresol concentration in the wastewater. During 24 h o-cresol concentration in the wastewater was suddenly increased from 100 to 300 and to 1000  $\text{mg L}^{-1}$ , on days 126 and 137 of operation, respectively (Fig. 2).

### 2.6. Analytical methods

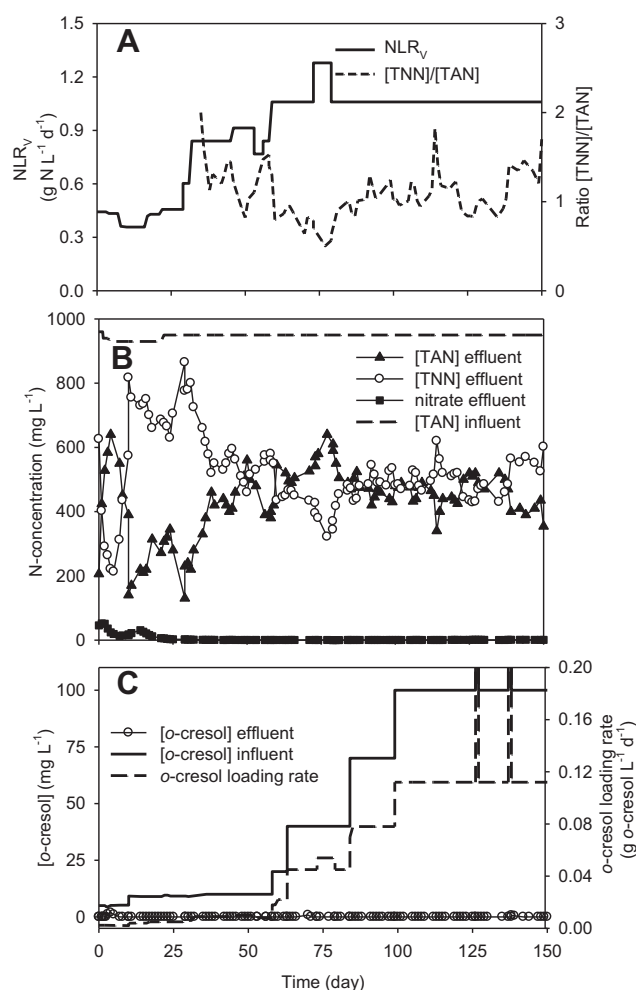
o-Cresol was determined by High Performance Liquid Chromatography (HPLC) as described by Martín-Hernández et al. (2009). The ammonium concentration measured as total ammonia nitrogen (TAN), the nitrite as total nitrite nitrogen (TNN) and nitrate concentrations were measured as detailed by Bartolí et al. (2010). VSS, total suspended solids (TSS) and

sludge volumetric index (SVI) were determined using the procedure described in Standard Methods (APHA, 1998). The granular biomass was characterized in terms of size, granule density and settling velocity. The size distribution of the granules was measured regularly by using image analysis with an optical microscope Zeiss Axioskop equipped with a video camera (iAi Protec). The digital image captured was further processed using Image-Pro Plus version 6.0 (Media Cybernetics, Inc.). The procedure followed was (i) to convert the original image to black and white for image processing, (ii) to define the threshold in order to delimit the area of interest in the image (i.e. the granules) and (iii) to export the selected data with the software to a worksheet. For each mean size determination, at least 50 granules were used. Density of the granular biomass was determined using the Dextran Blue method described by Beun et al. (2002). Settling velocity was determined by placing individual granule in a column containing the described wastewater and measuring the time spent to drop a height of 40 cm. The extracellular polymeric substances (EPS) were extracted from the granules using formaldehyde and NaOH and were analyzed according to Adav et al. (2008).

o-Cresol (concentrated solution, purity 99%) and ammonium chloride (purity 99.5%) were supplied by Panreac (Spain) and Carl Roth (Germany), respectively. All other chemicals and reagents were purchased from Sigma–Aldrich (Spain) at the highest purities available.

## 2.7. FISH analysis

The FISH–CLSM technique was used to identify betaproteobacterial ammonia-oxidising bacteria (BAOB), nitrite-oxidising bacteria (NOB) and possible heterotrophic bacteria able to degrade o-cresol in the geometry of sliced granules. Table 1 details the probes used in this study. The probes for identify possible heterotrophic bacteria able to degrade o-cresol were selected taking into account the characterisation of the PNP-degrading biomass used for bioaugmentation previously carried out by Suárez-Ojeda et al. (2011). FISH protocol was also adapted from the same authors. Entire granules were embedded in paraffin wax before their sectioning with a microtome. Slices with a thickness of 3  $\mu\text{m}$  were cut, and each single section was placed on the surface of poly-L-lysine coated microscopic slides. In order to obtain a better staining of the granule slices, the amount of the probe and hybridisation buffer were increased 4–5 fold, depending on the area



**Fig. 3 – Performance of the continuous granular airlift reactor treating a high-strength ammonium wastewater also containing o-cresol. (A) Volumetric nitrogen loading rate ( $\text{NLR}_v$ ) and  $[\text{TNN}]/[\text{TAN}]$  ratio; (B) Partial nitrification performance; (C) o-Cresol biodegradation performance throughout the experimental period. Bioaugmentation was performed on day 0.**

of the sliced granule. A Leica TCS-SP5 AOBs confocal laser scanning microscope (Leica Microsystems Heidelberg GmbH, Mannheim, Germany) was used. The confocal microscope was equipped with an HC PL APO CS 63x1.25 oil objective, several lasers for emission from 405 to 990 nm and a hybrid detector.

## 3. Results and discussion

### 3.1. Performance of the aerobic granular airlift reactor for simultaneous removal of ammonium and o-cresol

The results presented in this study correspond to the operation of an aerobic granular reactor with a high nitrification capacity (previously developed as explained in Section 2.3) and bioaugmented on day 0 (Fig. 2) with a PNP-degrading activated sludge and fed with a high-strength ammonium wastewater

**Table 1 – Probes employed in the FISH analysis for targeting specific microorganisms.**

Probe name	Specificity	Reference
Nso190	$\beta$ AOB	Mobarry et al. (1996)
NIT3	<i>Nitrobacter</i> sp.	Wagner et al. (1996)
KO 02	<i>Arthrobacter</i> sp.	Franke-Whittle et al. (2005)
ACA652	Genus <i>Acinetobacter</i>	Wagner et al. (1994)
UNIV1390	All organisms	Zheng et al. (1996)
EUBmix	Most bacteria, planctomycetales and verrucomicrobiales	Daims et al. (1999)



containing *o*-cresol. The performance of partial nitrification and *o*-cresol biodegradation is shown in Fig. 3. The reactor was able to maintain stable partial nitrification at low nitrate concentrations in the effluent (Fig. 3B) with full *o*-cresol biodegradation (Fig. 3C) for long-term (150 days). The balance of the N-species shows that the denitrification process in the aerobic granules was negligible throughout the experimental period. On day 35 onward, the [TNN]/[TAN] ratio in the effluent was steadily maintained between 1.0 and 1.5 during more than 100 days, being suitable for a subsequent anammox process (Fig. 3A). The reactor achieved a relatively high volumetric nitrogen loading rate ( $\text{NLR}_V = 1.1 \pm 0.1 \text{ g N L}^{-1} \text{ d}^{-1}$ ) in spite of the presence of *o*-cresol. This  $\text{NLR}_V$  value is comparable to those reported in the literature for conventional high-strength ammonium wastewaters at 30 °C (i.e. without containing any phenolic compound): Yamamoto et al. (2011) ( $0.7\text{--}2.6 \text{ g N L}^{-1} \text{ d}^{-1}$ ), Okabe et al. (2011) ( $1.0\text{--}1.8 \text{ g N L}^{-1} \text{ d}^{-1}$ ) and Bartroli et al. (2010) ( $0.75\text{--}6.1 \text{ g N L}^{-1} \text{ d}^{-1}$ ). *o*-Cresol was completely degraded throughout the operational period achieving a volumetric *o*-cresol loading rate ( $\text{oCLR}_V$ ) of  $0.11 \text{ g o-cresol L}^{-1} \text{ d}^{-1}$ .

The difference in oxygen affinity between AOB and NOB was determined as the key parameter to obtain nitrification in biofilm or granular reactors (Pérez et al., 2009). If strong oxygen limiting conditions are applied in a granular reactor, AOB outcompete NOB and nitrite oxidation is prevented. The key operational variable to be maintained for assuring strong oxygen limiting conditions in a granular reactor is the ratio between DO and TAN concentrations in the bulk liquid (Bartroli et al., 2010). The lower the [DO]/[TAN] ratio, the stronger the oxygen limiting condition in the biofilm (Jemaat et al., 2013). The control of this operational parameter allows for achieving and maintaining nitrification even at high DO concentration, as demonstrated with long term specific experiments (Bartroli et al., 2010). In that study, it was determined that nitrite oxidation was prevented for [DO]/[TAN] ratios lower than 0.25 in a granular airlift reactor.

In this study, the [DO]/[TAN] ratio was not automatically controlled but it was always maintained at very low values ( $0.003$  and  $0.010 \text{ mg O}_2 \text{ mg}^{-1} \text{ N}$ ) since DO concentration was  $2 \text{ mg O}_2 \text{ L}^{-1}$  and TAN concentration was always higher than  $200 \text{ mg N L}^{-1}$  (Fig. 3B). These strong oxygen limiting conditions imposed in the reactor for NOB allowed for maintaining partial nitrification throughout the study.

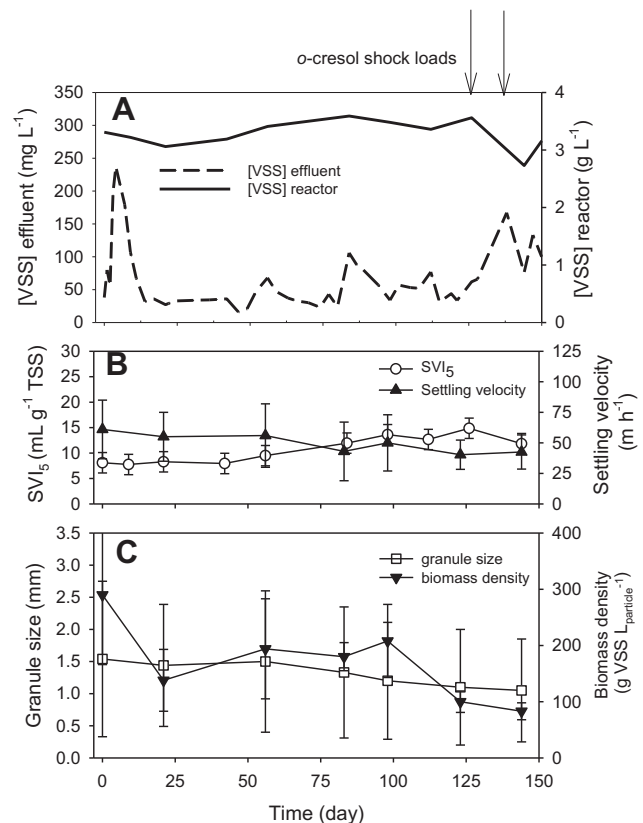
Bioaugmentation together with the operational strategy selected, by which *o*-cresol was progressively increased in the influent, prevented the accumulation of *o*-cresol in the reactor bulk liquid, as supported by the HPLC analyses showing concentrations of *o*-cresol lower than the detection limit ( $0.1 \text{ mg L}^{-1}$ ) throughout the experimental period (with the exception of the shock load events) and without detecting any potential intermediates. This was crucial for the stability of the nitrification process since the inhibition of nitrifiers, especially AOB, by phenolic compounds is well documented (Morita et al., 2007; Suárez-Ojeda et al., 2010; Milia et al., 2012), as already mentioned.

The granular biomass concentration in the reactor was maintained at around  $3\text{--}3.5 \text{ g L}^{-1}$  throughout the experimental period (with the exception of shock load event at day 137) (Fig. 4A). In the first ten days, an increase of biomass

concentration in the effluent was observed at around  $240 \text{ mg L}^{-1}$  (Fig. 4A). This occurrence was strongly related to the washout of a fraction of the bioaugmented PNP-degrading activated sludge that could not be retained in the reactor. On day 15 onward, the biomass in the effluent remained stable between  $40$  and  $90 \text{ mg L}^{-1}$  in most of the experimental period showing the stability of the granules.

Granular characteristics were not importantly affected throughout the operational period. The granule size, settling velocity, biofilm density and  $\text{SVI}_5$  remained rather steady, within the expected range for granular sludge, according to the review performed by Gao et al. (2011) (Fig. 4).  $\text{SVI}_5$  and settling velocity were steadily maintained in the range of  $7\text{--}14 \text{ mL g}^{-1}$  and  $40\text{--}60 \text{ m h}^{-1}$ , respectively. Only  $\text{SVI}_5$  was depicted in Fig. 4A since  $\text{SVI}_{30}$  values were identical to  $\text{SVI}_5$  values throughout the experimental period, being the ratio  $\text{SVI}_{30}/\text{SVI}_5$  always one. A slight decrease in biomass density was observed at the end of the experimental period and it may be linked to the progressive growth of heterotrophs related to *o*-cresol biodegradation over aerobic granules that decreased the compactness of the granular biomass.

EPS content in the granules was also monitored by determination of proteins (PN) and polysaccharides (PS) (Table 2). EPS is believed to be responsible for granule formation and stability; however, there is no consensus about the role played by the different EPS components: PS and PN (Zhu et al., 2012).



**Fig. 4 – (A) Concentration of volatile suspended solid in the reactor and in the effluent; (B)  $\text{SVI}_5$  values and settling velocity; (C) Granule size and biomass density in the continuous granular airlift reactor throughout the operational period.**

**Table 2 – Extracellular polymeric substances content of aerobic granular sludge during the simultaneous partial nitrification and o-cresol biodegradation.**

Time (day)	Polysaccharides (PS) (mg g <sup>-1</sup> VSS)	Protein (PN) (mg g <sup>-1</sup> VSS)	EPS (PS + PN) (mg g <sup>-1</sup> VSS)	Ratio PS/PN
0	27 ± 2	11 ± 1	38 ± 3	2.4
80	31 ± 9	6 ± 2	37 ± 11	5.2
143	26 ± 2	12 ± 4	38 ± 6	2.2

High PS content was noted to facilitate cell to cell adhesion and strengthen the microbial structure through a polymeric matrix (Adav et al., 2008) while the extracellular PN would be essential for maintaining structural stability of EPS matrix of aerobic granules (Xiong and Liu, 2013).

EPS concentration was remained rather steady throughout the operational period. PS was the dominant compound of the EPS in the granules, with PS/PN ratios always higher than 2 (Table 2). In general, the aerobic granules with a dominant heterotrophic activity show a higher content of PN than PS and consequently, the PS/PN ratios are lower than 1 (Adav et al., 2008; Zhu et al., 2012). However, the aerobic granules with a significant nitrifying activity show PS/PN ratios higher than 1 (Yang et al., 2005; Zhang et al., 2011). The results of this study agree with this general trend confirming that the aerobic granules had a significant nitrifying activity. In this sense, it is relevant that the values of PS and PN were almost identical at the beginning and at the end of the study (Table 2). Therefore, the treatment of o-cresol in the airlift reactor had not significant effect over the structural integrity of the granules.

Although the granular characteristics slightly changed during the simultaneous partial nitrification and o-cresol removal, the performance of the granular airlift reactor was maintained stable. This finding indicates that the ability of granular biomass in terms of contaminants biodegradation is unaffected in response to changes in granules characteristics.

A key feature for maintaining simultaneous partial nitrification and o-cresol removal at a long-term operation is the retention, development and attachment of the specialised biomass over the aerobic granules. To observe the morphological changes in the aerobic granules throughout the experimental period, a magnifying glass was used to obtain pictures of the granules on days 0, 90 and 123 (Fig. 5). On the first day, the original aerobic granules were characterised by a smooth and regular shape (Fig. 5A). After 90 and 123 days of operation, the outer surface of granules was covered with filamentous. The development of these filamentous structures was thought to be linked to the growth of heterotrophic bacteria responsible for o-cresol biodegradation (Fig. 5B and C). This was later confirmed by FISH–CLSM analysis of sliced granules (see Section 3.3).

### 3.2. o-Cresol shock load events

The performance of the reactor in front of o-cresol shock load events was also explored (Fig. 6). The theoretical accumulation of o-cresol in the bulk liquid of the reactor considering no biodegradation is also depicted in Fig. 6 to easily assess the impact of the shock load events. In the first shock load event on day 126 (300 mg o-cresol L<sup>-1</sup> in the influent during 24 h), o-

cresol accumulated up to 2 mg L<sup>-1</sup> in the reactor bulk liquid for the first 2 h but later, it was fully degraded (Fig. 6A). Meanwhile, partial nitrification remained unaffected. On day 137, a second shock load event was performed (1000 mg o-cresol L<sup>-1</sup> in the influent during 24 h), and in the first 4 h, o-cresol built up to 20 mg L<sup>-1</sup> in the reactor bulk liquid. Similarly, to the previous shock load event, o-cresol was fully degraded (Fig. 6B) after few hours. During this event, partial nitrification was stably maintained and surprisingly after the shock load event, the nitrification rate was slightly increased at around 14% and maintained until the end of the experimental period. It is also essential to highlight that in both shock load events, besides a small o-cresol accumulation of few hours, high removal efficiency of o-cresol still took place in the reactor. During the shock load of 1000 mg o-cresol L<sup>-1</sup>, the reactor achieved an oCLR<sub>v</sub> of 0.8 g o-cresol L<sup>-1</sup> d<sup>-1</sup>. This result indicates that the airlift reactor could be operated at oCLR<sub>v</sub> higher than the achieved oCLR<sub>v</sub> during normal operation (0.11 g o-cresol L<sup>-1</sup> d<sup>-1</sup>).

After the shock load event on day 137, the biomass concentration in the reactor decreased to about 2.8 g L<sup>-1</sup> (Fig. 4A). This decrease of biomass was strongly linked to a noticeable biomass washout occurred between day-127 and 137. Several days after the 300 mg o-cresol L<sup>-1</sup> shock load event, the biomass in the effluent increased to 150 mg L<sup>-1</sup> compared to the 50 mg L<sup>-1</sup> prior to the shock load event (Fig. 4A). A possible explanation for this increase in biomass concentration in the effluent might be the increase in heterotrophic bacteria growth because of the amount of organic substrate available during the shock load events. Thus, more detachment of biomass from granules is expected, causing more biomass washout after the shock load events, as observed in this experiment (Fig. 4A). Nevertheless, the biodegradation in the reactor was maintained stable in spite of this biomass washout.

Studies on the o-cresol inhibitory impact over ammonium oxidation have been reported by Radniecki et al. (2010) and Dyreborg and Arvin (1995) who estimated that 0.5 mg o-cresol L<sup>-1</sup> and 1.3 mg o-cresol L<sup>-1</sup> resulted in 50 and 100% inhibition, respectively. In the present study, nitrification inhibition was not observed despite o-cresol built up to 20 mg L<sup>-1</sup> during the last shock load event (Fig. 6B). These findings suggest that bioaugmentation and progressive growth of heterotrophs able to degrade o-cresol in the aerobic granules enhanced the development of aerobic granules with the ability to withstand to shock loads of toxic compounds.

The simultaneous presence of AOB and heterotrophic bacteria in the aerobic granules was not a problem from the point of view of the competition for oxygen between both populations. On one hand, the oxygen consumed for COD oxidation was only a 14% of the total oxygen consumed in the

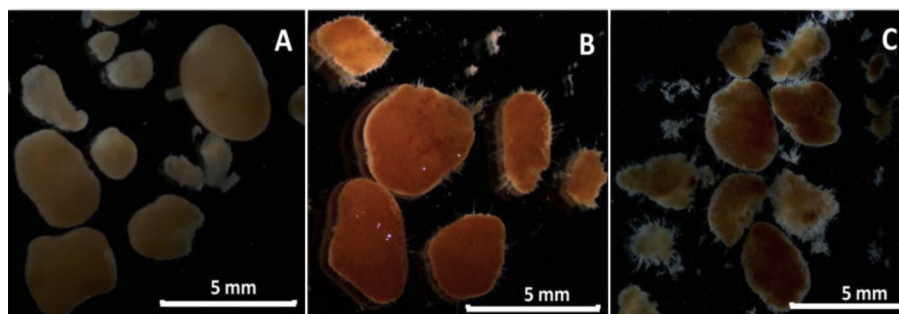


Fig. 5 – Changes in the morphology of the granules during the simultaneous partial nitrification and *o*-cresol biodegradation. (A) Day 0; (B) day 90; (C) day 123. Scale bar = 5 mm.

airlift reactor and, on the other hand, the influent [COD]/[TAN] ratio was around 0.25 and the airlift reactor can be considered as basically nitrifying because the competition for oxygen is clearly inclined towards nitrifying population in reactors with [COD]/[TAN] ratios below 0.5 (Carrera et al., 2004).

The present findings are consistent with the studies demonstrating that nitrifying bacteria embedded in microbial granules were effectively protected against the inhibitory effect of phenolic compounds present in the wastewater (Liu et al., 2005; Jiang et al., 2010).

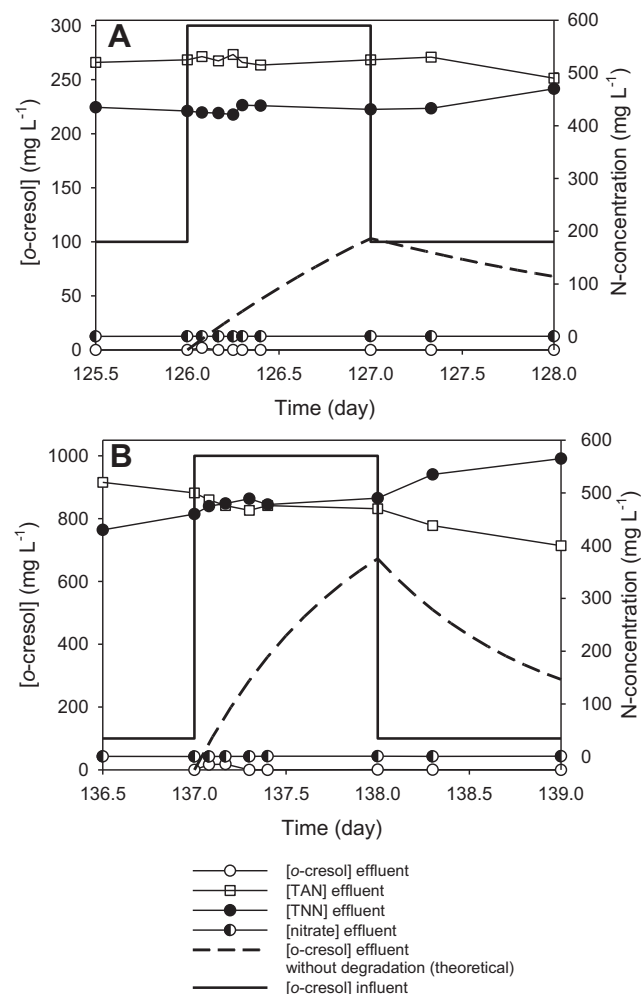


Fig. 6 – Effect of shock loading events on the performance of partial nitritation and *o*-cresol removal. The theoretical accumulation of *o*-cresol concentration in the reactor considering no biological degradation is also depicted for guiding purpose. (A) Day 126; (B) day 137.

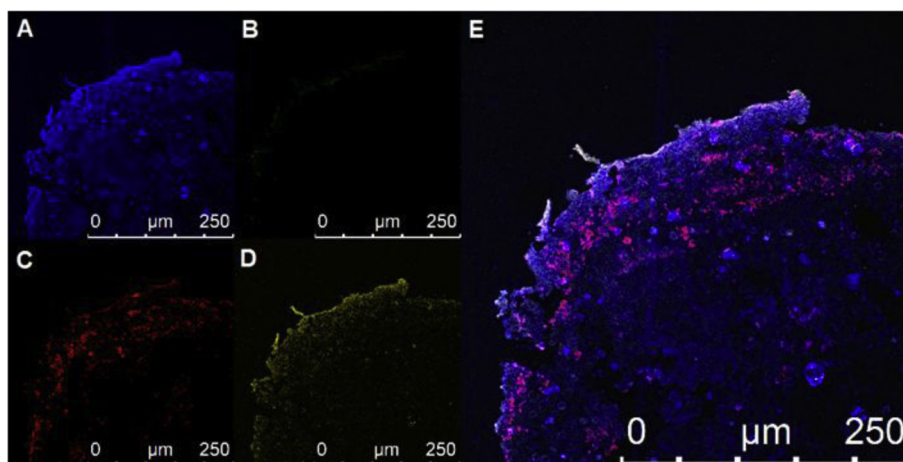
### 3.3. Identification of microbial species in the granular biomass

The results obtained by FISH help to identify that only *Acinetobacter* genus (Fig. 7D) and  $\beta$ AOB (Fig. 7C) were the populations with high occurrences in the granules, whereas *Nitrobacter* sp. (Fig. 7B) was also detected, but at a very low occurrence. Moreover, *Arthrobacter* sp. was not detected in our samples. The *Acinetobacter* genus is believed to be the responsible for *o*-cresol biodegradation; meanwhile, the low occurrence of *Nitrobacter* sp. confirms that NOB were out-competed by AOB in the granular biomass due to the imposed [DO]/[TAN] ratio, that guarantee a strong oxygen limiting conditions for NOB, as previously demonstrated (Bartolí et al., 2010). *Acinetobacter* genus tends to locate at the outer layer (Fig. 7D) in fact, some filaments are visible as in Fig. 5.  $\beta$ AOB tends to locate at the inner layers, although some of them are also located at the outer layer (Fig. 7C).

### 3.4. Practical implications

The results of this study show that the treatment of industrial wastewaters containing both, high ammonium and phenolic concentrations through biological nitrogen removal via nitrite is plausible. The most important points in this process would be: (i) the use of granular biomass which allows for maintaining different microbial populations into the same aerobic reactor, (ii) the use of bioaugmentation to ease the development of the different microbial populations in the granules, (iii) the use of the appropriated [DO]/[TAN] ratio to achieve and to maintain stable partial nitrification, leading to an effluent suitable for a subsequent anammox reactor and (iv) the complete removal of the phenolic compound in the aerobic granular reactor to guarantee the absence of this compound in the subsequent anammox reactor.

Moreover, the continuous airlift reactor has been proved as a good technological option for this kind of aerobic treatment. The use of a continuous reactor and granular biomass, which



**Fig. 7** – FISH–CLSM image of a sliced granule collected at the end of the experimental period (Bar = 250  $\mu\text{m}$ ). A) Blue: all bacteria (UNIV1390 and EUBmix); B) green: *Nitrobacter* sp. (NIT3); C) red:  $\beta$ AOB (Nso190); D) yellow: *Acinetobacter* genus (ACA652) and E) Merge Image. Centre of the granules is on the bottom right corner. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

is a special type of biofilm without carrier material, allowed the application of a low air flow rate. In this sense, the upflow velocity used in this study ( $0.4 \text{ cm s}^{-1}$ ) was below other velocities reported in the literature for aerobic granular reactor working as sequencing batch reactors (SBRs) and performing partial nitrification ( $2 \text{ cm s}^{-1}$ , Bao et al., 2009;  $0.6\text{--}1.2 \text{ cm s}^{-1}$ , Song et al., 2013). Therefore, the energy requirements can be reduced by comparing it to other systems.

#### 4. Conclusions

- The simultaneous partial nitrification and *o*-cresol biodegradation was successfully accomplished in a single reactor with aerobic granular biomass.
- A suitable effluent for a subsequent anammox process was stably maintained for more than 100 days.
- Bioaugmentation strategy enhanced the formation of a granular sludge with a special architecture in which an external layer of heterotrophs was degrading *o*-cresol and at the same time, it would help to protect AOB against the potentially inhibitory effect of *o*-cresol.
- The presence of *o*-cresol in the influent had a minor impact on the time course granules size, biomass density, EPS content and settling velocity.
- These results suggest that aerobic continuous granular airlift reactors are a promising technology for simultaneous removal of high-strength ammonium wastewaters containing recalcitrant compounds.

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