Partial nitritation 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 nitritation 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±25 mg $N\text{-}NH_4^+$ 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 mg L^{-1}. The results showed that stable partial nitritation 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 mg L^{-1} were applied to assess the capabilities of the system. Despite these shock load events, the partial nitritation 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.

Keywords

Nitritation; industrial wastewater; phenolic compounds; aerobic granules

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 nitritation 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 nitritation 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 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, inhibition by phenol (Yang et al., 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 nitritation reactor followed by an anammox reactor. The partial nitritation 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 nitritation 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 nitritation 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 (Figure 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 cm s^{-1}. Air flow rate in the reactor was regulated at 250 ± 50 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 ± 0.3 mg O_2 L^{-1} and pH was maintained at 8.3 ± 0.2 by a regular addition of $NaHCO_3$. The temperature in the reactor was maintained at 30 ± 1 ºC 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 μm 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 g L^{-1} NH_4Cl (950 ± 25 mg $N\text{-}NH_4^+$ L^{-1}) and the following compounds and micronutrients (concentrations are expressed in mg L^{-1}): CH_3COONa, 48.0; glucose, 12.5; sucrose, 11.9; $CaCl_2 \cdot 2H_2O$, 88.0; KH_2PO_4, 41.0; NaCl, 176.0; $MgCl_2 \cdot 7H_2O$, 198.0; $FeSO_4 \cdot 7H_2O$, 4.0; $MnSO_4 \cdot H_2O$, 3.0; $ZnSO_4 \cdot 7H_2O$, 4.0; $CuSO_4 \cdot 5H_2O$, 2.0; and H_3BO_3, 0.02; $CO(NH_2)_2$, 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 Figure 2.

2.3. Achievement of an aerobic granular biomass with a high nitritation 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 nitritation, following the strategy described by Bartrolí 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 ± 0.1 g N $L^{-1}d^{-1}$, low nitrate concentration (average of 3 ± 1 mg N L^{-1}) and the following biomass characteristics: mean size (mm) 1.5 ± 1.2, settling velocity (m h^{-1}) 61 ± 24, sludge volumetric index (SVI_5) (mL g^{-1} TSS) 8.1, ratio SVI_{30}/SVI_5 1.0 and biomass density (g VSS $L^{-1}_{particle}$) 290 ± 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±2 %) and genus *Acinetobacter* (31±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 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 wash out 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 (Figure 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 mg L^{-1}, on days 126 and 137 of operation, respectively (Figure 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 Bartrolí 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 and Lee, (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 (βAOB), 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 μm 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

227 slices, the amount of the probe and hybridisation buffer were increased 4 to 5 fold,
228 depending on the area of the sliced granule. A Leica TCS-SP5 AOBS confocal laser
229 scanning microscope (Leica Microsystems Heidelberg GmbH, Mannheim, Germany)
230 was used. The confocal microscope was equipped with a HC PL APO CS 63x1.25 oil
231 objective, several lasers for emission from 405 to 990 nm and a hybrid detector.
232
233 3. Results and discussion
234
235 3.1. Performance of the aerobic granular airlift reactor for simultaneous removal of
236 ammonium and *o*-cresol
237
238 The results presented in this study correspond to the operation of an aerobic granular
239 reactor with a high nitritation capacity (previously developed as explained in section
240 2.3) and bioaugmented on day 0 (Figure 2) with a PNP-degrading activated sludge and
241 fed with a high-strength ammonium wastewater containing *o*-cresol. The performance
242 of partial nitritation and *o*-cresol biodegradation is shown in Figure 3. The reactor was
243 able to maintain stable partial nitritation at low nitrate concentrations in the effluent
244 (Figure 3.B) with full *o*-cresol biodegradation (Figure 3.C) for long-term (150 days).
245 The balance of the N-species shows that the denitrification process in the aerobic
246 granules was negligible throughout the experimental period. On day 35 onward, the
247 [TNN]/[TAN] ratio in the effluent was steadily maintained between 1.0 and 1.5 during
248 more than 100 days, being suitable for a subsequent anammox process (Figure 3.A).
249 The reactor achieved a relatively high volumetric nitrogen loading rate (NLR_V =1.1 ±
250 0.1 g N $L^{-1}d^{-1}$) in spite of the presence of *o*-cresol. This NLR_V value is comparable to
251 those reported in the literature for conventional high-strength ammonium wastewaters at

252 30 ºC (i.e. without containing any phenolic compound): Yamamoto et al. (2011) (0.7-
253 2.6 g N L^{-1} d^{-1}), Okabe et al. (2011) (1.0-1.8 g N L^{-1} d^{-1}) and Bartrolí et al. (2010) (0.75-
254 6.1 g N L^{-1} d^{-1}). *o*-Cresol was completely degraded throughout the operational period
255 achieving a volumetric *o*-cresol loading rate ($oCLR_V$) of 0.11 g *o*-cresol L^{-1} d^{-1}.
256
257 The difference in oxygen affinity between AOB and NOB was determined as the key
258 parameter to obtain nitritation in biofilm or granular reactors (Pérez et al., 2009). If
259 strong oxygen limiting conditions are applied in a granular reactor, AOB outcompete
260 NOB and nitrite oxidation is prevented. The key operational variable to be maintained
261 for assuring strong oxygen limiting conditions in a granular reactor is the ratio between
262 DO and TAN concentrations in the bulk liquid (Bartrolí et al., 2010). The lower the
263 [DO]/[TAN] ratio, the stronger the oxygen limiting condition in the biofilm (Jemaat et
264 al., 2013). The control of this operational parameter allows for achieving and
265 maintaining nitritation even at high DO concentration, as demonstrated with long term
266 specific experiments (Bartrolí et al., 2010). In that study, it was determined that nitrite
267 oxidation was prevented for [DO]/[TAN] ratios lower than 0.25 in a granular airlift
268 reactor.
269
270 In this study, the [DO]/[TAN] ratio was not automatically controlled but it was always
271 maintained at very low values (0.003 and 0.010 mg O_2 mg^{-1} N) since DO concentration
272 was 2 mg O_2 L^{-1} and TAN concentration was always higher than 200 mg N L^{-1} (Figure
273 3.B). These strong oxygen limiting conditions imposed in the reactor for NOB allowed
274 for maintained partial nitritation throughout the study.
275

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 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 nitritation 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 to 3.5 g L^{-1} throughout the experimental period (with the exception of shock load event at day 137) (Figure 4.A). In the first ten days, an increase of biomass concentration in the effluent was observed at around 240 mg L^{-1} (Fig. 4.A). 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 to 90 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 SVI_5 remained rather steady, within the expected range for granular sludge, according the review performed by Gao et al. (2011) (Figure 4). SVI_5 and settling velocity were steadily maintained in the range of 7-14 mL g^{-1} and 40-60 m h^{-1}, respectively. Only SVI_5 was depicted in Figure 4.A since SVI_{30} values were identical to SVI_5 values throughout the experimental period, being the ratio SVI_{30}/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). 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; Zhan 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 nitritation 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 nitritation 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 (Figure 5). On the first day, the original aerobic granules were characterised by a smooth and regular shape (Fig. 5.A). 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. 5.B and 5.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 (Figure 6). The theoretical accumulation of *o*-cresol in the bulk liquid of the reactor considering no biodegradation is also depicted in Figure 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^{-1} in the influent during 24 h), *o*-cresol accumulated up to 2 mg L^{-1} in the reactor bulk liquid for the first two hours but later, it was fully degraded (Figure 6.A). Meanwhile, partial

nitritation remained unaffected. On day 137, a second shock load event was performed (1000 mg *o*-cresol L^{-1} in the influent during 24 h), and in the first four hours, *o*-cresol built up to 20 mg L^{-1} in the reactor bulk liquid. Similarly, to the previous shock load event, *o*-cresol was fully degraded (Figure 6.B) after few hours. During this event, partial nitritation was stably maintained and surprisingly after the shock load event, the nitritation 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^{-1}, the reactor achieved an $oCLR_V$ of 0.8 g *o*-cresol L^{-1} d^{-1}. This result indicates that the airlift reactor could be operated at $oCLR_V$ higher than the achieved $oCLR_V$ during normal operation (0.11 g *o*-cresol L^{-1} d^{-1}).

After the shock load event on day 137, the biomass concentration in the reactor decreased to about 2.8 g L^{-1} (Figure 4.A). 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^{-1} shock load event, the biomass in the effluent increased to 150 mg L^{-1} compared to the 50 mg L^{-1} prior to the shock load event (Figure 4.A). 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 (Figure 4.A). Nevertheless, the biodegradation in the reactor was maintained stable in spite of this biomass washout.

375 Studies on the *o*-cresol inhibitory impact over ammonium oxidation have been reported
376 by Radniecki et al. (2010) and Dyreborg and Arvin, (1995) who estimated that 0.5 mg
377 *o*-cresol L^{-1} and 1.3 mg *o*-cresol L^{-1} resulted in 50 and 100 % inhibition, respectively. In
378 the present study, nitritation inhibition was not observed despite *o*-cresol built up to 20
379 mg L^{-1} during the last shock load event (Figure 6.B). These findings suggest that
380 bioaugmentation and progressive growth of heterotrophs able to degrade *o*-cresol in the
381 aerobic granules enhanced the development of aerobic granules with the ability to
382 withstand to shock loads of toxic compounds.
383
384 The simultaneous presence of AOB and heterotrophic bacteria in the aerobic granules
385 was not a problem from the point of view of the competition for oxygen between both
386 populations. On one hand, the oxygen consumed for COD oxidation was only a 14% of
387 the total oxygen consumed in the airlift reactor and, on the other hand, the influent
388 COD/TAN ratio was around 0.25 and the airlift reactor can be considered as basically
389 nitrifying because the competition for oxygen is clearly inclined towards nitrifying
390 population in reactors with COD/TAN ratios below 0.5 (Carrera et al., 2004).
391
392 The present findings are consistent with the studies demonstrating that nitrifying
393 bacteria embedded in microbial granules were effectively protected against the
394 inhibitory effect of phenolic compounds present in the wastewater (Liu et al., 2005;
395 Jiang et al., 2010).
396
397 3.3. Identification of microbial species in the granular biomass
398

399 The results obtained by FISH help to identify that only *Acinetobacter* genus (Figure
400 7.D) and βAOB (Figure 7.C) were the populations with high occurrences in the
401 granules, whereas *Nitrobacter* sp. (Figure 7.B) was also detected, but at a very low
402 occurrence. Moreover, *Arthrobacter* sp. was not detected in our samples. The
403 *Acinetobacter* genus is believed to be the responsible for *o*-cresol biodegradation;
404 meanwhile, the low occurrence of *Nitrobacter* sp. confirms that NOB were outcompeted
405 by AOB in the granular biomass due to the imposed [DO]/[TAN] ratio, that guarantee a
406 strong oxygen limiting conditions for NOB, as previously demonstrated (Bartrolí et al.,
407 2010). *Acinetobacter* genus tends to locate at the outer layer (Figure 7.D) in fact, some
408 filaments are visible as in Figure 5. βAOB tends to locate at the inner layers, although
409 some of them are also located at the outer layer (Figure 7.C).
410
411 3.4. Practical implications
412
413 The results of this study show that the treatment of industrial wastewaters containing
414 both, high ammonium and phenolic concentrations through biological nitrogen removal
415 via nitrite is plausible. The most important points in this process would be: (i) the use of
416 granular biomass which allows for maintaining different microbial populations into the
417 same aerobic reactor, (ii) the use of bioaugmentation to ease the development of the
418 different microbial populations in the granules, (iii) the use of the appropriated
419 [DO]/[TAN] ratio to achieve and to maintain stable partial nitrification, leading to an
420 effluent suitable for a subsequent anammox reactor and (iv) the complete removal of the
421 phenolic compound in the aerobic granular reactor to guarantee the absence of this
422 compound in the subsequent anammox reactor.
423

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 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 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 cm s^{-1}, Bao et al., 2009; 0.6-1.2 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 nitritation 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.

5 Acknowledgements

This study was supported by the Spanish Ministerio de Economía y Competitividad through the ONLYBIO project (CTQ2011-24745/PPQ). The authors are members of the GENOCOV research group (2009 SGR 815). Z. Jemaat wants to thank Ministry of Higher Education of Malaysia and the Universiti Malaysia Pahang for his SLAB fellowship.

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622 Figure Captions and Table legends

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

626

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

630

631 Figure 3. Performance of the continuous granular airlift reactor treating a high-strength
632 ammonium wastewater also containing *o*-cresol. (A) Volumetric nitrogen loading rate
633 (NLR_v) and [TNN]/[TAN] ratio; (B) Partial nitritation performance; (C) *o*-Cresol
634 biodegradation performance throughout the experimental period. Bioaugmentation was
635 performed on day 0.

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637 Figure 4. (A) Concentration of volatile suspended solid in the reactor and in the
638 effluent); (B) SVI_5 values and settling velocity; (C) Granule size and biomass density in
639 the continuous granular airlift reactor throughout the operational period.

640

641 Figure 5. Changes in the morphology of the granules during the simultaneous partial
642 nitritation and *o*-cresol biodegradation. (A) day 0; (B) day 90; (C) day 123. Scale bar =
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644

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646 cresol removal. The theoretical accumulation of *o*-cresol concentration in the reactor
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649

650 Figure 7. FISH-CLSM image of a sliced granule collected at the end of the experimental
651 period (Bar = 250 μm). A) Blue: all bacteria (UNIV1390 and EUBmix); B) green:
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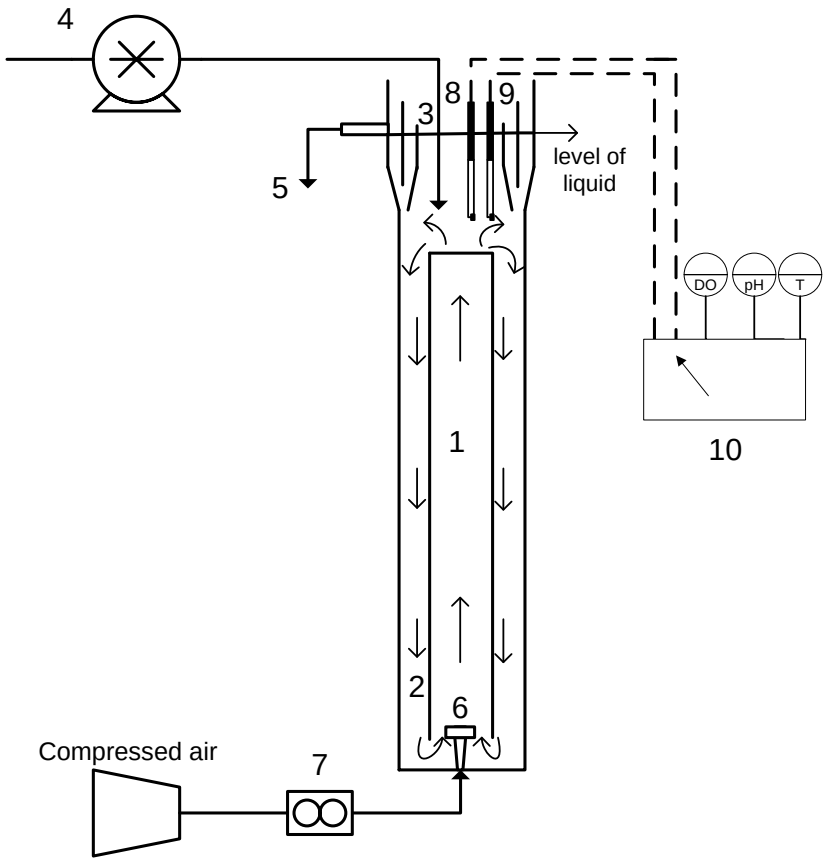
Table 1. Probes employed in the FISH analysis for targeting specific microorganisms.

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

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

Time (day)	Polysaccharides (PS) (mg g^{-1} VSS)	Protein (PN) (mg g^{-1} VSS)	EPS (PS +PN) (mg g^{-1} 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

Figure



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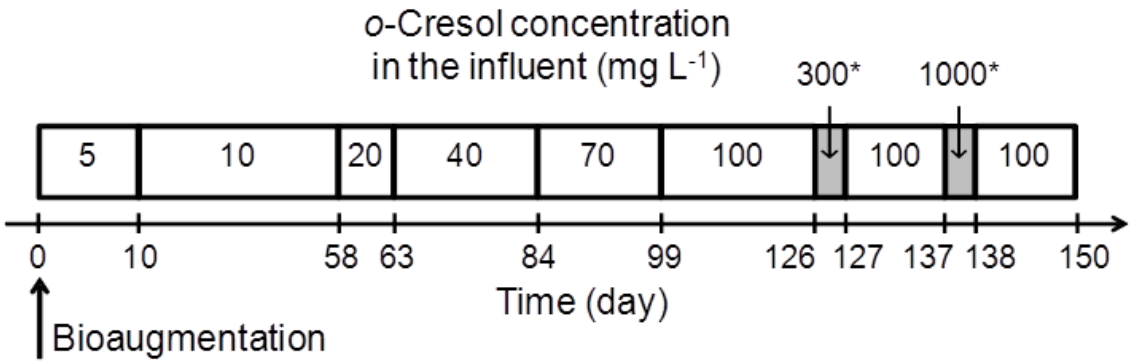

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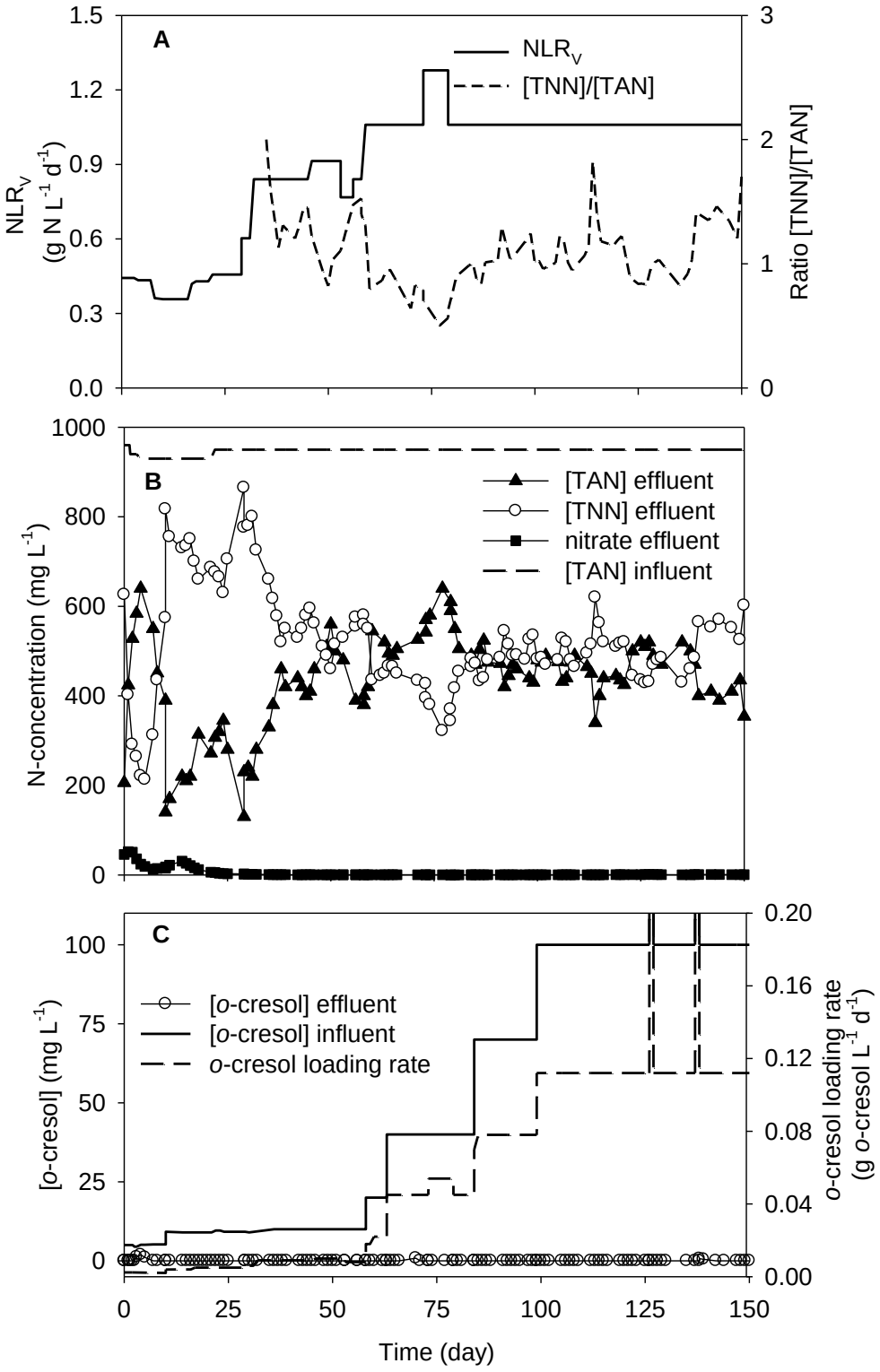

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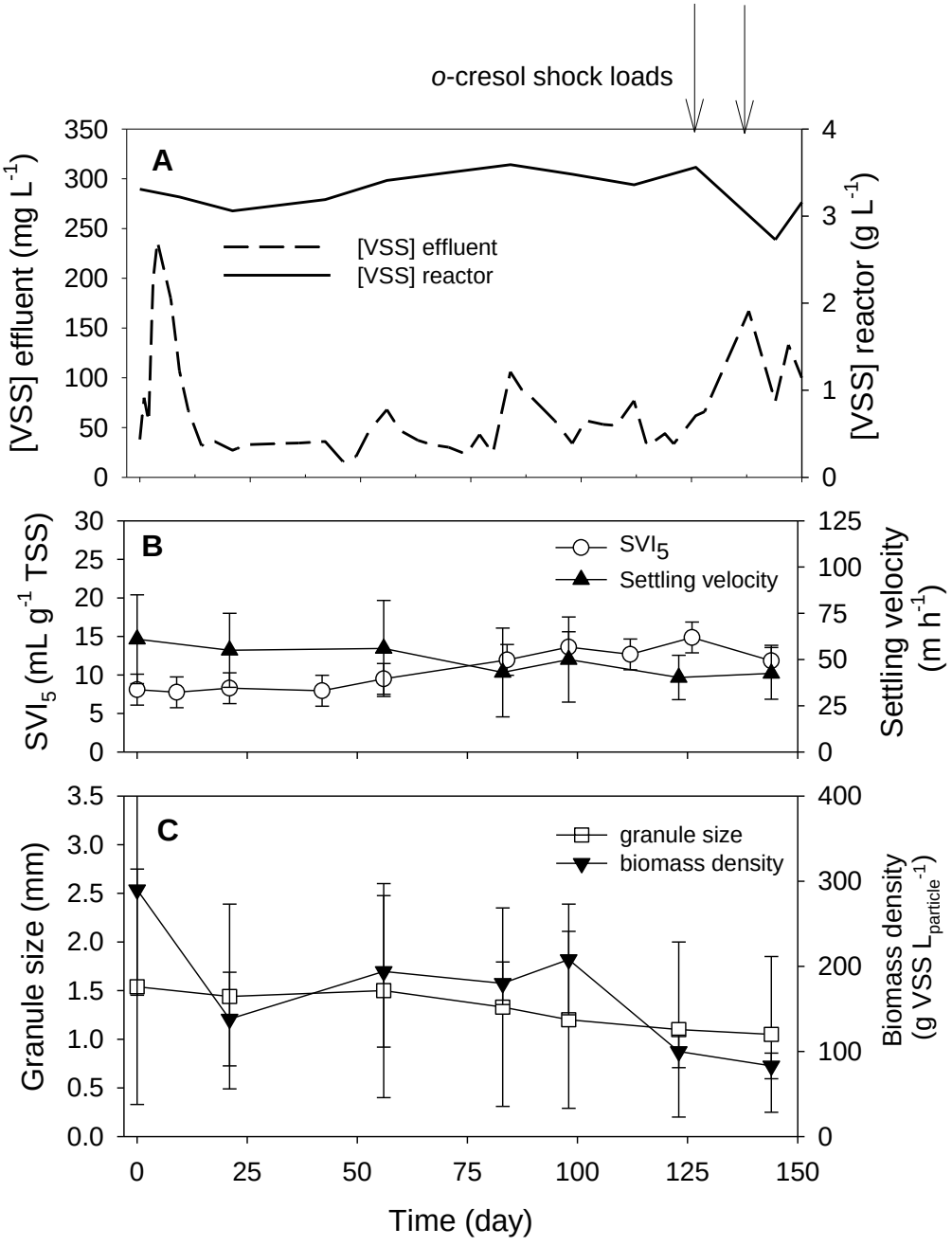

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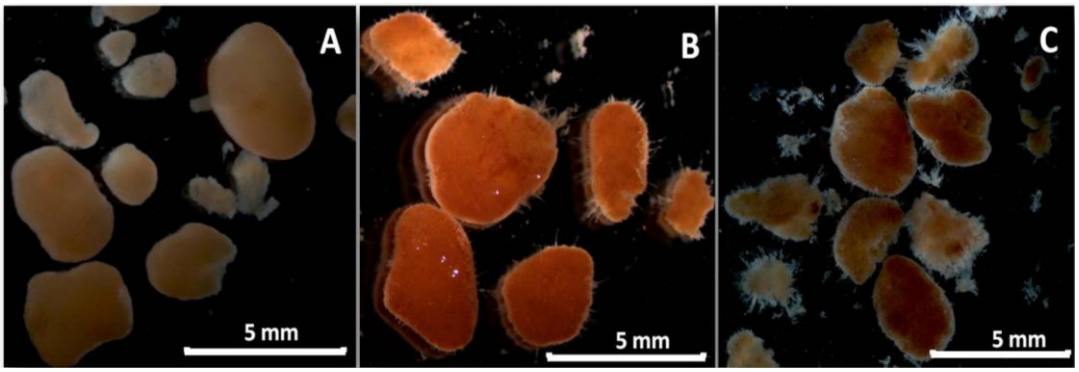

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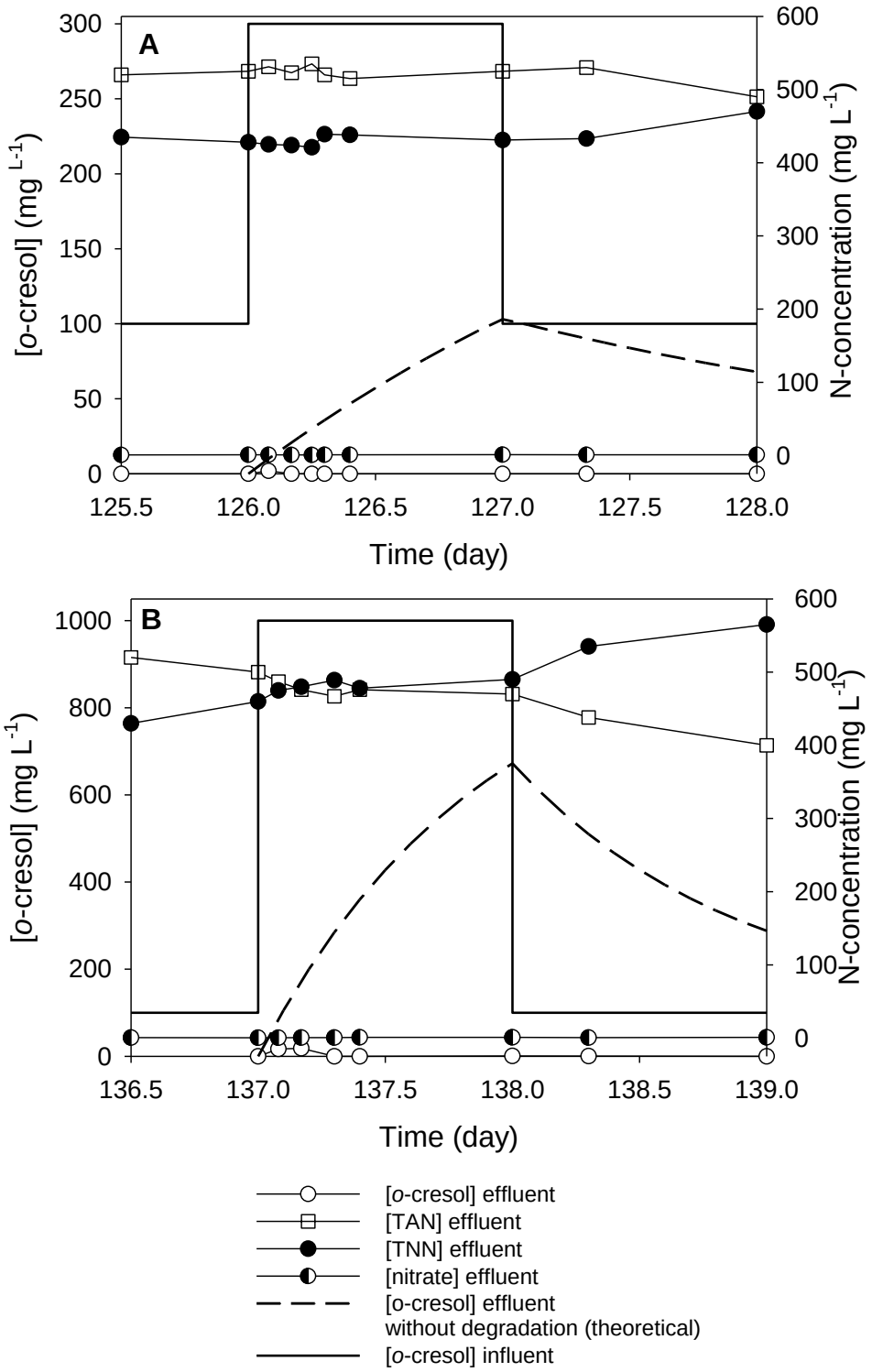

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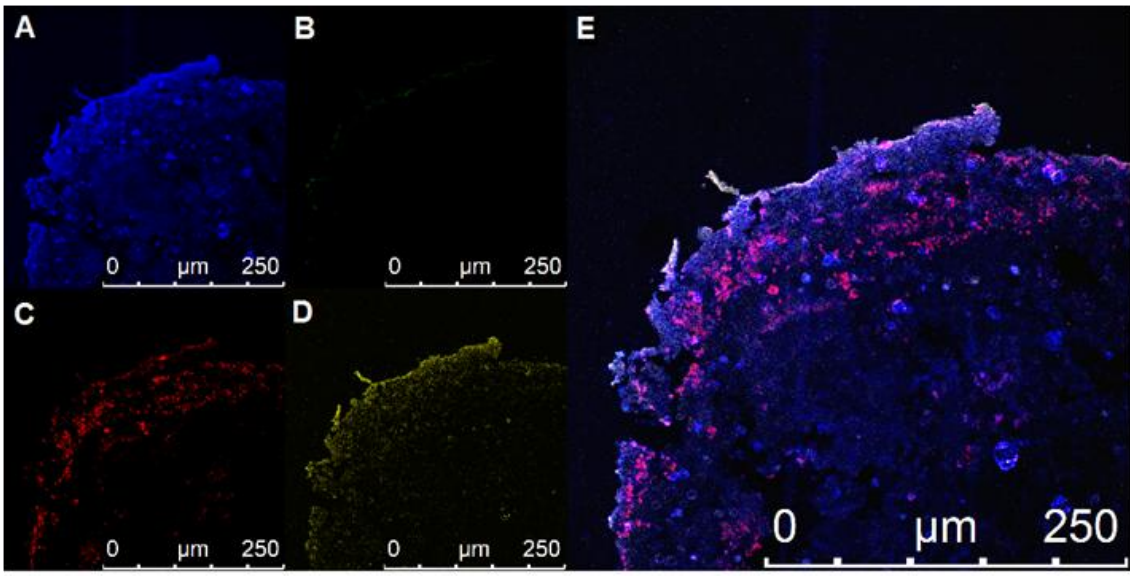

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