Sequentially alternating pollutant scenarios of phenolic compounds in a continuous aerobic granular sludge reactor performing simultaneous partial nitritation and *o*-cresol biodegradation

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Abstract

Industrial wastewater treatment plants must operate properly during the transient-state conditions often found in the industrial production. This study presents the performance of simultaneous partial nitritation and *o*-cresol biodegradation in a continuous aerobic granular reactor under sequentially alternating pollutant (SAP) scenarios. Three SAP scenarios were imposed during the operation of the granular reactor. In each one, a secondary recalcitrant compound (either *p*-nitrophenol (PNP), phenol or 2-chlorophenol (2CP)) were added for a short period of time to the regular

influent containing only ammonium and *o*-cresol. Partial nitritation and *o*-cresol biodegradation were not inhibited by the presence of PNP or phenol and both compounds were fully biodegraded. On the contrary, the presence of 2CP strongly inhibited both processes within two days. However, the reactor was recovered in a few days. These findings demonstrate that treatment of complex industrial wastewaters with variable influent composition is feasible in a continuous aerobic granular reactor.

Keywords

Partial nitrification; aerobic granules; *p*-nitrophenol; phenol; 2-chlorophenol

1. Introduction

Chemical, coke, petrochemicals and refineries industries produce wastewaters containing ammonium and several phenolic compounds (Milia et al., 2012). Often, these effluents are treated using physico-chemical methods, such as adsorption, absorption, incineration and advanced oxidation process (Ahmaruzzaman, 2008; Kim and Ihm, 2011). However, these methods are costly and may not entirely remove the contaminants (Oller et al., 2011). Biological treatment could be a potential solution since it could provide a complete and simultaneous biodegradation of these complex wastewaters at low investment and operational costs. Among all the biological alternatives, an autotrophic biological nitrogen removal (BNR) treatment, i.e. partial nitritation plus anammox, could be regarded as the technology with the cheapest costs and the lowest environmental foot-print for treating ammonium-rich wastewaters (Ahn, 2006). Therefore, a possible technological option for treating these industrial wastewaters could be a two-sludge system composed by an aerobic granular reactor,

where partial nitritiation and phenolic compounds degradation take place simultaneously, followed by an anammox reactor (Jemaat et al., 2014). The aerobic reactor should guarantee an effluent suitable for the subsequent anammox stage, i.e. an effluent with a correct nitrite/ammonium ratio (around one) and without phenolic compounds.

Nevertheless, any biological treatment can confront several problems when facing variable influent characteristics due to: i) temporary stops of the upstream production process, i.e. microorganisms will suffer from starvation due to influent interruption to the industrial wastewater treatment plant (Torà et al., 2011) or ii) variable production schedules, i.e. microorganisms will be exposed to variable influent composition in which the wastewaters are characterized by the presence of sequentially alternating pollutants (SAP) scenarios (Sipma et al., 2010). Although SAP scenarios often occur in full scale industrial wastewater treatment plants, studies on the performance of the biological treatment systems in front of these scenarios have not received much attention (Osuna et al., 2008; Sipma et al., 2010). In particular, studies on the presence of secondary recalcitrant compounds during SAP periods in a biological treatment, especially on the performance of a BNR process have not been reported so far.

The presence of recalcitrant compounds during SAP scenarios can be problematic in activated sludge systems since microorganisms could be inhibited by the presence of these toxic compounds resulting in biomass washout, thereby decreasing the treatment effectiveness and in some cases, causing a complete failure of the treatment system. (Emanuelsson et al., 2008; Sipma et al., 2010). To minimise these effects, aerobic granular sludge systems could be an alternative to overcome the activated sludge

limitation when facing SAP events. Aerobic granular sludge has proven to be feasible, resistant and resilient to dynamic changes in wastewater compositions compared to activated sludge (Jiang et al., 2010; Maszenan et al., 2011; Jemaat et al., 2014). The granular sludge possesses a unique structure assembled by a consortium of microorganisms wherein various species perform different and specific roles in the biodegradation of contaminants during wastewater treatment (Beun et al., 1999). This structure could contribute to create concentration gradients of potentially toxic or inhibitory compounds inside the granules, buffering their overall impact over more sensitive bacteria species (Maszenan et al., 2011).

Therefore, the aim of this study was to investigate the response in front of SAP scenarios of a continuous aerobic granular sludge reactor performing partial nitritation and *o*-cresol removal. Three SAP scenarios were designed to mimic the situation of variable production schedules encountered in an industry resulting in the presence of secondary recalcitrant compounds in the wastewater. *p*-Nitrophenol (PNP), phenol and 2-chlorophenol (2CP) were selected as model phenolic compounds since they are commonly found in the wastewaters produced by coke, chemical, petrochemical and refineries industries. Moreover, these compounds have different behaviour in front of a biological treatment since: (i) PNP is a chemical uncoupler of bacterial metabolism (Low et al., 2000), (ii) phenol is as a biogenic substrate (Aktas, 2012) and (iii) 2CP is, in most cases, a non-growth substrate and an inhibitory compound (Satoh et al., 2005). The investigation is focused both on the performance of the aerobic granular reactor, as well as on the impact on granular sludge characteristics in the course of SAP events.

2. Materials and Methods

2.1. Reactor set-up and operating conditions

The continuous granular sludge reactor performing simultaneous partial nitritation and *o*-cresol removal described in Jemaat et al. (2014) was utilized in this study. The reactor was an airlift type and had a working volume of 2.6 L. 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. 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 and pH were maintained at 2 mg O_2 L^{-1} and 8.1 ± 0.4 in the reactor, respectively. Air flow rate in the reactor was regulated by a rotameter (Aalborg, USA) whereas pH was regulated by the addition of $NaHCO_3$ into the reactor. The temperature in the reactor was maintained at 30 ± 1.0 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).

2.2. Wastewater

The reactor was fed with a synthetic wastewater containing a high-strength ammonium concentration of 3.63 g L^{-1} NH_4Cl (950 ± 25 mg N-NH_4^+ L^{-1}) and *o*-cresol (the primary recalcitrant compound) at a concentration of 100 ± 5 mg L^{-1}. The following compounds and micronutrients were are supplied (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; H_3BO_3, 0.02; $CO(NH_2)_2$, 12.0 and yeast extract, 2.0.

2.3. Description of the sequentially alternating pollutant (SAP) scenarios

Three SAP scenarios were designed and carried out. In each one of them, a secondary recalcitrant compound was added to the influent for a short period of time (details are given in Table 1). Three phenolic compounds namely, PNP, phenol and 2CP were selected as the secondary recalcitrant compound. In each SAP scenario, the wastewater regularly treated in the bioreactor suddenly suffers the addition of a secondary recalcitrant compound to reach a concentration of 15 mg L^{-1} of this phenolic compound. After several days, wastewater composition was set back to its previous composition, containing only the primary recalcitrant compound (*o*-cresol) and the high ammonium concentration.

2.4. Analytical methods

o-Cresol, PNP, phenol and 2CP concentrations were determined by High Performance Liquid Chromatography (HPLC) (UltiMate 3000, Dionex Corporation) using an Agilent Zorbax SB-C18 (4.6 x 100 mm, 3.5 μm) column and a UV detector set at 254 nm, the flow rate was 1.875 mL min^{-1} and the column temperature was maintained at 30 ºC (Martín-Hernández et al., 2009). The mobile phases were ultrapure water containing H_2SO_4 at pH 1.41 and HPLC-grade methanol following a gradient elution.

The gradient started from 100% of acidified water and progressively changed to 50:50 v/v of water:methanol in 18 min, then it remained isocratic until 20 min. The injection volume was 20 μL and the maximum pressure in the column was approximately 290000 hPa. The ammonium concentration measured as total ammonia nitrogen (TAN = $N\text{-}NH_4^+$ + $N\text{-}NH_3$) was analyzed using a continuous flow analyzer based on potentiometric determination. The nitrite, measured as total nitrite nitrogen (TNN = $N\text{-}NO_2^-$ + $N\text{-}HNO_2$) and nitrate concentrations were measured with ionic chromatography (ICS-2000 Integrated Reagent-Free IC System, DIONEX).

Volatile suspended solids (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, biomass density and settling velocity. The size distribution of the granules was measured 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. The density of the granular biomass was determined using the Dextran Blue method described by Beun et al. (2002). The 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 (Bartrolí et al., 2010). Also, the extracellular polymeric substances (EPS) were extracted from the

granules using formaldehyde and NaOH and were analyzed according to Adav and Lee (2011).

Chemicals used in this study were: *o*-cresol and 2CP in concentrated solutions (purity 99%) and PNP in granular form (purity 99%). They were supplied by Panreac (Spain). Ammonium chloride (purity 99.5%) was supplied by Carl Roth (Germany). Phenol and all the others chemicals and reagents were purchased from Sigma-Aldrich (Spain) and the highest purities available were employed.

3. Results and discussion

3.1. Effect of PNP as secondary recalcitrant compound

Before the addition of PNP as secondary phenolic compound (first SAP scenario), partial nitritation was maintained with an average TNN/TAN concentrations ratio of 1.4 in the effluent (Figure 1.a). Nitrate concentration was always below 1 mg $N\text{-}NO_3^-$ L^{-1} and simultaneously, complete *o*-cresol removal was maintained. The average volumetric nitrogen loading rate (NLR_V) and volumetric *o*-cresol loading rate ($oCLR_V$) were 1.1 g N L^{-1} d^{-1} and 0.11 g *o*-cresol $L^{-1}d^{-1}$, respectively. Consequently, the reactor was producing an effluent suitable for a subsequent anammox reactor.

In the first SAP scenario, PNP was added to the influent in a concentration of 15 mg L^{-1} from day 11 to day 37 (Figure 1.c). Immediately, PNP was accumulated in the reactor up to 13 mg L^{-1} (day 22). However, complete PNP degradation was attained on day 33 onwards, when PNP concentration was below HPLC detection limit (0.1 mg PNP L^{-1}).

p-Nitrocatechol, an intermediate of the aerobic PNP degradation (Martín-Hernández et al., 2009), was detected at a concentration of 3 mg L^{-1} from day 14 to day 24 (Figure 1.c). On day 28, *p*-nitrocatechol disappeared. The reactor was able to achieve a volumetric PNP loading rate of 14 mg PNP L^{-1} d^{-1}.

Partial nitritation and *o*-cresol removal remained unaltered during the first SAP scenario (Figure 1.b), even in the period in which PNP and *p*-nitrocatechol were simultaneously accumulated in the reactor (from day 14 to day 28). In fact, the maximum PNP concentration accumulated in the airlift reactor (13 mg L^{-1}) is enough to inhibit the nitritation in an activated sludge system (Jemaat et al., 2014). However, the nitritation was not inhibited in the airlift reactor probably due to the buffer effect produced by the granular structure of the biomass (Goh et al., 2009; Maszenan et al., 2011, Jemaat et al., 2014). During the first half of the SAP event (from day 13 to day 22), the TAN oxidation into TNN was maintained to ca. 62 % with low nitrate concentration in the effluent (Figure 1.b). The NLR_V and $oCLR_V$ remained unaltered during this period at 1.1 g N $L^{-1}d^{-1}$ and 0.11 g *o*-cresol $L^{-1}d^{-1}$, respectively while the TNN/TAN concentrations ratio was of 1.5 (Figure 1.a). In the second half of SAP event (after day 22), the TAN oxidation into TNN was slightly increased by ca. 10 % (Figure 1.b). Consequently, the TNN/TAN concentrations ratio was shifted from an average of 1.5 to 2.0 (Figure 1.a). Complete removal of *o*-cresol was always maintained during this SAP scenario (Figure 1.c). On day 45, the NLR_V was increased purposely to decrease the TNN/TAN concentrations ratio from 2.0 to around 1.2 for maintaining a suitable effluent for an anammox process (Figure 1.a). After the change in NLR_V, the partial nitritation was stably maintained with an average 52 % of TAN oxidation and nitrate concentration below 1 mg $N\text{-}NO_3^-$ L^{-1}. Again, complete *o*-cresol

removal was stably maintained. The achieved NLR_V and $oCLR_V$ were 1.2 g N $L^{-1}d^{-1}$ and 0.12 g *o*-cresol $L^{-1}d^{-1}$, respectively.

In the aerobic biodegradation of PNP and *o*-cresol, the ring fissions of both compounds are catalyzed by a similar ring-cleaving dioxygenases following, then, a similar biodegradation route (McLeod and Eltis, 2008). Therefore, the biodegradation of PNP could be performed by the same microbial consortium degrading *o*-cresol after a short adaptation period.

Biomass concentrations in the reactor and in the effluent were maintained at 3.1 ± 0.5 g VSS L^{-1} and 60 ± 20 VSS mg L^{-1} throughout the first SAP scenario, respectively. Most of the granular sludge characteristics were basically unaffected by the first SAP scenario since size, SVI_5, SVI_5/SVI_{30} ratio and settling velocity were remained rather steady (Table 2, period I and IV). On the contrary, EPS content of the granules changed during the addition of PNP throughout the SAP scenario from 38 ± 5 mg g^{-1} VSS on day 11 at the beginning to 52 ± 5 mg g^{-1} VSS on day 32 at the end. Moreover, EPS content decreased again to 29 ± 3 mg g^{-1} VSS on day 52 after 15 days without addition of PNP in the influent. These changes in the EPS content are probably caused by the presence of PNP in the reactor and its effect over the microorganisms present in the granules. The increase of the EPS content after several days of contact between the aerobic granules and PNP could be due to a higher EPS production as protection tool against a metabolic uncoupler compound such as PNP. In spite of this, overall results show the stability and resistance of aerobic granules towards the presence of PNP as secondary recalcitrant compound during a SAP scenario.

3.2. Effect of phenol as secondary phenolic compound

Before starting the second SAP scenario, the aerobic granular reactor was fed for 16 days with an influent containing only TAN and *o*-cresol to eliminate any influence of the first SAP scenario.

In the second SAP scenario, phenol was added to the influent in a concentration of 15 mg L^{-1} from day 52 to day 73 (Figure 2.c). Neither phenol nor any potential intermediates of the known degradation routes were detected during this SAP scenario, showing that 100 % of phenol degradation was attained. The reactor achieved a volumetric phenol loading rate of ca. 19 mg phenol L^{-1} d^{-1}. Similarly to the previous SAP scenario, partial nitritation and *o*-cresol removal were stably maintained (Figures 1.b and 1.c). The TAN oxidation was maintained at average 52 % of TNN accumulation (Figure 2.b), a similar performance to that achieved before the SAP event. The NLR_V and TNN/TAN concentrations ratio were retained at around 1.2 g N L^{-1} d^{-1} and 1.1, respectively while an $oCLR_V$ of 0.12 g *o*-cresol $L^{-1}d^{-1}$ was remained unchanged (Figure 2.a).

The full degradation of phenol observed in the present study is possibly linked to the ability of heterotrophic bacteria degrading *o*-cresol, to also degrade phenol. In this case, it is indeed possible that phenol degradation is following the same metabolic pathway than *o*-cresol. Masunaga et al. (1986) reported that the main degradation route of *o*-cresol was following the 3-methyl catechol pathway. Then, ring fission is catalyzed by ring-cleaving dioxygenases following the same pathway for phenol biodegradation (McLeod and Eltis, 2008). Moreover, Saravanan et al. (2008)

postulated that phenol is a simpler carbon source than *m*-cresol, and therefore, it could be easily utilized by the mixed culture without any lag phase in its biodegradation, explaining why no accumulation of phenol was detected in the reactor during the SAP scenario.

The granular biomass concentration in the reactor remained basically constant around 3.0 ± 0.2 g L^{-1} during the second SAP scenario. Granular sludge characteristics were not importantly affected by the presence in the wastewater of phenol as secondary recalcitrant compound. Granule size, SVI_5, SVI_5/SVI_{30} and settling velocity were inside the common ranges reported for granular sludge (Table 2, periods IV and VII) (Gao et al., 2011). Differently from the first SAP scenario with PNP, EPS content of the granules remained rather steady during the addition of phenol throughout the SAP scenario from 29 ± 3 mg g^{-1} VSS on day 52 at the beginning to 26 ± 6 mg g^{-1} VSS on day 71 at the end. Consequently, the performance of the granular airlift reactor was unaffected showing the stability of the granular biomass in the presence of phenol as a secondary recalcitrant compound during an SAP event.

3.3. Effect of 2CP as secondary phenolic compound

Before starting the third SAP scenario, the aerobic granular reactor was fed for 17 days with an influent containing only TAN and *o*-cresol to eliminate any influence of the second SAP scenario.

In the third SAP scenario, 2CP was added to the influent in a concentration of 15 mg L^{-1} from day 90 and 2CP was accumulated to 9 mg L^{-1} in the airlift reactor in only 2 days

(Figure 3.c). About 25 % of 2CP was disappearing at that moment, but an unidentified intermediate was detected during this period indicating incomplete 2CP oxidation. Simultaneously, partial nitritation and *o*-cresol degradation were clearly inhibited. In 2 days, TAN and *o*-cresol accumulated in the reactor at 860 mg N L^{-1} and 24 mg L^{-1}, respectively (Figures 3.b and 3.c), corresponding to an inhibition of ca. 90 % of nitritation and 25 % of *o*-cresol biodegradation. As a consequence of the strong inhibition recorded, the SAP event was finalized on day 93. At this moment, the granules were left to settle down at the bottom of the reactor and the supernatant was discarded and replaced with wastewater only containing micronutrients (i.e. without TAN or *o*-cresol). Then, the reactor operation was restarted with an influent containing only TAN without any phenolic compound and with a low NLR_V of 0.2 g N L^{-1} d^{-1}.

The restart-up of the airlift reactor was mainly focused on the recovery first, of nitritation and then, of simultaneous partial nitritation and *o*-cresol removal. From day 93, the reactor was fed continuously with a low NLR_V of 0.2 g N L^{-1} d^{-1}. Since no reactivation of nitritation was observed on day 102 and TAN was accumulated at ca. 600 mg N L^{-1}, the continuous mode of operation was switched to batch mode. Upon the reactivation of nitritation activity, TAN oxidation rate was estimated to be 100 mg N L^{-1} d^{-1}, based on this estimation, continuous operation was restarted with an initial NLR_V set at 0.3 g N L^{-1} d^{-1} on day 107. The NLR_V was increased progressively by keeping always the DO/TAN concentrations ratio lower than 0.01 mg O_2 mg^{-1} TAN until the partial nitritation producing an effluent suitable for a subsequent anammox process was achieved (Figure 3.b). This strategy was used to prevent the oxidation of nitrite into nitrate during the reactivation period as suggested by Bartrolí et al. (2010) and Jemaat et al. (2013). The results showed that 14 days were required for

reactivation of the nitritation and 3 weeks for full recovery of partial nitritation (Figure 3.c). At the end of the recovery period, the reactor achieved a NLR_V of 0.95 g N L^{-1} d^{-1} with 56 % TNN accumulation and TNN/TAN concentrations ratio of 1.2 (Figures 3.a and 3.b). Although the NLR_V achieved after the recovery period was slightly lower compared to that before the SAP event, the specific loading rate achieved (0.4 g N g^{-1} VSS L^{-1} d^{-1}) was quite similar. These results indicate that the granular sludge reactor was able to recover its previous performance after being exposed to a highly inhibitory compound. Once the steady state condition of partial nitritation was accomplished (from day 131), the reactor was re-fed with *o*-cresol, the primary recalcitrant compound (Figure 3.c).

With the reactivation strategy used, the granular reactor had a smooth restart-up of the nitritation process and *o*-cresol was totally degraded from the first day in which *o*-cresol was re-fed into the reactor. Within 4 days, the specific volumetric *o*-cresol loading rate ($oCLR_S$) reached a similar $oCLR_S$ applied prior to the third SAP scenario, i.e. 0.10 g *o*-cresol g^{-1} VSS L^{-1} d^{-1}. Considering that only TAN was fed into the reactor during the restart-up, the heterotrophic bacteria degrading *o*-cresol were starved for a period of 40 days. Possible explanations of the fast *o*-cresol degradation might be that i) *o*-cresol degrading bacteria survived in endogenous conditions obtaining carbon and nitrogen from cell lysis, EPS and TAN (Wang et al., 2006), and ii) granular sludge buffers the negative effects of starvation (Buitrón and Moreno-Andrade, 2011), which would yield short reactivation periods.

The biomass concentration in the reactor slightly decreased from 3.0 to 2.8 g VSS L^{-1}, at the end of third SAP event. This biomass decrease was linked to the wash out of

biomass from the reactor due to the severe inhibition during the SAP period. Nevertheless, most of the granular sludge characteristics were not importantly affected during the SAP scenario with 2CP and after the reactivation period. A slight increase in granule size, SVI_5, granule density and settling velocity was observed (Table 2, period VII and XI). Similarly to the first SAP scenario with PNP, the EPS content increased significantly with the addition of 2CP from 17 ± 2 mg g^{-1} VSS on day 90 at the beginning of the third SAP scenario to 54 ± 6 mg g^{-1} VSS on day 103 at the end. Microbial cells in biofilms produce more EPS to protect themselves from the presence of toxic substances (Sheng et al., 2010). In this case, 2CP acted as a strong inhibitor and the aerobic granules responded with an increase in their EPS content.

Studies on the recovery of partial nitritation after such severe inhibition caused by exposure to phenolic compounds in aerobic granular sludge are not reported to the best of our knowledge. The closest and comparable study was reported by Lim et al. (2012). In their study, the nitrogen removal efficiency in an activated sludge sequencing batch reactor was recovered in 52 days after stopping the introduction of 30 mg 2,4-dichlorophenol L^{-1}. In the present study, a considerably shorter recovery time (21 days) compared to the one reported by Lim et al. (2012) could be linked to the use of aerobic granular and the reactivation strategy imposed during the recovery period. By using granules, the 2CP penetration was probably limited to the external layer, inhibiting only the bacteria allocated in here, whereas the bacteria present in the deeper parts of the biofilm were still alive as found by Satoh et al., (2005) in another granular reactor. The strategy applied during the recovery period was able to i) avoid the problems during start-up due to substrate inhibition; ii) suppress nitrate

accumulation to obtain and easily maintain partial nitritation; thus a fast recovery of the reactor performance was attained.

3.4. Practical implications

This study shows that the applicability of a two-step autotrophic BNR process to treat complex industrial wastewaters containing ammonium and phenolic compounds can be affected by the variability of the characteristics of the influent.

In the first step, an aerobic granular reactor performing simultaneous partial nitritation and *o*-cresol removal can successfully deal with SAP scenarios depending on the nature of the recalcitrant compound added during the SAP event. If the recalcitrant compound can be considered as a growth substrate, such as phenol, or even a biodegradable metabolic uncoupler, such as PNP, the aerobic granular biomass is able to deal with the variability of the influent characteristics. However, if the recalcitrant compound can be considered as a highly inhibitory substance, such as 2CP, the aerobic granular biomass will fail with the wastewater treatment.

In the second step, an anammox reactor will be completely influenced by the performance of the first step in front of the SAP scenarios. If the recalcitrant compound present during an SAP event is completely removed from the beginning, such as in the case of phenol, the anammox reactor will not be affected. If the recalcitrant compound is not completely removed from the beginning, such as PNP or 2CP, this compound will reach the anammox reactor for a period of time and the anammox biomass will be affected since the phenolic compounds can be inhibitors for anammox bacteria (Yang et al., 2012; Martinez-Hernández et al., 2013). Nevertheless,

the effect of most phenols, such as PNP or 2CP, over the anammox bacteria has not been specifically determined and further, an anammox reactor can be adapted to receive phenol as demonstrated by Toh and Ashbolt (2002). More research effort devoted to investigate the effect of phenols on the anammox bacteria and their possible adaptation is required.

4. Conclusions

The simultaneous partial nitritation and *o*-cresol removal in an aerobic granular reactor was unaffected and stably maintained during the SAP scenarios with PNP and phenol as secondary recalcitrant compounds. Moreover, both phenols were totally degraded. However, the presence of 2CP in a third SAP scenario highly inhibited partial nitritation and *o*-cresol degradation within two days. Nevertheless, the reactor could be rapidly re-activated, fully recovering both processes in a few days. The applicability of aerobic granular biomass for the treatment of complex industrial wastewaters with variable influent characteristics depends on the nature of compounds added in the SAP scenarios.

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Figure Captions

Figure 1. Performance of the aerobic granular reactor simultaneously treating ammonium and *o*-cresol under sequentially alternating pollutant (SAP) scenario study with *p*-nitrophenol (PNP). (A) Volumetric nitrogen loading rate (NLR_V), volumetric *o*-cresol loading rate ($oCLR_V$) and TNN/TAN concentrations ratio. (B) Nitritation performance. (C) Removal performance of *o*-cresol, PNP and *p*-nitrocatechol. The theoretical accumulation of PNP in the reactor considering no biological degradation is also depicted for guiding purpose (dashed line).

Figure 2. Performance of the aerobic granular reactor treating simultaneously ammonium and *o*-cresol under sequentially alternating pollutant (SAP) scenario study with phenol. (A) Volumetric nitrogen loading rate (NLR_v), volumetric *o*-cresol loading rate ($oCLR_V$) and TNN/TAN concentrations ratio. (B) Nitritation performance. (C) Removal performance of *o*-cresol and phenol.

Figure 3. Performance of the aerobic granular reactor treating simultaneously ammonium and *o*-cresol under sequentially alternating pollutant (SAP) scenario study with 2-chlorophenol (2CP). (A) Volumetric nitrogen loading rate (NLR_V), volumetric *o*-cresol loading rate ($oCLR_V$) and TNN/TAN concentrations ratio. (B) Nitritation performance. (C) Removal performance of *o*-cresol and 2CP. Between day 102 until 107, the concentrations of TAN, TNN and nitrate were measured from samples withdrawn from the reactor bulk liquid.

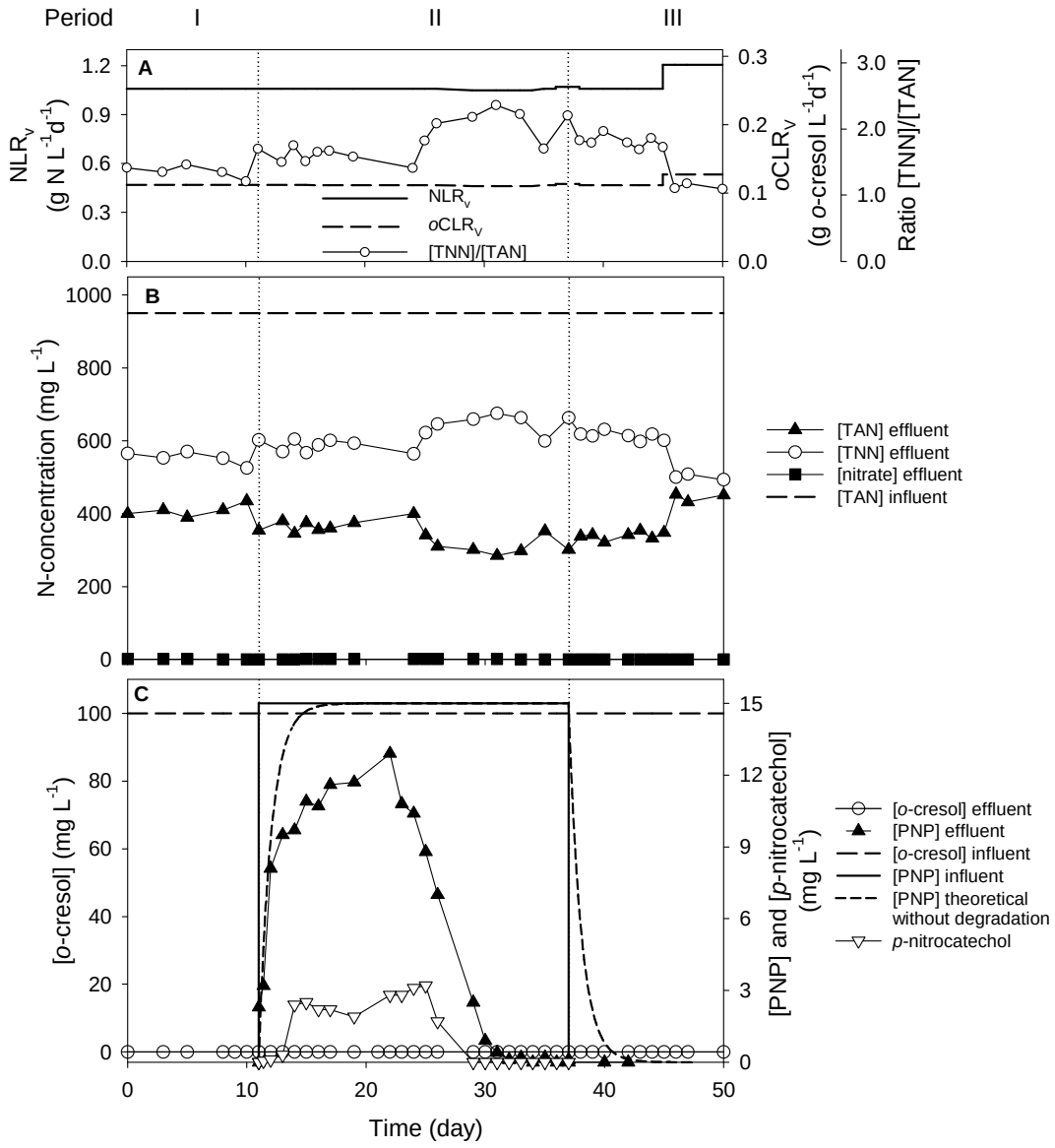
Period
I
II
III
A
B
C
NLR$_v$ (g N L^{-1}d^{-1})
oCLR$_V$ (g o-cresol L^{-1}d^{-1})
Ratio [TNN]/[TAN]
NLR$_v$
oCLR$_V$
[TNN]/[TAN]
N-concentration (mg L^{-1})
[TAN] effluent
[TNN] effluent
[nitrate] effluent
[TAN] influent
[o-cresol] (mg L^{-1})
[PNP] and [p-nitrocatechol] (mg L^{-1})
[o-cresol] effluent
[PNP] effluent
[o-cresol] influent
[PNP] influent
[PNP] theoretical without degradation
p-nitrocatechol
Time (day)

Figure 1.

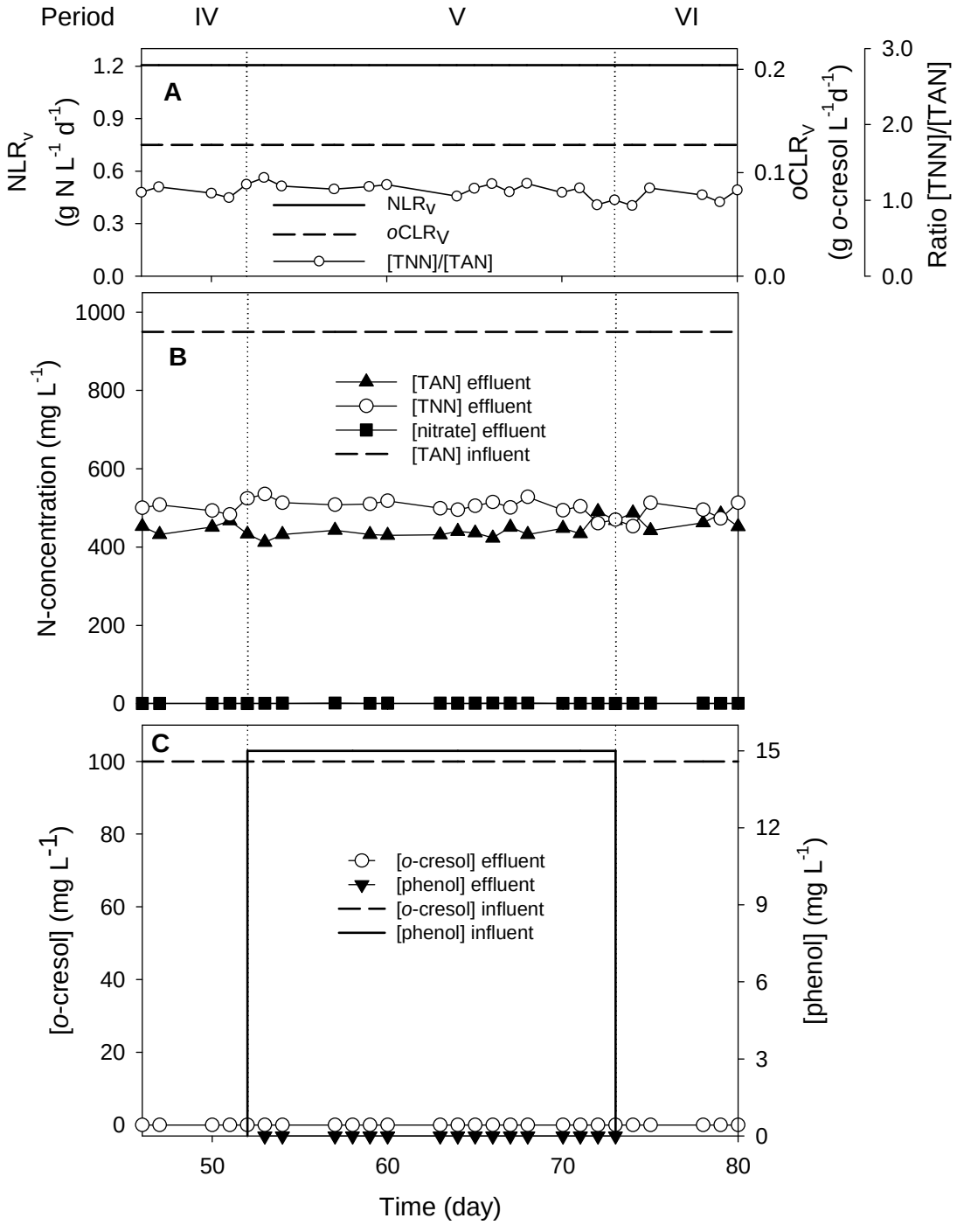
Period
IV
V
VI
A
B
C
NLR$_v$ (g N L^{-1} d^{-1})
oCLR$_V$ (g o-cresol L^{-1}d^{-1})
Ratio [TNN]/[TAN]
NLR$_V$
oCLR$_V$
[TNN]/[TAN]
N-concentration (mg L^{-1})
[TAN] effluent
[TNN] effluent
[nitrate] effluent
[TAN] influent
[o-cresol] (mg L^{-1})
[phenol] (mg L^{-1})
[o-cresol] effluent
[phenol] effluent
[o-cresol] influent
[phenol] influent
Time (day)

Figure 2.

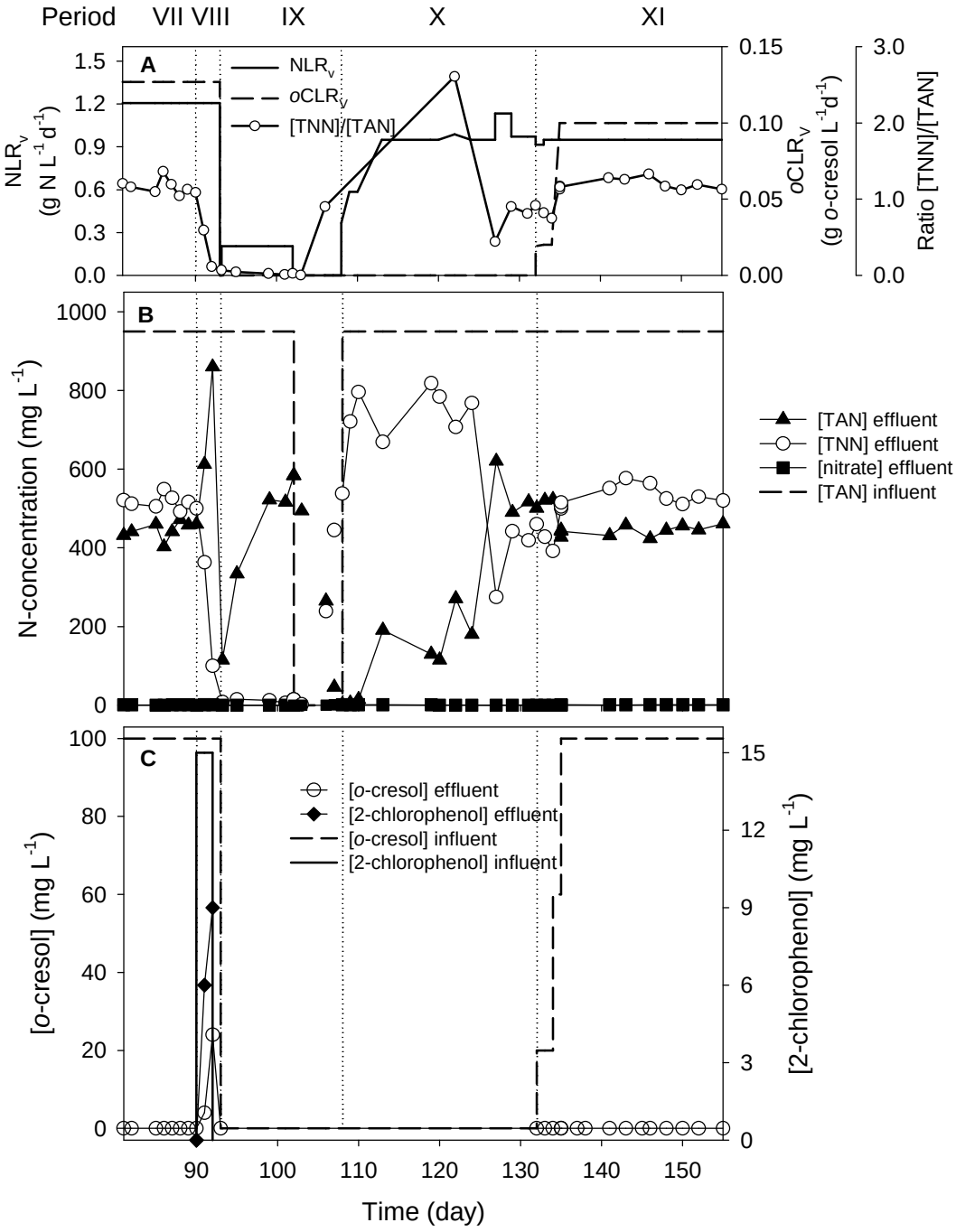
Period
VII VIII
IX
X
XI
A
B
C
NLR$_v$
oCLR$_v$
[TNN]/[TAN]
(g N L^{-1}d^{-1})
(g o-cresol L^{-1}d^{-1})
Ratio [TNN]/[TAN]
N-concentration (mg L^{-1})
[TAN] effluent
[TNN] effluent
[nitrate] effluent
[TAN] influent
[o-cresol] (mg L^{-1})
[2-chlorophenol] (mg L^{-1})
[o-cresol] effluent
[2-chlorophenol] effluent
[o-cresol] influent
[2-chlorophenol] influent
Time (day)

Figure 3.

Table 1. Main compounds present in the wastewater during the continuous operation of aerobic granular sludge reactor under sequentially alternating pollutant (SAP) scenarios study. Concentrations of each substrate are: TAN, 950 mg N L^{-1}; *o*-cresol, 100 mg L^{-1}; *p*-nitrophenol (PNP), 15 mg L^{-1}; phenol, 15 mg L^{-1}; 2-chlorophenol (2CP), 15 mg L^{-1}.

Time (days)	Substrate in the influent	Period	SAP scenarios
0-10	TAN + *o*-cresol	I	
11-36	TAN + *o*-cresol + PNP	II	SAP with PNP
37-45	TAN + *o*-cresol	III	
46-51	TAN + *o*-cresol	IV	
52-72	TAN + *o*-cresol + phenol	V	SAP with phenol
73-80	TAN + *o*-cresol	VI	
81-89	TAN + *o*-cresol	VII	
90-92	TAN + *o*-cresol + 2CP	VIII	SAP with 2CP
93-107	TAN [a]	IX	
108-131	TAN [b]	X	
132-155	TAN + *o*-cresol	XI	

[a]due to reactor failure caused by 2CP addition, the reactor was restarted in batch mode, before switching the operation to continuous mode

[b]reactor resumed in continuous mode

Table 2. Summary of the aerobic granules characteristics during the sequentially alternating pollutant scenarios. Periods: I: initial values; IV: after SAP with PNP; VII: after SAP with phenol; XI: after reactor recovered its normal activity.

	Period I	Period IV	Period VII	Period XI
Granule size (mm) [mean size, range]	[1.1, 0.4-1.8]	[1.0, 0.2-1.8]	[0.9, 0.3-1.5]	[1.1, 0.3-1.9]
Biomass density (g VSS $L_{particle}^{-1}$)	83 ± 10	91 ± 20	73 ± 15	94 ± 13
Settling velocity (m h^{-1})	43 ± 14	53 ± 12	39 ± 16	50 ±18
Sludge volumetric index (SVI_5)	11 ± 5	14 ± 5	14 ± 5	16 ± 5
Ratio SVI_5/SVI_{30}	1.0	1.0	1.0	1.0