Aerobic biodegradation of a mixture of monosubstituted phenols in a sequencing batch reactor

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Abstract

A sequencing batch reactor (SBR) was inoculated with *p*-nitrophenol-degrading activated sludge to biodegrade a mixture of monosubstituted phenols: *p*-nitrophenol (PNP), PNP and *o*-cresol; and PNP, *o*-cresol and *o*-chlorophenol. Settling times were progressively decreased to promote biomass granulation. PNP was completely biodegraded. The PNP and *o*-cresol mixture was also biodegraded although some transitory accumulation of intermediates occurred (mainly hydroquinone and catechol). *o*-Chlorophenol was not biodegraded and resulted in inhibition of *o*-cresol and PNP biodegradation and complete failure of the SBR within a few days. The biomass had very good settling properties when a settling time of 1 min was applied: sludge volume index (SVI_5) below 50 mL g^{-1}, SVI_5/ SVI_{30} ratio of 1 and average particle size of 200 µm.

Keywords

Aerobic biodegradation; *p*-nitrophenol; *o*-chlorophenol; *o*-cresol; sequencing batch reactor

1. INTRODUCTION

Phenolic compounds play an important role in the chemical industry and are wide spread in the environment. Many phenol derivatives are highly toxic [1] and their co-existence in wastes from industrial plants such as oil refineries, coking plants, chemical-synthesis factories, pharmaceuticals, plastic industries, textiles manufacturing and many others is well established [2]. Within the chemical industry, the most prevalent phenolic compounds are nitrophenols, cresols and chlorophenols, which are commonly used for preparing disinfectants, herbicides and fungicides and as synthesis precursors in the manufacture of paracetamol, mesalazine, 2-benzyl-4-chlorophenol and phenacetin.

Treatments for these organic pollutants are mainly physicochemical, although many have serious drawbacks, such as high installation and running costs, formation of secondary hazardous by-products or incomplete mineralization [3-5]. However, biodegradation has become an emerging technological option to degrade such compounds with effective mineralisation and economical processing [6,7]. Bacteria have been used in several studies on the degradation of phenolic compounds [6-8, among others]. Some genus of Gram-negative bacteria: *Pseudomonas*, *Sphingomonas*, *Acinetobacter*, *Ralstonia* and *Burkholderia* and Gram-positive genus *Rhodococcus* have been reported to degrade xenobiotic aromatic compounds [9,10].

Importantly, Martín-Hernández *et al.* [6] reported the enrichment of an activated sludge able to biodegrade *p*-nitrophenol (PNP) up to 0.26 g PNP g^{-1} VSS d^{-1} through two different metabolic pathways: through hydroquinone and through 4-nitrocatechol. This PNP-degrading sludge was mainly composed of *Acinetobacter* and *Arthrobacter* bacteria [10] and can biodegrade mixtures of dihydroxybenzenes [8]. Aerobic biological processes have also been reported to remove cresols [7,11] and chlorophenols [12,13]. Recently, Nielsen and Ingvorsen [14] demonstrated that a strain of *Citricoccus*, capable of effectively degrading PNP, was also able to degrade (and grow on) *p*-

chlorophenol and phenol, but not on *o*-nitrophenol, *m*-nitrophenol or *o*-chlorophenol. Few studies have been done on the aerobic biodegradation in continuous reactors of mixtures of phenols with different substituents (nitro, methyl and chloro functional groups). Gallego et al. [7] reported the simultaneous biodegradation of phenol, *o*-chlorophenol and *m*-cresol in an aerobic fluidized-bed reactor with the biomass supported on activated carbon. Recently, Silva et al. [15] reported the simultaneous removal of *o*-cholorophenol, phenol, *p*-cresol and *p*-hydroxybenzaldehyde under nitrifying conditions. In both these studies, high-strength phenolic wastewaters (≈ 200 mg C L^{-1}) were treated.

Aerobic granular biomass has been employed to remove nitrophenols [16-18], cresols [19] and chlorophenols [20], and shows several advantages relative to activated sludge processes, such as a larger capacity to withstand toxic substrates and stressful loadings, higher biomass retention, no requirement for a settler and enhanced biodegradation at high-loading rates [21]. To the best of our knowledge, aerobic granules have not been used for simultaneous removal of mixtures of phenols with different functional groups.

This study investigates the aerobic biodegradation of a mixture of monosubstituted phenols (≈ 200 mg C L^{-1} in the influent) by using aerobic granular biomass.

2. MATERIALS AND METHODS

2.1 Batch tests with a PNP acclimated sludge

Batch experiments were performed to assess the possibility of biodegrading different phenolic compounds with activated sludge acclimated to consume PNP [6]. The tested compounds were *o*-nitrophenol, *o*-cresol, *p*-cresol, *o*-chlorophenol and *p*-chlorophenol. Each compound was tested as the sole carbon source in the batch reactor then, binary mixtures of PNP and each of the compounds were used.

The batch reactor consists of a magnetically stirred vessel with a working volume of 1 L.

Continuous aeration was achieved from the bottom through a microdiffuser and a non-limiting dissolved oxygen (DO) concentration (above 4 mg O_2 L^{-1}) was maintained. The temperature of the vessel was controlled at 25±0.5 ºC with a thermostatic bath. The pH was continuously monitored with a pH probe (WTW-Sentix 81) and maintained (proportional control) at 7.5±0.2 by addition of acid or base with an automatic micro-burette (Crison MultiBurette 2S). DO concentration was measured with a WTW-CellOx 325 probe.

2.2. Experimental set-up

2.2.1. Reactor and operational strategy for a long-term experiment

The reactor employed for a long-term experiment was a SBR working as a bubble column with a volume of 2 L operated at room temperature (20-25ºC). The pH of the SBR was not adjusted and ranged between 7 and 8. Its height to diameter ratio was 12, which is relatively high to obtain high flow velocities, and facilitate the aerobic granule formation and selection [22]. At start-up, the air-flow rate was 0.9 NL min^{-1} and increased to 2.5 NL min^{-1} after 42 d. This flow-rate was constant until the end of the experiment and was enough to maintain high shear-stress in the reactor, which is a key factor for granulation [23].

The exchange volume was fixed at 25% throughout the operational period. For first 54 d, the total cycle time was 6 h and the hydraulic retention time (HRT) was 1 d. From day 55 until the end of the experiment, the total cycle time was increased to 12 h and the HRT was 2 d. Settling time was also modified to select a fast-settling biomass and promote granulation of the activated sludge.

The reactor was inoculated with 1 L of the same enriched PNP-degrading activated sludge previously used for the tests described in Section 2.1. The initial concentration of biomass in the reactor was 4 g VSS L^{-1}.

The feeding medium was a synthetic wastewater prepared with a constant total organic carbon (TOC) concentration of 400 mg C L^{-1}. For the first 148 d, 25% of the TOC was supplied by sucrose,

25% by glucose and 50% by PNP (200 mg C L^{-1}). From days 148 to 162, *o*-cresol was introduced into the feeding composition, supplying 10% of the TOC (40 mg C L^{-1}), whereas the PNP concentration was reduced to 40% of TOC (160 mg C L^{-1}). From days 162 to 265, PNP and *o*-cresol provided 25% TOC each (100 mg C L^{-1} each). Finally, from day 265 to the end of the operation, 5% of the TOC was provided by *o*-chlorophenol (20 mg C L^{-1}), and PNP and *o*-cresol supplied 22.5% each (90 mg C L^{-1} each). Micronutrients were also added to the feed according to Martín-Hernández *et al.* [6].

2.2.2. Observed and synthesis growth yield calculations

Observed and synthesis growth yields were calculated when stable operation of the reactor had been achieved. Observed growth yield (Y_{obs}) was calculated as the variation of the biomass [measured as volatile suspended solids, (VSS)] in the reactor plus the biomass loss in the effluent divided by the total consumption of organic matter (measured as TOC) in the same period. Synthesis growth yield (Y) was calculated by using the following equation:

$$Y_{obs} = \frac{Y}{(1 + b_H \cdot SRT)} \qquad (1)$$

where:

b_H: decay rate coefficient for heterotrophs (d^{-1})

SRT: sludge retention time (d)

2.3. Analytical and microbiological methods

Phenols and their metabolic intermediates were analysed by HPLC (UltiMate 3000, Dionex Corporation), with an Agilent Zorbax SB-C18 column and a UV detector [6]. TOC was measured with an OI Analytical TOC Analyzer (Model 1020A) equipped with a non-dispersive infrared

detector and a furnace maintained at 680 ºC. Total suspended solids (TSS) and VSS, as well as, sludge volumetric index after 5 and 30 min of settling (SVI_5, SVI_{30}, respectively) were determined according to Standard Methods [24]. Particle sizes were measured with a Malvern Mastersizer 2000 particle size analyser.

Fluorescence in-situ hybridization (FISH) identification was performed according to Suárez-Ojeda *et al.* [10]. In situ hybridization was carried out over polycarbonate filters and two pre-treatment steps were used to improve permeation through the cell wall. The probes used were UNIV1390, targeting all bacteria; Burkho, for Burkholderiaceae family; KO 02, for *Arthrobacter* sp.; ACA652, targeting the genus *Acinetobacter*; and Ppu, for *Pseudomonas* sp.

3. RESULTS AND DISCUSSION

3.1. Batch tests with a PNP-degrading activated sludge

A PNP-degrading activated sludge, composed of 26±2 % *Arthrobacter* sp. and 31±10 % genus *Acinetobacter* [10], was employed in batch tests in which each phenolic compound was the sole organic carbon source. These experiments did not show any significant degradation in the short-term except, for the PNP (data not shown). After that, PNP was supplied in all tests together with one of the other compounds. This strategy would determine if non-specific enzymes were responsible for PNP biodegradation and if they might be able to degrade other phenols to some extent, as well as, providing information about hypothetical inhibitory effects caused by each compound on PNP biodegradation. This hypothesis was based on the fact that the kinetics of biodegradation of a phenolic compound could be altered by the presence of other phenols that microorganisms can oxidize [25]. Moreover, the kinetics could be influenced by the formation and accumulation of metabolic intermediates [26-28]. For example, Bordel et al. [26] found that the aerobic biodegradation of benzene by *Pseudomonas putida* F1 could be inhibited by the accumulation of catechol, an intermediate in the metabolic pathway. In that case, catechol could act

as an inhibitor for benzene oxidation or as a self-inhibitor for catechol oxidation. Vázquez-Rodriguez et al. [27] also found that the aerobic biodegradation of phenol can be modelled through the formation of metabolic intermediates that could act as inhibitors of the phenol oxidation or as self-inhibitors of oxidations.

No inhibitory effect on PNP degradation was observed and the specific PNP removal rate was not affected by the presence of monosubstituted phenols (Figure 1). The average specific PNP removal rate was 0.29±0.04 g C_{PNP} g^{-1} VSS d^{-1}. This value is about two times the maximum specific removal rate obtained by Martín-Hernández *et al.* [6], which could be explained by an increase in PNP degradation rate of this culture caused by long-term acclimation. However, regarding the other phenolic compounds used as co-substrates, very small decreases in concentrations were observed, with differences between the initial and final values of $\simeq$ 1-2 mg L^{-1}, which can be attributed to concurrent volatilization and degradation. These low biodegradation rates could be a consequence of the inhibitory effect caused by the accumulation of hydroquinone, a metabolic intermediate of PNP biodegradation (Figure 1).

Pramparo *et al.* [8] performed similar tests with the same PNP-degrading activated sludge and three dihydroxybenzene isomers (catechol, resorcinol and hydroquinone) and found that the biomass consumed single isomers as well as binary or ternary mixtures. However, they reported that biodegradation rates were significantly different and influenced by the presence of other isomers. Pramparo *et al.* [8] proposed that the first step in biodegradation was the rate-limiting (controlling) step. The first bioconversion is different depending on the substituent present for each phenolic compound or the position of the substituent group and therefore may affect the global degradation rate. A similar explanation might be applicable to these results, suggesting that specific enzymes could be related to this rate-limiting step.

3.2. Performance of the aerobic SBR

3.2.1. Removal of monosubstituted phenols

In the previous section, the simultaneous degradation of monosubstituted phenols was not achieved within a short time-scale and the results suggested that an acclimation process for the PNP-degrading activated sludge should be carried out. Consequently, the next objective was to investigate if the PNP-degrading biomass was able to simultaneously remove PNP, *o*-cresol and *o*-chlorophenol in continuous operation. Additionally, granulation of the activated sludge was promoted to achieve aerobic granules with superior properties relative to those in an activated sludge.

The SBR operation began with PNP as the sole phenolic compound in the influent with sucrose and glucose as biodegradable co-substrates to support biomass growth (Figure 2A). The removal efficiency of PNP and TOC was 100% during this period except for a single accumulation of PNP on day 54 (Figure 2B and 2C). PNP concentration reached a value of 24 mg L^{-1} in the SBR during this incident and was sufficient to cause substrate inhibition [29]. Consequently, the phenolic loading rate was reduced by increasing the HRT to 2 d to prevent further PNP accumulations. After increasing the HRT, no further accumulations occurred and the SBR achieved steady-state conditions in terms of TOC and PNP removal.

On day 148, *o*-cresol was introduced into the feed (Figure 2A). Initially, *o*-cresol only represented 20% of the total phenolic load (the rest being PNP) but after 14 d, the influent concentration of *o*-cresol was increased to 100 mg C L^{-1} and the concentration of PNP was decreased to 100 mg C L^{-1} so that *o*-cresol became half of the phenolic load (Figure 2A). By using this method, *o*-cresol was totally consumed. However, the introduction of *o*-cresol caused the accumulation of intermediates of PNP and *o*-cresol biodegradation, mainly hydroquinone and catechol (Figure 2C), despite of the total phenolic loading rate remaining the same as when PNP was the sole phenolic compound. Hydroquinone is a well-known metabolic intermediate of aerobic PNP biodegradation and was identified as one of the intermediates formed and consumed by the PNP-degrading activated sludge

used as inoculum in this study [6]. Catechol is a metabolic intermediate in the aerobic biodegradation of *o*-cresol and also in the biodegradation of other phenolic compounds [9,18]. Both intermediates have been described as inhibitory or toxic compounds for a non-acclimated heterotrophic biomass at concentrations as low as 4 mg L^{-1} [11]. Nevertheless, the accumulation of up to 15 mg L^{-1} of both compounds did not cause any significant effect (Figure 2C) probably owing to the acclimation of the biomass to these compounds [11] or/and the protection effect of granulation [21]. After 220 d, hydroquinone and catechol had almost disappeared from the SBR and a removal efficiency of 100% in terms of TOC, PNP and *o*-cresol was maintained for 40 d.

On day 265, *o*-chlorophenol was introduced in the influent as 10% of the phenolic loading rate (20 mg C L^{-1}, Figure 2A). Although the results of the batch tests did not suggest any short-term inhibitory or toxic effect of this compound with PNP-degrading biomass (Figure 1B), the SBR performance was immediately affected. *o*-Cresol biodegradation was almost completely inhibited with concentrations in the SBR reaching 70 mg L^{-1} (Figure 2C). PNP biodegradation was less affected and concentrations reached 10 mg L^{-1}. However, *o*-chlorophenol was barely biodegraded (Figure 2C). This compound has been described as highly toxic for non-acclimated heterotrophic bacteria [30] and likely the cause of complete failure of the aerobic SBR after 10 d of *o*-chlorophenol accumulation (Figure 2C).

3.2.2. Granulation process

The strategy for achieving granulation of the inoculated activated sludge was progressive reduction of the settling time in the SBR (Figure 3). During the first 40 d the biomass concentration was almost constant (Figure 3A) despite of the reduction in settling time from 30 to 3 min. This means that the inoculum had very good settling properties, for example, a very low SVI_5 although average particle size was small ($\approx$ 150 µm) (Figure 3B).

Because the average particle size was under the threshold to be defined as granular biomass (200

µm, [31]), the settling time was reduced to 2 min to induce granulation. The effect was a significant decrease in biomass concentration and consequently, an increase in biomass concentration in the effluent (Figure 3A). However, the average particle size did not significantly change with this settling time (Figure 3B). When the settling time was reduced to 1 min the average particle size after one month was 200 µm (Figure 3B). At this time, the SVI_5 was low ($\approx$ 50 mL g^{-1}) and the SVI_5/SVI_{30} ratio was 1. In view of these results, the biomass might be considered as aerobic granules. The specific phenolic loading rate reached a constant and high value of 0.4 g C g^{-1} VSS d^{-1} (Figure 3C). This value is about three-fold higher than that reported by Martín-Hernández et al. [6] for PNP-degrading activated sludge. The specific loading rate is also higher than previously achieved in (i) batch tests with flocculent biomass (0.29 g C g^{-1} VSS d^{-1}, Section 3.1), (ii) the value reported for a granular SBR fed with PNP (0.24 g C g^{-1} VSS d^{-1} [16]) and (iii) the value reported for a granular SBR fed with *o*-nitrophenol (0.01 g C g^{-1} VSS d^{-1} [18]). The higher specific removal rate achieved in this study could be due to the selection of a biomass with faster growth rate than used in previous studies. This selection was made through the progressive shortening of the settling time (Figure 3) and favouring the formation of small granules. The size of these granules, together with the very short settling time produced sludge with a high active fraction, i.e. high specific removal rate.

Nevertheless, the biomass concentration in the SBR was low ($\approx$ 300 mg VSS L^{-1}) and a progressive increase in the settling time was planned to increase the biomass concentration in the reactor maintaining the properties of the granules. An increase of the settling time to 5 min did not cause any significant change in biomass concentration, SVI_5 or average particle size. At a settling time of 10 min, the biomass concentration in the reactor reached almost 3000 mg VSS L^{-1} in a few days. However, under these conditions the properties of the biomass deteriorated. The SVI_5 increased to 125 mL g^{-1} and the average particle size decreased to 120 µm with the loss of granular properties to become a flocculent biomass. On day 210, the settling time was reduced again to 5 min and the

SVI_5 decreased appreciably within a few days and the average particle size augmented to 170 µm. However, the introduction of *o*-chlorophenol in the influent on day 265 resulted in destabilization of the SBR and total loss of the biomass (Figure 3A).

In summary, the biomass reported in this study can only be considered as granular for the period of 100 d during which the settling time was 1 or 2 min and the SBR was fed with a mixture of PNP and *o*-cresol. This granular biomass had a SVI_5 lower than 50 mL g^{-1}, a SVI_5/SVI_{30} ratio of 1 and an average particle size of 200 µm. However, the biomass concentration in the reactor was very low. When the settling time was increased to grow the biomass concentration, the granular biomass properties were lost. Suja et al. [17] reported that formation of aerobic granules fed only with PNP was not possible because PNP is a chemical uncoupler which interferes with the granulation process. In this study, sucrose and glucose were used as easily biodegradable co-substrates, analogously to the work of Yi et al. [16] in which aerobic granules were formed with an influent containing PNP and glucose. However, Yi et al. [16] inoculated their reactor with activated sludge from a wastewater treatment plant, whereas in this study the inoculum was a PNP-degrading activated sludge cultivated for more than 4 years in a lab-scale SBR. Suja et al. [17] suggested that PNP might inhibit many microbial species involved in granulation. In this study, the PNP-degrading activated sludge might lack these species. Suja et al. [17] also reported that aerobic granules fed with acetate could lose their structural integrity when fed PNP.

3.3. Observed and synthesis growth yields calculation and microbial characterization

Observed (Y_{obs}) and synthesis (Y) growth yields were calculated at certain points during operation with PNP as the sole phenolic compound and also with PNP and *o*-cresol as phenolic compounds in the influent (Table 1). Y_{obs} values were obtained from experimental data of solids formation and organic matter consumption and Y values were calculated according to equation (1) and by using the calculated Y_{obs} values, the operational SRT in the SBR (3 d) and an appropriate decay rate

coefficient (b_H=0.2 d^{-1} at 20ºC [32]). The results show that the higher the percentage of *o*-cresol in the influent, the higher the growth yields (Table 1) because *o*-cresol can be considered as a biogenic substrate for heterotrophic biomass. However, PNP is a well-known chemical uncoupler of oxidative phosphorylation that reduces the growth yield of a heterotrophic population because it dissociates anabolism from catabolism. The energy generated from the catabolism-associated substrate consumption with PNP is greater than the anabolism requirement which results in excessive energy spilling [33,34].

The synthesis growth yield obtained by Martín-Hernández et al. [6] for an enriched PNP-degrading activated sludge fed only with PNP was Y=0.20 g VSS g^{-1} PNP (0.053 g VSS g^{-1} COD_{PNP}) whereas the typical synthesis growth yield for heterotrophs fed with easily biodegradable organic matter is Y=0.57 g $COD_{biomass}$ g^{-1} COD (0.40 g VSS g^{-1} COD [32]). The synthesis growth yield obtained with PNP as the sole phenolic compound in the influent (0.26 g VSS g^{-1} COD) was almost equal to that obtained from a mixture of 90% PNP and 10% *o*-cresol (0.27 g VSS g^{-1} COD). These values are significantly higher than those obtained with PNP as the sole organic carbon source by Martín-Hernández et al. [6] because in our study, half of the organic loading rate was composed of easily biodegradable compounds (sucrose and glucose). However, the synthesis growth yields of this study are significantly lower than those for heterotrophic biomass consuming biodegradable compounds and this is because of the chemical uncoupler effect of PNP. The reduction of biomass production owing to the PNP effect was 35% with respect to growth yields and similar to values reported in the literature (30-40%) [33,34].

FISH analyses were performed during the removal of PNP and *o*-cresol to identify the microbial population (Figure 4). *Acinetobacter* sp., *Arthrobacter* sp. and *Pseudomonas* sp. were identified: the first two species were the same as those described for the PNP-degrading activated sludge used as inoculum in this study [10]. These three species have been widely described as phenolic-degrading bacteria [9,25,35], therefore, it was not surprising that they were present and active in the SBR of

this study.

4. CONCLUSIONS

The simultaneous biodegradation of *p*-nitrophenol and *o*-cresol is feasible in an aerobic sequencing batch reactor with high removal efficiency. Transient accumulations of some intermediates, mainly hydroquinone and catechol, were detected. The addition of *o*-chlorophenol in the influent, jointly with PNP and *o*-cresol, caused complete failure of the SBR.

Small granules (200 µm) were obtained with a settling time of 1 min and settling properties were good: the sludge volume index (SVI_5) was below 50 mL g^{-1} and the SVI_5/SVI_{30} ratio was 1. Nevertheless, the granular sludge concentration was rather poor.

The phenolic-degrading species in the granules were identified through FISH analysis as *Pseudomonas* sp., *Acinetobacter* sp. and *Arthrobacter* sp.

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FIGURES CAPTIONS AND TABLE LEGENDS

Figure 1. Time course of phenolic compounds concentration through batch tests with a *p*-nitrophenol degrading activated sludge. A) Batch test with *p*-nitrophenol and *o*-cresol as substrates. B) Batch test with *p*-nitrophenol and *o*-chlorophenol as substrates.

Figure 2. A) Time course of *p*-nitrophenol, *o*-cresol and *o*-chlorophenol concentrations (measured as TOC) in the influent of the SBR. B) Time course of total phenols concentration (measured as TOC) in the effluent of the SBR. C) Time course of *p*-nitrophenol, *o*-cresol, *o*-chlorophenol, hydroquinone and catechol concentrations in the effluent of the SBR.

Figure 3. A) Time course of biomass concentration in the SBR and its effluent. B) Time course of the average particle size and SVI_5. C) Time course of the volumetric and specific phenolic loading rates applied to the SBR.

Figure 4. FISH images. Merge CLSM image obtained from simultaneous in-situ hybridisation with: A) Cy3-5'-end-labelled ACA652 probe (*Acinetobacter* sp.), B) FITC-5'-end-labelled KO 02 probe (*Arthrobacter* sp.), C) Texas Red-5'-end-labelled Ppu probe (*Pseudomonas* sp.). In blue: Cy5-5'-end-labelled UNIV1390 probe (all bacteria).

Table 1. Observed and synthesis growth yield coefficients calculations.

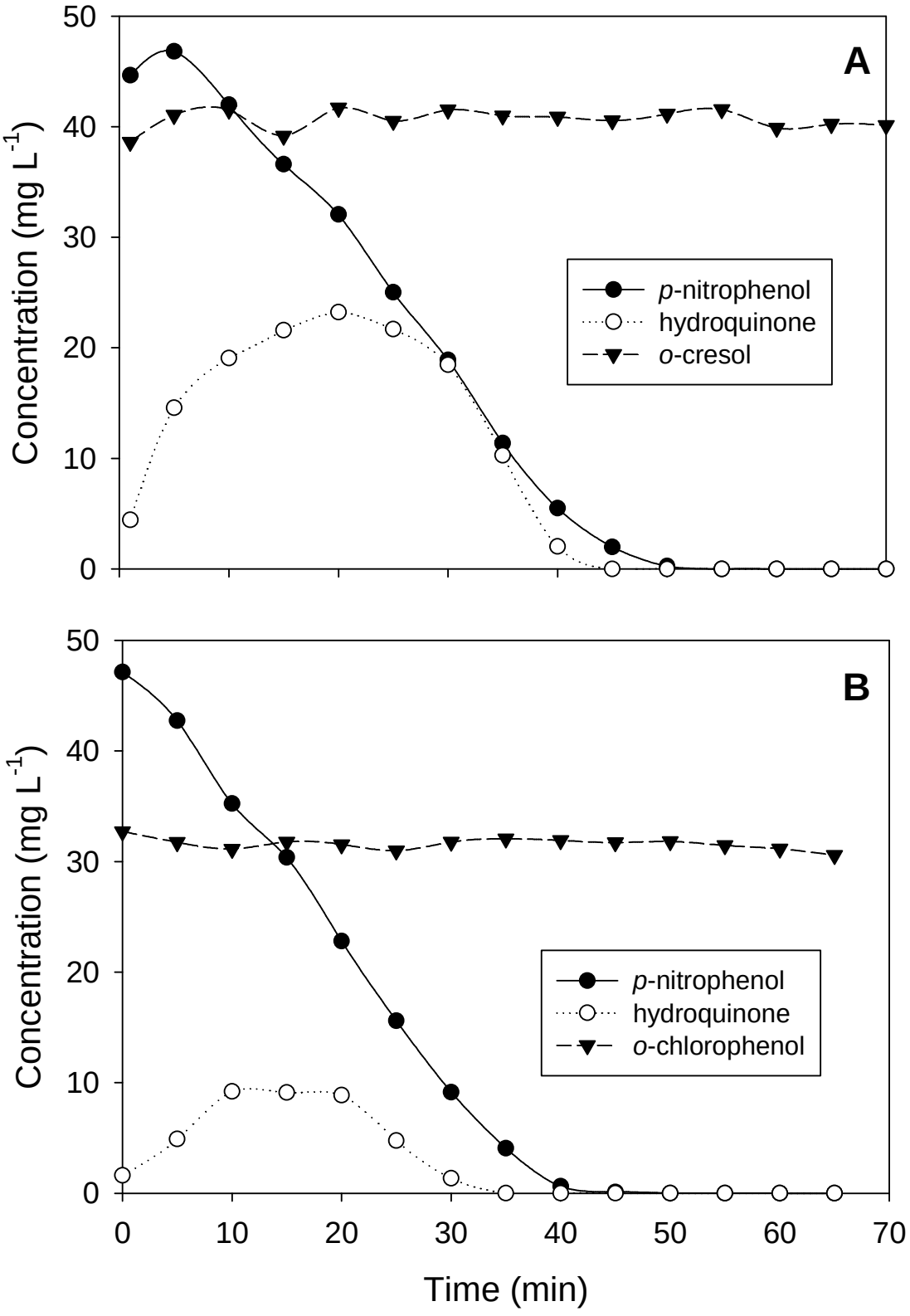

A
B
Concentration (mg L-1)
Time (min)
p-nitrophenol
hydroquinone
o-cresol
o-chlorophenol
0
10
20
30
40
50
60
70

Figure 1

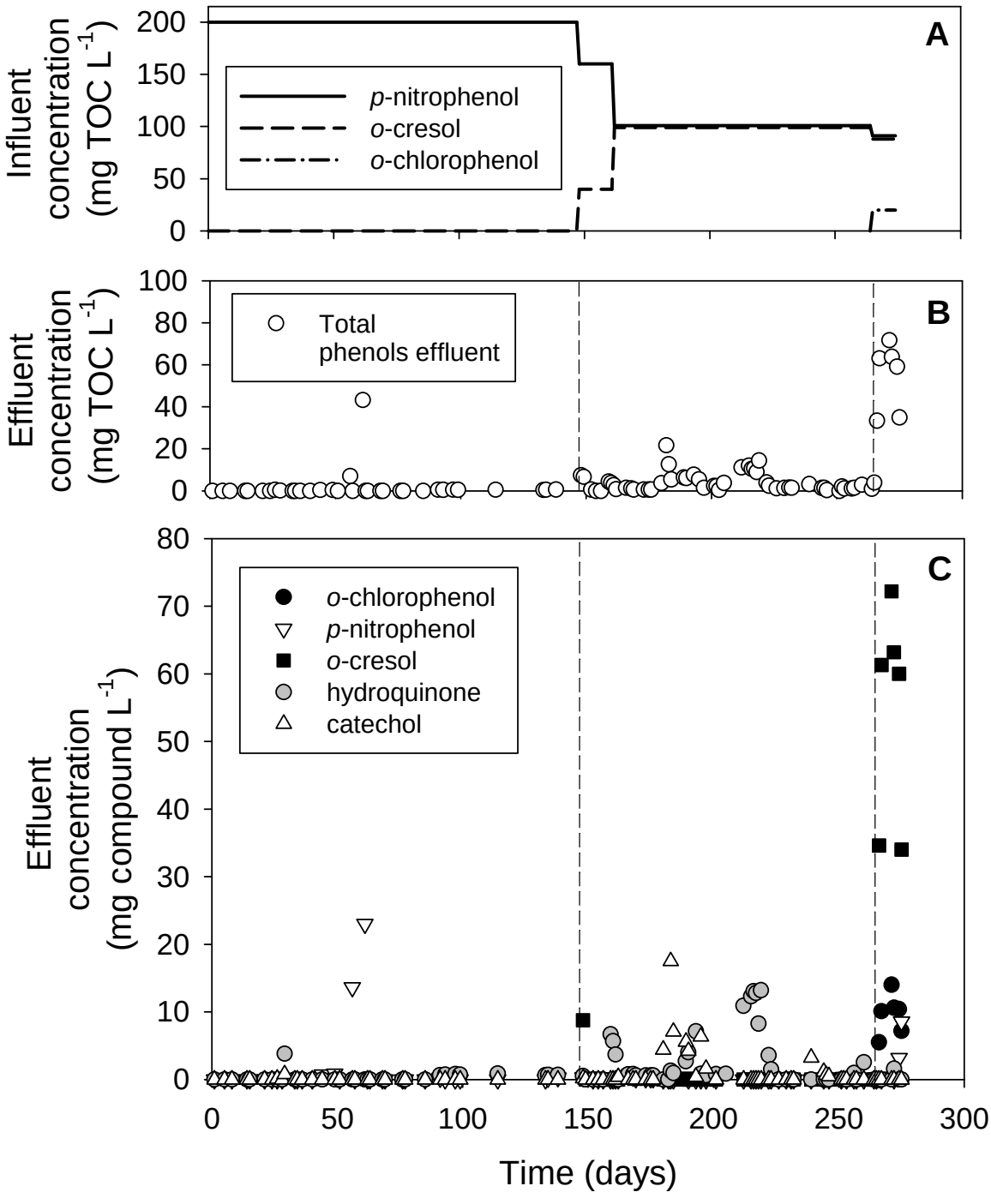

A
Influent concentration (mg TOC L-1)
200
150
100
50
0
p-nitrophenol
o-cresol
o-chlorophenol
B
Effluent concentration (mg TOC L-1)
100
80
60
40
20
0
Total phenols effluent
C
Effluent concentration (mg compound L-1)
80
70
60
50
40
30
20
10
0
o-chlorophenol
p-nitrophenol
o-cresol
hydroquinone
catechol
0
50
100
150
200
250
300
Time (days)

Figure 2.

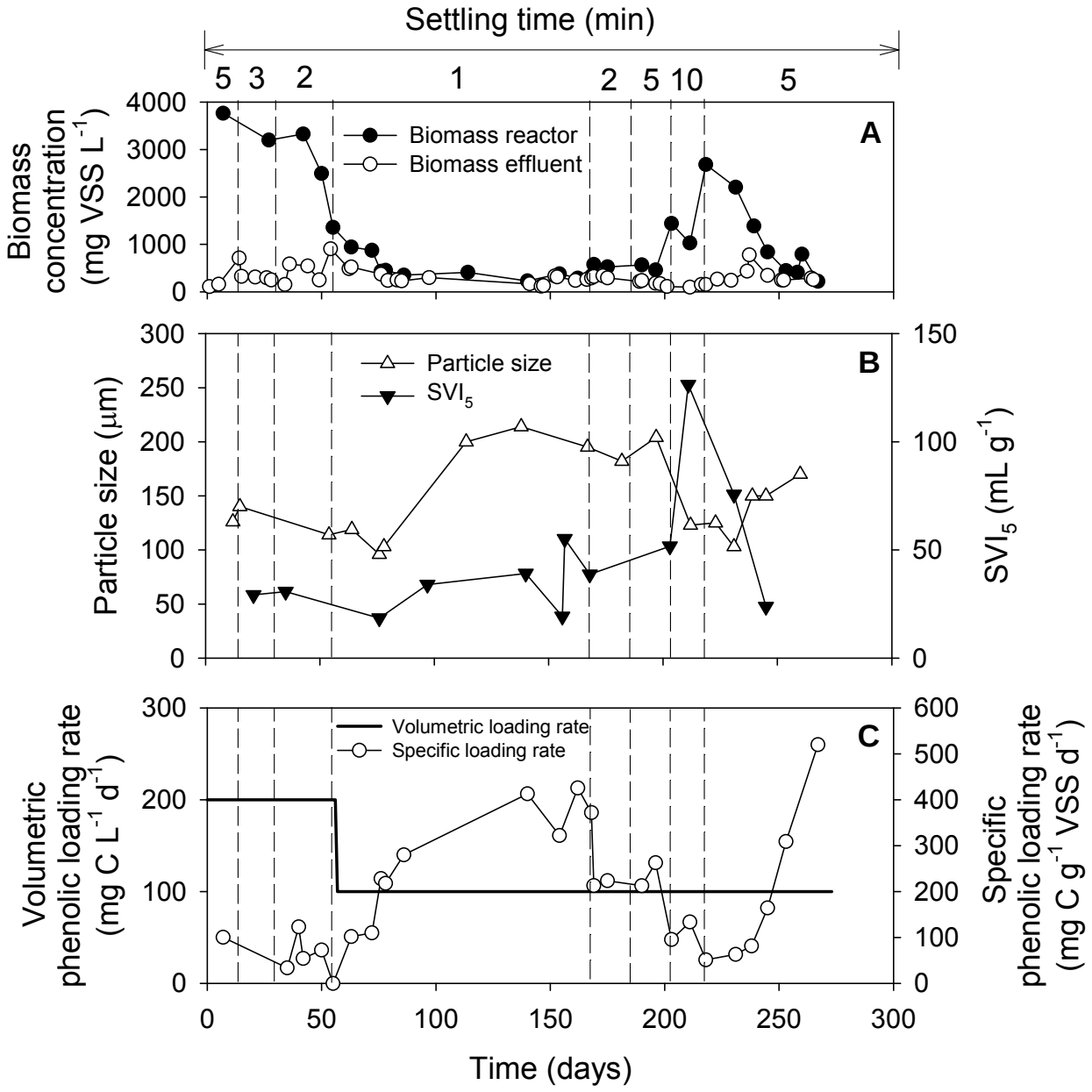
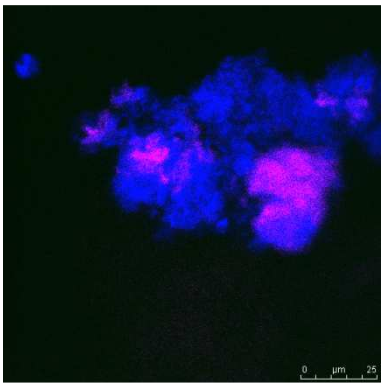
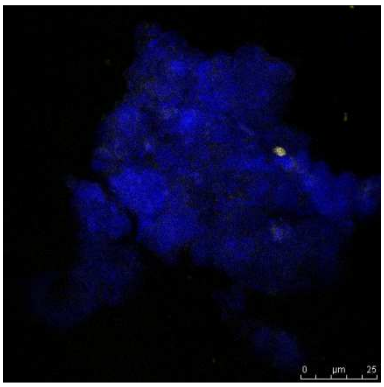

Settling time (min)
5 3 2 1 2 5 10 5
Biomass concentration (mg VSS L-1)
Biomass reactor
Biomass effluent
A
Particle size (μm)
SVI5 (mL g-1)
Particle size
SVI5
B
Volumetric phenolic loading rate (mg C L-1 d-1)
Specific phenolic loading rate (mg C g-1 VSS d-1)
Volumetric loading rate
Specific loading rate
C
Time (days)

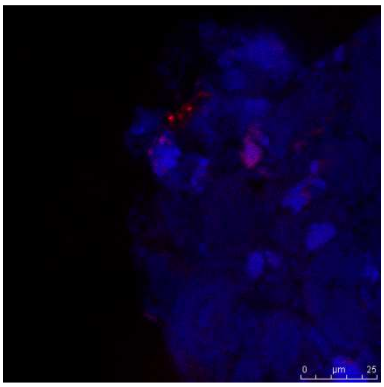
Figure 3.



A) *Acinetobacter* sp. (pink)



B) *Arthrobacter* sp. (yellow)



C) *Pseudomonas* sp. (pink)

Figure 4.

Table 1.

	Phenols in the feeding	Observed growth yield coefficient (Y_{obs})		Synthesis growth yield coefficient (Y)
Operation day		(g VSS g^{-1} TOC)	(g VSS g^{-1} COD)	(g VSS g^{-1} COD)
140-154	PNP	0.50	0.16	0.26
154-162	90% PNP + 10% *o*-cresol	0.54	0.17	0.27
162-168, 175-190, 245-260 (weighted average)	50% PNP + 50% *o*-cresol	0.76	0.24	0.38