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Long-term performance and stability of a continuous granular airlift reactor treating a high-strength wastewater containing a mixture of aromatic compounds

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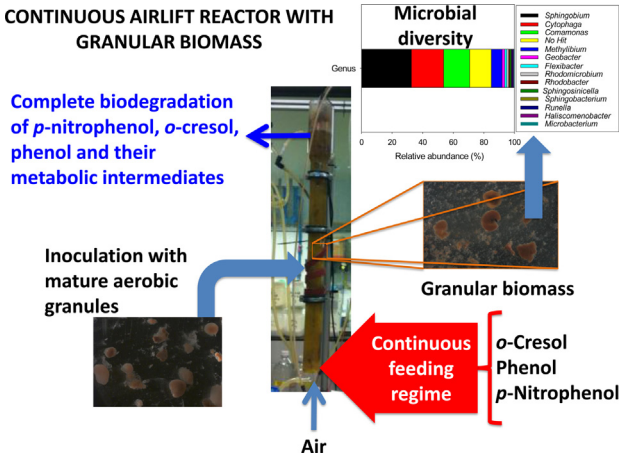
HIGHLIGHTS

- Aerobic biodegradation of a mixture of aromatics is feasible in a granular reactor.
- Applied organic loading rate is a key parameter for an optimal reactor performance.
- Stable mature aerobic granules were maintained 400 days in a continuous reactor.
- *Sphingobium*, *Cytophaga* and *Comamonas* were the main genera in the aerobic granules.

GRAPHICAL ABSTRACT



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ABSTRACT

Continuous feeding operation of an airlift reactor and its inoculation with mature aerobic granules allowed the successful treatment of a mixture of aromatic compounds (*p*-nitrophenol, *o*-cresol and phenol). Complete biodegradation of *p*-nitrophenol, *o*-cresol, phenol and their metabolic intermediates was achieved at an organic loading rate of 0.61 g COD L^{-1} d^{-1}. Stable granulation was obtained throughout the long-term operation (400 days) achieving an average granule size of 2.0 ± 1 mm and a sludge volumetric index of 26 ± 1 mL g^{-1} TSS. The identified genera in the aerobic granular biomass were heterotrophic bacteria able to consume aromatic compounds. Therefore, the continuous feeding regimen and the exposure of aerobic granules to a mixture of aromatic compounds make possible to obtain good granulation and high removal efficiency.

1. Introduction

Industrial wastewaters from agro-industries, coking plants, petrochemicals, pharmaceuticals, oil refineries, coal gasification processes, disinfectant/pesticides/fungicides and chemicals

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manufacturing are complex matrices composed by several recalcitrant/toxic compounds, such as aromatic compounds [1,2]. Industrial wastewaters are often treated by physico-chemical processes. However, these technologies have serious drawbacks [3,4]: (i) high costs due to the required conditions of temperature and pressure and the use of some chemicals, (ii) incomplete degradation of the recalcitrant/toxic organic compounds and (iii) generation other hazardous by-products (secondary pollutants).

Biological processes can satisfactorily overcome some of the disadvantages of physico-chemical processes. Technologies based on flocculent biomass, such as activated sludge systems, are the main biological processes implemented at full-scale, however its practical application for treating complex industrial wastewaters is rather limited because activated sludge systems are known to be inhibited by aromatic compounds [4]. To overcome the inhibition caused by organic compounds, a promising alternative to activated sludge systems is the application of reactors with aerobic granular biomass [5]. The application of aerobic granules allows retaining slow growing microorganisms and protects them from high concentrations of pollutants due to the diffusion gradients generated along the granule [5] that favour the gradual adaptation of microorganisms to stressing conditions.

In the past, several studies showed that aerobic granules can be used to treat single aromatic compounds in sequencing batch reactors (SBRs), for example: phenol [6–8], nitrophenols [9–12], chlorophenols [13–15] and cresols [16]. In most of these studies, a readily biodegradable organic compound was used as co-substrate for biomass growth. However, to the best of the authors' knowledge aerobic granular-based technologies have been hardly applied to treat more complex wastewaters composed by a mixture of aromatic compounds. This could be related to several facts: (i) the granular biomass is mostly applied in SBRs, thus it can be inhibited because high concentrations of aromatic compounds can be accumulated at the beginning of the SBR cycle; this inhibition can be increased since the accumulation of several aromatic compounds can produce synergistic effects and (ii) the formation of aerobic granules from floccular biomass is not an easy task when some specific aromatics such as nitrophenols or chlorophenols are treated. For instance, Fernández et al. [9] and Suja et al. [10] failed to reach a stable aerobic granular SBRs at long-term treating aromatic compounds since *p*-nitrophenol (PNP) seemed to inhibit many microbial species involved in granulation [10,17]. Therefore, new strategies should be applied to achieve successful treatment of wastewaters containing mixtures of aromatics compounds with aerobic granular reactors. An alternative way to develop aerobic granules to treat aromatic compounds can be the inoculation with aerobic granules treating readily biodegradable substrates and its adaptation to consume aromatic compounds [8,10,13,18]. Furthermore, the use of a continuous reactor, such as an airlift reactor, instead of a SBR allows preventing inhibitory effects caused by the aromatic compounds and enhancing the granulation process.

Therefore, the aim of this study is to demonstrate that a wastewater containing a mixture of aromatic compounds (*p*-nitrophenol, phenol and *o*-cresol) can be successfully treated by an aerobic granular-based technology provided that: (i) mature aerobic granules are used as inoculum and (ii) a continuous airlift reactor is chosen instead of SBR.

2. Materials and methods

2.1. Reactor

An airlift reactor made of glass (2.6 L of working volume) was used. The reactor configuration was as follows: the internal diameter of 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 up-flow velocity of 0.12–0.24 cm s^{-1}. Airflow rate in the reactor was regulated manually between 100 and 200 mL min^{-1} by a rotameter (Aalborg, USA) and it was enough to ensure an appropriate flow in the airlift reactor. The reactor was equipped with dissolved oxygen (DO) (Crison DO 6050), temperature (Crison Pt1000) and pH probes (Crison pH 5333) that were connected to a data monitoring system (Crison Multimeter 44). DO was not automatically controlled but varied between 4.0 and 5.0 mg O_2 L^{-1} according to the applied airflow rate. A Programmable Logic Controller (PLC) coupled to a Supervisory Control and Data Acquisition (SCADA) system regulated temperature, pH and feeding. pH was maintained at 8.0 ± 0.2 by a regular addition of $NaHCO_3$ whereas temperature in the reactor was maintained at 30 ± 0.5 °C using a temperature controller coupled with a belt-type heating device (Horst, Germany). Feeding to the reactor was made with a membrane pump (ProMinent Gamma/L).

2.2. Inoculum

Mature aerobic granules from an airlift performing simultaneous partial nitrification and biodegradation of *p*-nitrophenol (PNP) was used as inoculum [19]. The characteristics of the inoculated granular biomass were: 2.4 ± 0.6 mm of mean granule size, 68 ± 23 m h^{-1} of settling velocity, 10 ± 1 mL g^{-1} TSS (total suspended solids) of sludge volumetric index (SVI) at 30 min (SVI_{30}), an SVI_{30}/SVI_5 ratio of 1.0 and a biomass density of 12.5 ± 2 g VSS $L^{-1}{}_{particle}$. The inoculum was mainly composed by ammonia-oxidizing bacteria (AOB) and *p*-nitrophenol-degraders (basically *Acinetobacter* genus). The amount of inoculum used was the same as the reactor volume (2.6 L) with a concentration of 2.8 g VSS (volatile suspended solids) L^{-1}.

2.3. Wastewater composition and operational conditions

The airlift reactor was continuously fed with a high-strength synthetic wastewater composed by a mixture of aromatic compounds (*p*-nitrophenol, phenol and *o*-cresol) and a readily biodegradable mixture of glucose and sucrose. The operational period was divided in 3 phases, where the wastewater complexity was progressively increased. During phase I (the first 181 days of operation), *p*-nitrophenol was the only treated aromatic compound and its concentration in the influent was increased stepwise from 39 ± 1 mg PNP L^{-1} (63 mg COD L^{-1}) to 371 ± 10 mg PNP L^{-1} (598 mg COD L^{-1}). Therefore, the total organic matter concentration in the wastewater (including *p*-nitrophenol, glucose and sucrose) was increased from 121 ± 4 mg COD L^{-1} to 1161 ± 5 mg COD L^{-1}. In this phase, the hydraulic retention time (HRT) varied between 1.2 to 10.4 days. Phase II started on day-181, after the addition of phenol in the influent. During phase II, phenol concentration in the influent was increased (in one step) from 114 ± 1 mg phenol L^{-1} (336 mg COD L^{-1}) to 598 ± 2 mg phenol L^{-1} (1417 mg COD L^{-1}) whereas *p*-nitrophenol concentration remained at 371 ± 10 mg PNP L^{-1} (598 mg COD L^{-1}). Throughout phase II, the total concentration of organic matter in the influent increased from 1842 ± 6 mg COD L^{-1} to 2530 ± 2 mg COD L^{-1} and the HRT ranged from 1.2 to 1.8 days. In phase III (from day-260 to day-400), an *o*-cresol concentration of 103 ± 3 mg *o*-cresol L^{-1} (259 mg COD L^{-1}) was treated simultaneously with *p*-nitrophenol (371 ± 10 mg PNP L^{-1}) and phenol (598 ± 12 mg phenol L^{-1}). Therefore, the influent concentration was maintained in this phase at 3486 ± 10 mg COD L^{-1} and the HRT was increased stepwise from 1.8 to 5.7 days. During the whole operation, the average SRT in the airlift was 39 ± 11 d.

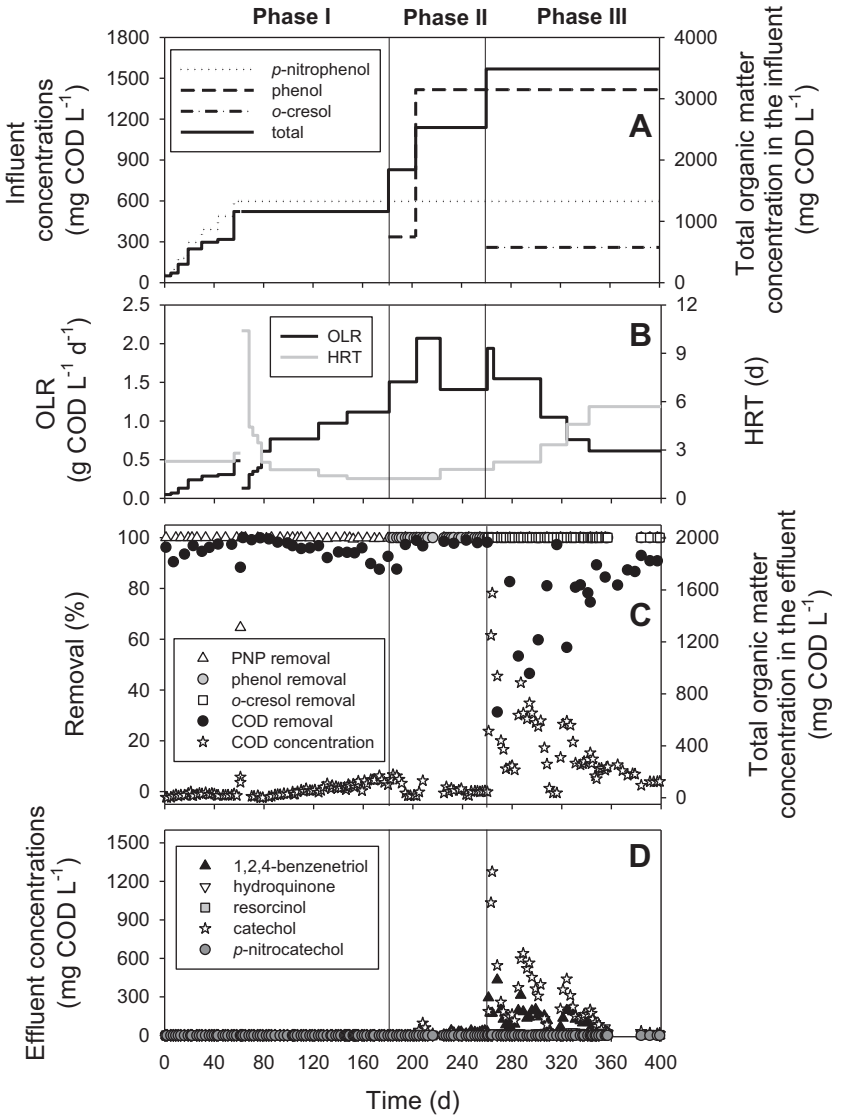

Fig. 1. Performance of the granular airlift reactor treating a complex mixture of aromatic compounds. Phase I: treating *p*-nitrophenol, phase II: treating *p*-nitrophenol and phenol, phase III: treating *p*-nitrophenol, phenol and *o*-cresol. A: influent concentrations in terms of COD, B: organic loading rate (OLR) and hydraulic retention time (HRT) applied, C: *p*-nitrophenol, phenol, *o*-cresol and COD removals and COD concentrations in the effluent and D: metabolic intermediates as COD concentrations in the effluent.

Throughout the operational time, sucrose and glucose were added as co-substrates, in a *p*-nitrophenol:(glucose + sucrose) ratio of 0.5 (as COD), being the proportion between glucose and sucrose of 1:1 (as COD). The composition of the micronutrients in the synthetic wastewater was maintained constant along the whole experimental period and was as follows (expressed as $mg\,L^{-1}$): 88 of $CaCl_2 \times 2H_2O$; 106 of NH_4Cl; 41.0 of KH_2PO_4; 176.0 of NaCl; 198.0 of $MgCl_2 \times 7H_2O$; 4.0 of $FeSO_4 \times 7H_2O$; 3.0 of $MnSO_4 \times H_2O$; 4.0 of $ZnSO_4 \times 7H_2O$; 2.0 of $CuSO_4 \times 5H_2O$; 0.02 of H_3BO_3; 12.0 of $CO(NH_2)_2$ and 1.0 of yeast extract.

2.4. Samples and analytical methods

Samples were regularly withdrawn from the influent and 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.

Phenol, *o*-cresol, *p*-nitrophenol and their metabolic intermediates (1,2,4-benzenetriol, hydroquinone, catechol, resorcinol and *p*-nitrocatechol) were measured by high performance liquid chromatography (HPLC), using a UltiMate 3000 (Dionex Corporation) with a Agilent Zorbax SB-C18 (4.6 mm × 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 °C. The mobile phases were acidified water (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 remained isocratic until 20 min. The injection volume was 20 μL and the maximum pressure in the column was approximately 290,000 hPa. Total organic carbon (TOC) was measured with an OI Analytical TOC Analyser (Model 1020A) equipped with a non-dispersive infrared (NDIR) detector and with a furnace maintained at 680 °C. Chemical oxygen demand (COD) was theoretically calculated using the HPLC and TOC experimental data and the theoretical combustion reaction of each aromatic compound (see Supporting information).

Total and volatile suspended solids (TSS and VSS, respectively) were determined for samples of the effluent and of inside the reactor, according to methods 2540 D and 2540 E of the standard methods for examination of water and wastewater [20].

The granular biomass was characterised in term of SVI, size, density and settling velocity. Both, SVI_5 and SVI_{30} were determined

using the method 2710 D as described in standard methods [20]. The size distribution of the granules was measured regularly by using image analysis with an optical microscope (Zeiss Axioskop equipped with an iAi Protec video camera). The captured digital image was further processed using Image-Pro Plus version 6.0 (Media Cybernetics, Inc.) and the mean Feret diameter of the granules was calculated. The Feret diameter is an average value between the shortest and the longest measured segment in the granule. Density of the granular biomass was determined using the Dextran Blue method [21]. Settling velocity was determined by placing individual granules in a column containing distilled water and measuring the time spent to drop a height of 30 cm. The granular sludge morphology was analysed using image analysis with an optical microscope.

2.5. Microbial diversity analysis

Identification of the microbial population at the end of the reactor operation (day-380) was performed using next-generation sequencing. Total genomic DNA of the aerobic granular biomass was extracted and purified using a PowerBiofilm™ DNA Isolation Kit (MoBio Laboratories, USA); in accordance with the manufacturer's instructions. Paired-end sequencing of the extracted DNA was performed on Illumina MiSeq platform by Research and Testing Laboratory (Lubbock, Texas, USA). Bacterial 16S rRNA variable regions V2-V4 were targeted using the primer pair 341F-907R. More details can be found in Supporting information. Biodiversity analysis and phylogenetic classification were performed with the methodology explained in detail in Supporting information. Relative abundances of reads were determined by taxonomic level. Indices of biological diversity were calculated at 97% of similitude with the following results: number of reads: 2011, Chao1: 490, Shannon: 2.85 and Eubacteria: 0.53. As can be seen in Fig. S1 in Supporting information, good coverage of diversity was reached.

2.6. Observed growth yields

Observed growth yields were calculated at different steady-state conditions. Each observed growth yield was calculated as the variation of the biomass in the reactor plus the biomass loss in the effluent divided by the total consumption of organic matter in the same period.

3. Results and discussion

3.1. Performance of the granular airlift reactor

The performance of the granular airlift reactor is shown on Fig. 1. As stated previously, the operation of the airlift reactor was divided in three different phases in which the wastewater complexity was progressively increased (see Fig. 1A). The organic loading rate (OLR) was manipulated by changing the organic matter concentration in the influent and/or the HRT (see Fig. 1B). Throughout the reactor operation, the concentration of organic matter in the influent was progressively increased from 121 ± 4 mg COD L^{-1} to 3486 ± 10 mg COD L^{-1}. Also, the HRT ranged between 1.2 to 10.4 days (by the increase/decrease of the inflow) to avoid inhibition or destabilization of the granular reactor.

3.1.1. Biodegradation of p-nitrophenol

From the beginning of the reactor operation until day 181, the only treated aromatic compound was *p*-nitrophenol. The OLR was progressively increased when stable *p*-nitrophenol removal was achieved. During the first 58 days of the reactor operation, the OLR was incremented by increasing the *p*-nitrophenol concentration in the influent up to 598 mg COD L^{-1} while the HRT was maintained at 2.4 days. From day-58 onwards, *p*-nitrophenol concentration in the influent was maintained at 598 mg COD L^{-1} and the HRT was varied to achieve the expected OLR. On day 58, a small accumulation of *p*-nitrophenol (82 mg COD L^{-1}) took place, reducing the COD removal from 97 to 88% (Fig. 1C). Therefore, the inflow was stopped and the reactor was operated in batch mode during 2 days to consume the accumulated *p*-nitrophenol. This accumulation of *p*-nitrophenol can be related to an overload of the airlift reactor. No metabolic intermediates (1,2,4-benzenetriol, hydroquinone, resorcinol, catechol or *p*-nitrocatechol) were accumulated (Fig. 1D). On day-61, the continuous feeding operation was restarted and an OLR of 0.1 g COD L^{-1} d^{-1} was applied. From day-61 to day-181, the OLR was progressively increased up to 1.1 g COD L^{-1} d^{-1}. Neither metabolic intermediates nor *p*-nitrophenol were accumulated in this period, achieving a COD removal of 92 ± 2%. This means that the COD concentration in the effluent (115 ± 10 mg COD L^{-1}) was not related to aromatic compounds or their intermediates. One might hypothesise that this organic matter is related to the secretion of extracellular polymeric substances (EPS), cell lysis products and/or loss of the integrity of the cell wall of non-adapted microorganisms [22–24].

The achieved OLR (1.1 g COD L^{-1} d^{-1}) was higher than the reported range for granular SBRs treating *p*-nitrophenol (0.1–1.0 g COD L^{-1} d^{-1}) [9–12]. Therefore, the results of this study show that the continuous operation is better than SBR operation in terms of applied OLR for a granular biomass treating *p*-nitrophenol, due to the low *p*-nitrophenol concentration in the bulk liquid during the airlift operation.

3.1.2. Simultaneous biodegradation of p-nitrophenol and phenol

On day-181, phenol was introduced in the influent to increase the wastewater complexity. Firstly, the phenol concentration in the feeding was 336 mg COD L^{-1}, increasing the OLR to 1.5 g COD L^{-1} d^{-1}. Phenol and *p*-nitrophenol were completely degraded, no metabolic intermediates were accumulated and a higher COD removal than in phase I was achieved (99 ± 1%, Fig. 1C). On day-203, phenol concentration in the influent was increased up to 1417 mg COD L^{-1}, resulting in an increase on the OLR up to 2.1 g COD L^{-1} d^{-1}. This change did not affect the *p*-nitrophenol and phenol removal, however it produced a transient accumulation of metabolic intermediates: catechol, resorcinol and 1,2,4-benzenetriol in the reactor (Fig. 1D). Catechol appeared in higher concentrations than resorcinol and 1,2,4-benzenetriol. After 7 days, resorcinol and 1,2,4-benzenetriol disappeared and catechol remained at low concentrations (13 mg COD L^{-1}), with COD removal and OLR of 94% and 2.1 g COD L^{-1} d^{-1}, respectively. However, some unidentified peaks were detected in the HPLC analyses indicating the accumulation of some unidentified organic compounds. Then, the OLR was decreased to 1.4 g COD L^{-1} d^{-1}, and this change slightly improved the COD removal up to 98%. It is well-known that catechol is a metabolic intermediate of the aerobic biodegradation of several aromatic compounds [24,25]. The aerobic biodegradation of *o*-cresol can produce 4-metilresorcinol [26–28], which could be degraded to resorcinol. 1,2,4-benzenetriol is a metabolic intermediate from the aerobic biodegradation of *p*-nitrophenol [29,30]. Accumulation of metabolic intermediates can produce inhibition to a non-acclimated aerobic heterotrophic biomass [31]. Nevertheless, the results of this study show that the granular biomass was not inhibited by these metabolic intermediates.

3.1.3. Simultaneous biodegradation of p-nitrophenol, phenol and o-cresol

On day-260, *o*-cresol (259 mg COD L^{-1}) was introduced as the third aromatic compound in the influent, increasing the OLR to 1.9 g COD L^{-1} d^{-1}. As soon as *o*-cresol was added, some metabolic

intermediates started to accumulate again (Fig. 1D) reaching a total concentration of 1580 mg COD L^{-1}. Despite this, the aromatic compounds of the influent: *p*-nitrophenol, phenol and *o*-cresol were completely degraded. Therefore, on day-265, the OLR was again decreased to 1.6 g COD L^{-1} d^{-1} by increasing the HRT. This action helps to decrease the concentration of metabolic intermediates, but complete biodegradation was still not achieved since catechol, 1,2,4-benzenetriol and resorcinol remained at 356, 169 and 10 mg COD L^{-1}, respectively. After 40 days since the OLR decrease, no further improvement of the biodegradation of the metabolic intermediates was detected and COD removal was close to 60%. This data suggests that incomplete biodegradation of the metabolic intermediates could be related to the instability of the biological process due to an oversized OLR and/or the presence of this specific mixture of aromatics in the influent. Therefore, to try to reach steady-state conditions and to remove the metabolic intermediates, OLR was reduced stepwise by increasing the HRT. After achieving an OLR of 0.6 g COD L^{-1} d^{-1}, no further accumulation of metabolic intermediates occurred and the airlift reactor achieved steady-state conditions, in terms of COD, *p*-nitrophenol, phenol, *o*-cresol and metabolic intermediates removal (Fig. 1).

3.2. Effect of the applied OLR on the aromatic compounds removal

The effect of the applied OLR on the aromatic compounds removal is shown on Fig. 2. In the case of treating only *p*-nitrophenol, an increase in the OLR from 0.1 to 1.1 g COD L^{-1} d^{-1} only caused a small decrease in the COD removal from 100 to 92%. Neither *p*-nitrophenol nor metabolic intermediates were detected at the lowest COD removal. In the case of simultaneous biodegradation of *p*-nitrophenol and phenol, higher OLR than in the case of only *p*-nitrophenol could be applied: 1.5–2.1 g COD L^{-1} d^{-1}, also with a higher COD removal (94–98%) than in the previous case. However, small amounts of metabolic intermediates, mainly catechol, were accumulated (50–90 mg COD L^{-1}). In the case of the wastewater with the highest complexity (*p*-nitrophenol, phenol and *o*-cresol), a significant reduction of the applied OLR had to be carried out to achieve COD removals higher than 90%. At the maximum OLR applied in this case (1.9 g COD L^{-1} d^{-1}), only 68% of COD removal was achieved due to the high accumulation of metabolic intermediates (around 1600 mg COD L^{-1} of total concentration, Fig. 1C). However, complete biodegradation of *p*-nitrophenol, phenol and *o*-cresol was obtained. Only with the reduction of the OLR to 0.6 g COD L^{-1} d^{-1}, a COD removal higher than 90% was reached and no *p*-nitrophenol, phenol or *o*-cresol nor metabolic intermediates were accumulated.

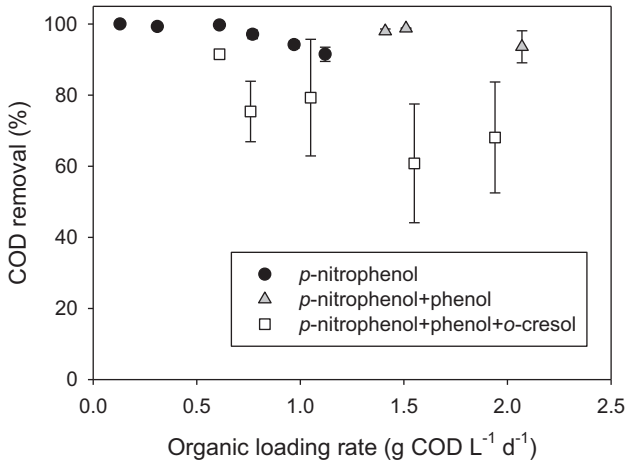

Fig. 2. Effect of the organic loading rate over the organic matter removal.

The aromatic compounds of the influent were always completely degraded in the granular airlift reactor, regardless of the applied OLR. This fact suggests that the biodegradation of these compounds by the aerobic granular biomass was not inhibited by the metabolic intermediates accumulated in some periods of the reactor operation.

Regarding the detected metabolic intermediates, the main one was catechol that was significantly accumulated when the three aromatic compounds were treated together. This fact suggests that catechol consumption could be the bottleneck of the biodegradation of a mixture of *p*-nitrophenol, phenol and *o*-cresol since a common metabolic pathway could be used by the granular biomass to degrade these aromatic compounds. It could be possible that a lack of specific enzymes caused the incomplete mineralization of the metabolic intermediates when the wastewater complexity increased. Therefore, the OLR should be carefully controlled in the treatment of complex wastewaters to avoid the accumulation of metabolic intermediates and the subsequent reduction of the COD removal.

3.3. Morphological and functional characterization of the aerobic granular biomass

The successful performance of the granular airlift reactor was related to the stable granulation throughout the operational period. The morphological and functional characteristics of the aerobic granules were determined in each phase of the reactor operation (Table 1).

Throughout the operational period, the SVI_5 increased from 8 ± 2 mL g^{-1} TSS to 26 ± 1 mL g^{-1} TSS, as more aromatic compounds were added to the influent. In spite of the increase in the SVI_5, its values always remained below 50 mL g^{-1} TSS, which is a typical value for good granule settleability [19,32,33].

No clear influence of the wastewater complexity over the average granule size, settling velocity or granules density was found (Table 1). Considering the high standard deviation of their values, the average granule size, settling velocity and granules density can be considered similar in all the phases. The settling velocity remained in the range considered proper for aerobic granules: 30–90 m h^{-1} [5,33]. Besides, the values of granules density indicated good granule compaction [5,33]. The granules density of the inoculum was higher than the reported ones in this study. This result could be related to the high amount of nitrifying bacteria in the inoculum since nitrifiers have a higher density than heterotrophs [34].

Proper granules density and low SVI values indicate that the biomass separation from the treated wastewater is very high, which is very important to achieve successful operation and high effluent quality of a granular reactor [35]. Throughout the operational period, the solids concentration in the effluent remained stable at 47 ± 27 mg TSS L^{-1}, confirming the good biomass separation from the treated wastewater. The stability of the morphological characteristics can be also appreciated in the pictures of granules showed in Supporting information (Fig. S2).

The biomass concentration in the reactor was always low (Table 1). Treating only *p*-nitrophenol, the biomass concentration was 1.1 ± 0.1 g VSS L^{-1} and it increased to 1.6 ± 0.1 g VSS L^{-1} when phenol and *o*-cresol were introduced in the influent. These low biomass concentrations could be related to the presence of *p*-nitrophenol since this aromatic compound is a chemical uncoupler of oxidative phosphorylation, which reduces the growth yield, because it dissociates anabolism from catabolism [36,37] avoiding a high biomass production. To confirm or reject this hypothesis, observed growth yields were calculated (Table 2). The obtained yields were significantly lower than the reported growth yields

Table 1
Performance of the airlift reactor and characteristics of the aerobic granules in the different operational phases of this study.

Aromatic compounds in the wastewater	Aromatic compounds loading rate [g COD L^{-1} d^{-1}]	Total OLR [g COD L^{-1} d^{-1}]	COD removal [%]	SVI_5 [mL g^{-1} TSS]	Settling velocity [m h^{-1}]	Granules size [mm]	Granules density [g L^{-1}]	Biomass concentration [g VSS L^{-1}]	Reference
p-Nitrophenol + ammonium	0.03	0.03	100	11	68 ± 23	2.4 ± 0.6	210	2.8	Inoculum [19]
p-Nitrophenol	0.5	1.1	90 ± 2	8 ± 2	78 ± 27	2.1 ± 1.0	17 ± 3	1.1 ± 0.1	This study
p-Nitrophenol + phenol	1.1	1.4	98 ± 1	18 ± 1	50 ± 22	1.7 ± 0.7	21 ± 2	1.6 ± 0.1	This study
p-Nitrophenol + phenol + *o*-cresol	0.4	0.6	92 ± 1	26 ± 1	61 ± 33	2.0 ± 0.5	14 ± 2	1.6 ± 0.1	This study

Table 2
Observed growth yields along the performance of the granular airlift reactor.

Aromatic compounds in the wastewater	Observed growth yield (g VSS g^{-1} COD)
p-Nitrophenol	0.03 ± 0.01
p-Nitrophenol + phenol	0.05 ± 0.01
p-Nitrophenol + phenol + *o*-cresol	0.04 ± 0.01

for heterotrophic biomass consuming readily biodegradable substrates [38], confirming the uncoupler effect of *p*-nitrophenol.

In summary, the morphological and functional characteristics of the aerobic granules did not show significant deterioration when a complex mixture of aromatic compounds was treated.

Comparing the results of this study with the reported ones by Fernández et al. [9], it seems that the strategy applied in this study was better to achieve a stable granular reactor treating a wastewater containing a mixture of aromatics than the proposed in that study. The differences between both studies were: (i) the use of mature aerobic granules as inoculum (in this study) instead of the formation of aerobic granules from a specialized floccular biomass (as in Fernández et al. [9]) and (ii) the use of a continuous airlift reactor (in this study) instead of using a SBR (as in Fernández et al. [9]). With the strategy applied in this study, mature aerobic granules were maintained at long-term, there were less episodes of accumulation of aromatic compounds in the reactor and the applied OLR was higher.

3.4. Microbial characterization of the aerobic granular biomass

Fig. 3 shows the analysis of bacterial composition at the class and genus level carried out by pyrosequencing. The most abundant classes were Alphaproteobacteria (43%), Cytophagia (23%), Betaproteobacteria (14%) and "No Hit" (17%), which represented the 97% of the analysed DNA sequences. These classes are members of the Proteobacteria and Bacteroides phyla, which are the dominant phyla in wastewater treatment plants treating petroleum refinery (characterized by high concentration of aromatic compounds) and pharmaceutical wastewaters [39]. At genus level, the

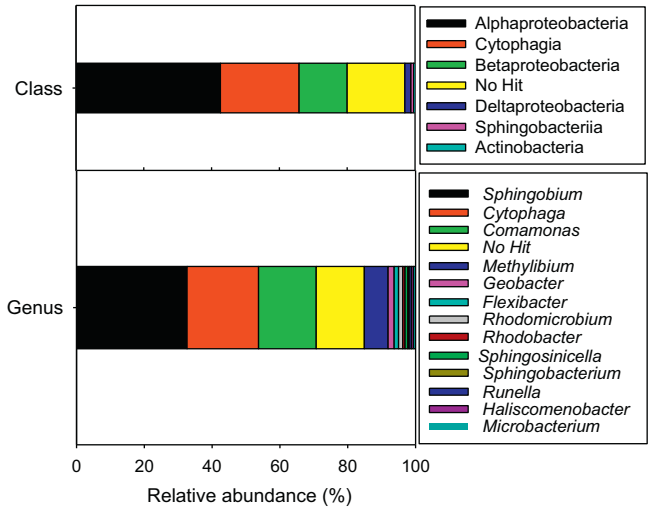

Fig. 3. Microbial diversity at class and genus level in the aerobic granules treating simultaneously *p*-nitrophenol, phenol and *o*-cresol in the airlift reactor. The percentages are referred to the detected relative abundance of bacteria and it was defined as the number of sequences affiliated with a particular taxon divided by the total number of sequences of the library.

main identified genera were *Sphingobium* (33%), *Cytophaga* (21%), *Comamonas* (14%) and "No Hit" (17%), representing 85% of the analysed DNA sequences.

The microbial characterization of the mature aerobic granules at day-380 shows that the main bacterial group present in the inoculum (*Acinetobacter* genus) was washed-out from the granules. *Sphingobium, Cytophaga* and *Comamonas* have the capacity to degrade several aromatic compounds [40–42]. Thus, the microbial characterization of the aerobic granular biomass treating *p*-nitrophenol, phenol and *o*-cresol revealed that the main identified genera are heterotrophic bacteria able to degrade several aromatic compounds, which corroborates the high removal efficiency of the aerobic granular reactor.

4. Conclusions

The aerobic biodegradation of a mixture of aromatic compounds (*p*-nitrophenol, phenol and *o*-cresol) in a continuous airlift reactor with granular biomass was successfully accomplished. The applied organic loading rate should be carefully taken into account to avoid the accumulation of metabolic intermediates when the treated wastewater contains several aromatic compounds.

The use of: (i) mature aerobic granules as inoculum and (ii) a continuous feeding regime seems to be a good strategy to achieve successful treatment of a wastewater containing a mixture of aromatic compounds by an aerobic granular-based technology. *Sphingobium, Cytophaga* and *Comamonas* were the main identified genera in the aerobic granular biomass and they were probably the responsible of aerobic biodegradation of aromatic compounds.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhazmat.2015.10.031.

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