Biodegradation of a high-strength wastewater containing a mixture of ammonium, aromatic compounds and salts with simultaneous nitritation in an aerobic granular reactor

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Abstract

Long-term operation (390 days) of a continuous airlift reactor with aerobic granular biomass was successfully applied to treat a highly complex wastewater composed of: ammonium (1000 mg N L^{-1}), *o*-cresol (100 mg L^{-1}), phenol (100 mg L^{-1}), quinoline (50 mg L^{-1}) and salts (16 g salts L^{-1}). High nitrogen loading rate (1.1 g N L^{-1} d^{-1}) and organic loading rate of 0.7 (g COD L^{-1} d^{-1}) were achieved for the simultaneous nitritation and complete biodegradation of the aromatic compounds. The successful operation of the granular airlift reactor can be related to (i) the growth of specialized microorganisms in the aerobic granules and (ii) the continuous feeding regime. Aerobic granules were maintained stable in spite of the high salinity conditions. Dissolved oxygen (DO) concentration and DO/ammonium concentrations ratio were the key parameters to select a suitable effluent for anammox or heterotrophic denitrification via nitrite. Besides, nitrous oxide emissions were related to the DO concentration in the reactor.

Keywords: partial nitrification; phenolic compounds; salinity; nitrous oxide

Introduction

Nowadays, the satisfactory and cost-effective treatment of industrial wastewaters from chemical, petrochemical, coke plant and refineries is a huge challenge since they are complex matrices, containing several organic and inorganic compounds, such as toxic/recalcitrant organic compounds (like aromatic compounds), ammonium and several inorganic salts [1,2]. Industrial wastewaters are often treated by physico-chemical processes; however, these technologies have serious drawbacks [3]: (i) high costs due to the high temperature and pressure conditions applied and chemical requirements, (ii) do not allow complete degradation of the aromatic compounds and (iii) they may produce other hazardous by-products (secondary pollutants).

Biological processes can overcome some of the disadvantages of physico-chemical treatments. However, biological processes can be inhibited by aromatic compounds [4]. Biological treatments based on floccular biomass (such as activated sludge systems) are the main biological technology used at full-scale. Nevertheless, its application for treating complex industrial wastewaters is limited because activated sludge systems are known to be highly inhibited by aromatic compounds [2-5]. Therefore, biological systems with floccular biomass: (i) are poor adjustable to fluctuations in the organic loading, (ii) need large reactor volumes because low loading rates can be applied and (iii) its settling capacity is limited, which means large secondary clarifiers. Biological treatments based on aerobic granules represent an alternative to treat these complex industrial wastewaters [6].

Ammonium can be removed by the application of Biological Nitrogen Removal (BNR) processes via nitrite [7,8], where ammonium is oxidized until nitrite (nitritation) and subsequently, nitrite can be reduced by heterotrophic denitrification via nitrite (denitritation) or by anammox (anaerobic ammonium oxidation) processes [7,8]. Nitrification is usually the

bottleneck for BNR in many wastewater treatment processes because nitrifying bacteria are very sensitive to several factors, such as inhibition by aromatic compounds. Biodegradation of aromatic compounds and nitritation can be simultaneously performed in a single aerobic granular reactor due to the substrates gradients occurring through the granules and the high biomass concentration that can be achieved in this kind of reactor [6,9]. Therefore, a two-stage process composed by a first aerobic granular reactor followed by a second anoxic granular reactor has been proposed as a feasible technology to treat complex industrial wastewaters [9,10]. Until now, this option has only been tested with wastewaters containing ammonium and a single aromatic compound: *o*-cresol [9] or *p*-nitrophenol [10]. However, it is well-known that industrial wastewaters can be composed by a mixture of several aromatic compounds; thus nitritation and biodegradation of a mixture of aromatic compounds should be tested to confirm the technological feasibility of this option. Furthermore, the effect of several parameters should be established to guarantee this feasibility: (i) operation at high salinity conditions, (ii) determination of nitrous oxide emissions from nitritation at different dissolved oxygen (DO) concentrations in the reactor and (iii) assessment of the stability of the morphological characteristics of the aerobic granules at long-term. Therefore, the objective of this study is to demonstrate the feasibility of the biodegradation of a high-strength wastewater containing a mixture of ammonium, aromatic compounds and salts with simultaneous nitritation in an aerobic granular reactor. In this sense, nitrous oxide emissions, stability and characteristics of the aerobic granular biomass at long-term operation and the microbial diversity in the granules will be assessed.

2BMaterials and methods

Reactor

An airlift reactor made of glass (2.6 L of working volume) was used (Figure 1). 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. At the top of the reactor, a gas-solid-liquid separator allowed the separation of the aerobic granules from the treated wastewater. Compressed air was supplied through an air diffuser placed at the bottom of the reactor at an upflow velocity of 0.2-0.3 cm s^{-1}. Air flow rate in the reactor was regulated manually between 150 to 250 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 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 and varied between 0.5 and 4.0 mg O_2 L^{-1} (excluding the first 25 days) according to the applied airflow (Figure 2A). 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 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).

Inoculum

Aerobic granular sludge from an airlift reactor performing partial nitritation and *o*-cresol biodegradation was used as inoculum. The biomass characteristics were as follows: 1.0-1.5 mm of mean granule size, 40-60 m h^{-1} (67-100 cm min^{-1}) of settling velocity, sludge

volumetric index at 5 min (SVI_5) of 7-14 mL g^{-1} TSS and SVI_{30}/SVI_5 ratio of 1.0. The biomass was under starvation period in mineral medium during 30 days at room temperature prior to the start-up of the reactor. More information can be found in Jemaat et al. [9].

Wastewater composition and operational conditions

The airlift reactor was fed continuously with a synthetic wastewater. Throughout the operational period, ammonium concentration was of 1000 ± 39 mg $N\text{-}NH_4^+$ L^{-1} (3818 mg NH_4Cl L^{-1}) in the influent and different aromatic compounds were also added. During the first 60 days of operation, hydraulic residence time (HRT) was varied between 6.3 to 0.9 days (by increasing the inflow rate) to increase the applied organic (OLR) and nitrogen (NLR) loading rates. From day-60 onwards, HRT was maintained at 0.9 days. The complexity of the treated wastewater was increased stepwise by introducing the aromatic compounds as follows:

i) Phase I (from day-22 to day-114). Ammonium (1000 ± 39 mg N L^{-1}) and *o*-cresol (100 ± 6 mg L^{-1}) were simultaneously treated.

ii) Phase II (from day-114 to day-195). Phenol was added to the influent; therefore ammonium (1000 ± 39 mg N L^{-1}), *o*-cresol (100 ± 6 mg L^{-1}) and phenol were treated together. Phenol concentration in the influent was increased stepwise from 25 ± 1 to 100 ± 3 mg L^{-1}.

iii) Phase III (from day-195 to day-332). Quinoline was added to the influent; therefore ammonium (1000 ± 39 mg N L^{-1}), *o*-cresol (100 ± 6 mg L^{-1}), phenol (100 ± 3 mg L^{-1}) and quinoline were treated together. Quinoline concentration in the influent was increased stepwise from 15 ± 2 to 50 ± 4 mg L^{-1}.

The aim of the first three phases was to achieve an effluent containing only nitrite, removing completely *o*-cresol, phenol and quinoline for a subsequent denitritation stage.

iv) Phase IV (from day-333 to day-390). The influent was the same than in phase III but the aim was to achieve an effluent with a nitrite/ammonium ratio close to 1 for a subsequent anammox stage, removing completely *o*-cresol, phenol and quinoline.

The chemical oxygen demand to nitrogen ratio (COD/N) in the influent increased from 0.25 to 0.64 throughout the experimental period. The composition of the micronutrients in the synthetic wastewater was (expressed as mg L^{-1}): 48 CH_3COONa; 12.5 $C_6H_{12}O_6$; 11.9 $C_{12}H_{22}O_{11}$; 41.0 KH_2PO_4; 176.0 NaCl; 198.0 $MgCl_2x7H_2O$; 4.0 $FeSO_4x7H_2O$; 3.0 $MnSO_4xH_2O$; 4.0 $ZnSO_4x7H_2O$; 2.0 $CuSO_4x5H_2O$; 0.02 H_3BO_3; 12.0 $CO(NH_2)_2$ and 2.0 yeast extract. Acetate, glucose and sucrose were added in low amount as readily biodegradable organic matter. Their contribution in the influent composition was low and only represented: 24, 14 and 11 % of the total COD in the phases I, II and III-IV, respectively. Sodium bicarbonate (11.2 g $NaHCO_3$ L^{-1}) was added to the influent to ensure enough buffer capacity. As a consequence of the high NH_4Cl and $NaHCO_3$ concentrations, the influent had high salinity conditions (16 g salts L^{-1}, corresponding to a conductivity of 15 mS cm^{-1}).

Kinetic batch experiments

Several batch experiments were carried out around day-300 to have a deep understanding of the biological processes occurring in the airlift reactor. The experiments were performed in batch mode in a reactor of 300 mL of reaction volume (500 mL of total volume). Air was flowed through a pressure manoreductor and a gas flowmeter to ensure a constant airflow and was supplied from the bottom of the reactor through a diffuser in order to mix the reaction media and maintain the granular structure of the biomass. DO concentration ranged between 2 to 4 mg O_2 L^{-1}. The pH was maintained at 8.0, using phosphate buffer. Temperature was set at

150 30 ± 1 °C. Aerobic granular biomass from the airlift reactor was used and washed three times
151 with the phosphate buffer before being used in the experiment. A pulse of substrate
152 (ammonium and/or the aromatic compound) was added, and nitrite, nitrate, aromatic
153 compounds and any metabolic intermediate concentrations were followed throughout the
154 reaction time. Four different conditions were studied: (i) only ammonium (60 mg N L^{-1}), (ii)
155 *o*-cresol (13 mg L^{-1}) and phenol (11 mg L^{-1}), (iii) ammonium (60 mg N L^{-1}), *o*-cresol (10 mg
156 L^{-1}) and phenol (10 mg L^{-1}) and (iv) ammonium (60 mg N L^{-1}) and quinoline (15 mg L^{-1}).
157
158 Samples and analytical methods
159
160 Ammonium, nitrite, nitrate, conductivity, aromatic compounds (phenol, *o*-cresol and
161 quinoline) and metabolic intermediates of the aerobic biodegradation of the aromatic
162 compounds (1,2,4-benzenetriol, hydroquinone, catechol, resorcinol and *p*-nitrocatechol) were
163 determined for the influent and the effluent. Prior to the analysis, the samples were filtered
164 through 0.20 µm membrane of hydrophilic Durapore®.
165 Ammonium was measured by a continuous flow ammonia analyser (Hach Lange AMTAX
166 sc), based on potentiometric determination. Nitrate and nitrite concentrations were analysed
167 with ionic chromatography using an ICS-2000 Integrated Reagent-Free IC system (DIONEX
168 Corporation), which performs ion analyses using suppressed conductivity detection. Phenol,
169 *o*-cresol, quinoline, 1,2,4-benzenetriol, hydroquinone, catechol, resorcinol and *p*-nitrocatechol
170 were measured by High Performance Liquid Chromatography (HPLC) (the methodology is
171 explained in detail in Supplementary Material). For each aromatic compound, the chemical
172 oxygen demand (COD) was determined as the theoretical oxygen demand, which was
173 calculated as the amount of oxygen required to oxidize a compound to its final oxidation
174 products. Conductivity was measured with a CRISON conductivity device (micro CM 2100)

with independent temperature probe and conductivity cell, and the values are given at 25 °C, according to the method 2510 B of the Standard Methods [11]. Total and volatile suspended solids (TSS and VSS, respectively) were determined in the reactor and in the effluent, according to method 2540 D and 2540 E of the Standard Methods [11], for TSS and VSS, respectively.

The granular biomass was characterised in terms of SVI, size, granule density and settling velocity. SVI was determined using the procedure described in the method 2710 D of the Standard Methods [11]. The size of the granules was measured regularly by using image analysis with an optical microscope (Zeiss Axioskop equipped with a video camera (iAi Protec)), according to Jemaat et al. [9] (more details can be found in Supplementary Material). The distribution of the granules size was measured by a laser particle size analysis system (Mastersizer 2000, Malvern instruments). Density of the granular biomass was determined using the Dextran Blue method [12]. 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.

Granules morphology was measured by using image analysis with an optical microscope (Zeiss Axioskop equipped with a video camera (iAi Protec)) and by Scanning Electron Microscope (SEM). The methodology for SEM can be obtained from Julian et al. [13] (more details can be found in Supplementary Material).

Off-gas grab samples were collected from the headspace of the airlift reactor to determine the nitrous oxide (N_2O) emissions. Nitrous oxide concentration in the gas phase was measured off-line in a gas chromatograph. N_2O emissions were determined at several DO concentrations in the bulk liquid (0.5, 1.0, 1.5 and 4.0 mg O_2 L^{-1}) during the period between day-310 and day-330. The detailed procedure and calculations of nitrous oxide emissions can be found in Supplementary Material.

Microbial diversity analysis

Identification of the microbial population at the day-300 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 Roche 454 GS-FLX+ 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 Supplementary Material. Biodiversity analysis and phylogenetic classification was performed with the methodology explained in detail in Supplementary Material. 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: 9494, Chao1: 334, Shannon: 2.85 and Eubacteria: 0.51. As can be seen in Figure S1 in Supplementary Material, good coverage of diversity was reached. Fluorescence in-situ hybridization (FISH) coupled with Confocal Laser Scanning Microscopy (CLSM) was used to identify betaproteobacterial ammonia oxidizing (β-AOB) and nitrite oxidizing bacteria (NOB). FISH protocol was adapted from Suárez-Ojeda et al. [14] (more details can be found in Supplementary Material).

Results and discussion

Reactor performance: removal capacity and nitrous oxide emissions

The performance of the aerobic granular airlift reactor treating a complex wastewater composed by ammonium and several aromatic compounds (*o*-cresol, phenol and quinoline) at high salinity (16 g salts L^{-1}) is presented in Figure 2. In general, throughout the 390 days of operation, the granular airlift reactor was able to maintain stable nitritation and complete biodegradation of the aromatic compounds. During the first 21 days of operation, the reactor was operated in batch mode, adding pulses of ammonium (100 mg N L^{-1}) and *o*-cresol (100 mg L^{-1}) at regular intervals of 24-48 hours. In this way, the activity of the biomass could be properly reactivated from starvation to perform simultaneous nitritation and biodegradation of *o*-cresol. On day-21, the operation in continuous feeding was started (Phase I). DO and [DO]/[N-NH_4^+] ratio (Figure 2A) were manipulated to achieve successful nitrite accumulation (Figure 2C) and biodegradation of *o*-cresol (Figure 2D). NLR and OLR were increased stepwise until reach 1.1 g N L^{-1} d^{-1} and 0.3 g COD L^{-1} d^{-1}, respectively (Figure 2B). From day-60, the effluent was mainly composed by nitrite (942 ± 32 mg N L^{-1}) and low concentrations of ammonium and nitrate, 26 ± 19 mg N L^{-1} and 10 ± 4 mg N L^{-1}, respectively (Figure 2C). The *o*-cresol removal was always complete without accumulation of intermediates (Figure 2D).

On day-114, phenol was added to the influent (Phase II). Phenol concentration was increased stepwise from 25 ± 1 to 100 ± 3 mg L^{-1} and *o*-cresol was maintained at 100 mg L^{-1}, reaching an OLR of 0.5 g COD L^{-1} d^{-1}. The NLR was maintained at 1.1 g N L^{-1} d^{-1}. During this phase, neither *o*-cresol or phenol nor metabolic intermediates (1,2,4-benzenetriol, hydroquinone, catechol, resorcinol and *p*-nitrocatechol) were accumulated in the reactor (Figure 2D). The effluent was mainly composed by nitrite (838 ± 43 mg N L^{-1}) and the concentrations of ammonium and nitrate remained low at 27 ± 18 mg N L^{-1} and 13 ± 6 mg N L^{-1}, respectively (Figure 2C). The operation of the granular airlift reactor was maintained during other 60 days at a NLR and OLR of 1.1 g N L^{-1} d^{-1} and 0.5 g COD L^{-1} d^{-1}, respectively.

From day-195, quinoline was added to the influent (Phase III). Quinoline concentration was increased stepwise from 15 ± 2 to 50 ± 4 mg L^{-1} and phenol and *o*-cresol were keep at 100 mg L^{-1} each one, reaching an OLR of 0.7 g COD L^{-1} d^{-1}. Complete biodegradation of *o*-cresol, phenol and quinoline took place and no metabolic intermediates were detected (Figure 2D). The NLR was kept unchanged at 1.1 g N L^{-1} d^{-1}. As in the previous phases, the aerobic granular reactor was able to oxidize ammonium to nitrite (844 ± 50 mg N L^{-1}) instead of nitrate (4 ± 1 mg N L^{-1}); the ammonium concentration in the effluent was 25 ± 27 mg N L^{-1}. At the end of this phase, a nitrogen mass balance (see Figure S2 in Supplementary Material) confirmed that the main biological processes related to ammonium removal were: nitritation (83.8 % of the incoming ammonium), denitrification (8.0 %) and nitrous oxide emissions (4.1 %). Based on this information, there was virtually not denitrification in the aerobic granular reactor of this study. However, the simultaneous nitrification-denitrification (SND) process has been reported in other aerobic granular reactors operated at high loading rates and with readily biodegradable organic matter sources (as acetate) [15,16]. The low extent of denitrification in the aerobic granular reactor of this study could be related to: (i) the organic matter sources (*o*-cresol, phenol and quinoline) are recalcitrant compounds and maybe the aerobic biodegradation of these compounds is favoured compared to their anoxic biodegradation, (ii) the average granule size reported in granular reactors with SND (3.5-5.0 mm [15,16]) was significantly higher than that achieved in this study (1.0 mm), allowing the growth of denitrifying bacteria in the inners layer of the granules and (iii) SND is not favoured in a continuous feeding regimen as the applied in the airlift reactor of this study. However, alternating oxic-anoxic conditions and the use of sequencing batch reactors can favour the SND process [16].

In the last phase, from day 333-onward (Phase IV), the aim was to achieve an effluent suitable for a subsequent anammox reactor, i.e., an effluent composed by a nitrite/ammonium

concentrations ratio close to 1.0 and without aromatic compounds and metabolic intermediates. The strategy to achieve this new effluent was to maintain the NLR and OLR at 1.1 g N L^{-1} d^{-1} and 0.7 g COD L^{-1} d^{-1}, respectively but decreasing the DO concentration in the reactor (by decreasing the airflow) from 1.0 to 0.5 mg O_2 L^{-1} to limit AOB activity and force the accumulation of ammonium (Figure 2C). After this change, the nitrite concentration decreased to 499 ± 45 mg N L^{-1} while the ammonium concentration increased to 419 ± 60 mg N L^{-1}. Therefore, the nitrite/ammonium concentrations ratio in the effluent was steadily maintained between 1.0-1.2 during this phase (phase IV, Figure 2C). Considering that the biomass concentration in the reactor was maintained at 3.8 ± 0.6 g VSS L^{-1} throughout the operational period, the specific NLR in the reactor was 0.29 g N g^{-1} VSS d^{-1}.

Along the reactor operation, the salinity in the effluent remained stable at 14 ± 1 mS cm^{-1}. The difference in the oxygen affinity between AOB and NOB was determined to be the crucial parameter to obtain nitritation in biofilm or granular reactors [17]. If strong oxygen limiting conditions are applied in a granular reactor, AOB outcompete NOB and nitrite oxidation is prevented. To achieve this, the key operational variable to be maintained is the ratio between DO and ammonium concentrations in the bulk liquid [17]. The lower the [DO]/[N-NH_4^+] ratio, the stronger the oxygen limiting condition in the biofilm [18]. The control of this operational parameter allowed achieving and maintaining nitritation even at high DO concentration, as previously demonstrated with long-term specific experiments [17]. In this study, the [DO]/[N-NH_4^+] ratio was not automatically controlled but it was always maintained at very low values (0.05 and 0.75 mg O_2 mg^{-1} N, Figure 2A). These strong oxygen limiting conditions imposed in the reactor for NOB allowed maintaining partial nitritation throughout the study.

Four batch experiments were carried out to determine the effect of the simultaneous presence of ammonium and aromatics on the aerobic granular biomass. The results of these batch tests

299 are shown in Figure 3. In the first test (Figure 3A), only ammonium was added and only
300 nitrite was produced at a specific nitrite production rate of 0.21 ± 0.04 g N g^{-1} VSS d^{-1}. In the
301 second test (Figure 3B), *o*-cresol and phenol were simultaneously added and consumed at the
302 specific degradation rates of 0.30 ± 0.01 g COD g^{-1} VSS d^{-1} and 0.35 ± 0.01 g COD g^{-1} VSS
303 d^{-1}, respectively. In the third test, ammonium, *o*-cresol and phenol were added together
304 (Figure 3C). Nitrite was not produced until *o*-cresol and phenol were almost completely
305 degraded. Specific nitrite production and *o*-cresol and phenol biodegradations rates were 0.25
306 ± 0.06 g N g^{-1} VSS d^{-1}, 0.19 ± 0.05 g COD g^{-1} VSS d^{-1} and 0.24 ± 0.04 g COD g^{-1} VSS d^{-1},
307 respectively. Finally, in the fourth test, ammonium and quinoline were added together (Figure
308 3D). In this case, nitrite was simultaneous produced with the biodegradation of quinoline.
309 Specific nitrite production and quinoline biodegradation rates were 0.44 ± 0.05 g N g^{-1} VSS
310 d^{-1} and 0.14 ± 0.03 g COD g^{-1} VSS d^{-1}, respectively.
311 These results show the benefits of using a continuous feeding regime in an aerobic granular
312 reactor treating simultaneously ammonium and aromatic compounds [9]. Conventionally,
313 granular biomass is used in sequencing batch reactors (SBRs) where high substrate
314 concentrations can be achieved at the beginning of the SBR cycle. In that case, nitritation
315 could not start until the aromatic compounds were completely consumed, causing an increase
316 in the duration of the SBR cycle and, consequently, a reduction of the efficiency [19,20]. On
317 the contrary, continuous operation can be a suitable option since the concentration of the
318 aromatic compounds inside the reactor (bulk liquid) is expected to be (always) low due to the
319 high removal efficiency, reducing their toxic/inhibitory effect over nitritation or the oxygen
320 limitation caused by the degradation of the organic compounds, which is in agreement with
321 the data of the kinetic tests and the airlift reactor performance.
322 Nitrous oxide production were measured at several DO concentrations in the bulk liquid (0.5,
323 1.0, 1.5 and 4.0 mg O_2 L^{-1}) during the period between day-310 and day-330 when the airlift

reactor was treating ammonium, *o*-cresol, phenol and quinoline producing a suitable effluent for denitritation (Figure 4). At 0.5 and 1.0 mg O_2 L^{-1}, the lower the DO, the higher the N_2O emissions were. However, between 1.0 to 4.0 mg O_2 L^{-1}, the N_2O emissions remained stable at 4 % of N-converted. N_2O can be produced as an intermediate product of nitritation, mainly due to low DO concentration in the aerobic reactor [21-23]. The observed trend in the N_2O emissions was similar than the obtained by Pijuan et al. [21] in another granular airlift reactor performing nitritation of a real reject wastewater. They also found a significant increase of the N_2O emissions at low DO concentrations (up to 7 % of N-converted at 1 mg O_2 L^{-1}) and a constant percentage of N_2O emissions (2 % of N-converted) for DO concentrations higher than 4 mg O_2 L^{-1}. In this study, the percentage was constant (4 % of N-converted) from 1 mg O_2 L^{-1} and the maximum value was 11 % of N-converted at 0.5 mg O_2 L^{-1}. The difference in the percentages of N_2O emissions between both studies could be related to differences on: average granular diameter, pH in the reactor, applied airflow, reactor size, etc. The obtained results indicate that the granular airlift reactor of this study should be operated at DO higher than1.0 mg O_2 L^{-1} to minimize the N_2O emissions. In this sense, it should be noted that the application of partial nitritation for subsequent anammox step could be limited by the high N_2O emissions (11 % of N-converted) at low DO (0.5 mg O_2 L^{-1}).

Reactor performance: stability of the aerobic granular biomass

The morphological characteristics of the aerobic granular biomass were evaluated throughout the experimental period. Table 1 shows the average size, density and settling velocity of the granular biomass in each of the four phases of the reactor operation.

The average granules size decreased from 2.7 ± 0.8 to 1.0 ± 0.1 mm but the percentage of biomass with a diameter higher than 0.2 mm was always higher than 95%, which means that

the biomass of the airlift reactor can be considered as mature and stable aerobic granules. Moreover, density of the granules increased from 57 to 97 g L^{-1} but there was not a significant change of the settling velocity.

Throughout the operational period, there was a decrease of the average diameter and an increase of the granule density. These facts can be related to the supplied aeration because the airflow was increased together with the increase of the OLR to maintain the DO concentration high enough to avoid DO limitation for organic matter biodegradation.

The biomass inside the reactor was maintained at 3.8 ± 0.6 g VSS L^{-1} by withdrawing granular sludge periodically and achieving a sludge retention time (SRT) of 107 ± 46 days. The granules had a low VSS/TSS ratio (0.31 ± 0.04), which could be related to the high salinity (16 g salts L^{-1}) and the high SRT in the reactor. Throughout the reactor operation, SVI_5 and SVI_{30} remained at the same low value (12 ± 2 mL g^{-1} TSS). This value is below 50 mL g^{-1} TSS, which is considered as a typical value for aerobic granules [9,24,25]. Therefore, the value of the SVI_{30}/SVI_5 ratio was always one. Moreover, throughout the experimental period, VSS and TSS concentrations in the effluent were 79 ± 31 mg VSS L^{-1} and 61 ± 26 mg TSS L^{-1}, respectively. These values indicate that a good separation of the biomass was achieved in the reactor since the biomass concentration in the reactor was 3.8 g VSS L^{-1}.

Pictures of the granules were made to study their morphological characteristics. In phase I, some granules presented a smooth and regular shape and other granules showed an outer surface covered with filaments, possibly due to the presence of filamentous microorganisms (Figures 5A and 5B). In subsequent phases, when the OLR was increased due to the introduction of phenol and quinoline in the influent, almost all the granules were covered with filamentous microorganisms (see Figures 5C-5H and also Figure S3 in Supplementary Material). Commonly, the growth of bacteria with filamentous morphology is related to problems with the settling properties of the sludge [26]. However, in spite of the growth of

filamentous bacteria over the surface, the aerobic granules maintained a very good settleability throughout the study.

Another important fact is the stability of the granular biomass at high salinity conditions. The main salts in the influent were chloride and bicarbonate, giving a salinity of 16 g salts L^{-1}. As explained before, the aerobic granules presented good settleability at long-term and consequently, they can be considered also resistant to a high content of salts.

Bacterial populations in the aerobic granular biomass

The bacterial communities involved in the simultaneous nitritation and biodegradation of *o*-cresol, phenol and quinoline at high salinity were analysed by pyrosequencing on day-300 (Figure 6). The most abundant groups at class level were: Betaproteobacteria (80%), Gammaproteobacteria (10%), Alphaproteobacteria (5%) and Flavobacteria (3%); which represented the 98% of the analysed sequences. *Comamonas* (65%), *Nitrosomonas* (11%), *Pseudomonas* (5%), *Stenotrophomonas* (4%), *Bradyrhizobium* (3%) and *Burkholderia* (3%) were the most abundant genera (Figure 6); representing 91% of the analysed sequences. *Comamonas*, *Nitrosomonas* and *Burkholderia* genera belong to Betaproteobacteria whereas *Pseudomonas* and *Stenotrophomonas* genera belong to Gammaproteobacteria and *Bradyrhizobium* genus belong to Alphaproteobacteria.

Moreover, on day-300 FISH analyses for AOB and NOB were performed. Positive hybridization was obtained for β-AOB and the FISH quantification revealed that β-AOB was 71 ± 21 % of the total bacterial population (see Figure S4 in Supplementary Material). However, NOB was not detected by FISH.

Regarding the biodegradation of aromatic compounds, *Comamonas*, *Pseudomonas*, *Stenotrophomonas*, *Burkholderia* and *Bradyrhizobium* were the main heterotrophic genera

399 detected and they have been described to be able to biodegrade several aromatic compounds,
400 such as phenol, quinoline and cresols under aerobic conditions [27-30]. Therefore, the results
401 of the pyrosequencing are consistent with the reactor performance and the main identified
402 bacteria can be considered as the responsible microorganisms for biodegradation of phenol, *o*-
403 cresol and quinoline.
404 It is also remarkable that the main abundant genus in the aerobic granules (*Comamonas*) is
405 able to grow as filamentous bacteria under specific conditions [31,32]. This fact could explain
406 the significant growth of filamentous bacteria over the surface of the aerobic granules (Figure
407 5). Probably, these filaments were mainly composed by *Comamonas.*
408 Regarding the oxidation of ammonium, only an AOB genus was detected by pyrosequencing
409 in the aerobic granules (*Nitrosomonas*) while no NOB genus was detected by pyrosequencing
410 or by FISH. This result confirms that the applied [DO]/[N-NH_4^+] ratio (below 0.75 mg O_2
411 mg^{-1} N) is a proper condition to achieve the wash-out of NOB and to accumulate nitrite
412 (nitritation) instead of nitrate (conventional nitrification), even with the simultaneous
413 biodegradation of aromatic compounds.
414 However, the percentage *Nitrosomonas* measured by pyrosequencing (11%) seems to be low
415 to explain the high nitritation capacity of the aerobic granules. Two facts could explain this
416 result: (i) on one hand, the pyrosequencing relates the presence of bacteria and the FISH
417 reveals not only the presence but also the activity of bacteria [33]. In previous studies, it has
418 been described for AOB communities that the most abundant species may not necessarily be
419 the most active ones [33]. The same fact could have happened in this study where
420 *Nitrosomonas* was much less abundant than *Comamonas* (Figure 6). Nevertheless,
421 *Nitrosomonas* could be the most active bacteria, as the FISH analysis revealed; (ii) on the
422 other hand, nitrification is usually attributed to autotrophic bacteria (AOB and NOB).
423 However, some heterotrophic bacteria can also perform heterotrophic nitrification.

Heterotrophic nitrification is still poorly understood and it is usually described as the oxidation of ammonium until nitrate by heterotrophic bacteria [34]. *Comamonas* and *Pseudomonas* have been described as heterotrophic nitrifiers genera [34,35]. However, nitrate was hardly produced in this study while high nitrite accumulation took place (Figure 2C). This means that, if *Comamonas* and *Pseudomonas* played a significant role in the oxidation of ammonium in this study, the heterotrophic nitrification was stopped in nitrite instead of nitrate. *Alcaligenes faecalis* has been identified as a heterotrophic bacterium with the capacity to biodegrade phenol and to oxidize ammonium until nitrite [36], i.e. as a species able to perform heterotrophic nitritation. In the same way, *Comamonas* and *Pseudomonas* genera could also perform heterotrophic nitritation in the conditions applied in this study.

According to the above exposed results, the successful operation of the granular airlift reactor for simultaneous nitritation and biodegradation of aromatic compounds can be related to the: (i) growth of specialized microorganisms in the aerobic granules and (ii) the continuous feeding regime.

Conclusions

Simultaneous nitritation and biodegradation of a mixture of aromatic compounds (*o*-cresol, phenol and quinoline) at high salinity were successfully achieved in a continuous airlift granular reactor.

A suitable effluent for denitritation or for anammox was attained by manipulating the DO concentration and the DO/N-NH_4^+ concentrations ratio in the bulk liquid.

DO concentration had a significant effect on the N_2O emissions and DO higher than 1.0 mg O_2 L^{-1} should be maintained to avoid high N_2O emissions.

448 *Comamonas* and *Pseudomonas* were the main heterotrophic genera detected and they were
449 probably the responsible of biodegradation of aromatic compounds. *Nitrosomonas* genus was
450 the only AOB detected in the aerobic granules while NOB were not detected.
451
452 **Acknowledgements**
453
454 This work was supported by the Ministerio de Economía y Competitividad (Spanish
455 Government) through the ONLYBIO project (CTQ2011-24745/PPQ). The authors are
456 members of the GENOCOV group (2009 SGR 815, www.genocov.com). Carlos Ramos
457 wants to thank the Universitat Autònoma de Barcelona for his pre-doctoral fellowship.
458
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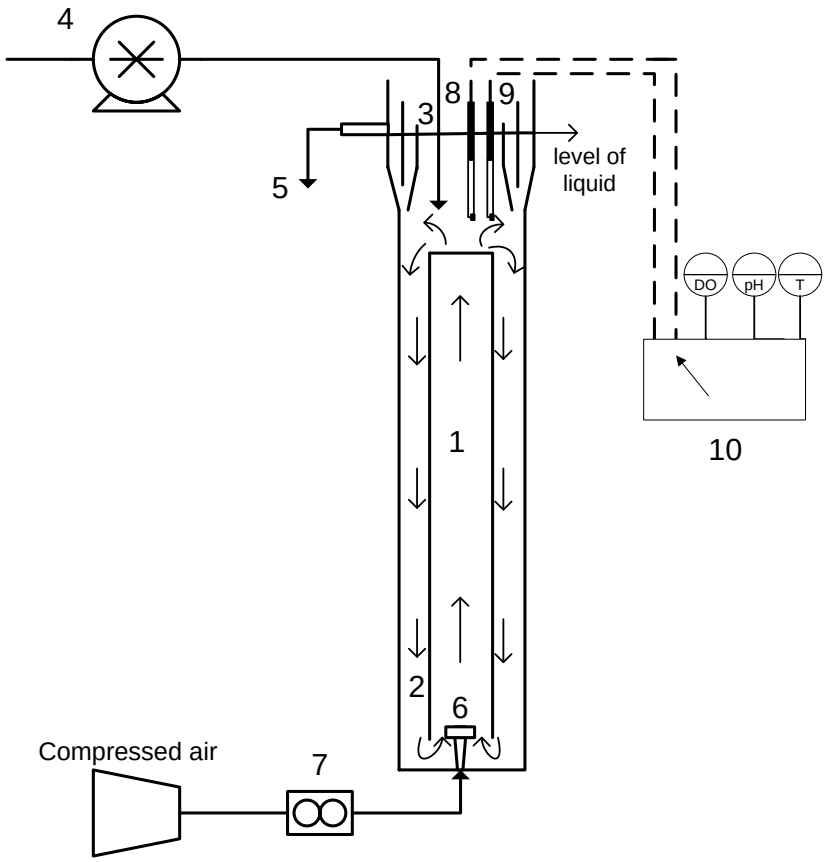

Figure 1. Experimental set-up of the continuous granular airlift reactor. (1) riser; (2) down comer; (3) separator; (4) feed pump; (5) effluent port; (6) air sparger; (7) rotameter; (8) pH probe; (9) DO probe; (10) monitoring panel.

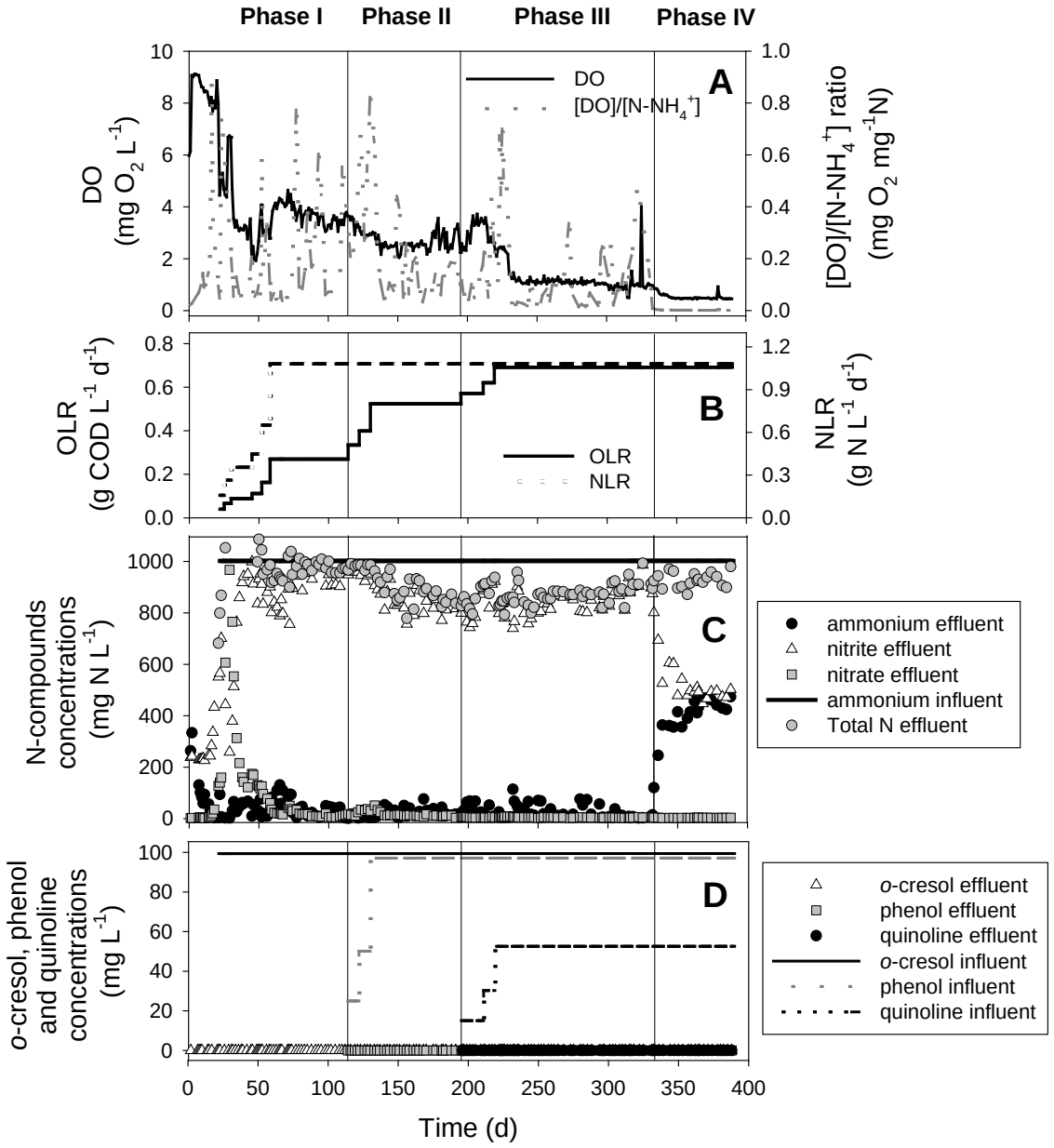

Figure 2. Operational conditions applied and performance of the granular airlift reactor treating a wastewater composed by high concentrations of ammonium and aromatic compounds at high salinity (16 g salts L^{-1}). DO: dissolved oxygen concentration, NLR: nitrogen loading rate and OLR: organic loading rate. Phase I: simultaneous nitritation and biodegradation of *o*-cresol, phase II: simultaneous nitritation and biodegradation of *o*-cresol and phenol, phase III: simultaneous nitritation and biodegradation of *o*-cresol, phenol and quinoline and phase IV: simultaneous partial nitritation and biodegradation of *o*-cresol, phenol and quinoline.

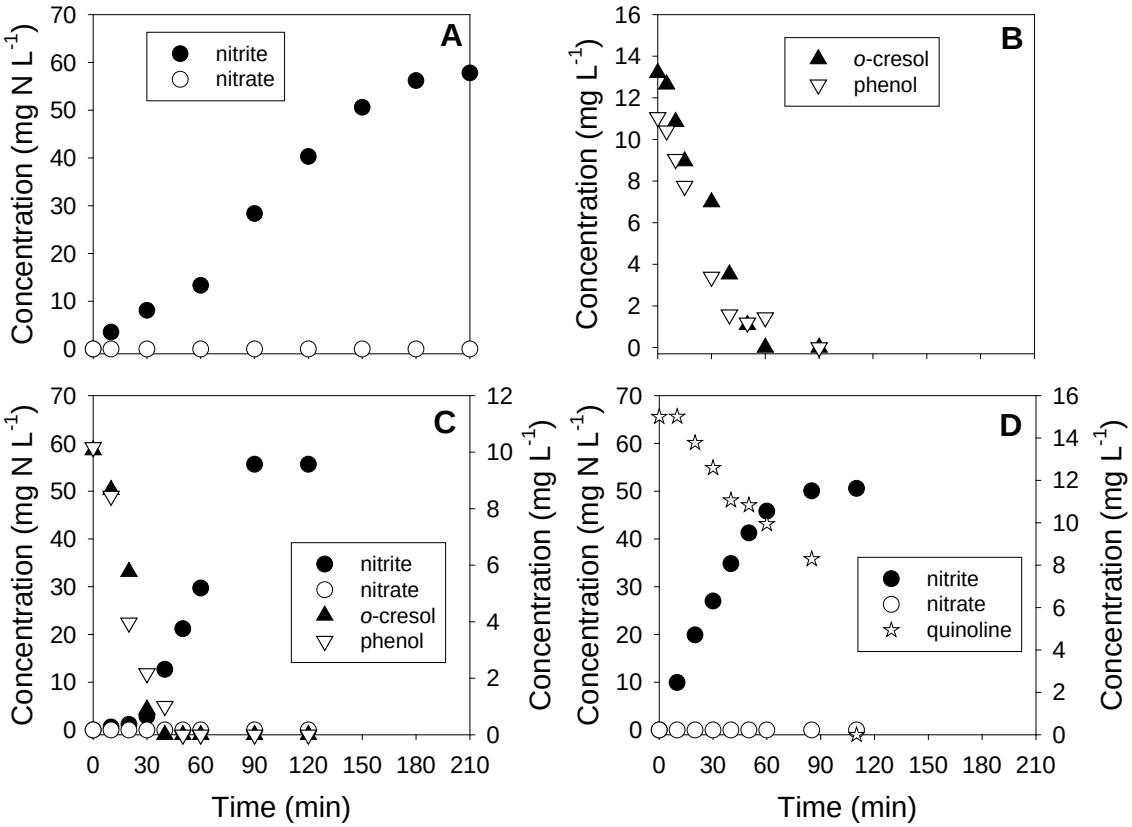

Figure 3. Kinetic study of the granular biomass performing nitritation and biodegradation of aromatic compound. A: ammonium (60 mg N L^{-1}); B: ammonium (60 mg N L^{-1}) plus *o*-cresol (13 mg L^{-1}) and phenol (11 mg L^{-1}); C: ammonium (60 mg N L^{-1}) plus *o*-cresol (10 mg L^{-1}) and phenol (10 mg L^{-1}); D: ammonium (60 mg N L^{-1}) plus quinoline (15 mg L^{-1}).

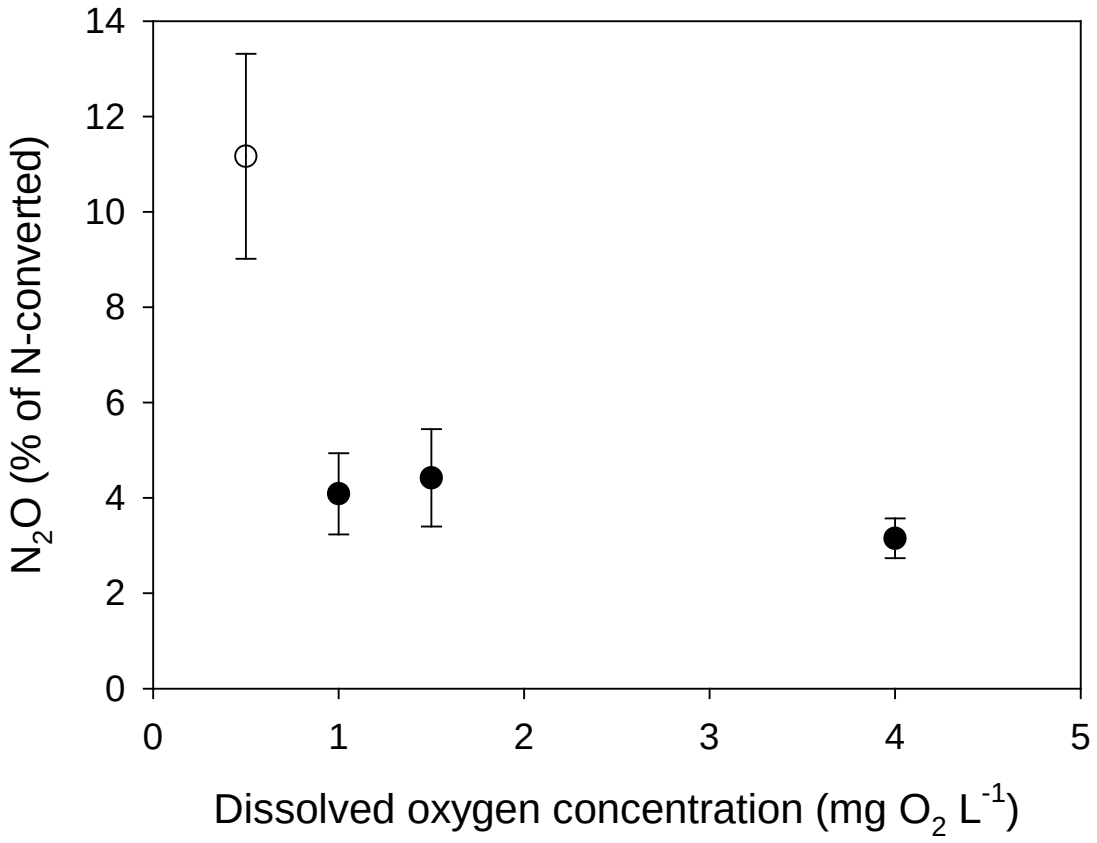

Figure 4. Nitrous oxide production under different dissolved oxygen concentrations in the granular airlift reactor treating ammonium, *o*-cresol, phenol and quinoline at high salinity (16 g salts L^{-1}).

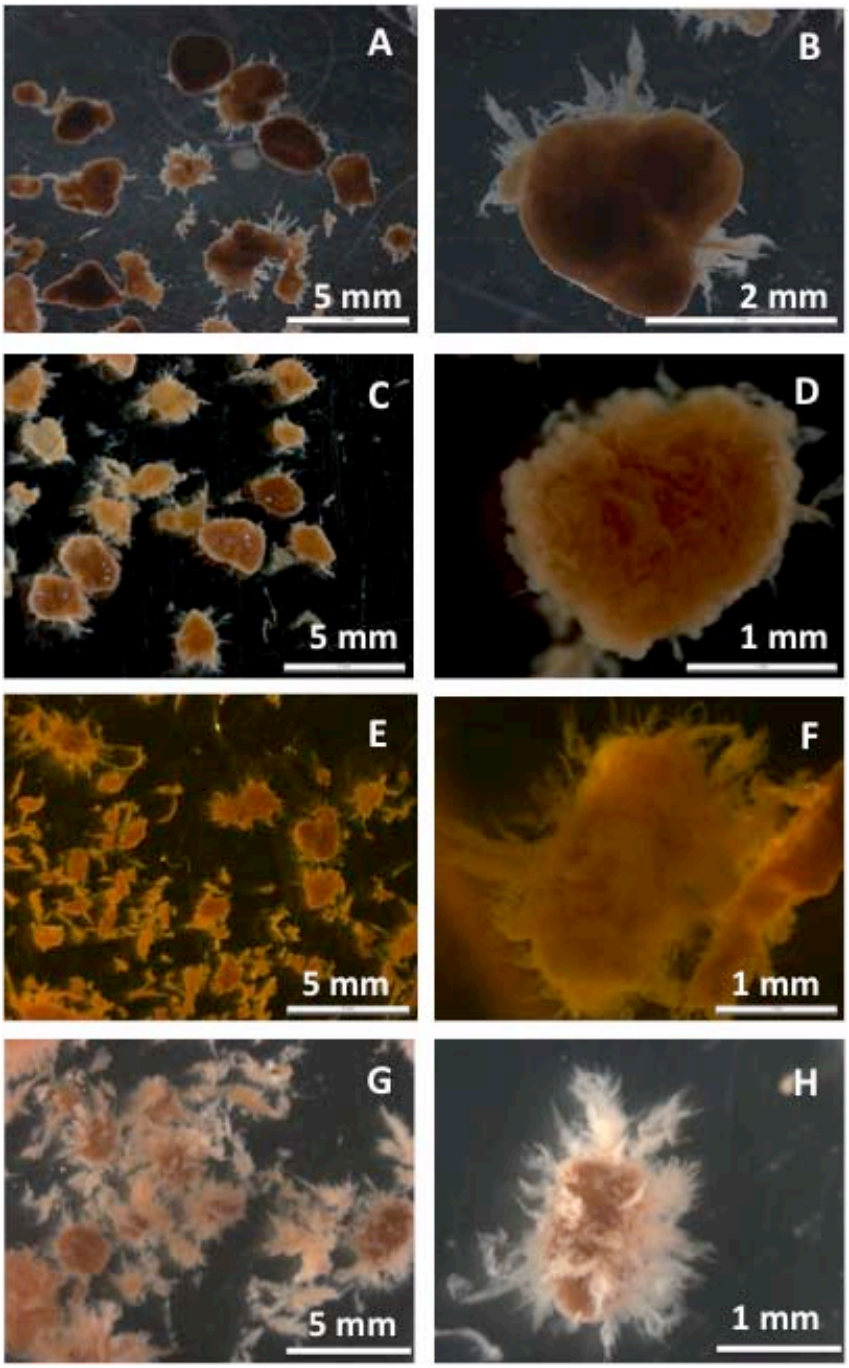

Figure 5. Pictures of the granules in the airlift reactor treating a wastewater composed by high ammonium and aromatic compounds concentrations. A and B treating ammonium and *o*-cresol with 97 % nitrite in the effluent; C and D treating ammonium, *o*-cresol and phenol with 97 % nitrite in the effluent; E and F treating ammonium, *o*-cresol, phenol and quinoline with 97 % nitrite in the effluent and G and H treating ammonium, *o*-cresol, phenol and quinoline with 54 % nitrite in the effluent.

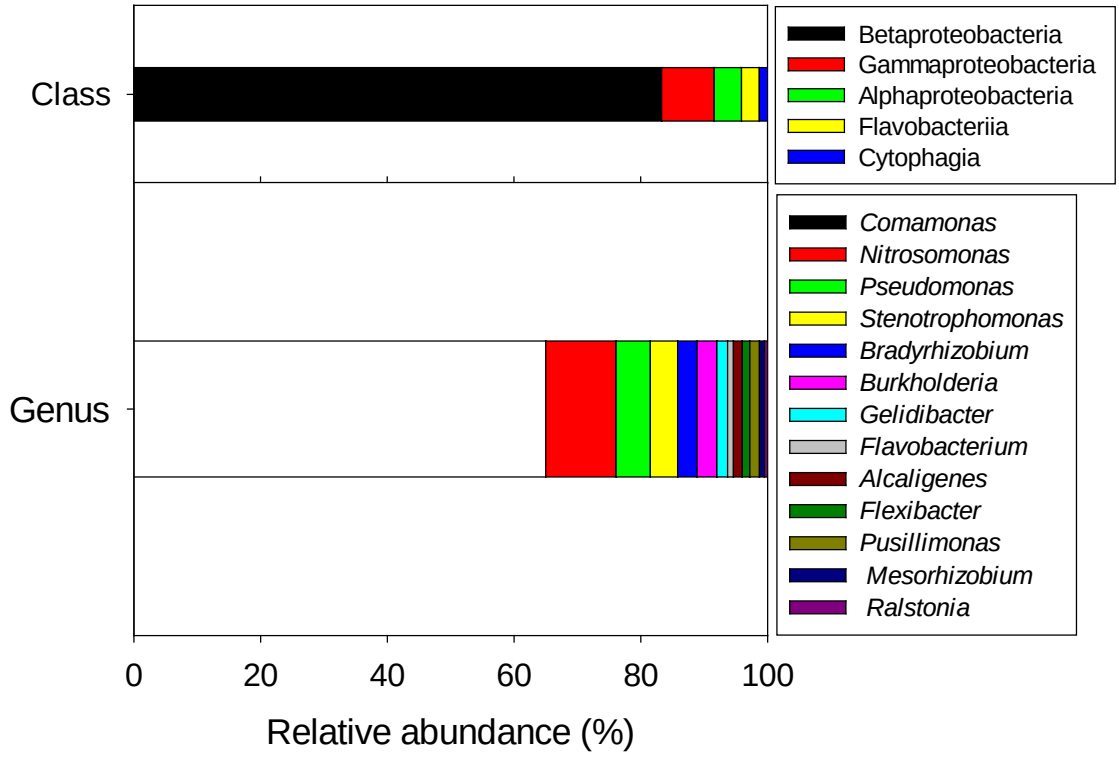

Figure 6. Bacterial distribution at genus and class level in the aerobic granules in the airlift reactor at day-300. The percentages are referred to the relative abundance of bacteria defined as the number of sequences affiliated with that taxon divided by the total number of sequences of the sample.

Table 1. Morphological characterization of the granular biomass along the operation of the airlift reactor performing nitritation and biodegradation of aromatic compounds. The nitrogen loading rate was always 1.1 g N L^{-1} d^{-1}.

Phase	Organic compounds in the influent	OLR (g COD L^{-1} d^{-1})	Nitrite effluent (%)	Settling velocity (m h^{-1})	Average granule size (mm)	Granule density (g VSS $L_{particle}^{-1}$)
I	*o*-cresol	0.3	97	57 ± 19	2.7 ± 0.8	57 ± 0
II	*o*-cresol + phenol	0.5	97	50 ± 23	2.4 ± 0.6	73 ± 4
III	*o*-cresol + phenol	0.7	97	35 ± 9	1.5 ± 0.4	95 ± 3
IV	+ quinoline	0.7	55	52 ± 17	1.0 ± 0.1	97 ± 3