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Acetate-fed aerobic granular sludge for the degradation of 4-chlorophenol

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ABSTRACT

Chlorinated phenols are considered a critical environmental problem, due to their extreme toxicity and their widespread use both in industrial and agricultural activities.

In this study, aerobic granular sludge was initially developed into an acetate-fed Granulated Sequencing Batch Reactor (GSBR) and then used for the degradation of low chlorinated 4-mono-chlorophenol (4CP), with readily biodegradable sodium acetate (NaAc) as co-substrate. Influent 4CP concentration ranged between 0 and 50 mg/l, with a maximum volumetric organic loading rate of 0.20 kg_{4CP}/m^3 d (0.32 $kg_{COD\text{-}4CP}/m^3$ d). Differences in granules shape and size were observed with 4CP dosed in the influent at different concentrations, and the effects of such toxic compound on acetate removal were evaluated, with both unacclimated and acclimated biomass.

Aerobic granules grown on acetate as carbon source proved to be an interesting solution for the degradation of 4CP, showing good resistance to high 4CP concentrations in the influent even if unacclimated (short term effects). Moreover, the monitoring of intermediate products and the evaluation of chloride release due to 4CP degradation proved that acclimated granular sludge could completely remove 4CP (long term effects), with high specific removal rates.

1. Introduction

Chlorinated phenolic compounds are characterized by their high toxicity even at low concentrations and their widespread use both in industrial and agricultural activities [1]: they can be found in industrial effluents from pulp and paper manufacture, preparation of wood preservatives, oil refining activities and textile industries, as well as components of many pesticides and biocides [2,3].

Due to the strong C-Cl bound, chlorinated phenols are resistant to biodegradation and then persistent in the environment, with their toxicity and bioaccumulative potential increasing with the degree of chlorination [4] and consequent chlorophenol lipophilicity [5].

In particular, 4-mono-chlorophenol (4CP) can originate from wastewater chlorination processes or from the breakdown of pesticides and chlorinated aromatic compounds [6]. Since chlorophenols aerobic biodegradation rate decreases with the increase of chlorine atoms in the molecule [7], the presence of only one chlorine atom in 4CP molecular structure makes its complete biodegradation possible under aerobic conditions.

Conventional wastewater treatment systems based on activated sludge have some disadvantages, e.g. low flexibility with respect to fluctuating loading rates, a large area requirement for reactors and secondary clarifiers, a relatively low volumetric conversion capacity and a high surplus biomass production [8].

Distinctive features of the aerobic granular sludge technology are [9,10]: the higher biomass concentration inside the reactors, the coexistence of a heterogeneous biomass and the lower sludge production due to high biomass retention. Since separation of sludge and effluent occurs inside the reactors, without any supplementary sedimentation tank, very small footprints are required.

Moreover, aerobic granular sludge already showed promising results on phenol degradation [11,12]: diffusive processes involved in granule-shaped biomass led to the formation of a phenol concentration gradient within the granules, able to protect the microorganisms in the inner layers thus allowing microbial activity and substrate utilization [13].

Such positive results being related to intrinsic granules characteristics [11], acetate-fed aerobic granules were used in this study to degrade toxic 4CP. Though 4CP was reported to be biodegraded as the sole carbon and energy source by either pure [14] and mixed [15] cultures, readily biodegradable sodium acetate was used in this study as co-substrate in order to guarantee a sufficiently high volumetric organic loading rate, which is considered an important parameter in order to obtain a stable granulation process [16,17]. If only 4CP was used as organic substrate, its influent concentration would be extremely high in order to keep a sufficient volumetric organic loading rate.

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2. Materials and methods

2.1. Reactor set-up

A glass GSBR with a working volume of 4.2 l and an internal diameter of 10.4 cm was used to carry out the experiments. The reactor was started-up with conventional activated sludge from a municipal wastewater treatment plant as inoculum. Air was supplied by a membrane pump and introduced via a fine bubble aerator at the bottom of the reactor, with a constant airflow of 10 N l/min (the corresponding superficial gas velocity was 1.96 cm/s).

The reactor was operated at 20 °C in 3-h cycles, each cycle consisting of 2 min influent addition (pulse feed), 171.5 min aeration with DO concentration at 100% of saturation value, 1.5 min settling and 5 min effluent withdrawal.

Effluent was discharged at 25 cm from the bottom of the reactor, with a volumetric exchange ratio of 50% and a corresponding Hydraulic Retention Time (HRT) of 6 h. Due to the short settling time applied, only particles with a settling velocity higher than 10 m/h could avoid washout from the GSBR.

The Sludge Retention Time (SRT) was not controlled during the experiments, thus varying depending on sludge settling properties.

The pH was constantly monitored and controlled within the range 6.8–7.2 by dosing 1 M H_2SO_4 or 1 M NaOH via peristaltic pumps.

A schematic representation of the GSBR is shown in Fig. 1.

2.2. Influent composition

The GSBR was started-up with a synthetic influent containing readily biodegradable sodium acetate (NaAc) as the sole carbon and energy source.

The synthetic influent, dosed into the reactor by two peristaltic pumps, consisted of 300 ml Medium A, 300 ml Medium B and 1500 ml tap water, with a total volume of 2.1 l.

The composition of the influent media was (A) NaAc 7 g/l, 4CP from 0 to 0.35 g/l, $MgSO_4{\cdot}7H_2O$ 0.60 g/l and (B) K_2HPO_4 2.30 g/l, KH_2PO_4 0.9 g/l, KCl 0.24 g/l, NH_4Cl 1.06 g/l, trace elements solution 6.125 ml/l (according to Vishniac and Santer [18]).

The applied volumetric organic loading rates were 4.0 $kg_{NaAc}/(m^3\ d)$ and from 0 to 0.20 $kg_{4CP}/(m^3\ d)$; considering that 1 kg_{COD} equals to 1.28 kg_{NaAc} [11] and 0.62 kg_{4CP} [19], volumetric organic loading rates in terms of COD were 3.125 $kg_{COD\text{-}NaAc}/(m^3\ d)$ and from 0 to 0.32 $kg_{COD\text{-}4CP}/(m^3\ d)$.

2.3. Analytical methods

The concentration of 4CP was determined by HPLC-UV using a DIONEX P680 pump equipped with a C8 column and a UV detector (preferred wavelength, 218 nm), the mobile phase consisting of acetonitrile/water (34/66, v/v).

The concentration of NaAc was determined by ion-chromatography using a DIONEX ICS-90 with an AS14A Ion-PAC 5 μm column.

Samples were drawn twice per week from both influent and effluent, as well as at the beginning of the aerobic phase. In order to determine NaAc and 4CP concentration profiles, samples were periodically taken at regular intervals during a cycle; the maximum removal rates were evaluated from the steepest part of each profile.

All samples were filtered (0.45 μm) and diluted with de-ionized water before analysis.

The maximum specific removal rates were then calculated as the ratio between the maximum removal rate and the VSS concentration in the GSBR.

The concentration of chloride ions was determined with an ORION chloride electrode (mod. 97-17B) and an ORION millivoltmeter (mod. 90-01): 25 ml filtered (0.45 μm) samples were diluted with distilled water (1:1) for a final sampling volume of 50 ml, and 1 ml ISA solution ($NaNO_3$ 5 M) was then added before analysis.

Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS) were determined by standard methods [20].

The SRT was calculated by dividing the amount of biomass in the reactor by the amount of biomass removed with the effluent per day.

The biomass density was determined as described by Beun et al. [21]; the SVI_8 was determined by reading the height of the settled bed after 8 min settling and calculated from the settled bed volume and the TSS concentration in the reactor. The ratio SVI_8/SVI_{30} could be calculated.

The presence of intermediate products deriving from 4CP biodegradation was determined by spectrophotometry. Samples were filtered (0.45 μm) and scanned at different wavelengths, thus determining the maximum absorbance as described by Bali and Şengül [22].

The amount of 4CP lost due to volatility was determined under the same operating conditions as in the GSBR, but without biomass. Samples were taken at the beginning and at the end of a cycle, and differences in 4CP concentrations between samples were evaluated.

3. Results

After a stable granulation was achieved with NaAc as the only carbon and energy source, 4CP was dosed in the influent with different concentrations. Different experimental Phases refer to changes in 4CP concentrations, as summarized in Table 1 (NaAc concentration in the influent was kept at 1 g_{NaAc}/l for the whole research, as co-substrate). Days refer to GSBR start-up with only NaAc as organic substrate.

According to previous studies [23,24], a pulse feed configuration was proved to be very important for a stable granulation under aerobic conditions. Such a configuration was used in this study in order to maximize the length of the aerated phase and, thus, the favorable conditions for 4CP oxidation. A similar GSBR cycle configuration was already successfully used for the aerobic degradation of phenol [11–13].

3.1. Phase A (days 0–119)

The GSBR was inoculated with conventional activated sludge and started-up with readily biodegradable NaAc as the sole carbon and energy source in order to achieve a stable granulation. Since acetate-fed aerobic granules were proved to be a valid microbial seed for the cultivation of stable phenol-degrading granules [11], and better than conventional activated sludge [12], the latter was not immediately fed with 4CP.

Granules developed quite soon, and the settling time could be decreased at1.5 min (corresponding to a minimum settling velocity of 10 m/h) on day 14.

NaAc removal efficiencies of 99 ± 100% were observed during the whole Phase A, while NaAc maximum specific removal rates reached a steady state condition from day 82, with average values of 0.60 ± 0.10 g_{NaAc}/g_{VSS} h.

VSS concentration in the reactor reached a steady state condition on day 89, with an average value of 3.21 ± 0.09 g_{VSS}/l and a VSS/TSS ratio of 0.94 ± 0.005. SVI_8 under steady state conditions

Table 1
Organization of experimental Phases, referring to 4CP dosed in the influent.

PHASE A	PHASE B	PHASE C	PHASE D
days 0–119 (0 mg_{4CP}/l)	days 120–131 (50 mg_{4CP}/l)	days 132–198 (30 mg_{4CP}/l)	days 265–295 (50 mg_{4CP}/l)

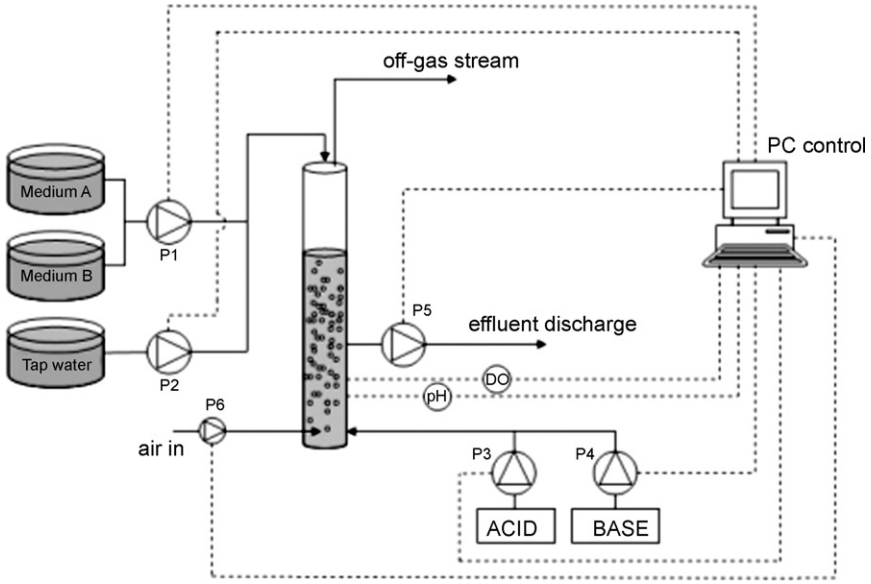

Fig. 1. Schematic representation of the GSBR.

was 81.6 ± 5.3 ml/g_{TSS}, very low with respect to the SVI_{30} measured for the activated sludge used as inoculum (150 ml/g_{TSS}). Observed maximum granules density was 48 g_{TSS}/l_{gran}.

3.2. Phase B (days 120–131)

In order to evaluate the short term toxic effects of 4CP on unacclimated granular sludge, 4CP was dosed in the influent at its maximum concentration (50 mg/l, corresponding to a volumetric organic loading rate of 0.32 $kg_{COD\text{-}4CP}/m^3$ d) together with NaAc (1 g/l) starting from day 120.

A decrease in acetate removal efficiency and acetate removal rates was observed. While acetate maximum specific removal rates dropped immediately after 4CP dosage (up to −90% with respect to Phase A), acetate removal efficiency decreased only after 4CP accumulation occurred. Table 2 shows the increasing 4CP concentration in the reactor immediately after the feeding phase (expected 4CP concentration was 25 mg/l) and in the effluent, during Phase B.

As shown in Fig. 2, acetate removal efficiency of 99–100% was completely restored on day 127, while 4CP removal efficiency never exceeded 32% (day 127). 4CP removal during Phase B was partly due to biological activity and partly due to volatilization (which was determined to be less than 4–5%) and possible adsorption on biomass.

As shown in Fig. 3, VSS concentration in the reactor did not show any particular change after 4CP dosage in the influent (average values of 3.53 ± 0.20 g_{VSS}/l, with respect to 3.21 ± 0.09 g_{VSS}/l during Phase A), so the temporary decrease in NaAc removal efficiency could be directly linked to the decrease in biomass activity due to 4CP inhibiting effect. NaAc maximum specific removal rates were only partially restored within few days (0.31 g_{NaAc}/g_{VSS} h on day 131). Average VSS/TSS ratio was 0.94 ± 0.002, indicating a very stable condition.

Table 2
4CP progressive accumulation in the GSBR during Phase B.

Day	4CP (mg/l, reactor)	4CP (mg/l, effluent)
120	23.65	21.25
121	32.54	22.91
124	37.98	28.14
127	37.07	25.36
131	39.76	32.74

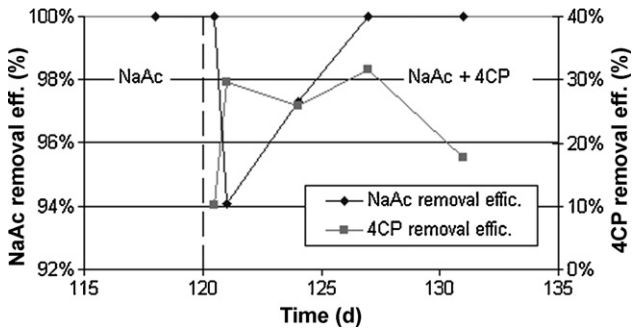

Fig. 2. NaAc and 4CP removal efficiencies during Phase B. Dashed line represents 4CP first dosage in the reactor.

Good granules settleability was maintained after 4CP dosage in the influent. Average SVI_8 values of 60.1 ± 9.4 ml/g_{TSS} were observed during Phase B, even lower than those obtained in the last part of Phase A, while observed granules density during Phase B increased up to 80 g_{TSS}/l_{gran} (day 131). A yellow color of the bulk liquid was observed few cycles after 4CP dosage in the influent and it persisted in the bulk liquid during the whole Phase B. A similar yellow bulk liquid was observed in previous studies [22,25,26] and spectrophotometric analysis confirmed (maximum absorbance was determined at 380 nm under neutral pH conditions) it was due to the presence of 5-chloro-2-hydroxymuconic

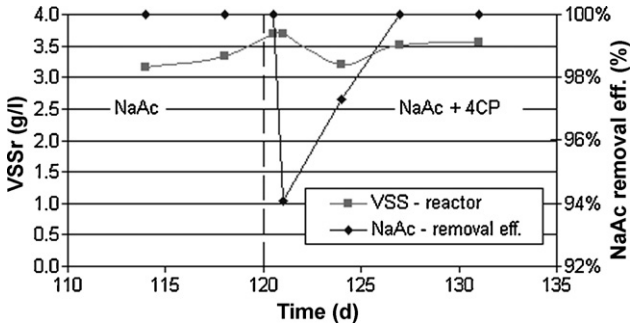

Fig. 3. VSS concentration in the reactor and NaAc removal efficiencies after 4CP dosage in the influent. Dashed line represents 4CP first dosage in the reactor.

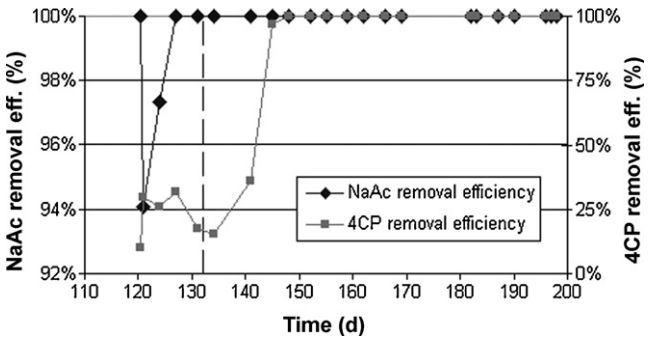

Fig. 4. NaAc and 4CP removal efficiencies during Phase B and Phase C. Dashed line represents the beginning of Phase C.

semialdehyde, an intermediate product of 4CP aerobic degradation.

3.3. Phase C (days 132–198)

Even if complete NaAc removal was restored on day 127, in order to avoid 4CP accumulation in the system, its concentration in the influent was decreased at 30 mg/l (corresponding to a volumetric organic loading rate of 0.19 $kg_{COD\text{-}4CP}/m^3$ d) on day 132.

As shown in Fig. 4, NaAc removal efficiencies did not change (99–100%) with respect to Phase B, while 4CP removal efficiencies increased up to 100% and did not vary during the whole Phase C.

Referring to NaAc and 4CP maximum specific removal rates, steady state conditions were reached on day 152 and 155 respectively; average values for NaAc and 4CP maximum specific removal rates under steady state conditions were $0.46 \pm 0.03\ g_{NaAc}/g_{VSS}$ h and $3.40 \pm 0.47\ mg_{4CP}/g_{VSS}$ h.

Differently from Phase B, the formation and subsequent complete degradation of 5-chloro-2-hydroxymuconic semialdehyde could be observed: the yellow color in the bulk liquid reached its maximum intensity around 30 min from the beginning of the aerated phase, and then completely disappeared before the end of the cycle. A chloride release of $0.27 \pm 0.02\ mg_{Cl}/mg_{4CP}$ due to 4CP degradation, very close to its stoichiometric value of 0.28 mg_{Cl}/mg_{4CP} [27] was observed.

During Phase C, average VSS concentration in the GSBR was $5.53 \pm 1.08\ g_{VSS}$/l, while the VSS/TSS ratio was 0.94 ± 0.003. Starting from day 162 (steady state conditions) low VSS concentrations in the effluent were observed, with an average value of $0.03 \pm 0.002\ g_{VSS}$/l.

According to the aforementioned experimental observations, granules improved their compact and dense structure, as also confirmed by TSS concentration and SVI_8 trends shown in Fig. 5. In particular, observed SVI_8 values were low (average value of 33.7 ± 6.0 ml/g_{TSS} considering the whole Phase C, and

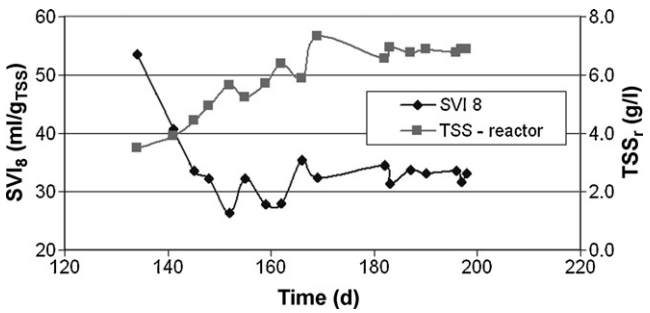

Fig. 5. Profiles of TSS concentration in the reactor and SVI_8 during Phase C.

31.8 ± 2.8 ml/g_{TSS} considering Phase C under stable conditions, from day 152).

3.4. Phase D (days 265–295)

As aerobic granules showed a good acclimation to 4CP, its concentration in the influent was gradually increased up to 50 mg/l (reached on day 265).

NaAc and 4CP removal efficiencies did not show any change (99–100% and 100% respectively), while average values of maximum specific removal rates were $0.39 \pm 0.06\ g_{NaAc}/g_{VSS}$ h and $4.65 \pm 0.99\ mg_{4CP}/g_{VSS}$ h, respectively. In particular, observed average 4CP maximum specific removal rates ($4.65 \pm 0.99\ mg_{4CP}/g_{VSS}$ h) were found to be competitive with those described in previous studies [22] (3.35 mg_{4CP}/g_{VSS} h, with a high density fed-batch reactor and glucose as growth substrate), even though direct comparisons between different technologies are always difficult since the high amount of published data often lacks in terms of uniformity.

During Phase D, formation and subsequent complete degradation of 5-chloro-2-hydroxymuconic semialdehyde, together with an almost stoichiometric chloride release, could be observed.

Average values of VSS concentration in the GSBR and VSS/TSS ratio were $4.25 \pm 0.21\ g_{VSS}$/l and 0.93 ± 0.004 respectively. Observed granules density was 70 g_{TSS}/l_{gran}, while average SVI_8 was 72.5 ± 2.7 ml/g_{TSS}. Average VSS concentration in the effluent was $0.06 \pm 0.002\ g_{VSS}$/l, under steady state conditions.

4. Discussion

According to Jiang et al. [13], a stronger microbial aggregation, i.e. the development of denser granules, is a valid defensive strategy against inhibiting effects of toxic compounds. Moreover, since substrate penetration inside granules is diffusion limited, biomass in the inner layers will experience a concentration of 4CP definitely lower than that in the bulk liquid, thus being protected against 4CP toxic effects. This could explain the increase in granules density observed during Phase B, which led, together with the almost complete washout of floc shaped biomass, to a low increase in TSS and VSS concentration in the GSBR (even though biomass was not acclimated) and to the consequent decrease in SVI_8 values with respect to Phase A.

Since VSS and TSS concentration during Phase A and Phase B were similar, the decrease in SVI_8 values could be related to a more compact structure and/or to a higher washout of fluffy and bad settling biomass. The latter possibility seems to be also confirmed by the increase in average VSS concentration in the effluent ($0.09 \pm 0.009\ g_{VSS}$/l during Phase B, $0.07 \pm 0.020\ g_{VSS}$/l during Phase A).

The formation of 5-chloro-2-hydroxymuconic semialdehyde as intermediate product of 4CP degradation was previously reported by Bali and Şengül [22]: the aerobic degradation of 4CP involves its transformation into 4-chlorocatechol, followed by ring meta-cleavage and resulting in the production of 5-chloro-2-hydroxymuconic semialdehyde. As shown in Fig. 6, the degradation can proceed further with chloride release and it ends with the TCA-cycle.

The presence of 5-chloro-2-hydroxymuconic semialdehyde in the GSBR proved that degradation of 4CP due to biological activity took place during Phase B. Anyway its persistence in the reactor and the negligible chloride release during the GSBR cycle confirmed that biological degradation of 4CP was only partial.

When 4CP influent concentration was lowered (Phase C), the absence of 5-chloro-2-hydroxymuconic semialdehyde in the effluent, together with the almost stoichiometric chloride release,

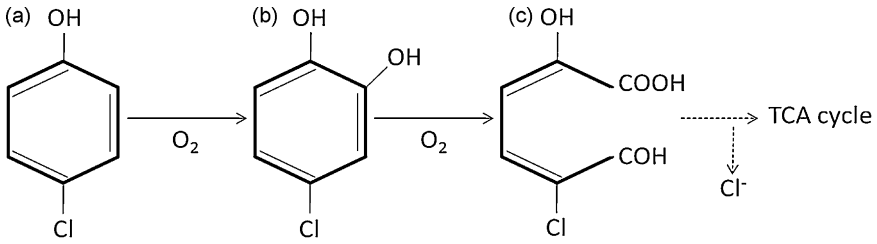

Fig. 6. Proposed 4CP aerobic degradation pathway, with (a) 4CP, (b) 4-chlorocathecol, (c) 5-chloro-2-hydroxymuconic semialdehyde (Bali and Şengül, 2002).

proved the complete dechlorination of 4CP as well as the negligible adsorption on biomass. The effects of volatilization were also taken into account.

The high increase in VSS concentration inside the GSBR observed during Phase C, with respect to Phase A and B, can be explained only if further biomass acclimation (leading to higher granules density) and the complete washout of floc shaped biomass are considered. In particular, during Phase C maximum granules density was 100 g_{TSS}/l_{gran} (very high with respect to 48 g_{TSS}/l_{gran} measured during Phase A), thus proving that microbial self aggregation reached its maximum intensity. On the other hand, low VSS concentrations in the effluent under steady state conditions (from day 162), with an average value of 0.03 ± 0.002 g_{VSS}/l (0.07 ± 0.02 g_{VSS}/l and 0.09 ± 0.009 g_{VSS}/l during Phase A and B respectively) could be clearly related to the complete washout of floc shaped biomass.

During Phase D, the formation and subsequent complete degradation of 5-chloro-2-hydroxymuconic semialdehyde, followed by an almost stoichiometric chloride release, proved that complete 4CP dechlorination was reached, even at its maximum concentration. As shown in Fig. 7, chloride release stopped around 40 min before the end of the cycle: even though 4CP losses due to volatilization were taken into account (<4–5%), observed chloride release (0.27 mg_{Cl}/mg_{4CP}) was anyway slightly lower than stoichiometric values expected (0.28 mg_{Cl}/mg_{4CP}). The amount of chloride not released due to biological activity might be related to 4CP adsorbed on biomass, which could be considered negligible (<4%).

With respect to Phase C (5.53 ± 1.08 g_{VSS}/l), VSS concentration was lower and it could be related to an increase in VSS concentration in the effluent (0.06 ± 0.002 g_{VSS}/l) and a decrease in granules density (70 g_{TSS}/l_{gran}), while floc-shaped biomass was not present under these conditions. Consequently, SVI_8 increased to an average value of 72.5 ± 2.7 ml/g_{TSS}.

Possible explanations for such behavior can be related to the complete biomass acclimation and to the gradual, slow increase in 4CP influent concentration. Sudden changes in 4CP influent concentration from 0 mg/l (Phase A) to 50 mg/l (Phase B), and then to 30 mg/l (Phase C), pushed unacclimated biomass to strongly enhance microbial self aggregation as a defensive strategy. Also changes in Extracellular Polymeric Substances (EPS) amount and composition (in terms of polysaccharides, PS, and proteins, PN) under different toxic compounds concentrations may influence granules structure and density: an increase in EPS production is commonly associated with the development of more compact and dense granules [28]. A strong increase in EPS production was observed [11] when a high phenol concentration was suddenly applied to unacclimated granular sludge (confirming the increase in granules density observed during Phases B and C); in the same study, a very low increase in EPS production was observed when the increase in phenol concentration was low (with unacclimated biomass). Such results may suggest that as biomass acclimation proceeds, the effects of gradual increase in 4CP influent concentration in terms of EPS production are lower than those related to a stepwise increase in influent concentration.

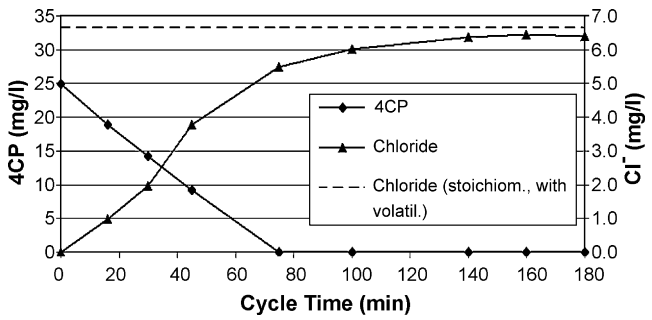

Fig. 7. Chloride concentration profiles measured during Phase D (dashed line represents the theoretical value of chloride release expected when the effects of volatilization are taken into account).

Moreover, gradual changes in 4CP influent concentration made biomass acclimation easier, and allowed a complete shift in microbial population. Clear changes in microbial population were observed by Tay et al. [12] in phenol-degrading aerobic granular sludge (previously developed with acetate as carbon source).

With a more specific biomass, developed under these conditions, self aggregation as a defensive strategy may play a less important role and aerobic granules could grow less compact and dense, still maintaining a good settling ability and high 4CP removal efficiencies.

The observed loss of biomass (related to lower granules density and to the consequent higher biomass washout) would be accompanied by lower biological activity (i.e. lower specific removal rates) if it was due to the inhibiting effects of 4CP: as previously reported, 4CP specific removal rates observed during Phase D were even higher than those observed during Phase C, thus meaning that the gradual increase in 4CP influent concentration did not negatively affect granular sludge in terms of biological activity, but only in terms of morphology and settling properties.

Table 3 sums up aerobic granular sludge main features during different experimental Phases: in particular, SVI_8 and density values observed during Phase D, even if worse than those determined during Phase B and C, were better than those observed during Phase A (no 4CP in the influent). This fact suggests that microbial self aggregation always plays a role when toxic compounds are present, this role being more or less important according to how changes in 4CP influent concentration are applied. SRT varied widely during the whole research (in brackets, SRT values observed in the second half of Phase C, days 166–198), reflecting different behaviors in biomass settling and subsequent washout.

Differences in granules size and density become evident when pictures taken during different experimental Phases are compared, as shown in Fig. 8. During Phase C (4CP influent concentration set at 30 mg/l), granules were characterized by the highest density (100 g_{TSS}/l_{gran}) and appeared smaller, more compact and uniform in size than those observed during Phase A, without 4CP in the influent (observed density, 48 g_{TSS}/l_{gran}).

Table 3
SVI_8, density, SRT and VSS effluent concentration values measured during different experimental Phases.

Parameter	PHASE A (0 mg_{4CP}/l)	PHASE B (50 mg_{4CP}/l)	PHASE C (30 mg_{4CP}/l)	PHASE D (50 mg_{4CP}/l)
SVI_8 (ml/g_{TSS})	81.6 ± 5.3	60.1 ± 9.4	31.8 ± 2.8	72.5 ± 2.7
Density (g_{TSS}/l_{gran})	48	80	100	70
VSS_e (g_{VSS}/l)	0.07 ± 0.02	0.09 ± 0.009	0.03 ± 0.002	0.06 ± 0.002
SRT (d)	12.6 ± 3.6	10.5 ± 0.8	33.9 ± 17.7 (47.4 ± 1.8)	15.39 ± 2.75

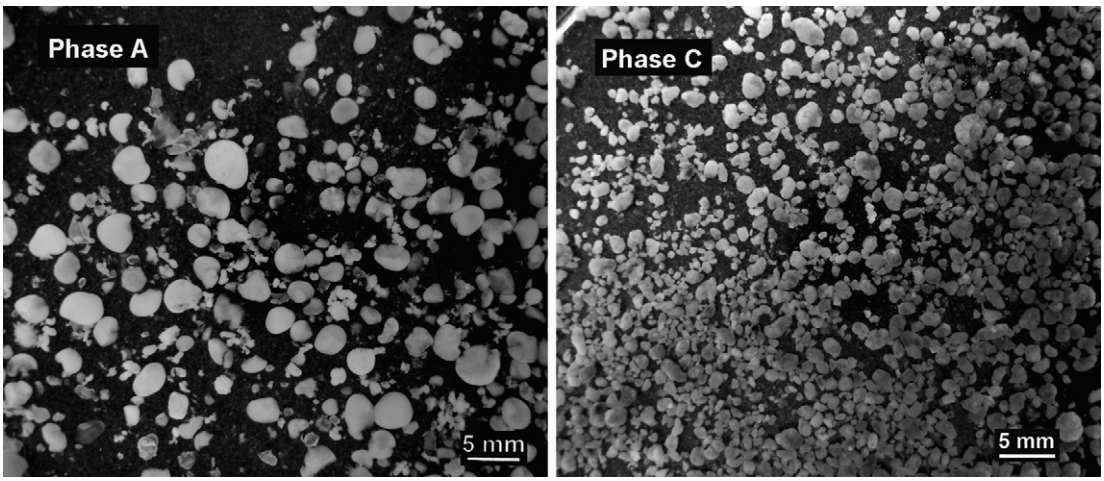

Fig. 8. Images of granular sludge taken during Phase A (minimum density) and Phase C (maximum density).

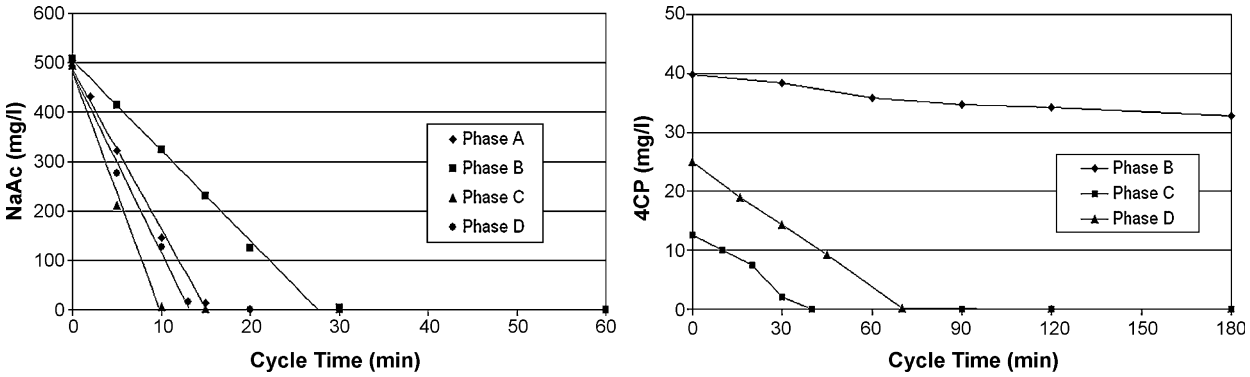

Fig. 9. NaAc concentration profiles during Phase A, B, C and D (left) and 4CP concentration profiles during Phase B, C and D (right).

Fig. 9 provides a comparison between biomass behaviors under different 4CP concentrations in the influent (in terms of biological activity), referring to both NaAc and 4CP. Fast and complete NaAc removal was achieved with no 4CP in the influent (Phase A); with unacclimated biomass (Phase B) and a similar biomass concentration, NaAc removal rates definitely dropped, reflecting an actual decrease in biological activity due to 4CP inhibition, with the latter not being completely removed from the system.

With higher biomass concentrations than the previous two phases, in Phases C and D NaAc removal rates increased though the specific removal rates were indeed lower than in Phase A (as described at the end of this section, Table 4). With unacclimated biomass (Phase B), 4CP complete removal was not achieved. However, during Phases C and D no 4CP could be detected in the effluent; profiles maximum slopes (representing the removal rates) were similar during Phase C and Phase D, and the differences in maximum specific removal rates are due to different VSS concentrations in the GSBR, reported in Fig. 10 and previously discussed.

With acclimated biomass, 4CP was completely removed from the bulk liquid within 70 min even at its maximum concentration in the influent (Phase D).

During the four experimental Phases, granules maintained a good settleability and compactness, with a SVI_8/SVI_{30} ratio of 1.02 ± 0.03.

Table 4 shows average NaAc and 4CP maximum specific removal rates during Phase A (only NaAc), B, C and D. Surprisingly, 4CP specific removal rates during Phase D are even higher than those

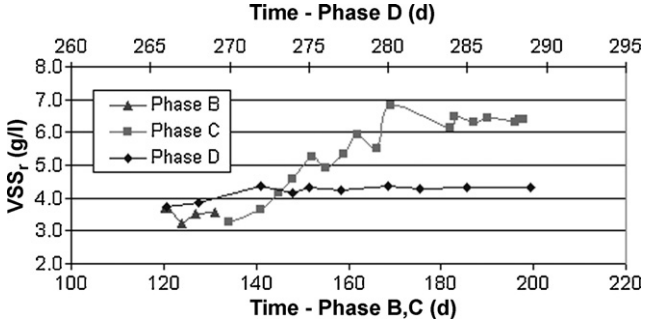

Fig. 10. Profiles of VSS concentration in the reactor during Phase B, C and D.

Table 4
NaAc and 4CP maximum specific removal rates during different experimental Phases.

Compound	PHASE A	PHASE B	PHASE C	PHASE D
NaAc (g_{NaAc}/g_{VSS} h)	0.60 ± 0.10	0.26 ± 0.16	0.46 ± 0.03	0.39 ± 0.06
4CP (mg_{4CP}/g_{VSS} h)	–	0.82 ± 0.44	3.40 ± 0.47	4.65 ± 0.99

measured during Phase C, showing a complete biomass acclimation to the toxic compound. Acetate removal was strongly inhibited during Phase B with unacclimated biomass and high 4CP influent concentration (50 mg/l). Just lowering 4CP influent concentration (Phase C) led to an increase in NaAc specific removal rates, which could not reach the values observed during Phase A (without 4CP): when 4CP clearly exerted its inhibiting effect on unacclimated biomass, both NaAc and 4CP specific removal rates were the lowest observed (Phase B). During Phase D, after that 4CP influent concentration was gradually increased up to 50 mg/l, observed decrease in NaAc specific removal rates with respect to Phase C was not accompanied by the same trend in 4CP specific removal rates (which was determined to be the highest for the whole research), as if microorganisms shifted their preference towards 4CP instead of NaAc.

5. Conclusions

On the basis of the results presented and discussed in this study, the following conclusions can be drawn:

- As expected, aerobic granules grown on acetate as the sole carbon and energy source proved to be a valid inoculum for the cultivation of stable 4CP degrading granular sludge.
- Under high 4CP influent concentration (Phase B), unacclimated aerobic granules were able to withstand the inhibiting toxic effect. A similar behavior by aerobic granules was also observed in previous studies for phenol degradation, and likely related to the high biomass density and to diffusive processes involved [11–13]; in a direct comparison with conventional activated sludge [12], aerobic granules grown on acetate showed faster acclimation to high 4CP influent concentrations and were proved to be a better inoculum for the development of phenol-degrading aerobic granules. From a parallel experimentation (data not included), we observed that activated sludge in a conventional SBR required lower initial 4CP influent concentrations and a longer time for complete acclimation than granular sludge.
- In our study, NaAc removal efficiencies were completely restored within few days after 4CP dosage, while NaAc maximum specific removal rates could be only partially restored. The progressive accumulation of 4CP in the GSBR was observed, together with the formation and accumulation in the system of 5-chloro-2-hydroxymuconic semialdehyde as dead-end product.
- Decreasing 4CP concentration in the influent at 30 mg/l (Phase C) was enough to allow biomass to acclimate: complete 4CP removal was observed, together with an increase in NaAc maximum specific removal rates. 5-chloro-2-hydroxymuconic semialdehyde was completely degraded and an almost stoichiometric chloride release was observed, thus proving the complete dechlorination of 4CP. Similar results were obtained with the same acclimated biomass and high 4CP concentration in the influent (Phase D, 50 mg/l).
- Long term exposure of acclimated granular sludge to 4CP did not compromise biological activity, even though the way 4CP influent concentration was changed (stepwise or gradually) seemed to play a role in terms of granules properties (i.e. density and settling ability).
- Average 4CP maximum specific removal rates obtained during Phase D were found to be competitive with those described in previous studies [22].
- Since toxic phenol is often used as co-substrate for the degradation of chlorinated phenols [27,29–31], with the optimal phenol/4CP ratio of 4:1 or higher [27], the successful use of NaAc (not toxic) as co-substrate seems to be a valid option both from the economical and sanitary point of view.
- Moreover, since the volumetric organic loading rate is not an independent parameter for aerobic granulation, but it is strictly related both to superficial air velocity and settling time [21], further investigations will be carried out in order to determine the optimal influent NaAc/4CP ratio, thus minimizing acetate consumption.
- Even though biomass washout cannot be considered negligible during the whole experimentation, specially during Phase A and B, it could be reduced by temporarily increasing the settling time as in conventional SBRs. For granular sludge, settling time can be increased without losing the selective pressure if other process parameters (i.e. organic loading rate and superficial air velocity) are properly modified [21].

Further investigations on shifts in microbial composition under different substrates applied (i.e. only NaAc, or NaAc + 4CP) must be carried out in order to better understand the process and optimize its operating parameters, as well as on the effects of gradual and stepwise changes in 4CP influent concentration on EPS production/composition, which may influence granules characteristics.

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