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4-Chlorophenol inhibition on flocculent and granular sludge sequencing batch reactors treating synthetic industrial wastewater

Mohammad Rafiee[a], Alireza Mesdaghinia[b,*], Mohammad Hossein Ghahremani[c], Simin Nasseri[a], Ramin Nabizadeh[a]

[a]*Department of Environmental Health Engineering, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
[b]*Department of Environmental Health Engineering, School of Public Health, Center for Environmental Research, Tehran University of Medical Sciences, Tehran, Iran*
Tel. +98 21 88954914; Fax: +98 21 8895 0188; email: mesdaghinia@sina.tums.ac.ir
[c]*Pharmacology Research Laboratory, Department of Toxicology-Pharmacology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran*

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ABSTRACT

Two laboratory-scale column-type sequencing batch reactors (SBRs) were used to investigate the inhibition of suspended flocs and aerobic granular biomass at COD_{4CP}/COD_{Total} ratios ranging from 0 to 1. The inhibitory concentrations of 4-chlorophenol (4CP) were determined as a function of substrate consumption rate (q_{max}) and specific oxygen uptake rate (SOUR), as well as of chemical oxygen demand (COD) and 4CP removal efficiencies. Monoethylene glycol was used as a biogenic substrate to simulate industrial wastewater. Granular SBR (GSBR) showed high efficiency in both COD and 4CP removal, at concentrations of up to 620 mg/L; however, the process ceased at 200 and 550 mg-4CP/L in the suspended flocs SBR (FSBR). Other than dosing various concentrations of 4CP in the system, the lack of sufficient dedicated time led to the accumulation of inhibitory compounds over time. At inhibitory concentration levels, only partial degradation of 4CP occurred, which resulted in 5-chloro-2-hydroxymuconic semialdehyde accumulation in the system. Assimilatory removal of 4CP was negligible at the normal performance of FSBR, but reached considerable values in GSBR, or at the time of cessation of FSBR performance. The bulk liquid color evolution in the case of repeated feast and famine conditions, both after process cessation and in the absence of 4CP in the feed, emphasized the crucial role of the biodegradable organic compound to enhancement of 4CP removal. An increasing trend of q_{max} was not only observed at any definite concentration of 4CP as the cycles proceeded, but also with the feed ratio of $4CP/COD_{Total}$ in both reactors. In comparison with FSBR, higher q_{max} was observed in GSBR, as biomass completely adapted to 4CP, suggesting that the granular bioreactor is limited by diffusion rather than biomass.

Keywords: Sequencing batch reactor; Aerobic granular sludge; Inhibition; 4-Chlorophenol; Mono ethylene glycol; CHMS; Assimilation

1. Introduction

Biological processes involved in domestic and industrial wastewater treatment deal with living organisms and are therefore subject to disturbance by inhibitory or toxic agents. The effect of toxicity is manifested in a lower degradation rate and, usually, alteration of biomass activity [1]. Chlorinated phenolic compounds are considered to be a major group of priority pollutants. They are characterized by their

*Corresponding author.

high toxicity, persistence, and their widespread use, both in industrial and agricultural activities. Owing to their persistence, chlorophenols have been classified as dangerous organic substances that affect human health and the environment [2–4]. Chlorophenols are also known for their inherent toxicity to micro-organisms and for their recalcitrant nature. They can be found in industrial effluents from pulp and paper manufacturing, preparation of wood preservatives, petroleum refining activities, the manufacture of plastics, resins, and textiles, the iron, steel, and textile industries, as well as being components of many pesticides and biocides [5,6]. Levels of chlorinated phenols have been reported as ranging from 150 μg/L [7] to 100–200 mg/L [8] in contaminated environments. Monochlorophenols are formed as an intermediate in the biodegradation of highly chlorinated phenols. The formation of monochlorophenols has also been reported during the chlorination of wastewater [9,10]. Ye and Shen [11] reported that, of all the monochlorophenols, acclimation and degradation of anaerobic sludge with 4-chlorophenol (4CP) was the most difficult to achieve. In the present study, 4CP was chosen as the target compound for removal from simulated industrial wastewater. It is a slowly biodegradable compound which exhibits the characteristic of cell growth inhibition in wastewater treatment plants. The recalcitrance of chlorophenols results from the carbon–halogen bond, which is only cleaved with great difficulty, and the stability of their aromatic structures results in their accumulation in nature [12,13].

Increasing concerns over the environment and health have led to the development of new bioremediation technologies capable of dealing with toxic pollutants. A variety of clean-up methods are available for toxic or inhibitory compounds, depending on the type of material contaminated. In contrast to physico-chemical options, bioremediation is a low cost alternative which, theoretically, produces no toxic end products [14,15]. The use of activated sludge as a suitable inoculum to treat toxic and inhibitory compounds, due to its microbial diversity, is recommended. However, an adequate acclimation has been found to be the key issue in achieving the degradation of recalcitrant and xenobiotic compounds [16]. Depending on the quantity and quality of the inoculum utilized, as well as the conditions under which acclimation is carried out, its duration ranges from relatively short periods (where induced enzymes are involved) to several weeks, months, or even years (where more fundamental changes are necessary). It has been demonstrated that the dynamic conditions of operation which are recognized as typical of discontinuous systems such as sequencing batch reactors (SBRs) favor an induction process able to develop specific metabolic pathways [17].

The novel environmental biotechnological process using aerobically grown microbial granules in SBRs is increasingly drawing the interest of researchers engaged in the field of biological wastewater treatment. The advantages of aerobic wastewater treatment using microbial granules are the formation of dense and strong microbial structures, excellent settleability, high retention of granulated biomass, diversity of physiological functions of micro-organisms in the granule, and micro-organisms' resistance to toxic substances inside the granule as compared to conventional activated sludge flocs [18–20]. The introduction of granular sludge sheds light on aerobic wastewater treatment of toxic aromatic pollutants; it has consequently received increasing interest in the context of wastewater treatment.

The aim of this investigation was to evaluate the inhibition of suspended flocs and aerobic granular biomass systems in the presence of 4CP. The inhibitory concentrations of 4CP were determined as a function of the decrease in substrate consumption rate, q_{max}, and SOUR.

Cosubstrates have been used to enhance xenobiotic uptake rates, as well as allowing higher concentrations of these recalcitrant compounds to be treated. Several works propose that the addition of growth substrates may aid in lowering both the toxicity and growth inhibition of xenobiotics on cells or act as inducing agents for biodegradative enzymes [21]. Biotransformation of xenobiotic toxic compounds, such as chlorophenols, is generally more complex, because these compounds cannot support cell growth and can only be transformed in the presence of a growth substrate. The growth substrate not only serves to sustain biomass production, but also acts as an electron donor for degradation of non-growth substrate such as chlorophenols [21]. In the present study, 4CP and monoethylene glycol (MEG) have been selected as the target pollutant and growth substrate, respectively. MEG is an industrially important solvent that is completely miscible with water in all proportions. While the largest use of MEG is in the automobile industry as an antifreeze, it is also used in paints, inks, etc. and in the manufacture of polyester fibers [22]. Additionally, it is used to guarantee a sufficiently high volumetric organic loading rate, which is considered as an important establishing parameter for a stable granulation process [23,24].

2. Materials and methods

2.1. Experimental set-up

Two laboratory-scale column-type reactors, each with a working volume of 2 L and housed in a temperature-controlled room at 25°C, were used. The internal diameter and total height of the column was 5 and 120 cm, respectively. The reactor working H/D ratio was approximately 20. Both reactors were operated in a sequencing batch mode. Five ports for sampling, removing effluent, and sludge withdrawal were placed at various distances from the reactor bottom. Effluent was discharged from the middle port of the reactor at a volumetric exchange ratio of 50%, thus 1 L was left in the reactor after effluent withdrawal. Four porous air stones were placed symmetrically at the bottom of the reactor for aeration and mixing at an airflow rate of 2–4 L/min (equivalent to a superficial gas velocity of 1.7–3.4 cm/s). The air flux was controlled by a flow meter. The reactors operated sequentially in 6-h cycles. Phase duration and operating conditions of each SBR are given in (Table 1). The working cycles were controlled by a PLC time controller (Omron, Japan).

The reactors were seeded by adding 1,000 mL of activated sludge (3,200 mg/L suspended solids) obtained from the aeration basin of a municipal treatment plant and were fed with synthetic wastewater which consisted of MEG as the main source of carbon and energy, and other nutrients. In order to increase the formation of biomass, decrease the setbacks imposed by the negative effects of toxic chemical wastewater and improve the system's stability, glucose and sodium acetate (NaAc) were used as the sole carbon source before adding MEG.

Nutrient composition used was as follows: K_2HPO_4, 58 mg/L; KH_2PO_4, 25 mg/L; $MgSO_4{\cdot}7H_2O$, 75 mg/L; NH_4Cl 191, $CaCl_2{\cdot}2H_2O$, 50 mg/L; and trace solution, 1 ml/L. The composition of the trace solution was: $FeCl_3{\cdot}6H_2O$, 1.5 g/L; $CuSO_4{\cdot}5H_2O$, 0.03 g/L; H_3BO_3, 0.15 g/L; $MnCl_2{\cdot}4H_2O$, 0.12 g/L; KI, 0.03 g/L; $Na_2MoO_4{\cdot}2H_2O$, 0.06 g/L; $ZnSO_4.7H_2O$, 0.12 g/L; and $CoCl_2{\cdot}6H_2O$, 0.15 g/L [25]. Sodium bicarbonate ($NaHCO_3$) was also dosed into the synthetic wastewater to maintain the suspension pH in a neutral range between 6.5 and 8.0. The concentration of 4CP was gradually increased from 0 to 550 mg/L by keeping the chemical oxygen demand (COD) content of the feed solution at approximately 1,000 mg/L (1 mg 4CP/L = 1.62 mg COD/L and 1 mL MEG/L = 1,430 mg COD/L).

Table 1
Phase duration of SBRs

Operational step	SBR	
	Suspended flocs	Aerobic granules
Fill (min)	5	5
React (min)	320	348
Settle (min)	30	2
Decant (min)	5	5
Idle (min)	0	0
Total cycle length	360	360

Reactor 2 was initially operated in identical mode to Reactor 1. After 21 days of operation, during which steady-state conditions were established in both reactors, the sludge settling time in Reactor 2 was reduced gradually from 30 to 15, 5, 3, and 2 min, consecutively, and the air flow rate was increased from 2 L/min to its maximum value (i.e. 4 L/min) in order to cultivate aerobic granules. The biomass structure changed significantly thereafter, and aerobic granules developed in the reactor. This reactor was subsequently operated as a granular aerobic sequencing batch reactor (GSBR).

Organic loading rate and hydraulic retention time (HRT) were 2 kg COD/m^3 d and 12 h, respectively. Sludge retention time (SRT) of 10 days was obtained by daily removal of 200 mL of mixed liquor.

2.2. Analytical methods

The concentration of 4CP was measured spectrophotometrically using the 4-aminoantipyrine method with a detection limit of 0.1 mg/L [26]. Samples were centrifuged or filtered on 0.45 μm mixed cellulose ester membrane filters (Schleicher & Schuell), following which they were acidified in order to stop 4CP biodegradation being caused by the residual biomass not retained in the filter. Samples were then analyzed by measuring the absorbance at 500 nm. Total suspended solids (TSS), volatile suspended solids (VSS), sludge volume index (SVI), dissolved oxygen (DO), and COD were determined according to standard methods [26], except for COD analysis, in which a high dose of $AgSO_4$ was added to the samples to eradicate chloride interference. For oxygen uptake rate (OUR) determination, mixed liquor samples with specific VSS concentrations were filled to overflowing in a 500 mL Erlenmeyer flask equipped with a gas-tight stopper. DO levels were measured using a DO meter (YSI 5000, USA) at time intervals of 30 s until DO reached either zero or a constant stable value. The first step in the procedure was the preaeration of the biomass suspension until a constant stable DO concentration was obtained. The oxygen supply was then turned off,and the respiration rate was detected from the oxygen depletion

profile. The specific oxygen uptake rate was evaluated from OUR data and VSS concentrations. Accordingly, q_{max} was calculated as $q = (dS/dt)_u/X$; where the derivative $(dS/dt)_u$ represents the substrate utilization rate, X is the concentration of biomass present, and q_{max} is the maximum specific substrate utilization rate.

Determination of 4CP degradation products was conducted by spectrometry. Filtered samples were scanned at different wavelengths and the maximum absorbance was determined. Thereafter, the absorbance of solution was measured at determined wavelengths.

Abiotic 4CP removal by adsorption on biomass was determined by centrifugation of approximately 25–50 mL sludge sample at the end of one SBR cycle. Supernatant was then removed. The 25–50 mL 0.1 N NaOH was added to the sludge samples and the mixture then shaken for 10–20 min to desorp 4CP from cell surfaces. The sample was centrifuged again and supernatant was further filtered. The supernatant was finally subjected to 4CP measurement.

3. Results and discussion

The study of microbial xenobiotic metabolism is mainly aimed at using micro-organisms to remove toxic or inhibitory pollutants by biodegradation. However, bioconjugation and bioaccumulation are also involved in bioremediation. Abiotic removal of a target compound may also occur. The bioremediation discussed in this paper is limited to bioaccumulation and biodegradation. Abiotic removal of 4CP is also discussed.

3.1. Biodegradation of 4CP

3.1.1. Start-up and acclimatization

After a stable operation was achieved with MEG as the sole carbon and energy source (at COD 1,000 mg/L) and active biomass established, the operational parameters (aeration rate and settling time) in GSBR were changed so as to achieve granulation. Mature and stable granules (1.5–2 mm) with very compact interiors appeared 52 days after operation. While it is well known that aerobic granulation is a gradual process from seed sludge to mature granules, with reference to the literature this was a fairly long time for granular sludge cultivation. This can be explained well by using the surface chemistry in MEG [22]. MEG penetrating inside the flocs seems to form loosely structured aggregates. However, as biomass was adapted to use MEG as the sole source of carbon and energy, compact and dense structures were formed. One major cause of well-compacted cultivated aerobic granules is the low volumetric organic loading rate, L_{org}, used in the present study (2 kg COD/m^3 d). Although the effect of substrate loading rate on the formation of aerobic granules was considered insignificant, the applied loading rate was lower than the reported range of L_{org} (2.5–15.0 kg COD/m^3 d) [27,29]. High L_{org} can raise the biomass growth rate, which in turn reduces the strength of the structure of aerobic granules [27,29].

4CP was initially dosed in the influent at a concentration of 5 mg/L. 4CP removal efficiencies of 78–94% were obtained during this initial adaptation phase, indicating a progressive improvement in the capability of the biomass to degrade the inhibitory compound. After a few cycles, the bulk liquid color in both reactors turned greenish-yellow, which lasted during the whole cycle. The color did not remain in the reactor for a long time, as it faded at the end of the eighth cycle. Thereafter, the appearance of color and, subsequently, its absence was noted at the end of the aeration phase in each cycle. The waxing and waning of similar colors have been reported in other studies [21,24–30]. The meta-cleavage intermediate by-product of 4CP degradation, 5-chloro-2-hydroxymuconic semialdehyde (CHMS), is known to be responsible for this observation [31]. For the sake of clarity, the maximum absorbance was determined at 380 nm, spectrophotometrically. According to the mechanism suggested by Bali and Sengül (2002, Fig. 1), the pathway for 4CP biodegradation under aerobic conditions involves its transformation into 4-chlorocatechol by the activity of phenol hydroxylase, followed by ring meta-cleavage which results in the production of CHMS [31]. CHMS seems to be the last dead-end metabolite in the mineralization process by pure cultures [32,33]; however, further degradation, especially in mixed cultures, ends at the TCA cycle with chloride release [21,24,33]. The appearance of CHMS in both reactors confirmed biological degradation of 4CP during the cycle. Nonetheless, its existence in the reactor effluent during the few initial cycles after 4CP dosage in the influent

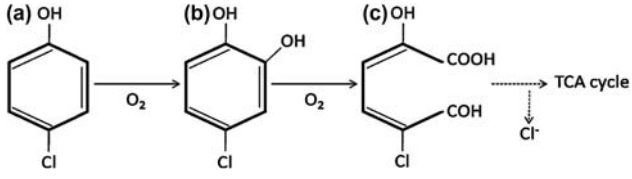

Fig. 1. 4-Chlorophenol degradation pathway under aerobic conditions with (a) 4CP, (b) 4-chlorocathecol, and (c) CHMS [31].

showed incomplete biological degradation of 4CP. This behavior, noted in the initial time lag, is accounted for the time necessary to develop new metabolic pathways which induce biomass acclimation and, as a result, the biodegradation of xenobiotic compounds in the early stages of reactor operation [34]. After a stable condition was achieved, 4CP was progressively dosed in the influent with different ratios of COD_{4CP}/COD_{Total} while the total COD was maintained at 1,000 mg/L by a contemporaneous decrease of biogenic substrate fraction. The details of acclimation are shown in (Fig. 2): complete biodegradation of 4CP occurred in both flocs SBR (FSBR) and GSBR. COD and 4CP removal efficiencies of 98–100% were noted along the progressive increase of 4CP loading rates after steady state conditions were established.

In all experiments, accumulation of greenish-yellow color increased in the reactor in parallel with the removal of 4CP, typically as shown in Figs. 3 and 4. The highest concentration of color was detected (at 380 nm) when 4CP was just completely removed in the bulk liquid. As illustrated in (Fig. 3), the maximum absorbance was typically observed in approximately 80 and 180 min for 4CP concentrations of 100 and 300 mg/L, respectively. Roughly similar trends were observed for other concentrations; i.e. maximum values of color and its rapid formation were detected at low concentrations of 4CP. On the other hand, color was gradually increased at higher concentrations, whereas a rapid decrease in color was observed in all experiments when biomass had been acclimated to the inhibitory compound. The later was more apparent at lower concentrations of 4CP. Such an observation can possibly explain the appearance of this color in the FSBR effluent at the inhibitory concentrations (200 and 550 mg/L) of 4CP, which led to the ceasing of reactor performance. Furthermore, at such high concentrations only a limited microbial community capable of purely partial degradation of 4CP could be viable and/or metabolize 4CP as a substrate. Fig. 5 depicts this assumption. This figure illustrates the FSBR performance at 36 cycles after initial inhibition of biomass function induced by a shock concentration of 4CP (200 mg/L), which forced a shift away from 4CP feed-

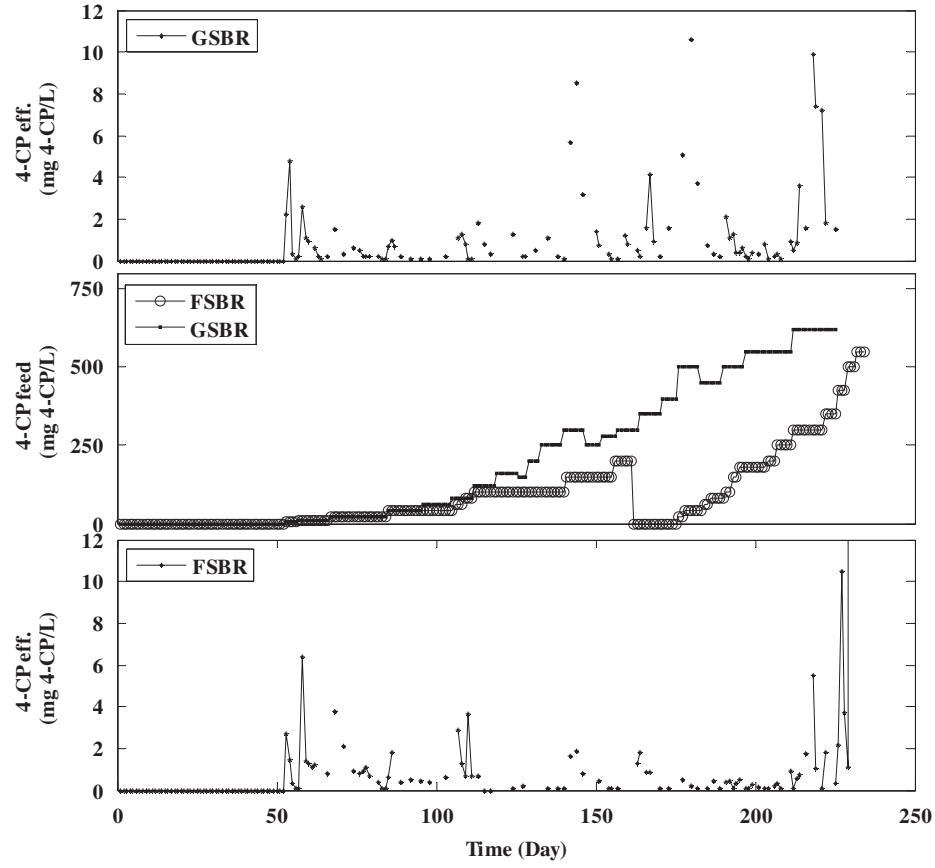

Fig. 2. Details of acclimatization procedure to the increasing 4CP loading rates.

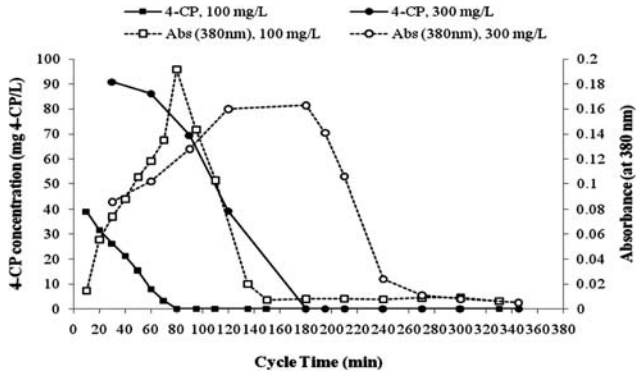

Fig. 3. Typical pattern of 4CP concentration and CHMS evolution profile at C_{4CP} concentrations of 100 and 300 mg/L (FSBR).

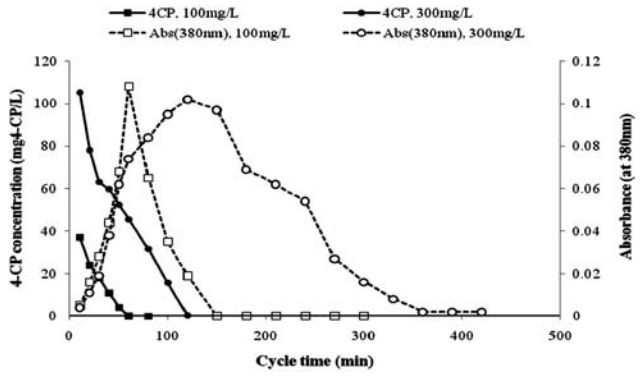

Fig. 4. Typical pattern of 4CP concentration and CHMS evolution profile at C_{4CP} concentrations of 100 and 300 mg/L (GSBR).

ing to totally biogenic substrate. It is possible to assume that this was due to the diffusion of adsorbed 4CP on biomass into the bulk liquid as the new feed was dosed into the system, and subsequently the diffused 4CP was biologically transformed into CHMS in the presence of biogenic substrate. However, biotransformation ceased once the biogenic substrate was wholly consumed and CHMS was accumulated in the system without a considerable evolution. It is clear from this figure that only partial degradation of 4CP with accumulation of CHMS took place in the system. Therefore, it can be concluded that the biomass behaved like a pure culture. Nongrowth substrates, such as 4CP, cannot support cell growth and can only be transformed by a specific community of microorganisms [35]. Variation in microbial population was observed (data not shown), and has also been observed in other studies, in proceeding to biological degradation of toxic or inhibitory compounds [36–38]. Although degradation of this by-product and complete removal of color was then achieved after acclimation of biomass, it has been widely reported as being a dead-end metabolite, especially in working with pure cultures [32,33]. Moreover, in both of these figures (Figs. 3 and 4), a steep decline in 4CP concentration is observed in the very initial reaction phase that is, to a major extent, attributed to the homogenization of the solution.

3.1.2. Biomass activity and maximum specific removal rate

After establishment of steady state conditions, complete degradation of 4CP, even at concentrations as high as 550 mg/L, was observed in the first 1–2.5 h in GSBR, whereas it occurred in 3–4 h in FSBR (Figs. 3 and 5). Previous studies have reported at least 8 h for complete degradation of 4CP in SBR [15]. Complete

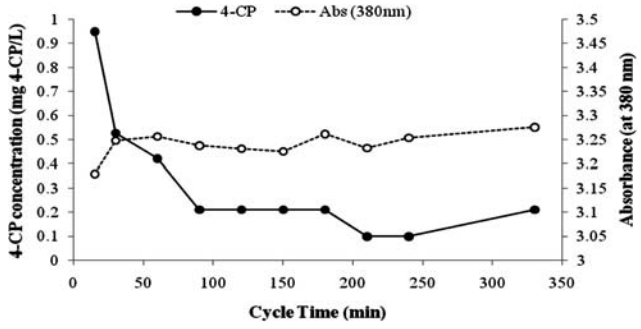

Fig. 5. Typical pattern of 4CP concentration and CHMS evolution profile 36 cycles after initial inhibition of flocculent biomass ($C_{4CP} = 0$ mg/L).

removal of 4CP within 70 min with acclimated granular biomass has also been reported at a maximum concentration of 50 mg/L [24]. This phenomenon can be related to the higher concentration of microbial population in GSBR and the short starvation period applied in the present study (especially in GSBR). The negative influence of the starvation period on the activity of the acclimated biomass has been demonstrated [18].

VSS concentration had a regular fluctuation, with an average of 2,985 and 5,470 mg/L in FSBR and GSBR, respectively. VSS concentrations did not change considerably during the operation of reactors; however, SOUR decreased immediately in each 4CP increment as 4CP organic loading rate increased. Moreover, owing to the inhibitory nature of 4CP on biomass growth, SOUR plummeted and then retrieved to somewhat less than its last value in both reactors (Fig. 6). Significantly higher SOUR values observed after the initial peak and before the endogenous phase at lower concentrations of 4CP (particularly 40 mg/L and, to some extent, 100 mg/L) are likely to be attributable to the consumption of biogenic substrate.

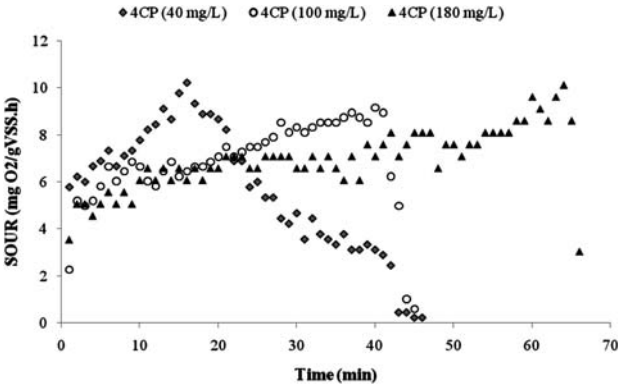

Fig. 6. Typical patterns of time course variation of biomass activity (SOUR) at different 4CP concentrations.

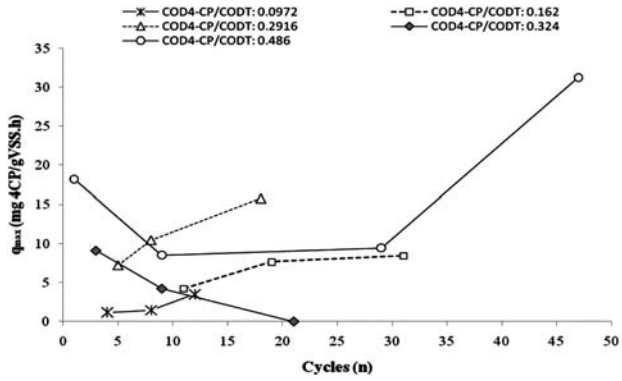

Fig. 7. Trends 4CP consumption rates, q_{max}, at different ratios of COD_{4CP}/COD_{total} (FSBR; COD_{4CP}/COD_{Total}: 0.0972 and 0.324 corresponds to 60 and 200 mg 4-CP/L, GSBR; COD_{4CP}/COD_{Total}: 0.162, 0.2916, and 0.486 corresponds to 100, 180, and 300 mg 4-CP/L).

An initial time lag at higher concentrations of 4CP (typically 180 mg/L) is necessary for biomass adaptation to the new environment, as expected, and the increasing trend of SOUR illustrates a progressive improvement in the biomass capability to degrade the available substrate. A similar behavior has also been reported for biodegradation of xenobiotic compounds in SBR reactors [36].

4CP concentrations lower than 0.1 mg/L were observed in the effluent for the whole experimental period after achieving steady state conditions, excluding the concentration of 200 mg/L at which the FSBR performance suddenly ceased. GSBR worked well even at concentrations of up to 620 mg/L, without any interruption in its performance. However, at increased concentrations of 4CP, q_{max} showed a progressive trend along the reaction phase in the first few cycles, which suggested that the granular bioreactor was limited by diffusion rather than biomass. It should be highlighted that the faster biodegradation in GSBR than FSBR was observed just when biomass had been completely adapted to 4CP. On the contrary, no meaningful difference was observed at the initial cycles at each definite concentration, which further emphasizes our assumption. This might be due to the fact that the toxic effects of 4CP on biomass can be severely limited by diffusional resistance in granules. Since mass transfer resistance is lower in flocs than in granules, it may be concluded that the detrimental effects of a toxic compound is moderated by a lightening mechanism, such as diffusional resistance in granular aggregates. The mass transfer barrier provided by a granule matrix, which produces lower local inhibitory compound concentrations on cells in the inner layers than the bulk value, is the most likely reason to account for the high degradation efficiency of similar biological structures, such as biofilms which degrade toxic or inhibitory compounds [22].

On the contrary, a decreasing followed by an increasing trend of q_{max} was observed at a definite concentration of 4CP as the cycles proceeded and also for the feed $4CP/COD_{Total}$ ratio in both reactors (Fig. 7). As shown, the maximum value of q_{max} was observed in GSBR equal to 31.25 mg4CP/gVSS.h. According to Tomei and Blackburn [34,39], possible explanations for such behavior can be related to the improvement of biomass capability in degrading the inhibitory substrate and also increasing the fraction of biomass involved in the 4CP degradation (X_{4CP}/X_{Total}) with increasing fraction of 4CP in the feed (COD_{4CP}/COD_{Total}). With a more specific biomass, developed under a suitable acclimation period, the rate of toxic compound biodegradation increased correspondingly.

3.3. Assimilation and bioaccumulation of 4CP

The contribution of assimilation in flocculent and granular sludge to 4CP removal showed a contradictory behavior.

3.3.1. Suspended flocs SBR

Adsorption on biomass was negligible at various concentrations of 4CP before reaching the concentration at which the process was ceased. Process cessation occurred at 200 and 550 mg/L in the FSBR (Fig. 2). It should also be noted that, after four cycles with a starting dosage of 200 mg/L, the suspended biomass was still active. However, the biomass activity was made worse by continuing high 4CP dosage into the system. Undoubtedly, this phenomenon was

compounded by the accumulation of inhibitory compounds in the system. The SOUR of the sludge at 8, 9, and 16 cycles after increasing the 4CP concentration to 200 mg/L is presented in (Fig. 8). SOUR profiles shown in this figure are characteristic of substrate inhibition originating from 4CP accumulation in the system. The decreasing trends of SOUR were observed as the cycles proceeded. As depicted, the biomass was fairly active; though as the cycles proceeded, its activity decreased due to 4CP accumulation in the system, and finally a tarry black color was noted both in effluent and sludge after 20 cycles at a starting dosage of 200 mg 4CP/L. The appearance of the sludge at this time was an indication of its inactivity, with a high inorganic state of biomass (VSS/TSS ratio of 0.64), which implied process inhibition on account of high inhibitory substrate loading rate (0.4 g 4CP/L d). The 4CP concentration thereafter was immediately lowered to zero and the biomass was allowed to retrieve itself. A greenish-yellow color, which continued for 16 cycles thenceforth without any revolution in light absorbance (at 380 nm), was nevertheless observed when the 4CP injection was blocked up (Fig. 5). Similarly, the obvious reason for this observation is the accumulation of 4CP, which suggested continuous desorption. The assumption was confirmed, as the concentration of 4CP in the effluent was 1.8 mg/L three days after stopping its injection into the influent, and COD removal efficiency never exceeded 81%. TSS gradually increased to 1,300 mg/L six cycles after reactor performance interruption and achieved 2,800 mg/L sixteen cycles later. At this point, feeding was completely stopped and the aeration phase was allowed to continue without interruption. The greenish-yellow color was totally removed after 18 h; however, interestingly it appeared again as the feeding was started. This trend was observed repeatedly for six days; i.e. the bulk liquid color turned to greenish-yellow by feeding MEG and was then removed completely by consuming the biogenic substrate when sufficient time was allowed for biomass adaptation. The most significant result of this is that 4CP removal was obviously enhanced in the presence of another biodegradable organic compound, namely cosubstrate. As biotransformation of chlorophenols is generally more complex, the use of cosubstrates has been demonstrated for enhancing their uptake rates as well as allowing the treatment of higher concentrations of these compounds [30,34,40,41].

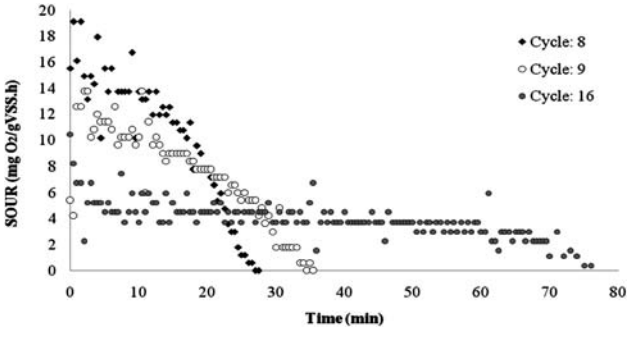

Fig. 8. Typical patterns of time course variation of biomass activity (SOUR) after 8, 9, and 16 cycles at an inhibiting concentration of 4CP (FSBR: $C_{4CP} = 200$ mg/L).

After establishment of steady state conditions (24 cycles later), the reactor was fed gradually with incremental concentrations of 4CP up to a concentration of 550 mg/L, which again led to complete cessation of reactor performance. Adsorption of 4CP on biomass was negligible at the normal performance level of the reactor, but it reached up to 24 and 19% at 200 and 550 mg/L, respectively; both resulted in cessation of reactor performance. This confirms that, at normal conditions of bioreactor performance, the main pathway for 4CP removal was through biodegradation.

3.3.2. Granular sludge SBR

The assimilatory removal of 4CP was fairly significant and reached a maximum value of 11.6% at the highest concentration of 4CP. Not only biomass exposure to higher concentrations of 4CP, but also the higher biomass concentration in GSBR than FSBR and its specific layered structure may be justified by this observation. The concentric aerobic, anoxic, and anaerobic layers arranged in sequence have been described for aerobically grown granules. On the other hand, as a general rule it is regarded that less chlorinated pollutants, such as monochlorophenols, are more susceptible to degradation by aerobic mechanisms [14]. Considering the diffusional flux of substrate in granular biomass, the major outcome of this knowledge is the accumulation of unmetabolized 4CP in the interior core of aerobically grown biomass at high concentrations of inhibitory compound.

4. Conclusion

From the results obtained in this research work, the following conclusions can be drawn:

- In comparison with other biogenic substrates, the cultivation of aerobic granules lagged behind other readily biodegradable substrates. This can be explained by the penetration of MEG inside the

flocs which results in the development of loosely structured aggregates.

- Aerobic granules, in comparison with suspended flocs, showed high persistence against the toxic effects of xenobiotic compound. Process cessation was observed in FSBR when inhibitory compound progressively accumulated in the system. At inhibitory concentrations, only partial degradation of 4CP took place and biomass behaved like a pure culture.
- Accumulation of CHMS increased in the reactor in parallel with the removal of 4CP. The highest concentration of CHMS was detected when 4CP was just completely removed in the bulk liquid. Maximum values of CHMS and its rapid formation were detected at low concentrations of 4CP. On the other hand, CHMS was gradually increased at higher concentration, whereas a rapid decrease of color was observed when biomass had been acclimated to the inhibitory compound.
- An increasing trend of q_{max} was observed at any definite concentration of 4CP as the cycles proceeded and also with the feed 4CP/COD_{total} ratio in both reactors. This was related to the improvement of biomass capability in degrading the inhibitory substrate and also increasing the fraction of biomass involved in the 4CP degradation. Not only biomass exposure to higher concentrations of 4CP, but also the higher biomass concentration in GSBR than FSBR and its specific layered structure may be justified to this observation.
- The bulk liquid color evolution through repeated feast and famine conditions, which are characteristic of SBR, emphasized the crucial role of the presence of another biogenic compound in the removal of 4CP.
- When complete adaptation of biomass is not allowed, bioaccumulation of inhibitory compound exceeded its capability in biodegradation, which in turn resulted in cessation of bioreactor performance. Lowering the 4CP concentration in the feed resulted in desorption of assimilated 4CP. This can be overcome by increasing reactor's reaction time.

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