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Effect of Starvation and Shock Loads on the Biodegradation of 4-Chlorophenol in a Discontinuous Moving Bed Biofilm Reactor

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Abstract The influence of starvation (defined as the period without substrate) and shock loads on the performance of a moving bed sequencing batch reactor degrading 4-chlorophenol (4CP) were investigated. The biomass was acclimated to biodegrade 100 mg/L of 4CP, and the colonization of the packing material was followed. Two starvation periods and two shock loads were studied. The degradation capacity of the suspended and the attached biomass present on the moving bed was also evaluated. The experiments showed that, after the starvation period, the specific degradation rate decreased from 30.5 to 28.5 and 20 mg 4CP/gVSS/h, when starvation periods of 24 and 48 h were applied, respectively. When two concentration peaks of 500 and 1,050 mg/L were applied, a loss of 6% and 8% on the specific degradation rate, respectively, was also observed. The moving bed thus showed great robustness against starvation periods and shock loads. Suspended biomass presented higher specific degradation rates, but attached biomass did not generate a metabolite that is inhibitory when it accumulates.

Keywords Moving bed · Biofilm · SBR · Shock loads · Starvation · 4-chlorophenol

Introduction

The first step to biodegrade toxic substances in a wastewater treatment plant is the acclimation of the microorganisms. Nevertheless, it has been shown that this acclimation is not permanent [1]. For instance, when containers and reactors in some industries are cleaned, a peak in the concentration of toxic substances (shock load) occurs in the wastewater treatment plant. There may also occur a decrease (or total absence) in the toxic concentration to which the microorganisms were acclimated; for example, when a different

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type of product is manufactured, the composition of the wastewater discharged changes, varying the composition. This last condition is here defined as starvation. These variations of the toxic substrate or starvation in relation to the toxic compound have an important effect on the sludge activity [2] and are characterized by stressful conditions for the microorganisms.

With the use of periodic aerobic processes as the sequencing batch reactor (SBR), the previous problems can be overcome. SBR is the general term applied to the discontinuous wastewater treatment technology where the volume in the reactor tank is variable in time [3]. In these systems, there is a periodic influent and discharge. The SBR process has three major characteristics: periodic repetition of a sequence of well-defined phases, planned duration of each process phase in accordance with the treatment result to be met, and progress of the various biological and physical reactions as a function of time. These batch systems can manage the continuous variations of pollutant concentrations present in industrial wastewater [1]. In fact, mass balances for batch systems describe the unstable behavior produced by the natural variations of volumetric flows and pollutant concentrations. Usually a SBR-type bioreactor operates under five well-defined phases: fill, react, settle, draw, and idle.

It has been observed that relatively short starvation periods have a negative effect on the degradation of 4-chlorophenol (4CP) for an activated sludge in a sequencing batch reactor [4]. The degradation time was found to increase six times (from 0.7 to 4.5 h) as a result of a starvation period of 24 h. This loss in the microbial degradation capacity was attributed to a decline in both the enzymatic activity and the viability of the suspended cells.

In the case of the shock loads, it has been reported that punctual increases in the concentration of inhibitory substrates produces inhibition of the microorganisms [5], reflected as low efficiencies regarding the removal of toxic compounds. Shock loads appear when the toxic concentration in the influent greatly increases as a consequence of changes upstream in the manufacturing process. A toxic shock load produces an increase in the reaction time, diminishes the efficiency, and may inhibit the biomass. Buitrón et al. [5] evaluated the degradation of 4-chlorophenol in a SBR operated with suspended biomass and reported that the treatment process was affected when the biomass was exposed to a shock load of 1,050 mg/L, since the degradation time increased 230%. Here, the mineralization was not totally completed and the activity, based on the specific oxygen uptake rate, decreased. Moreover, the process performance was affected for around 1 month after the 1,050 mg/L peak. The original degradation time and the activity of microorganisms after the peak was not recovered in the subsequent cycles operated with a lower 4CP concentration.

It has been observed that biofilm systems, such as the moving bed, improve the performance of the bioreactor [6]. The moving bed biofilm reactor (MBBR) has been successfully used in the biological treatment of different effluents, such as the wastewater from the pulp and paper industry [7], refineries [8], the lactic industry [9, 10], and in municipal wastewater treatment plants [11, 12], as well as in different conditions like mesophilic and thermophilic environments [13]. Hosseini and Borghei [14] reported the degradation of a mixture of phenolic compounds in a continuous moving bed reactor. No effects on the metabolic activity were found when low phenolic concentrations were degraded. Nevertheless, an increase in the phenol concentration to 220 mg/L produced a decrease in the physiological parameters, and therefore, inhibition occurred.

In the present work, the effect of the starvation and shock loads in the performance of a moving bed sequencing batch reactor degrading 4-chlorophenol is reported.

Materials and Methods

A sequencing batch reactor with a capacity of 7 L and an exchange volume of 57% (4 L) was used. The carriers were cylinders of high density polyethylene (BCN 009 plus, 2H Germany) having a total superficial area of 963 m^2/m^3, a contact area of 10.084 cm^2/cylinder, a density of 150 kg/m^3, and an average diameter of 7 mm. The reactor was packed with 1,450 cylinders (30% of the total useful volume) giving a total superficial area of 1.46 m^2, corresponding to a specific effective surface of 208.6 $m^2/m^3_{reactor}$. The packing was maintained in suspension with an airflow rate of 1.5 L/min, and the temperature was maintained at 20 °C. The moving bed sequencing batch reactor (MBSBR) was inoculated with microorganisms coming from a municipal activated sludge treatment plant (2,000 mgVSS/L).

Synthetic wastewater containing 4-chlorophenol (4CP) was used as the sole source of carbon and energy. Nutrients such as nitrogen, phosphorus, and oligoelements were added following the techniques recommended by the Association Française de Normalisation [15]. The substrate concentration was measured by taking samples and processing them off-line using the colorimetric technique of the 4-aminoantipyrine method [16]. Total and volatile suspended solid (TSS and VSS) analyses were determined according to the Standard Methods [16]. Dissolved organic carbon (DOC) was determined with a Shimadzu TOC-5050 and chemical oxygen demand (COD) was measured according to Standard Methods [16]. These analyses were performed to evaluate the 4CP degradation. The metabolite 5-chloro-2hydroxy-muconic acid semialdehyde, formed by an alternate degradation route of 4CP by the microorganisms and which can be inhibitory for the microorganisms, was determined by spectrophotometry at 380 nm using a HACH spectrophotometer according to [17]. The SBR was operated under the following strategy: pre-aeration (15 min), filling (5 min), reaction (variable in time depending on the degradation efficiency expected), settling (12 to 30 min), and draw (1 min). The pre-aeration phase was introduced in order to ensure the reduction of the carbon and metabolite that could have remained from the precedent cycle. The degradation time was established by looking at the dissolved oxygen concentration profile under a constant airflow rate [5]. Colonization of the packing material was followed by measuring the active biomass on the support by using the INT-F dehydrogenase test [18]. Each measurement was made in triplicate, and values were normalized by dividing the INT-F by the contact area of the carriers taken for the test.

The biomass was acclimated using a variable cycle strategy, i.e., the reaction phase duration was variable and stopped when the removal of 4CP was equal or greater than 95%, according to Moreno-Andrade and Buitrón [19]. At the beginning of the acclimation, the packing was introduced into the reactor in order to allow its colonization. Biomass was acclimated to 100 mg 4CP/L. After acclimation, the initial concentration was maintained. The experimental design considered two starvation conditions (24 and 48 h) and two shock loads (500 and 1,050 mg 4CP/L). At least three kinetics were obtained for each condition. The effects of starvation and shock loads on both the degradation time and the specific degradation rate (q) were evaluated. For each condition, a kinetic before, during, and after the perturbation was conducted.

Results and Discussion

By following the degradation kinetics during the acclimation process, it was possible to observe that, as a consequence of the acclimation, the time needed to remove up to 99% as

4CP and 95% as DOC (degradation time) decreased from 52 h in the first cycle to 1.75 h in the tenth cycle (Fig. 1). Acclimation was considered to be reached when three degradation times were relatively constant (± 10%). In this case, acclimation was achieved after ten reaction cycles or 8 days. During the acclimation, the suspended biomass increased its activity to degrade 4CP. In this sense, it has been reported that, during the acclimation, there is a selection and multiplication of specialized microorganisms with capacities for the degradation of 4-chlorophenol [19]. The colonization of the packing material was evaluated indirectly determining the dehydrogenase activity using the INT-F dehydrogenase test. A correlation curve between the INT-F and the suspended solids was obtained in order to quantify the attached biomass to the packing material. A relationship of 66 mg INT-F/gVSS was found. Figure 2 presents the evolution of the colonization of the packing material. During the acclimation, the biofilm was not observed in the carrier material, until after the eighth day of operation, when an increase in the dehydrogenase activity of microorganisms was detected in the support. After 38 days of the reactor operation, no significant changes in the degradation time or in the performance of the reactor were observed. Thus, it was considered that enough biomass was attached to the carriers, and the next phase of the experiment was conducted. The maximal value obtained from the carriers at this point was 0.0158 mgINT-F/cm^2. The biofilm amount after colonization per surface of carriers was 0.24±0.017 mgVSS/cm^2.

Influence of Starvation Periods

The influence of starvation on the degradation rates (q) was evaluated from the data of the kinetics obtained for each case. Since a direct relationship between the substrate concentration versus time was observed (zero order kinetic), q was calculated as the slope of the linear regression of the data (between the initial concentration and the concentration observed when 99% of the 4CP was removed). The absolute value of the result was divided by the total mass of VSS present in the reactor to obtain specific degradation rates. Considering the total surface area, the number of carrier materials, and the amount of biomass present at the end of the colonization period, the total mass of VSS was calculated to be 3.5 g.

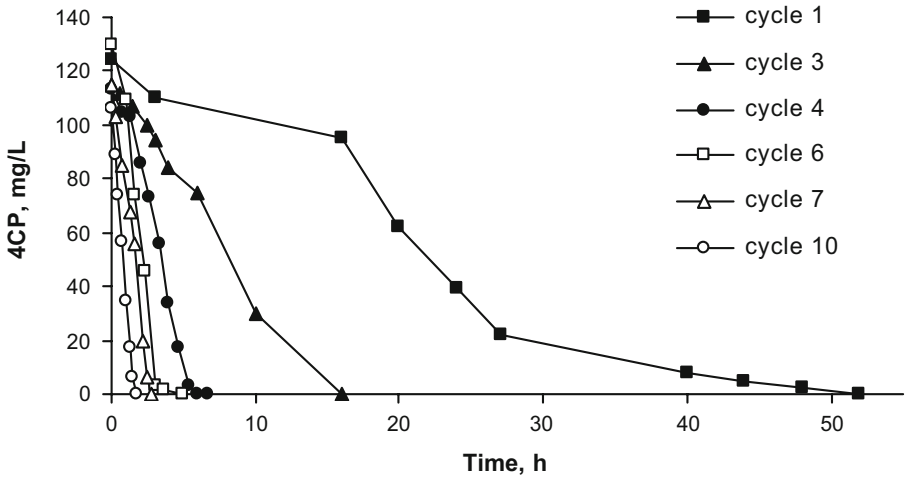

Fig. 1 Degradation kinetics of 4CP during the acclimation of the biomass

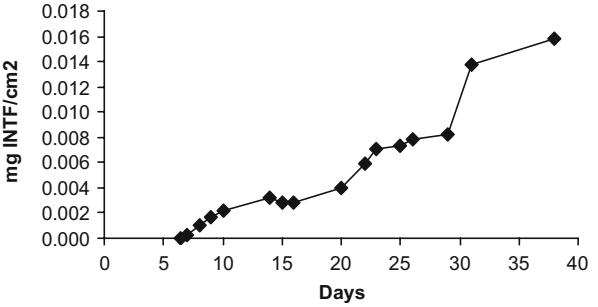

Fig. 2 Colonization evolution of the carrier material

It was found that after a starvation period of 24 h, only 6% of the specific degradation rate (q) was lost. It decreased from 30.5 to 28.5 mg 4CP/gVSS/h. For the case of 48 h of starvation, the loss on q was 33%, since this value went from 30.5 to 20 mg 4CP/gVSS/h (Table 1). It is possible to observe in Fig. 3 that the microorganisms' activity to degrade the 4CP is lower when a starvation period of 48 h is introduced: compared to that when the microorganisms were not exposed to the stress condition. The time needed to biodegrade the 4CP decreased from 3 to 2 h.

In a previous study, Buitrón and Moreno [1] also exposed activated sludge degrading 4CP to starvation periods. It was found that the degradation rate and the tolerance to the toxic compounds decreased when a suspended biomass degrading 4CP is subject to starvation. A reduction of 75% in the degradation activity was observed after 36 h of starvation. In that study, it was demonstrated that all the kinetic parameters decreased with an increment of the starvation time, and it was also observed that the more deacclimated the biomass is to the toxic substrate, the lower and narrower is its representative Haldane curve.

Previous studies dealing with 4CP degradation [5, 19] showed that proper acclimation can lead to similar operation parameters of the process, such as removal efficiencies and degradation times, independently of the source of the inocula used. Thus, it can be considered that although the composition of the microbial consortia could not have been the same, specific degraders responsible of the 4CP degradation were selected. The comparison of the previous results with those obtained in this study suggests that suspended biomass is more unstable than the biomass present in the moving bed, since for a higher starvation period, the microbial community present in the moving bed had a lower impact in their activity.

Table 1 Summary of the effect of starvation and concentrations peaks on the specific removal rate.

Condition	q Before perturbation, Mg4CP/gVSS/h	q During the peak degradation, mg4CP/gVSS/h	q After perturbation, mg4CP/gVSS/h	Effect, percent of reduction on q after the perturbation
24 h starvation	30.5±1.6	–	28.5±0.7	6.6
48 h starvation	30.0±0.8	–	20.0±1.1	33.3
500 mg/L peak	24.5±2.5	28.5±2.2	23.0±2.9	6.1
1,050 mg/L peak	27.0±1.1	14.6±1.8	25.0±1.4	8.0

Values represent the mean±SD of the kinetics.

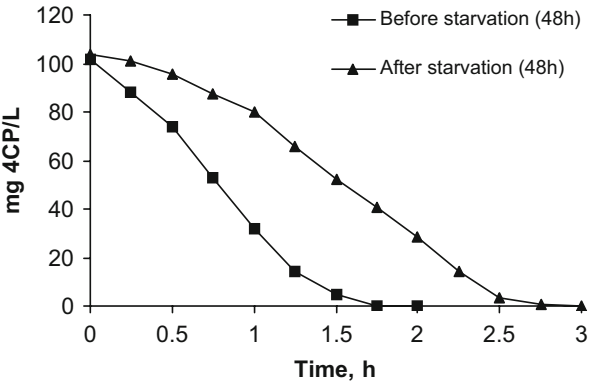

Fig. 3 Kinetics of 4CP degradation before and after the introduction of a starvation period of 48 h

Influence of Shock Loads

Two concentration peaks with an initial concentration of 500 and 1,050 mg/L in the mixed liquor were evaluated in the MBSBR. For both concentrations, no significant influence was found after the introduction of the perturbation (Table 1). Fig. 4 shows the degradation of 1,050 mg/L of 4CP and Fig. 5 shows the 4CP degradation kinetics before and after the shock load of 1,050 mg4CP/L. During the shock load of 1,050 mg4CP/L, the degradation of 4CP and DOC was reached in 29 h, with removal efficiencies greater than 99.9% and 98.9%, respectively. Comparing the kinetics for the cycles before and after the introduction of the shock load, a minimal reduction of the degradation activity can be observed, with a loss of 6% and 8% on the specific degradation rate for the concentration peaks of 500 and 1,050 mg/L, respectively.

These results contrast with those obtained by Buitrón et al. [5] when a suspended biomass system was tested against this kind of perturbation. In that case, an inhibition of 70% in the degradation rate was found after a toxic shock load of 1,050 mg/L was introduced, whereas in this study, in the moving bed reactor, the reduction was only 8%. This demonstrates that the moving bed can support higher concentrations of toxic compounds compared with systems using only suspended biomass.

Comparison of the Suspended and Attached Biomass Present in the MBSBR

In order to investigate the degradation capacity of the suspended and attached biomass on the MBSBR, the carrying material was separated from the suspended biomass. A parallel

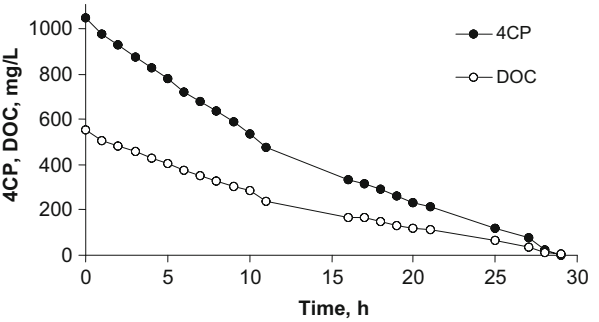

Fig. 4 Kinetics of 4CP and COD degradation of a peak of 1,050 mg 4CP/L

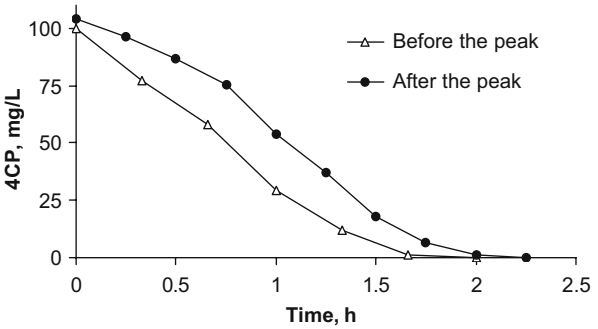

Fig. 5 Kinetics of 4CP degradation before and after the introduction of a peak of 1050 mg 4CP/L

set of kinetics was run to degrade the same initial substrate (S_0) to biomass (X_0) ratio (S_0/X_0=0.175), i.e., the same quantity of substrate degraded by the same quantity of biomass as in the combined system. It has been suggested that this parameter must be kept constant to obtain similar kinetic responses [20]. Results for the substrate (S) were normalized with respect its initial value (S_0) in order to visualize the behavior in each kinetic, since there were two initial concentrations in each experiment (to keep constant the S_0/X_0 ratio) as shown in Fig. 6. It was observed that the degradation rate was higher for the suspended biomass than for the attached one. When the quantity of biomass is taken into account, the specific degradation rate for the suspended biomass resulted to be 23 mg 4CP/gVSS/h, which is almost twice as high as the value for the biomass attached to the carrier material, which was 12 mg 4CP/gVSS/h.

The pathway proposed for the degradation of chlorophenols consists of initial monooxygenation to form chlorocatechols, which undergo ortho ring cleavage to chloromuconic acids, lactonization with loss of chloride, and further degradation (the β-ketoadipate pathway) [17]. There also exists the meta cleavage of chlorocatechol that is realized by the catechol 2-3-dioxygenases and produces the metabolite 5-chloro-2hydroxy-muconic acid semialdehyde. The presence of this metabolite is noticed during the reaction when a green-yellow coloration is observed. In this study, to compare the behavior of the two biomasses, the production of the metabolite was followed during the parallel kinetics by measuring the absorbance at 380 nm, and this is shown in Fig. 7. The area under the curves shown is an indication of the total metabolite produced during each reaction. The metabolite production is higher when only suspended biomass is used,

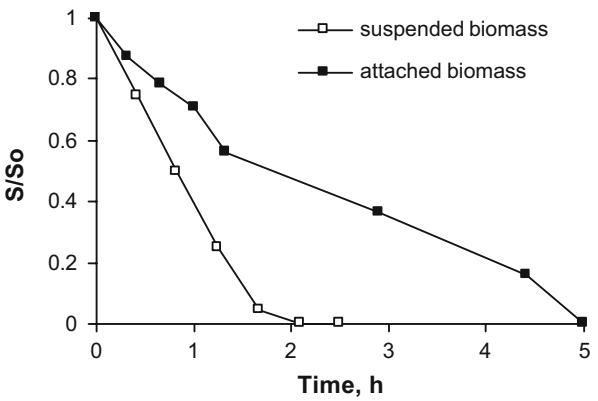

Fig. 6 Kinetics of 4CP degradation for suspended and attached biomass from the moving bed bioreactor, with substrate (S) normalized with respect to initial substrate concentration (S_0)

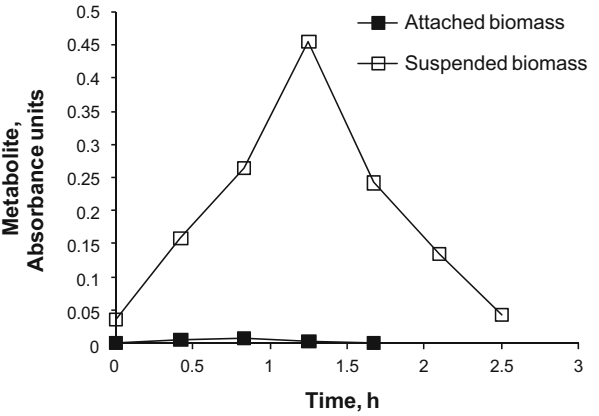

Fig. 7 Kinetics for metabolite production for suspended and attached biomass from the moving bed bioreactor

while only a very small quantity of metabolite was observed for the attached biomass during the degradation of the 4CP, even if the curve is integrated for the longer reaction duration. This observation suggests that the 4CP degradation by the microorganisms attached to the carrier do not take the meta cleavage. It has been observed that such metabolite is produced when the biomass is under stressful conditions, as before acclimation [19] or when shock loads are applied [5]. It was observed that the metabolite was produced but immediately degraded in the case of suspended biomass. The interesting point is that attached biomass did not generate this metabolite, suggesting that there are different types of microorganisms involved.

The results showed that suspended biomass is more susceptible to produce this metabolite, which is toxic when it accumulates [5], but more efficient in terms of the degradation rate. Attached biomass is less efficient than suspended biomass, but it is also less susceptible to produce the metabolite. In the moving bed reactor, there is a combination of suspended and attached biomass. The robustness observed when perturbations (starvation and shock loads) were introduced into the moving bed may be due to the presence of both, attached and suspended biomass that take part in complementary tasks.

Conclusions

The results clearly suggest an improvement in the robustness of the reactor by the application of the combination of suspended and attached biomass. The specific removal rate in a discontinuous process when applying a moving bed was not significantly affected during the starvation period of 24 h. If the starvation was extended for more than 24 h, the effect of the starvation is negative, but with less impact than in a comparable suspended biomass SBR. Shock loads have a minimal impact on the degradation rate since only 8% of reduction was observed when a 1,050-mg/L peak was introduced. A high degradation rate was found when the suspended biomass was studied alone. However, attached biomass demonstrates to have the capacity to produce less metabolite during 4CP degradation. This suggests that different types of microorganisms are present in the attached and the suspended biomass and that these complement each other in their biodegradation tasks.

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