

## EVOLUTION OF THE ACTIVITY OF THE MICROORGANISMS DEGRADING 4-CHLOROPHENOL WHEN EXPOSED TO STARVATION PERIODS

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### Abstract

The variation of the microbial activity during starvation in the degradation of 4-chlorophenol (4CP) was studied in a sequencing batch reactor. The microbial activity was followed by measuring the substrate uptake rate and the specific oxygen uptake rate. After the acclimation, the biomass was exposed to different periods of starvation to study the variation of this activity. The results showed that the starvation generates a decrease of microbial activity. A decrement from 21 to 44 % was observed on the removal rate and from 26 to 35 % on the microbial activity, measured as specific oxygen uptake rate. The extent of the deacclimation of starved microorganisms seems to be affected by the history of the culture. Degradation rates during the acclimation and deacclimation process followed an exponential model.

**Keywords:** microorganism activity; starvation; SBR; 4-chlorophenol

### INTRODUCTION

To biodegrade toxic substances microorganisms must be acclimated. This occurs when the microorganisms are put in contact with toxic compounds in a favorable environment (Aelion *et al.* 1989). However, it has been shown that this acclimation is not permanent (Buitrón and Moreno, 2002). Arbuckle and Kennedy (1989) found that activated sludge acclimated to degrade 4-chlorophenol (4CP) lost its ability when the compound was absent from the feed of the reactor (starvation period). It has been suggested that the lost in microbial activity is affected by the starvation period length, the type of compound, the culture history and the initial induction level (Babcock *et al.*, 1992). The exposition of the acclimated population to prolonged periods of starvation produces a decrease of the bacterial activity and even the death of some of them (Coello *et al.*, 2003). Buitrón *et al.* (1994) found a negative effect of starvation periods on 4CP degradation for an activated sludge in a sequencing batch reactor (SBR) system. In that study, the aeration was extended 20 to 23 hours after the toxic degradation had been completed. It was found that the degradation time increased 6 times (from 0.7 to 4.5 h) as a result of such a starvation period. This loss in the microbial degradation capacity was attributed to a decline in both the enzymatic activity and the viability of the suspended cells. On the other hand, other studies examined the effects of pre-starvation on the survival of cells (van Overbeek *et al.*, 1995). In some cases an improvement of survivability has been reported, although other studies showed no effects of the starvation treatment. It has been suggested that this inconsistency may have been due to the different starvation conditions used in these studies (van Overbeek *et al.*, 1995). Watanabe *et al.*, (2000) showed that during long starvation times there exists a decrease in the population density, respiration rate, dehydrogenase activity and in the phenol-oxygenating activity that may affect the performance of a wastewater treatment plant. In addition, some physiological changes occurred to starved cells. Microscopic observations of the microorganisms revealed a reduction in cell size, a change in cell shape, and a loss of the motility during long starvation times.

In the present work, the evolution of the kinetics and the respiratory activity during the starvation of a mixed culture of microorganisms was studied, when the microorganisms were previously acclimated to degrade 4-chlorophenol (4CP) in a discontinuous reactor.

## METHODOLOGY

An aerobic automated Sequencing Batch Reactor (SBR) system with a capacity of 7L and an exchange volume of 57% was used (fig 1). The airflow rate was 1.5 liters per minute and the temperature was maintained at 20 °C inside the reactor. The reactor was inoculated with microorganisms coming from a municipal activated sludge treatment plant. A synthetic wastewater containing 4CP was used as a sole source of carbon and energy. Nutrients such as nitrogen, phosphorus, and oligoelements were added following the techniques recommended by ANFOR (1985). The SBR was operated under the following strategy: a preaeration time of 15 min, a filling time of 5 min, a variable reaction time depending on the necessary time to reach a removal efficiency of 4-CP of 99%, a settling time of 30 min and a draw time of 1 min. Degradation time was determined using the dissolved oxygen concentration present in the reactor (Buitrón *et al.*, 2003).



**Figure 1** Experimental assembly showing the reactor set-up

The substrate concentration was measured taking samples and processing them offline using the colorimetric technique of the 4-aminoantipyrine (Standard Methods, 1992). Total and volatile suspended solids (TSS and VSS) analyses were determined according to the Standard Methods (1992). Dissolved organic carbon (DOC) was determined with a Shimadzu TOC-5050 and Chemical Oxygen Demand (COD) according to Standard Methods (1992). These analyses were performed to evaluate the mineralization of 4CP. The metabolite (5-chloro-2hydroxy-muconique acid semialdehyde), formed by an alternate degradation route of 4-CP by the microorganisms, and that can be inhibitory for the microorganisms (Commandeur and Parson, 1990), was also determined by spectrophotometry at 380 nm using a HACH spectrophotometer.

In order to follow the respirometric activity, the specific oxygen uptake rate (SOUR) was measured by placing 10 mL of the mixed liquor of the SBR harvested just after a degradation cycle in a mini-reactor of 160 mL. An oxygen-saturated solution with nutrients and substrate (acetate or 4CP) was added and dissolved oxygen measured was recorded. Endogenous respiration was measured adding only nutrients. SOUR was computed from the slope of the respirogram divided by the VSS concentration.

## Acclimation

The reactor was inoculated with activated sludge from a municipal wastewater treatment plant containing 2000 mgVSS/L. 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%. The biomass was acclimated to three different concentrations (50, 100 and 200 mg4CP/L) in order to have a stable reactor for each of the starvation experiments.

## Starvation

Acclimated biomass was exposed to different starvation periods. Starvation was introduced by maintaining the aeration some time after the degradation of 4CP was completed, i.e. microorganisms were kept under endogenous conditions. For each acclimated biomass, different sets of starvation periods were studied (Table 1).

Table 1. Starvation experiments

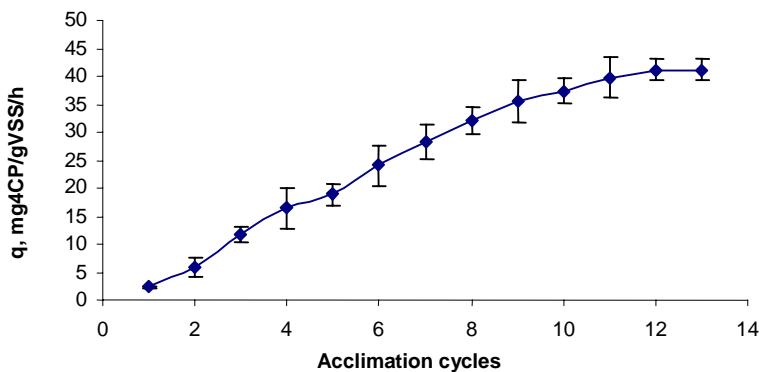
Biomass acclimated to 4CP concentration, mg/L	Starvation times, h
50 (ST50)	8, 12 and 24
100 (ST100)	12 and 24
200 (ST200)	12, 24 and 36

For each experiment the kinetics of 4CP degradation and the SOUR were followed before and after the starvation. Each set of conditions was made in the same reactor. Once a starvation time was studied, the biomass was allowed to recover its capacities before starvation by feeding the reactor and proceeding as in the acclimation phase in order to reach the degradation time observed before the starvation.

## RESULTS AND DISCUSSION

### Acclimation

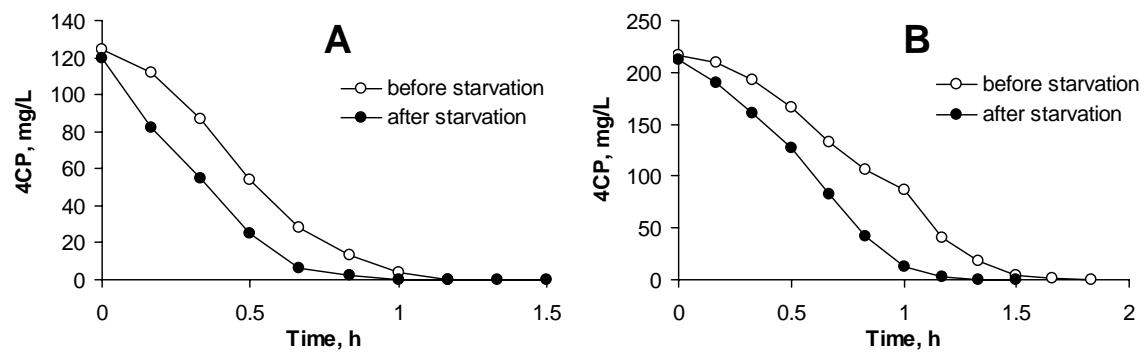
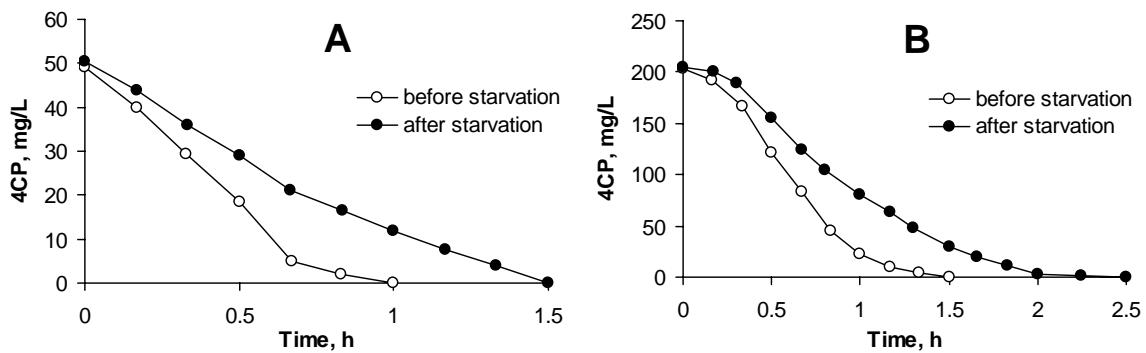
Figure 2 present an example of the evolution of the specific degradation rate ( $q$ ) during the acclimation of 200 mg/L to 4CP. The degradation rate was calculated by dividing the amount of degraded substrate by the degradation time and by the amount of biomass. Results are the average values of 3 different acclimation experiments. It can be observed that after the 12<sup>th</sup> cycle  $q$  reaches a steady state. At this point the biomass can be considered acclimated to the toxic compound.



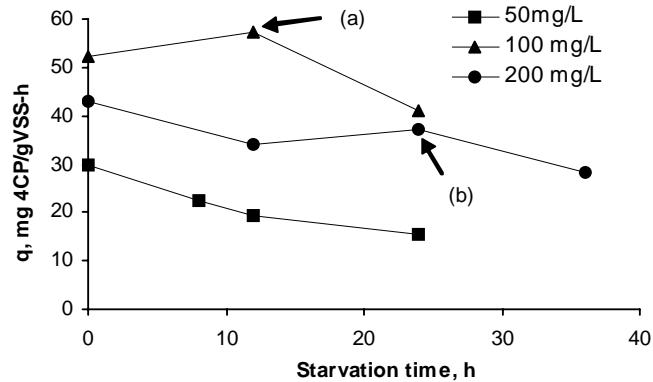
**Figure 2** Evolution of the specific degradation rate for the acclimation of activated sludge to 200 mg 4CP/L

### Starvation

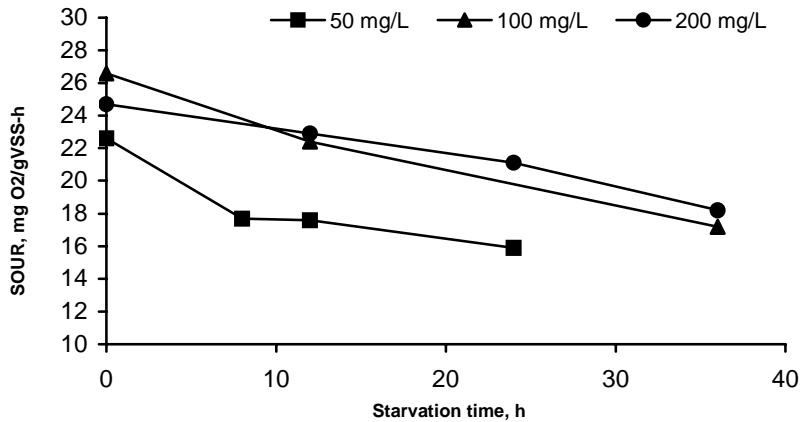
For the case of the biomass acclimated to 50 mg/L the starvation period always had a negative influence on the degradation time. In the other set of experiments (ST100 and ST 200) the influence of starvation periods did not present the same behavior. It was observed that short starvation periods not always produced a loss of activity, but on the contrary, the degradation time was shorter than before. Figure 3 and 4 present the kinetics obtained for the starvation experiments. The negative influence of starvation periods is shown in Figure 3. These experiments present the case where the introduction of a period without substrate has a negative effect for the biomass. In Figure 4 two examples where starvation decreases the degradation time are present.



Figures 5 and 6 present the influence of starvation on the specific removal rate  $q$  and on the SOUR. As shown, the starvation gradually decreases the microbial activity. In general, a decrement from 21 to 44 % was observed on  $q$  due to the introduction of starvation periods, and from 26 to 35 % of activity lost measured as SOUR (Figure 6). It was found that the substrate uptake rate was correlated with the negative exponential of starvation time, independently of the originally acclimated concentration (figure 5). Similar results were obtained by Arbuckle and Kennedy (1989). They found that the 4CP degradation rate decreased by 30% after one day, and 50% after two days of starvation.



**Figure 5** Influence of starvation time on the specific substrate removal rate. Results after starvation are compared with the initial condition before the perturbation. Points (a) and (b) represent the positive effect on the activity by starvation.



**Figure 6** Influence of starvation time on the specific oxygen uptake rate for SOUR. 4CP was used as the only substrate for the respirometric test. The values represent only the exogenous respiration.

It is interesting to point out that the strategy to conduct the experiments of starvation influence the results obtained. Buitrón *et al.*, (1994) starved the biomass in the same reactor applying several and consecutive starvation periods of 24 h. The degradation time increases up to 6 times from the initial value generating 80% of reduction on  $q$ . In the present study, the biomass in the reactor was acclimated, and then the starvation experiments were conducted for each initial concentration. Between each starvation experiment several cycles were conducted in order to recover the initial activity of the biomass and then the next starvation time was applied. This procedure generated a history in the culture. Apparently, by using this operation mode the deacclimation effect is diminished. It is possible to observe in Figure 5, for the case of ST100 (12 h) and ST200 (24h), that after a cycle of starvation, there exists even an increase in the value of  $q$  indicating a reduction of the degradation time. Thus, a positive influence of starvation is observed.

Previous studies have examined the effect of pre-starvation on the survival of inoculant cells. In this case an improved survivability has been reported (Van Elsas *et al.*, 1994). Another study documented that pre-starvation exerted no significant effects on the survival (Van Overbeek *et al.*, 1995). The inconsistency may have been due to the different starvation conditions used in those studies. The point to be considered is that during starvation there exists a decrease in the population density, respiration rate, dehydrogenase activity and in the phenol-oxygenating activity that may affect the performance of a wastewater treatment plant (Watanabe *et al.*, 2000). In addition, some physiological changes occurred to starved cells. Microscopic observations of the microorganisms revealed a reduction in cell size, a change in cell shape, and a loss of the motility during starvation. Watanabe *et al.* (2000) also demonstrated an increase in cell surface hydrophobicity and floc adhesion capacity due to starvation. Consequently, it is possible to consider that the microorganisms, subject to cycles of starvation-recovery-starvation, changed in such a way to protect themselves. There is a minimal reduction in the microbial activity in this case. Nevertheless, in practice, the industrial wastewater treatment plants are subject to random changes of toxic concentration increasing the risk of deacclimation by an important activity variation of the microorganisms. Thus starvation could negatively affect the performances of a continuous toxic wastewater treatment plants, since in their practical operation, when starvation appears, there is no time to allow the biomass to reacclimate because in these systems the hydraulic retention time is fixed.

## CONCLUSIONS

Starvation generates a decrease on the microbial activity. In general, a decrement from 21 to 44 % was observed on  $q$  due to the introduction of starvation periods, and from 26 to 35 % of activity lost

measured as SOUR. The extent of the deacclimation of starved microorganisms seems to be affected by the history of the culture. The effect of starvation on the degradation rate is less significant when microorganisms are starved, recovered and then starved again, than in the case where starvation is cyclically presented without a recovery period. In any case, there exist an activity decrement generated by the variation of the toxic and thus a deacclimation of the previously acclimated bacteria that could explain the variations on the performance of treatment of toxic industrial wastes. Furthermore the specific degradation rates during the deacclimation process seem to follow an exponential model.

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