Variation of the microbial activity during the acclimation phase of a SBR system degrading 4-chlorophenol

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Abstract The variation of microbial activity during acclimation to 4-chlorophenol (4CP) in an aerobic automated sequencing batch reactor was studied. The results show a reduction in degradation time as the acclimation process occurred. During acclimation for an initial concentration of 50 mg 4CP/L, degradation time was reduced from 40 h to 50 min after 10 cycles. In the case of an initial concentration of 100 mg/L, degradation time was reduced from 52 h to 1.16 h, also after 10 cycles. Doubling the initial concentration of a previously acclimated sludge produces only a slight increase in degradation time. It was found that as acclimation took place, the affinity of the consortia to biodegrade the toxic increased, whereas the ability to biodegrade acetate decreased. The evolution of the substrate uptake rate over time during the acclimation period was highly correlated with an exponential relationship. It was also observed that acclimation decreased both the production of a toxic metabolite and the sludge volumetric index.

Keywords Acclimation; aerobic biodegradation; 4-chlorophenol; microbial activity; SBR

Introduction

Many industrial processes generating wastewater containing toxic compounds are characterized by their variability. In the chemical, pharmaceutical, plastic, and petrochemical industries, for some cases, production processes are in batch. Because of the high variations in flow and concentration of contaminants in industrial wastewater, usual treatment processes do not obtain satisfactory removal efficiencies. Besides, due to its toxicity, biological treatment of industrial wastes containing a high concentration of phenols is difficult. The first step to biodegrade toxic substances in a wastewater treatment plant is the acclimation of the microorganisms. In a favorable environment, when microorganisms are put in contact with toxic compounds, acclimation to these compounds may occur (Aelion *et al*., 1989). Different mechanisms have been described to explain the acclimation phase. Wiggings *et al*. (1987) suggested that there is a selection and a multiplication of specialized microorganisms during this phase and physiological transformations occur in the metabolic system of the microorganisms, i.e., alterations at the enzymatic level, regulation and production, mutations, etc. In aerobic microbial communities, the acclimation periods range from several hours to several days (Wiggings *et al*., 1987). Moreno and Buitrón (2004) studied the influence of the acclimation strategy on the 4-chlorophenol (4CP) degradation in a sequencing batch reactor (SBR). The acclimation was performed using two strategies, the first one fixing the reaction time, independent of the removal efficiency (fixed time) and the second one fixing a removal efficiency of 90% as 4CP (variable time). The variable time strategy produced a microbial community with higher specific activity compared with that obtained for the fixed time strategy.

However, not much information is available concerning the activity evolution of a microbial consortium during the acclimation to toxic wastewater. The present work describes the evolution of the kinetics curves and the respiratory activity of a mixed culture of microorganisms during acclimation to 4CP in a sequencing batch reactor.

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Methodology

An aerobic automated SBR system with a capacity of 7 L and an exchange volume of 57% was used (Figure 1). The airflow rate was 1.5 L/min 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 the sole source of carbon and energy. Nutrients such as nitrogen, phosphorus, and oligoelements were added following the techniques recommended by AFNOR (1985). The SBR was operated under the following strategy: preaeration time (15 min), filling time (5 min), reaction time (variable depending on the necessary time to reach 99% of removal efficiency of 4-CP), settling time (12–30 min) and draw time (1 min). Degradation time was followed using the dissolved oxygen (DO) concentration present in the reactor (Buitrón *et al*., 2003).

Substrate concentration was measured taking samples and processing them offline using the colorimetric technique of 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 4CP mineralization. The metabolite (5-chloro-2hydroxy-muconic acid semialdehyde) formed by an alternate degradation route of 4-CP by the microorganisms, which can be inhibitory for the microorganisms, 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 SBR mixed liquor harvested just after a degradation cycle in a mini-reactor of 160 mL. An oxygen-saturated solution with nutrients and substrate (acetate or

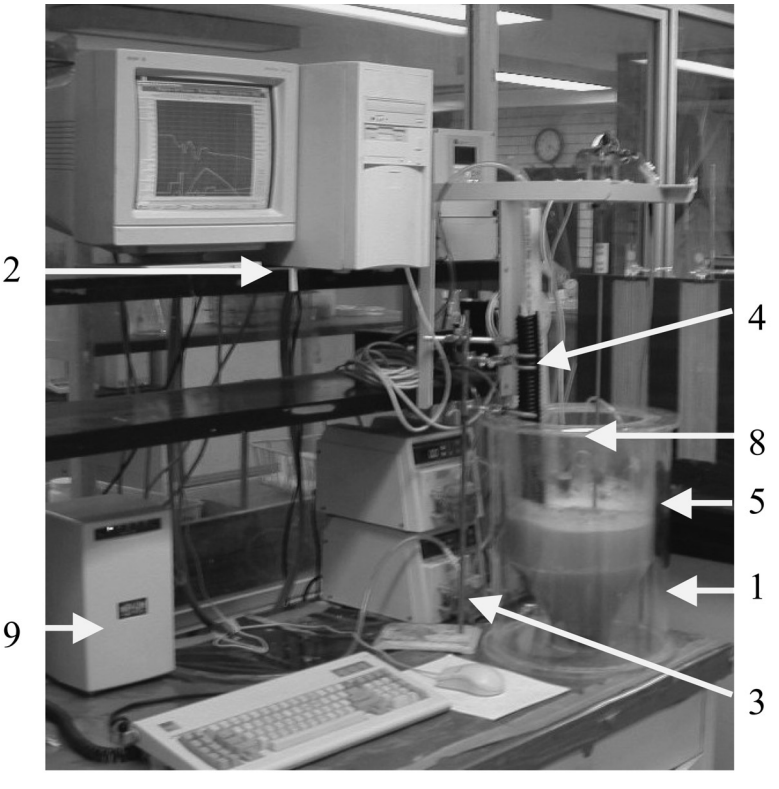

Figure 1 Automatized pilot reactor utilized for 4CP degradation. 1) Reactor, 2) PC, 3) feeding and drawing pumps, 4) temperature and dissolved oxygen sensor, 5) level switch, 9) no-break

4CP) was added and dissolved oxygen was measured and 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 2,000 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 99%. The experimental design considered three different sets of initial concentration of 4CP (50, 100 and 200 mg/L) named AC50, AC100 and AC200, respectively. Each experiment was initiated with fresh non-acclimated sludge, except for the case of AC200, in which the previously 100 mg/L acclimated sludge was used as inoculum.

Results and discussion

The degradation kinetics for the acclimation process was followed. For the two initial concentrations of 4CP used, acclimation was obtained in 10 degradation cycles. During the acclimation, 4CP was degraded with efficiencies higher than 99% as 4CP and 95% as DOC and COD. During the acclimation of the activated sludge, the relationship between residual 4CP concentration and incubation time gradually changed, and then stabilized. In the case of AC50, after 75 h, degradation time was reduced from 40 h to 50 min (from cycle 1 to cycle 10) (Figure 2A). For AC100 (Figure 2B) degradation times were reduced from 52 h to 1.16 h, after 125 h (cycle 1 to 10). It was observed that there is a proportional increase in the acclimation period when the initial concentration of the toxic was increased from 50 to 100 mg/L (75 vs 125 h).

Figure 3 shows the behavior of the DO as a function of time for the acclimation cycles. Note that during the first cycle practically there were no changes in oxygen concentration since the elimination of 4CP was very slow. In cycle 2 a minimum in the DO concentration is observed. This point corresponds to the maximal activity, and after that the 4CP concentration is minimal. It is possible to predict the end of the reaction phase observing the respirogram. We can consider that degradation of 4CP was completed once the DO has passed a minimum value and again reached the saturation value. As the acclimation took place the degradation time diminished correlating with the duration of each DO cycle.

When the pre-acclimated sludge of AC100 was exposed to an increase of 100% of the initial concentration, degradation was only slightly affected. The first degradation cycle for AC200 took only 2.5 h to remove 100% of the initial 4CP. After 5 h, degradation times were reduced to 1.75 h (from cycle 1 to cycle 6). This result indicated that during the AC100 acclimation the required microorganisms reproduced and they also developed the necessary enzymatic activity. Doubling the initial concentration only generates a proportional increase in the degradation time.

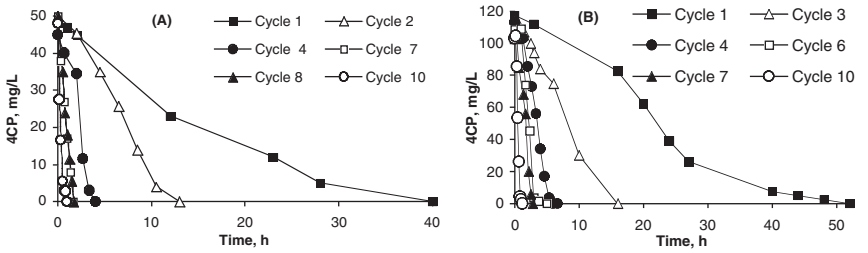

Figure 2 Degradation kinetics during the acclimation process. (A) acclimation to 50 mg4CF/L; (B) acclimation to 100 mg/L

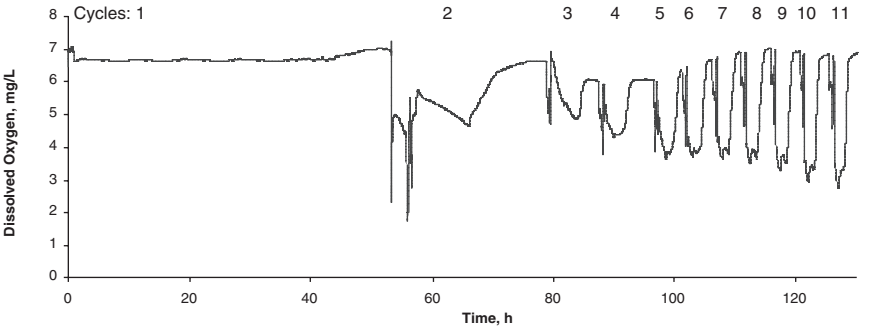

Figure 3 DO evolution as a function of acclimation time for AC 100

Once the biomass was completely acclimated to 4CP, when the compound was added again at 200 mg/L, the configuration of the degradation curve was similar, independently of the initial acclimation concentration. Nevertheless, care must be taken since an elevated concentration of the toxic compound may cause inhibition problems. Moreno-Andrade and Buitrón (2003) observed that inhibition is not only a function of the initial substrate concentration, but also of the initial biomass concentration. In general, a low biomass concentration will produce a greater inhibition. For this reason the acclimation of microorganisms is preferably done at a lower initial substrate to microorganism ratio. Once the microorganisms are acclimated, they can biodegrade concentrations of 4CP up to 1,400 mg/L (Buitrón *et al.*, 2003).

Microorganism activity

Figure 4 presents the evolution of the SOUR computed during the acclimation of microorganisms to 4CP. Two parallel experiments were carried out. First, the SOUR was measured feeding the mini-reactor with an easy-to-biodegrade substrate, namely acetate. A second experiment was conducted using 4CP as substrate. The substrate for each case was the sole source of carbon and energy, and for both cases, endogenous respiration was evaluated and taken into account for calculations.

As acclimation took place, an increase in the SOUR against 4CP was observed. On the contrary, a decrease on the SOUR occurred for the case of acetate. In Figure 4, a crossing point is observed at 60 h and 80 h for the cases of AC50 and AC100, respectively. We can consider that after this point affinity of the consortia is higher for the toxic compound than

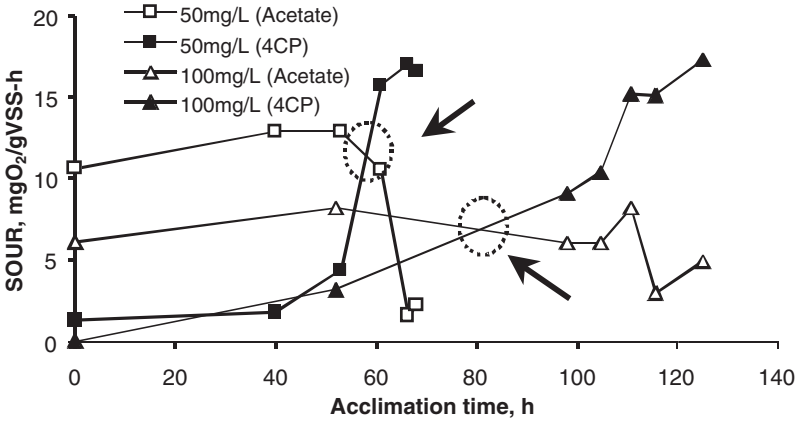

Figure 4 Evolution of the SOUR for the microorganisms acclimated to 4CP. Arrows indicate the crossing point when the activity to the consumption of 4CP is higher than the activity of acetate consumption

for an easy-to-biodegrade substrate such as the acetate. Thus, there is a point when the affinity of the microbial community shifts towards the toxic compound and afterwards, the degradation rate increases rapidly and stabilizes.

Figure 5 presents the specific substrate uptake rate, q_x, as a function of the acclimation time. The evolution of q_x over time during the acclimation period may be modeled with an exponential relationship. Similar results were observed when anaerobic sludge was acclimated to the degradation of chlorophenols (Ye and Shen, 2003). The exponential behavior observed in the present work suggested that the adaptation might have been caused by the growth of a new population. This effect is clear for activated sludge that was put in contact for the first time to 4CP (AC50, AC100). Note that, this behavior was not observed for the case where there was only an increase of toxic concentration (AC200), since the biomass was already acclimated. In this case a logarithmic relationship was found. The behavior was similar for the case of COD, as shown in Figure 6, indicating that during acclimation uptake of 4CP is correlated with the mineralization.

When a previously acclimated biomass was exposed to a higher concentration of 4CP, the acclimation time was lower than the time needed by a non-acclimated sludge, as shown in Figures 5 and 6 for q_x obtained for the case of AC200. In this case, a logarithmic relationship was obtained to explain the evolution of the q_x. This observation indicated that after the acclimation process was completed the q_x no longer increases exponentially, but seems stabilized in a maximal value. Care must be taken when acclimation is conducted; if this is

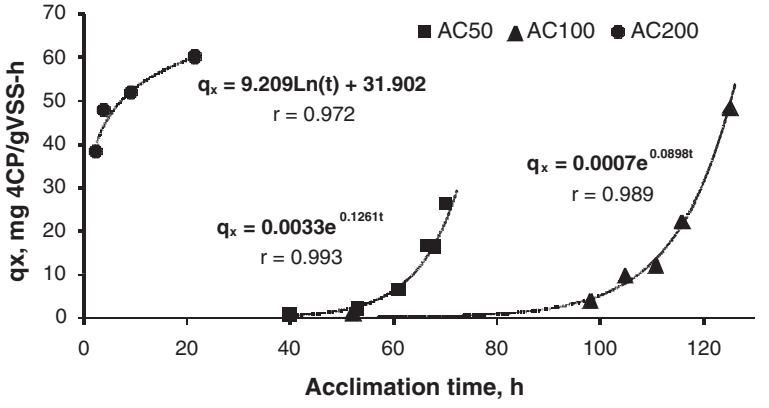

Figure 5 Evolution of the specific substrate uptake rate, q_x, as a function of the acclimation time. Degradation rate was evaluated dividing the concentration of 4CP by the time needed to degrade more than 99% of the original concentration and divided by the VSS present in the reactor

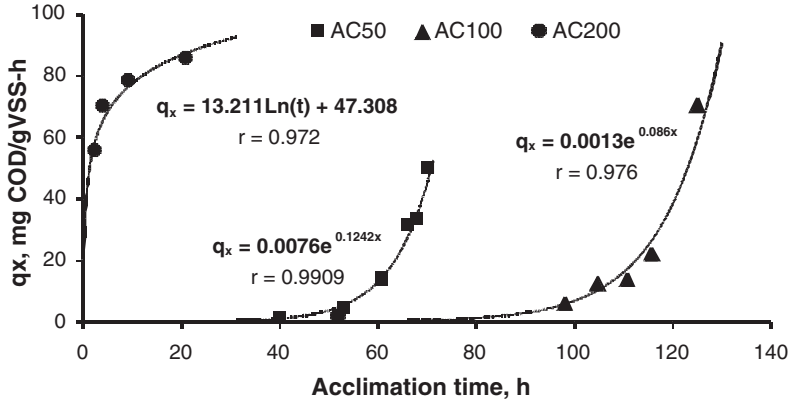

Figure 6 Evolution of the specific COD uptake rate, q_x, as a function of the acclimation time

done at an elevated concentration of the toxic compound (beyond the inhibition concentration) there will be problems and the acclimation process may not be successful.

Metabolite production in the acclimation

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) (Commandeur and Persons, 1990). When the biomass is stressed (for example under toxic shocks) the degradation of 4CP is poor, due to the accumulation of a toxic metabolite formed during another pathway of ring cleavage. The meta cleavage of chlorocatechol is realized by the catechol 2-3-dioxygenases and produces the metabolite 5-chloro-2hydroxy-muconic acid semialdehyde. In this case, during acclimation a green-yellowish coloration was observed. The spectral characteristics of this compound are similar to those reported by Westmeier and Rehm (1987) for precisely this metabolite.

The production of the metabolite was followed during the acclimation by measuring the absorbance at 380 nm. Figure 7 presents an example of the evolution of the metabolite production during the acclimation AC100. It is possible to observe that as the acclimation proceeds the metabolite production is less important.

To take into account the metabolite production, the absorbance generated during the degradation cycle was computed (area under each curve of Figure 7); the results are expressed as absorbance units multiplied by hours (Table 1). It is observed that higher quantities of metabolite were generated at the beginning of the acclimation. As the bacteria adapted, the toxic metabolite production diminished from 0.053 to 0.016 (for AC50) and from 0.110 to 0.018 absorbance units-hour. It has been observed that the appearance of this metabolite also indicates an operational problem, and thus deacclimation of the microorganisms (Buitrón *et al*., 2003).

Sludge volumetric index

As the acclimation proceeds, the sludge volumetric index (SVI) decreased (Figure 8). Microscopic observations of the sludge indicated that filamentous bacteria found in the inoculum used for the AC100 experiment were eliminated during acclimation to the toxic. In general, the microorganisms degrading 4CP presented excellent settling properties. Even for a problematic inoculum (AC100), the SVI decreased from 500 to 240 mL/g after 10 cycles, and to 70 after 20 more cycles (see the values for 200 mg 4CP/L).

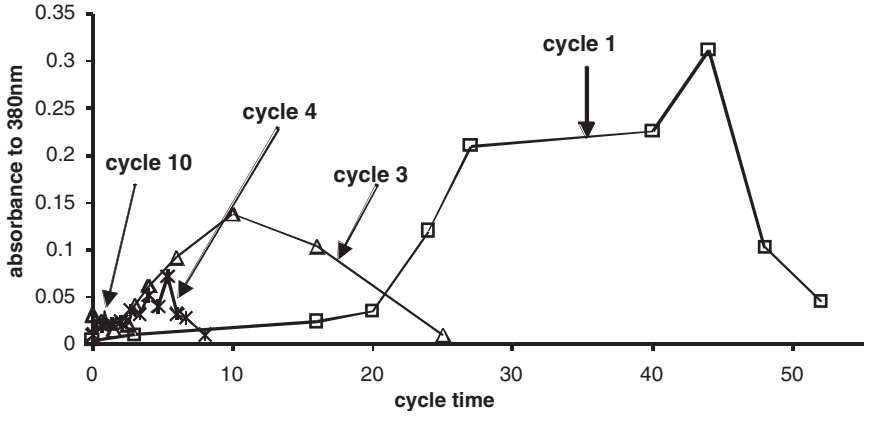

Figure 7 Kinetics of the metabolite production during acclimation AC100

Table 1 Metabolite production during the acclimation process

4CP concentration	Cycle number	Metabolite production (Abs*h)
AC50	1	0.053
(50 mg/L)	2	0.020
	4	0.016
	7	0.016
	8	0.016
	10	0.016
AC100	1	0.119
(100 mg/L)	3	0.026
	4	0.030
	6	0.016
	7	0.017
	10	0.018

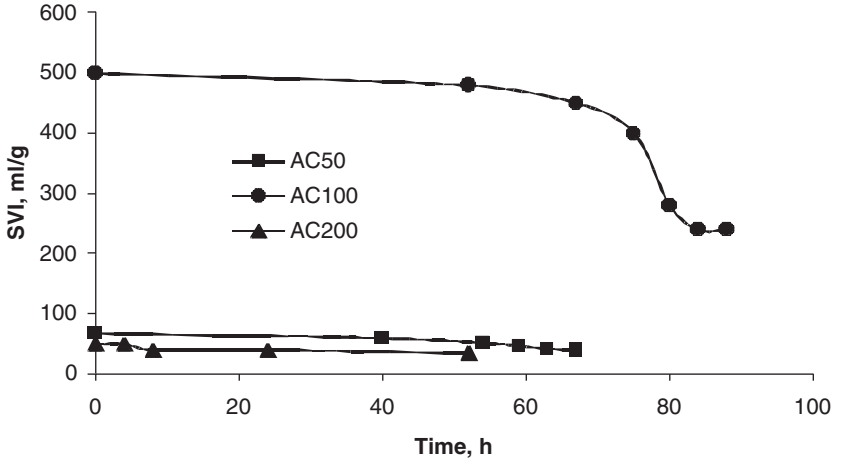

Figure 8 Variation of the SVI during the acclimation to 4CP

Conclusions

The results showed a reduction in the degradation time during the acclimation process. In the case of 50 mg 4CP/L, degradation time was reduced from 40 h to 50 min, after 71 h and from 52 to 1.16 h, after 105 h. By comparing the necessary time to acclimate the consortia to 50 and 100 mg/L, it is noted that doubling the initial concentration of the toxic only generates 25% increase of acclimation period (71 vs 105 h). As the acclimation proceeded, it was found that the affinity of the consortia to biodegrade the toxic increased, whereas the ability to biodegrade acetate decreased. The evolution of the substrate uptake rate over time during the acclimation period was highly correlated with an exponential relationship. The exponential behavior observed suggests that the adaptation might have been caused by the growth of a new population. There is a decrease of the toxic metabolite production as the bacteria adapt to 4CP degradation. Acclimation to 4CP also decreases the SVI, improving the settling properties of the sludge.

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