Biotechnology and Bioprocess Engineering 16: 000-000 (2011)
DOI 10.1007/s12257-010-0418-z

RESEARCH PAPER

Multisubstrate Monod Kinetic Model for Simultaneous Degradation of Chlorophenol Mixtures

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Received: 17 November 2010 / Revised: 7 May 2011 / Accepted: 23 May 2011

Abstract Chlorophenols (CPs) are persistent and highly toxic compounds rated as priority pollutants by the Environmental Protection Agency (EPA). Frequently, these compounds are present as mixtures of CPs in industrial wastewaters. Therefore the study of biodegradation on mixed pollutants is an important aspect of biodegradation and wastewater treatment. In this work, we studied the multisubstrate degradation of CPs by a mixed culture of *Pseudomonas aeruginosa* and a novel *Acromobacter* sp. capable of using pentachlorophenol (PCP), 2,4,6 trichlorophenol (2,4,6 TCP) and 2,3,5,6 tetrachlorophenol (2,3,5,6 TeCP) as the sole sources of carbon and energy. The main objective of this work was to evaluate the effect of substrate mixtures on the degradation kinetics of PCP. Batch experiments were conducted with each CP separately and in mixtures of PCP + 2,4,6 TCP, PCP + 2,3,5,6 TeCP, and PCP + 2,4,6 TCP + 2,3,5,6 TeCP. Based upon our results we have concluded that the simultaneous degradation of CPs is a key factor contributing to the improvement of PCP degradation. The kinetic parameters for PCP and 2,4,6 TCP were obtained by fitting the data to a Monod kinetics model. Using such parameters, the model was able to predict simultaneous multisubstrate degradation of PCP with others CPs.

Keywords: chlorophenols, simultaneous degradation, bacteria, modeling

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1. Introduction

The chemical industry produces highly toxic organic wastes, many of which are aromatic compounds resistant to natural biodegradation [1]. Chlorophenols (CPs) are used in wood preservation, pesticides, and fungicide formulations, and are rated as priority pollutants by the EPA [2]. The extensive use of pentachlorophenol (PCP) has led to the pollution of soil and groundwater. PCP toxicity stems from the fact that it is an oxidative phosphorylation inhibitor [3]. Moreover, PCP is recalcitrant to degradation because of its stable aromatic ring structure and high chlorine content.

Conventional methods such as solvent extraction, activated carbon adsorption, and chemical oxidation are usually expensive and may produce hazardous wastes [4]. Biodegradation is an attractive alternative to treat pollutants because innocuous byproducts such as carbon dioxide (CO_2) and water (H_2O) are obtained as final products [5].

Several studies have reported on the biodegradation of PCP by axenic or mixed cultures of bacteria [3,6] and fungi [7]. Although PCP degradation is very effective when using pure cultures, the ability of mixed cultures to survive in a non-sterile environment is important in applied biodegradation in the field [6].

One possible way to overcome slow kinetic limitations is to use additional substrates to facilitate the degradation of recalcitrant substances. Readily degradable substrates such as glucose, glutamate, or any other simple carbohydrate or amino acid can be added, however, this introduces additional costs. Furthermore, these substrates may facilitate the potential growth of indigenous bacteria that tolerate but do not degrade CPs. For example, Murialdo *et al.* [6] used glucose to improve PCP degradation while others proposed the use of toxic compounds to improve the degradation of another toxic compound. For example, the addition of

naphthalene improved biodegradation of phenanthrene and fluorene [8] and biodegradation of 4-chlorophenol by *Comamonas testosteroni* was enhanced in the presence of phenol [9]. Additionally, De los Cobos-Vasconcelos *et al.* [10] studied the degradation of PCP with 2-monochlorophenol (2-CP), 4-monochlorophenol (4-CP), 2-4 dichlorophenol (2-4 DCP), and 2-6 dichlorophenol (2,6 DCP). The degradation of PCP along with trichlorophenol (TCP) and tetrachlorophenol (TeCP) isomers was investigated by McAllister *et al*. [11] with favorable results. The enhanced removal rate may be due to the higher amount of biomass formed at the expense of the additional carbon source available, or to the presence of induced enzymes beneficial for the degradation of recalcitrant compounds. [12].

Many models predict multisubstrate degradation [13]; however, they can be quite complex. A simpler multisubstrate Monod kinetic model (MMKM) is widely used in applied research [8]. This model is fully predictive in the sense that the parameters can be estimated from independent measurements in sole-substrate systems.

The MMKM assumes that all the components in the mixture share common enzyme reaction pathways which are rate-limiting. The 2,4,6-TCP and the 2,3,5,6-TeCP, that were completely dechlorinated by *Flavobacterium* sp., were shown to be, in addition to PCP itself, inducers of the complete PCP degradation pathway [14].

This formulation is analogous to the theoretical multisubstrate enzyme kinetic expressions derived by several authors [15,16]. Furthermore, the use of this model as a predictive tool implies the assumption that the microbial community in the multisubstrate system is comparable to that in the sole-substrate systems with respect to physiologic state. This assumption is valid, even in a mixed culture system, provided that all the substrates are utilized by a common enzyme system.

The main objective of this work was to study for the first time the effect of some CP mixtures on the degradation kinetics of PCP. In this context we studied the effect of CP mixtures on the degradation kinetics of PCP by two PCP-degrading strains isolated in our laboratory [6,17]. In a previous work, Murialdo *et al.* [6] demonstrated that these strains were able to dechlorinate PCP completely. Subsequent work also demonstrated the absence of toxicity of the supernatant after degradation, towards a sensitive bacteria [18]. In this research, we studied the individual degradation of PCP, 2,4,6 TCP, and 2,3,5,6 TeCP by mixed culture in a batch reactor to evaluate the kinetic parameters for each substrate. We also studied the mixtures of PCP + 2,4,6 TCP, PCP + 2,3,5,6 TeCP, and PCP + 2,4,6 TCP + 2,3,5,6 TeCP to analyze the effect of the different mixtures on total PCP degradation. In addition, the ability of the MMKM as a predictive tool was evaluated.

2. Materials and Methods

2.1. Microorganisms and culture conditions

The mixed culture used in this work consisted of *P. aeruginosa* [6] and *Achromobacter* sp. [17] (1:1 ratio) which were isolated in our laboratory from soil contaminated with CPs [19,20]. These strains were selected because they showed the best CP degradation kinetics among the strains isolated [19].

The bacteria were grown in a liquid medium modified from that used by Stanlake and Finn [21].The stock culture (MS) contained the following (g/L) inorganic salts: K_2HPO_4, 0.65; K H_2PO_4, 0.17; NH_4CL, 0.05; $NaNO_3$, 0.05; and $MgSO_4 \cdot 7H_2O$, 0.1. In addition, a mixture of PCP (20 mg/L), 2,4,6 TCP (20 mg/L), and 2,3,5,6 TeCP (20 mg/L) were added. This was the only source of carbon and energy used. Before inoculation, all media were adjusted to pH 7 with sodium hydroxide (NaOH) and autoclaved at 121ºC for 15 min. The CPs were first converted to sodium salt by dissolving them in NaOH after which they were added directly to the MS medium.

The bacteria (*P. aeruginosa* and *Achromobacter* sp.) are able to degrade each CP separately. Prior to our study, they were acclimated for six months to the mixture of CPs (20 mg/L of each), plus MS stock culture medium. We considered the bacteria to be acclimated when the degradation of the CPs was consistent and without a lag phase. During acclimation, 90% of the liquid medium was discarded every six days (after checking that the CP had been degraded) and replenished with fresh sterile medium that contained a mixture of CPs and MS. The remaining 10% (v/v) served as inoculum.

2.2. Chemicals

Stock solutions of 5 g/L of PCP, 2,4,6 TCP, and 2,3,5,6 TeCP were prepared with the addition of NaOH to facilitate complete dissolution of the CPs in distilled water.

2.3. Batch reactions

Batch experiments were conducted in 250 mL Erlenmeyer flasks with a total reaction volume of 100 mL. For all the assays a 10% (v/v) stock culture in the exponential phase of microbial growth was used as inoculum (10^4 CFU/mL). Each reactor contained MS medium and PCP, 2,4,6 TCP, and 2,3,5,6 TeCP as the only source of carbon and energy. The reactors were sealed with cotton stoppers and shaken at 120 rpm in an orbital shaker in the dark at 25ºC. During all the tests the pH value was kept constant and equal to 7. The flasks were sampled (1 mL) periodically to measure CP removal and pH under aerobic conditions. These experiments were used to estimate the kinetic parameters for each CP in the concentration range of 0 ~ 50 mg/L. Batch experi-

ments were also performed with mixtures of PCP and 2,4,6 TCP; PCP and 2,3,5,6 TeCP; and PCP, 2,4,6 TCP, and 2,3,5,6 TeCP. All the runs were performed in duplicate simultaneously and the data are presented as mean values. As a control, a batch flask was cultivated in the absence of cells to rule out the degradation of PCP by non-biological means.

2.4. Analytical methods

The bacterial population was estimated by the plate count method at the beginning and end of every run to estimate the yield coefficient for every CP (Table 1).

CPs were analyzed using high-performance liquid chromatography (HPLC). The cell suspensions were clarified by centrifugation at 14,000 rpm for 5 min and the cell-free supernatant fraction was analyzed using HPLC with a Waters Model 590 pump, Model 484 ultraviolet (UV) detector, and Spherisorb (5 μm, OD 52, 4.6 × 250 mm) analytical column (Millipore Corporation, Milford, MA, USA). The UV detector was set at 219 nm. The mobile phase consisted of acetonitrile and 7 mM phosphoric acid (70:30, v/v) with a flow rate of 1 mL/min.

2.5. Kinetic parameters determination

The equation by which kinetic parameters were evaluated is formulated below, assuming that product formation is negligible:

$$\frac{\partial X\cdot V}{\partial t} = \mu_i \cdot X \cdot V \tag{1}$$

$$\frac{\partial S_i\cdot V}{\partial t} = \frac{1}{Y_{X/S_i}}\cdot \mu_i \cdot X \cdot V \tag{2}$$

where μ (h) is the specific growth rate, X (CFU/mL) is the biomass concentration, S_i (mg/L) is the substrate i concentration, Yx/s_i (CFU/mL /mg/L) is the yield coefficient of biomass from substrate i and V (L) is the total reaction volume. The specific growth rate is defined by the Monod model equation for each substrate (Eq. 3). This model relates the growth rate to the concentration of a single growth-controlling substrate *via* two parameters: the maximum specific growth rate (μmax_i) and the substrate affinity constant or Monod constant (Ks_i). These parameters were estimated by fitting Equation (4) to experimental data from individual batch degradation experiments. The yield Yx/s_i was evaluated from the data of substrate consumption and plate counts.

$$\mu_i = \frac{\mu\max_i \cdot S_i}{Ks_i + S_i} \tag{3}$$

$$\frac{\partial S_i}{\partial t} = -\frac{X}{Y_{X/S_i}}\cdot\frac{\mu\max_i \cdot S_i}{Ks_i + S_i} \tag{4}$$

2.6. Model description

In this work MMKM was used to simulate the simultaneous degradation of CP mixtures. This model is widely used in applied research [8] and it is fully predictive.

Substrate interactions may result from the dual effects of (i) competitive metabolism, in which one substrate inhibits the utilization of another because of competition for the active binding site of an enzyme and (ii) the growth of biomass at the expense of one or more of the multiple substrates present. The first effect will negatively impact the substrate biodegradation rate and the second will enhance it. MMKM has the capability to contemplate both of these interaction effects.

The model assumes that since all the substrates can serve as growth substrates, the total specific growth rate (μ_T) can be written as the summation of the individual specific growth rate (μ_i). The specific growth rate (μ_i) is related to the concentrations of the substrates by the multisubstrate Monod growth relationship (Eq. 6) [13]. Since the model proposed is fully predictive, the parameters, $\mu\max_i$ and Ks_i, obtained by fitting the single substrate degradation data with Equation (4) can be used for predictions.

$$\mu_T = \sum_{i=1}^{n} \mu_i \tag{5}$$

$$\mu_i = \frac{\mu\max_i \cdot S_i}{Ks_i + \sum_{j=1}^{n} \frac{Ks_i}{Ks_j} S_j} \tag{6}$$

This model (Eq. 6) is different from the substrate interaction models discussed by other authors [22,23], in which the summation in the denominator is replaced with $S_i + K_I$, where K_I is an empirical interaction parameter. The mathematical implication of the summation term in the denominator of Equation (6) is that μ_i in the multisubstrate case is less than the specific growth rate that would be predicted by sole-substrate Monod kinetics. However, since the total biomass growth rate in the multisubstrate case is larger than what would occur if there was only one substrate (Eq. 5), the actual substrate depletion rate can be enhanced. These two competing effects are taken into account in the substrate depletion rate equation (Eq. 8).

Substituting Equations 5 and 6 in the respective mass balances for substrate and biomass in batch systems, MMKM is formulated as follows:

$$\frac{\partial X}{\partial t} = \mu_T \cdot X = \left(\sum_{i=1}^{n} \mu_i\right)\cdot X \tag{7}$$

$$\frac{\partial S_i}{\partial t} = \frac{1}{Y_{X/S_i}}\cdot\frac{\mu\max_i \cdot S_i}{Ks_i + \sum_{j=1}^{n} \frac{Ks_i}{Ks_j} S_j}\cdot X \tag{8}$$

The differential mass balances for biomass (Eq. 7) and for each substrate (Eq. 8) were solved simultaneously with Mathcad 2001 Professional© 1986 ~ 2000 (MathSoft, Inc., Cambridge, MA, USA). The kinetic parameters obtained from individual tests were used to predict the multisubstrate degradation behavior in accordance with MMKM, using a fourth order Runge-Kutta routine which integrates at fixed steps to reach a solution.

3. Results and Discussion

3.1. Multisubstrate biodegradation

Batch experiments were conducted with each CP separately and in mixtures of PCP + 2,4,6 TCP, PCP + 2,3,5,6 TeCP, and PCP + 2,4,6 TCP + 2,3,5,6 TeCP. The initial concentration of each CP in each assay is shown in Table 1. The goal was to use an initial total CP concentration on the order of 40 ~ 45 mg/L. Because the actual concentrations did not depart from the initial target, we assert that both the physiologic response and the kinetic behavior are the same. This has been found experimentally by Murialdo [19] with the same bacteria and by González and Hu [24], who determined that using concentrations between 25 and 50 mg/L of PCP the specific growth rate and the cell yield of a *Flavobacterium* sp. were essentially the same in that interval of substrate concentration. Also, during the degradation, the death constant was essentially zero in that concentration range.

The removal efficiency and cell growth yield in the case of each CP was compared to the results obtained in the mixtures. In the simultaneous degradation experiment of PCP + 2,4,6 TCP an enhancement upon the removal of PCP was observed (Fig. 1A). The improvement in the PCP degradation in the presence of 2,4,6 TCP lies in the fact that 2,4,6 TCP has a higher yield coefficient (Table 3) and the biomass generated from it is able to degrade PCP. On the other hand, the degradation of 2,4,6 TCP in the presence of PCP is slower than when alone (Fig. 1B) due to the competitive inhibition between substrates. This has also been observed by Guha *et al.* [8].

The inhibition observed during the degradation of 2,3,5,6 TeCP alone (Fig. 1C) is due to the fact that the studied strains had been acclimated to just 20 mg/L of each CP and higher concentrations of 2,3,5,6 TeCP are lethal to non-acclimated bacteria. González and Hu [24] confirmed this experimentally. In the presence of 2,3,5,6 TeCP the degradation of PCP (Fig. 1A) was slower than in the case of PCP alone.

The high toxicity of CPs without chlorine in position 4 [25] could also be responsible for this detrimental effect. These observations agree with results obtained by Liu *et al.* [26] and Steiert *et al.* [14]. They conducted studies of microbial degradation with various CPs, and found that the toxicity increased with the degree of chlorination. Phenols with substitutions at 2 and 6 were the least toxic. However, according to the results showed by Yang *et al.* [27], the ability of *S. chlorophenolica* cells to degrade various CPs was not related to the chlorine ring substitution patterns of specific compounds. These authors explained that this phenomenon was due to the metabolic pathway of this strain. Although the metabolic pathway of the isolated strains (*P. aeruginosa* and *Achromobacter marplatensis*) is so far unknown, other authors [28] reported that PCP degradation is initiated by pentachlorophenol 4-monooxygenase (PcpB). The degradation of PCP and 2,4,6 TCP, is produced via hydroquinones as central intermediates. CP monooxygenases not related to PcpB have been reported to be involved in 2,4,6-TCP degradation, whereas in PCP degrading *Sphingomonas*, 2,6-dichlorohydroquinone is the ring-cleavage substrate, and 6-chlorohydroxyquinol is the ring-cleavage substrate in 2,4,6 TCP degradation by JMP134. In contrast, degradation of 2,4,6 TCP occurs via ring-cleavage of trihydroxylated intermediates.

The results of the ternary mixture degradation experiments are shown in Fig. 1. In this system PCP and 2,3,5,6 TeCP consumption reaches almost the same extent as 2,4,6 TCP consumption. In the mixture of all the tested CPs, a higher degradation rate was observed for PCP than with PCP alone (Fig. 1A), but it is lower than that observed in the binary system with 2,4,6 TCP. On the other hand, the degradation of 2,3,5,6 TeCP in a ternary mixture (Fig. 1C)

Table 1. Initial concentration of batch experiments and biomass determination

Assay	PCP (mg/L)	2,4,6 TCP (mg/L)	2,3,5,6 TeCP (mg/L)	Total CPs (mg/L)	X_0 (CFU/mL)	X_f (CFU/mL)
PCP	35.163	–	–	35.163	3.2×10^4	2.4×10^6
2,4,6 TCP	–	52.930	–	52.930	4.8×10^4	7.6×10^6
2,3,5,6 TeCP	–	–	45.094	45.094	4.1×10^4	ND
PCP + 2,4,6 TCP	18.372	17.197	–	35.569	3.6×10^4	3.7×10^6
PCP + 2,3,5,6 TeCP	19.633	–	17.883	37.516	4.6×10^4	ND
PCP + 2,4,6 TCP + 2,3,5,6 TeCP	17.676	16.053	15.929	49.658	4.5×10^4	3.7×10^6

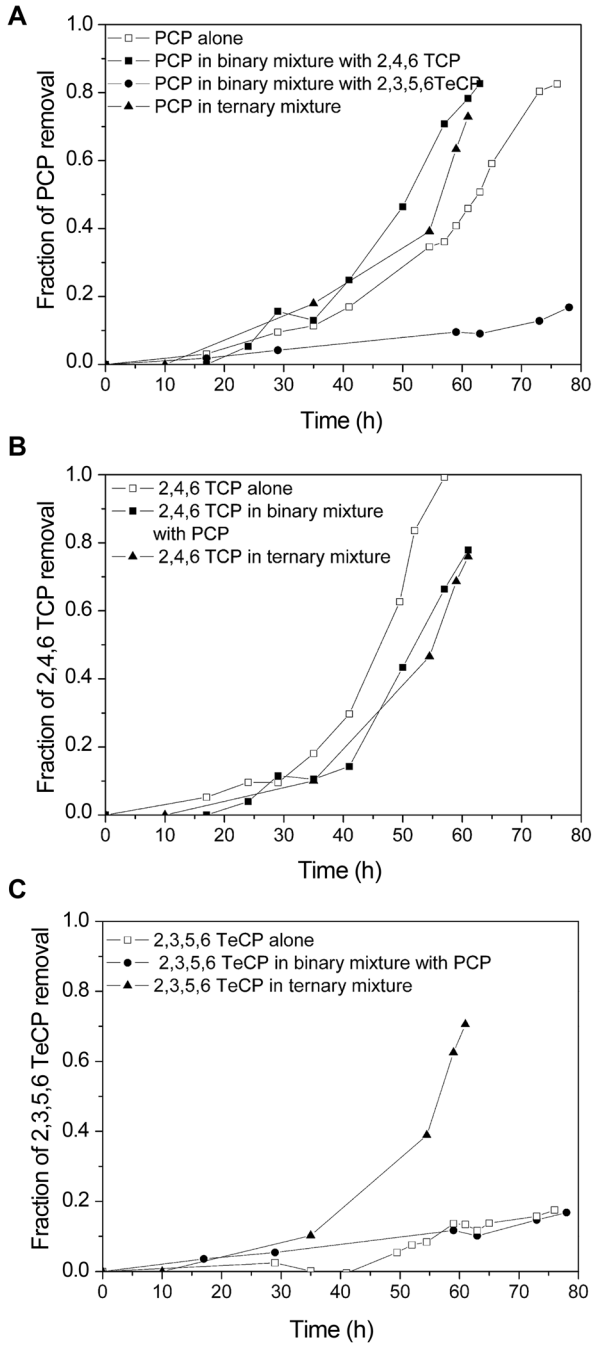

Fig. 1. (A) PCP biodegradation alone (open squares), PCP biodegradation in binary mixture with 2,4,6 TCP (filled squares), PCP biodegradation in binary mixture with 2,3,5,6TeCP (filled circles) and PCP degradation in ternary mixture (filled triangles). The results are shown as fraction of PCP removal. (B) 2,4,6 TCP biodegradation alone (open squares) 2,4,6 TCP biodegradation in binary mixture with PCP (filled squares) and 2,4,6 TCP biodegradation in ternary mixture. The results are shown as fraction of 2,4,6 TCP removal. (C) 2,3,5,6 TeCP biodegradation alone (open squares), 2,3,5,6 TeCP biodegradation in binary mixture with PCP (filled circles) and 2,3,5,6 TeCP in ternary mixture (filled triangles). The results are shown as fraction of 2,3,5,6 TeCP removal.

became possible due to the fact that the concentration was lower than the acclimation concentration at every moment. This result demonstrates that for PCP degradation (the most recalcitrant CP), the presence of other substrates is beneficial for its mineralization and total CP degradation (Table 2). The addition of less recalcitrant CPs is seen as a key step in this type of process for improving the degradation of PCP. Under aerobic conditions, the degradation pathways of chlorinated aromatics like CPs are more diverse than under anaerobic conditions as ring cleavage can occur either before or after removal of the chlorine substituents giving rise to a whole array of intermediates of varying toxicity [29]. Furthermore, if 2,4,6 TCP were an intermediate in PCP degradation, the process would be autocatalytic. These results are significant because in several industrial streams CPs are present in mixtures together with other CPs and aromatics compounds, which can enhance or decrease the PCP degradation rate.

Table 2 presents the total degradation rate, defined as the total CPs consumed during the time of operation. These results show how the addition of less recalcitrant CPs such as 2,4,6 TCP improves total CP degradation in comparison to PCP as the only source of carbon and energy. The same effect is observed with the ternary mixture. In this case total CP degradation improvement is greater than in the binary mixture due to the lower ratio of PCP to recalcitrant substrate. This result is relevant for field operations that cannot be performed under sterile conditions. Addition of a co-substrate that is consumed exclusively or preferentially by PCP degraders will give them a competitive advantage over the native flora that tolerate but do not degrade CPs.

These results support the idea of interplay between metabolic pathways with the chlorophenols studied. *Rhodococcus chlorophenolicum* PCP-1 degraded 2,3,5,6 TeCP similar to PCP, and 2,3,5,6 TeCP was hydroxylated to TeCH (Tetrahydroquinone) [30]. The preferred growth substrate was 2,4,6 TCP which is metabolized *via* 2,6 dichlorohydroquinone for several bacteria [30]. A minor bacterial transformation pathway for the higher chlorinated phenols starts by methylation of the hydroxyl group [30]. This transformation reaction is a detoxification mechanism, since the methylated products are less toxic than the corresponding CPs.

The difference between the degradation of the single CPs and their mixture are explained using the cometabolic principle. Three stable CPs (3,5-dichlorophenol (3,5 DCP), 3,4,5-trichlorophenol (3,4,5 TCP), and 2,3,5,6 TeCP), which could not be degraded when present as single substrates, were found to be biodegradable using the cometabolic technique [31]. When a lower chlorinated mono-

Table 2. Total degradation rate for different tests

Assay	Total degradation rate $\left(\frac{mg/L}{h}\right)$
PCP	0.4608
2,4,6 TCP	0.9264
PCP + 2,4,6 TCP	0.594
PCP + 2,4,6 TCP + 2,3,5,6 TeCP	0.81

chlorophenol was the cometabolite, only the medium 3,5 DCP was biodegraded, while the 2,3,5,6, TeCP was not degraded by the culture. However, all stable CPs (3,5 DCP; 3,4,5 TCP; 2,3,4,5 TeCP and PCP) were rapidly biodegraded when present simultaneously.

3.2. Kinetic parameters

The kinetic parameters for PCP and 2,4,6 TCP were obtained by fitting the Monod model to the experimental data. The results are shown in Table 3. These values adjust properly to experimental data, as seen in Figs. 2 and 3. The limit of detection of the analytical device is approximately 5 mg/L, for this reason the model prediction departs slightly from experimental values at low concentrations. Field *et al.* [32] summarized the kinetic parameters for different CPs from several strains, as pure and mixed cultures. The

Table 3. Kinetic parameters to CPs

	μmax (1/h)	*Ks* (mg/L)	$Y_{X/S}$ (CFU·mL^{-1}/mg·L^{-1})
PCP	0.0615	0.5894	0.79×10^5
2,4,6 TCP	0.0929	0.4771	1.43×10^5
2,3,5,6 TeCP	0.0523	0.71	0.63×10^5

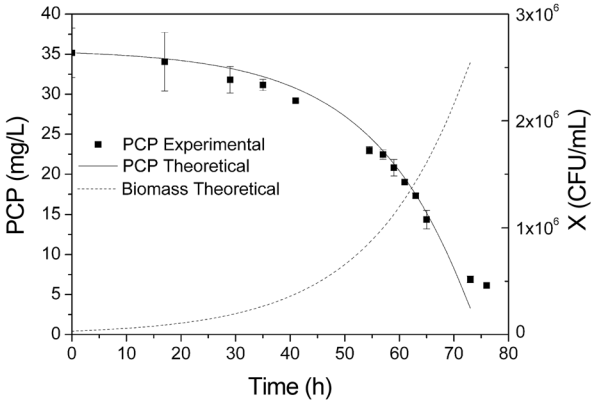

Fig. 2. Experimental PCP data fitted to Monod model, with PCP as the only source of carbon and energy. Filled square points are the experimental PCP concentration (mg/L), and the solid line is the theoretical PCP concentration (mg/L). The dashed line is the theoretical biomass growth (X) (CFU/mL).

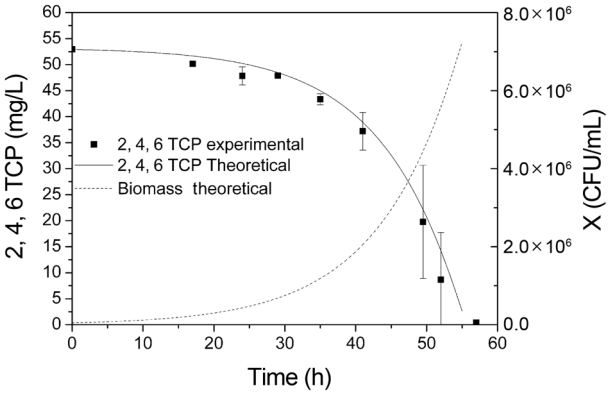

Fig. 3. Experimental 2,4,6 TCP data fitted to Monod model, with 2,4,6 TCP as the only source of carbon and energy. Filled square points are the experimental 2,4,6 TCP concentration (mg/L), and the solid line is the theoretical 2,4,6 TCP concentration (mg/L). The dashed line is the theoretical biomass growth (X) (CFU/mL).

PCP specific growth rate found in this work is similar to that reported by Kleka and Maier [22] by an enriched culture, but lower than other values obtained with pure cultures [32]. The 2,4,6 TCP specific growth rate agrees with that reported by Field *et al.* [32]. The values of Ks_i reported for CPs fluctuated in the range of 0.05 ~ 5, but the most common was around 0.5 ~ 1 [32]. The kinetic values for 2,3,5,6 TeCP were estimated based on the results for the ternary mixture (see simulation model section), where the total CP concentration was close to 45 mg/L and the concentration of 2,3,5,6 TeCP (16 mg/L) was such that it could be degradated.

3.3. Model simulation

The degradation of the binary mixture was simulated using MMKM with the parameters obtained by the fitting of

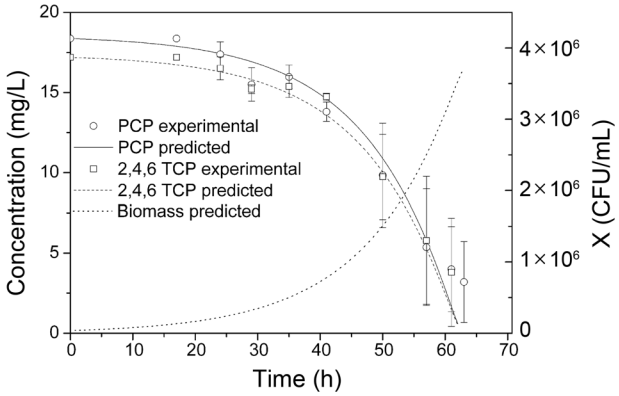

Fig. 4. MMKM mathematical prediction of PCP and 2,4,6 TCP simultaneous degradation. Circles are the PCP experimental concentration (mg/L) and squares are the 2,4,6 TCP experimental concentration (mg/L). Lines are the behavior predicted for MMKM: solid for PCP (mg/L), dashed for 2,4,6TCP (mg/L), and dotted for biomass growth (CFU/mL).

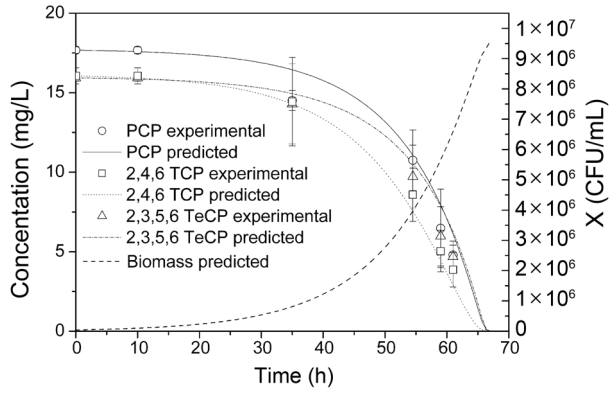

Fig. 5. MMKM mathematical prediction of the ternary mixture simultaneous degradation. Circles are the PCP experimental concentration (mg/L), squares are the 2,4,6 TCP experimental concentration (mg/L), and triangles are 2,3,5,6 TeCP concentration. Lines are the behavior predicted for MMKM: Solid for PCP (mg/L), dotted for 2,4,6TCP (mg/L), dot-dash for 2,3,5,6 TeCP (mg/L), and dash for biomass growth (CFU/mL).

individual tests. Fig. 4 presents the experimental mean value concentrations in multisubstrate degradation of PCP and 2,4,6 TCP and the theoretical behavior predicted by MMKM. The results presented show that the model can satisfactorily predict the multisubstrate degradation of PCP and 2,4,6 TCP with an average error of less than 10%. Assuming that the MMKM is able to represent the real behavior of the ternary mixture, the kinetic parameters for 2,3,5,6 TeCP (Table 3) were evaluated from experimental data from ternary mixture experiments. Fig. 5 shows the behavior of ternary mixtures using MMKM. The results obtained adjust to experimental data with an average error of 10%. The 2,4,6 TCP simulated degradation is higher than other CP degradation which agrees with experimental observations. These results demonstrate that MMKM is a powerful tool to predict the real behavior of a mixture of different CPs, knowing just the kinetic parameters from the different compounds alone, without any additional assays. The use of this model is particularly useful to design continuous and semi-continuous reactors and to optimize wastewater treatment and reactor operations.

4. Conclusion

Based on the results obtained from our research, we conclude the following:

(1) For PCP degradation (the most recalcitrant CP), the presence of other substrates is beneficial for its mineralization by the isolated strains with a positive impact in field bioremediations.

(2) The simultaneous degradation of CPs is a key factor contributing to the improvement of total CP degradation.

(3) MMKM is a useful tool to predict the behavior of CP mixtures.

(4) The model presented is able to predict the simultaneous degradation of PCP with other CPs in batch mode using results from single substrate experiments.

Acknowledgements

Support for this work was provided by grants from the National University of Mar del Plata (15/G247) and the ANPCyT (PICT N 00556). The authors are staff members of CONICET, CIC and UNMdP.

Nomenclature

Ks : the substrate affinity constant or Monod Constant (mg/L), kinetic parameter
S : Substrate concentration (mg/L)
t : time (h)
V : total volume of batch reactor (L)
X : biomass concentration (CFU/mL)
$Y_{X/S}$: yield coefficient biomass-substrate (CFU/mL/mg/L)
μ : specific growth rate (h)
μmax : maximum specific growth rate (h), kinetic parameter

Subscripts

i : referred to component i
j : referred to component j
T : total, referred to all the components

References

1. Chung, T. -P., H. -Y. Tseng, and R. -S. Juang (2003) Mass transfer effect and intermediate detection for phenol degradation in immobilized *Pseudomonas putida* systems. *Proc. Biochem. (Amsterdam, Neth.)*. 38: 1497-1507.
2. Office of Environmental Health Hazard, E. P. A. (2005) *Proposition 65*. State of California, USA.
3. Yang, C. -F. and C. -M. Lee (2008) Pentachlorophenol contaminated groundwater bioremediation using immobilized Sphingomonas cells inoculation in the bioreactor system. *J. Hazard. Mater.* 152: 159-165.
4. Atlow, S. C., L. Bonnadonna-Aparo, and A. M. Klivanov (1984) Dephenolization of industrial wastewaters catalyzed by polyphenol oxidase. *Biotechnol. Bioeng.* 26: 599-603.
5. Rutgers, M., A. M. Breure, J. G. V. Andel, and W. A. Duetz (1997) Growth yield coefficients of *Sphingomonas* sp. strain P5 on various chlorophenols in chemostat culture. *Appl. Microbiol. Biotechnol.* 48: 656-661.
6. Murialdo, S. E., P. M. Haure, M. R. Fenoglio, and J. F. Gonzalez (2003) Degradation of phenol and chlorophenols by mixed and pure cultures. *Water SA*. 29: 457-463.

7. Pallerla, S. and R. P. Chambers (1998) Reactor development for biodegradation of pentachlorophenol. *Catal. Today* 40: 103-111.
8. Guha, S., C. A. Peters, and P. R. Jaffe (1999) Multisubstrate biodegradation kinetics of Naphthalene, Phenanthrene, and Pyrene mixtures. *Biotechnol. Bioeng.* 65: 491-499.
9. Tobajas, M., V. M. Monsalvo, A. F. Mohedano, and J. J. Rodriguez (2010) Enhancement of cometabolic biodegradation of 4-chlorophenol induced with phenol and glucose as carbon sources by Comamonas testosteroni. *J. Environ. Manage*. In press: 1-6.
10. De Los Cobos-Vasconcelos, D., F. Santoyo-Tepole, C. Juárez-Ramírez, N. Ruiz-Ordaz, and C. J. J. Galíndez-Mayer (2006) Cometabolic degradation of chlorophenols by a strain of Burkholderia in fed-batch culture. *Enz. Microb. Technol.* 40: 57-60.
11. McAllister, K. A. (1996) Microbial degradation of pentachlorophenol. *Biodegradation*. 7: 1-40.
12. Alexander, M. A. (1999) *Biodegradation and bioremediation*. 2nd ed., Academic Press, San Diego.
13. Blanch, H. W. and D. S. Clark (1996) *Biochemical Engineering*. Marcel Dekker Inc., NY.
14. Steiert, J. G., J. J. Pignatello, and R. L. Crawford (1987) Degradation of chlorinated phenols by a pentachlorophenol-degrading bacterium. *Appl. Environ. Microbiol*. 53: 907-910.
15. Segel, I. H. (1975) *Enzyme kinetics*. John Wiley & Sons, Inc., NY.
16. Stringfellow, W. T. and M. D. Aitken (1995) Competitive metabolism of naphthalene, methylnaphthalenes, and fluorene by phenanthrene-degrading P*seudomonads*. *Appl. Environ. Microbiol.* 61: 357-362.
17. Gomila, M., L. Tvrzová, A. Teshim, I. Sedláček, N. González-Escalona, Z. Zdráhal, O. Šedo, J. F. González, A. Bennasar, E. R. B. Moore, J. Lalucat, and S. E. Murialdo (2010) Achromobacter marplatensis sp. nov., isolated from a pentachlorophenol contaminated soil. *Int. J. Syst. Evol. Microbiol.* 60: 249-266.
18. Ayude, M. A., E. Okada, J. F. González, P. M. Haure, and S. E. Murialdo (2009) Bacillus subtilis as a bioindicator to estimate pentachlorophenol toxicity and concentration. *J. Ind. Microbiol. Biotechnol.* 36: 765-768.
19. Murialdo, S. E. (2004) *Isolation and characterization of chlorophenol degrading microorganisms*. National University of Mar del Plata., Mar del Plata, Argentina.
20. Wolski, E. A., S. E. Murialdo, and J. F. Gonzalez (2006) Effect of pH and inoculum size on pentachlorophenol degradation by *Pseudomonas* sp. *Water SA.* 32: 93-98.
21. Stanlake, G. J. and R. K. Finn (1982) Isolation and characterization of a pentachlorophenol degrading bacterium. *Appl. Environ. Microbiol.* 44: 1421-1427.
22. Klecka, G. M. and W. J. Maier (1988) Kinetics of microbial growth on mixtures of pentachlorophenol and chlorinated aromatic compounds. *Biotechnol. Bioeng.* 31: 328-335.
23. Oh, Y. -S., Z. Shareefdeen, B. C. Baltzis, and R. Bartha (1994) Interactions between benzene, toluene, and p-xylene (btx) during their biodegradation. *Biotechnol. Bioeng.* 44: 533-538.
24. González, J. F. and W. -S. Hu (1985) Pentacholorophenol biodegradation: Simple models. *Environ. Technol.* 16: 287-293.
25. Chu, J. and E. J. Kirsch (1973) Utilization of halophenols by a pentachlorophenol metabolizing bacterium. *Dev. Ind. Micro.* 14: 264-273.
26. Liu, D., K. Thomson, and K. L. E. Kaiser (1982) Quantitative structuretoxicity relationship of halogenated phenols on bacteria. *Bull. Environ. Contam. Toxicol.* 29: 130-136.
27. Yang, C. -F., C. -M. Lee, and C. -C. Wang (2005) Degradation of chlorophenols using Pentachlorophenol-degrading bacteria sphingomonas chlorophenolica in a batch reactor. *Curr. Microbiol.* 51: 156-160.
28. Pieper, D. H. and W. Reineke (2004) Degradation of chloroaromatics by pseudomona(d)s. In: J. -L. Ramos (ed.). *Pseudomona(d)s*. Kluwer Academic/Plenum Publishers, NY.
29. Knackmuss, H. J. and W. Reineke (1988) Microbial degradation of haloartomatics. *Annu. Rev. Microbiol*. 42: 263-287.
30. van Agteren, M. H., S. Keuning, and D. B. Janssen (1998) Handbook on biodegradation and biological treatment of hazardous organic compounds. pp. 383-407. Kluwer Academic Publishers, The Netherlands.
31. Liu, D., R. J. Maguire, G. Pacepavicius, and B. J. Dutka (2006) Biodegradation of recalcitrant chlorophenols by cometabolism. In: N. W. R. Institute (ed.). *Environmental Toxicology and Water Quality*. Rivers Research Branch, Ontario, Canada.
32. Field, J. and R. Sierra-Alvarez (2008) Microbial degradation of chlorinated phenols. *Rev. Environ. Sci. Biotechnol*. 7: 211-241.