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ORIGINAL PAPER

Modeling chlorophenols degradation in sequencing batch reactors with instantaneous feed-effect of 2,4-DCP presence on 4-CP degradation kinetics

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Abstract Two instantaneously fed sequencing batch reactors (SBRs), one receiving 4-chlorophenol (4-CP) (SBR4) only and one receiving mixture of 4-CP and 2,4-dichlorophenol (2,4-DCP) (SBRM), were operated with increasing chlorophenols concentrations in the feed. Complete degradation of chlorophenols and high-Chemical oxygen demand (COD) removal efficiencies were observed throughout the reactors operation. Only a fraction of biomass (competent biomass) was thought to be responsible for the degradation of chlorophenols due to required unique metabolic pathways. Haldane model developed based on competent biomass concentration fitted reasonably well to the experimental data at different feed chlorophenols concentrations. The presence of 2,4-DCP competitively inhibited 4-CP degradation and its degradation began only after complete removal of 2,4-DCP. Based on the experimental results, the 4-CP degrader's fraction in SBRM was estimated to be higher than that in SBR4 since 2,4-DCP degraders were also capable of degrading 4-CP due to similarity in the degradation pathways of both compounds.

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Keywords 4-chlorophenol · 2,4-dichlorophenol · Biodegradation kinetics · Competent biomass · Modeling · Sequencing batch reactor

Abbreviations

2,4-DCP	2,4-dichlorophenol
4-CP	4-chlorophenol
a	COD fraction of chlorophenols in the feed of SBRs
CHMS	5-chloro-2-hydroxymuconic semialdehyde
COD	chemical oxygen demand
HRT	hydraulic retention time
K_i	self-inhibition constant
K_{ii}	competitive inhibition constant
K_s	half saturation constant
MLVSS	biomass concentration as mixed liquor volatile suspended solids
$MLVSS_s$	MLVSS of competent biomass
q_m	maximum specific degradation rate
SBR	sequencing batch reactor
SBR4	sequencing batch reactor receiving 4-CP
SBRM	sequencing batch reactor receiving mixture of 4-chlorophenol and 2,4-dichlorophenol
SDR	specific degradation rate on the basis of total biomass concentration
SDR_c	specific degradation rates on the basis of competent biomass concentration
SOCs	synthetic organic chemicals

SRT	sludge retention time
X	biomass concentration
Y	yield coefficient

Introduction

Widespread use of chemicals such as chlorophenols has a potentially negative impact on public health and the ecosystem (Mangat and Elefsiniotis 1999). Chlorophenols are introduced into the environment through various human activities such as waste incineration, uncontrolled use of wood preservatives, pesticides, fungicides, and herbicides, as well as via bleaching of pulp with chlorine and the chlorination of drinking water (Contrerasa et al. 2003). Some typical values of phenolic compounds reported for chemical industry wastewaters have been 400 mg l^{-1} for phenolic resin production, 50 mg l^{-1} for refineries, 12 mg l^{-1} for naphthalenic acid production, and 200 mg l^{-1} for shale dry distillation (Chen et al. 1997). Despite the recalcitrance of chlorophenols, efforts are still being made to treat them biologically for economic reasons and in expectation of few byproducts.

Sequencing batch reactors (SBRs) offer an attractive alternative to conventional biological wastewater treatment systems, mainly because of their simple and flexible operation and cost effectiveness for small-scale treatment facilities (Mangat and Elefsiniotis 1999; Chiavola et al. 2004). The key difference is that the reactor volume of SBRs varies with time but remains constant with traditional continuous flow systems (Mohan et al. 2005). Enforcement of controlled, short-term, non-steady conditions in SBRs may favor induction of enzymes to degrade biorefractory compounds (Tomei et al. 2004).

Mohan et al. (2005) investigated the performance of SBR in treating complex chemical wastewaters. The SBR showed relatively more efficient performance over conventional suspended growth systems. The authors stated that the enhanced performance with SBR over conventional suspended growth system may be due to enforced short-term unsteady state conditions coupled with periodic exposure of the microorganisms to defined process conditions which facilitate the required metabolic conditions for treating complex chemical effluents. Tomei et al. (2003) showed that removal kinetics of 4-nitrophenol in a SBR is well described by the typical substrate inhibition pattern as predicted by the Haldane equation. Also, they observed high-removal rates, short-acclimation times, and good settling characteristics in SBR. Similarly, Zhuang et al. (2005) demonstrated the utility of SBR to adapt micro-organisms toward biological removal of tert-butyl alcohol. The increases in tert-butyl alcohol loading produced better settling biomass and higher biomass concentrations with higher specific tert-butyl alcohol biodegradation rates. Specific tert-butyl alcohol biodegradation rates in the granules followed the Haldane substrate inhibition model. In another study, Mangat and Elefsiniotis (1999) conducted a bench-scale study with SBRs to investigate the effects of hydraulic retention time (HRT), the presence or absence of supplemental substrate and variation in feed concentration on the biodegradation potential of 2,4-dichlorophenoxyacetic acid. A long-acclimation period (about 4 months) was observed before 2,4-dichlorophenoxyacetic acid biodegradation was established. The 2,4-dichlorophenoxyacetic acid specific removal rates were affected by the type of supplemental substrate used (phenol or dextrose), being significantly lower in the case of dextrose.

Knowledge of microbial growth and substrate utilization kinetics is important to predict the fate of organic compounds in natural and engineered environments (Grady et al. 1996). Mixed microbial communities are used by environmental engineers (Templon and Grady 1988); therefore, the kinetics of microbial growth and substrate utilization should be studied using mixed culture in order to produce data that can be used in the design of real wastewater treatment plant. In literature, total biomass has generally been considered for growth and substrate degradation kinetics. However, models based on total biomass concentrations will fail to predict the performance of the bioreactor if it receives synthetic organic chemicals (SOCs) at varying concentrations (Magbanua et al. 1998) as it is known that

only a fraction of biomass in the community is responsible for the degradation of SOC of interest due to required unique metabolic pathway. Some researchers used the fraction of chemical oxygen demand (COD) contributed to the feed by that compound in the calculation of competent biomass fraction based on the assumption that specialists grows only on SOC of interest (Ellis et al. 1996; Magbanua et al. 1998; Hu et al. 2005a, b). Also, wastewater treatment systems receive multicomponent feed and the degradation of any individual organic constituent is synergistically or antagonistically impacted by the concurrent utilization of other substrates (Lu and Spetiel 1988; Lu and Tsai 1993).

Therefore, the goals of this work were to investigate chlorophenols degradation kinetics at varying feed 4-chlorophenol (4-CP) concentrations in the presence and absence of 2,4-dichlorophenol (2,4-DCP) through long-term operated instantaneously fed SBRs. By this way, the use of competent biomass in the degradation kinetics of chlorophenols at varying feed concentrations was deeply investigated.

Materials and methods

SBR experiments

A 2.5 l glass vessel with 2 l of working volume was used as SBR. Primary settling tank effluent of Ankara Domestic Wastewater Treatment Plant was used as initial inocula. The reactors were fed with synthetic medium once a day and at the end of each cycle excess sludge was wasted from mixed liquor to adjust the sludge retention time (SRT) as 10 days. After settling, 1.75 l of the treated effluent was drawn and around 0.25 l sludge was maintained for the next cycle, which gives 1.14 days of HRT. One of the reactors received 4-CP only (SBR4), whereas the other reactor received mixture of 4-CP and 2,4-DCP (SBRM). Chlorophenols were added to the feed at low concentrations (10 mg l^{-1} 4-CP and 5 mg l^{-1} 2,4-DCP) and the concentrations were gradually increased up to 200 mg l^{-1} 4-CP and 100 mg l^{-1} 2,4-DCP, which lasted around 5 months (Fig. 1). The maximum concentrations of chlorophenols were selected to represent a high-strength industrial wastewater. Concentrations of 2,4-DCP in the feed of SBRM was kept always one half of 4-CP concentration. Synthetic wastewater pH was 7.2 ± 0.2 and it was 6.5–6.8 at the end of one-day cycle. The reactors were operated in a water bath at 26 ± 1°C and aerated with air pumps to maintain a dissolved oxygen concentration of at least 3 mg l^{-1}. When the predetermined concentration of chlorophenols were reached, reactors were allowed at least two SRTs to reach steady state condition, which was also checked measuring the COD and mixed liquor suspended solids (MLVSS) concentrations at the end of three successive cycles. Samples were drawn from the reactors for the determination of chlorophenols, COD, 5-chloro-2-hydroxymuconic semialdehyde (CHMS) and MLVSS at different time intervals.

The synthetic wastewater used in the experiments contained various 4-CP and 2,4-DCP concentrations and 400 mg l^{-1} peptone. Peptone was

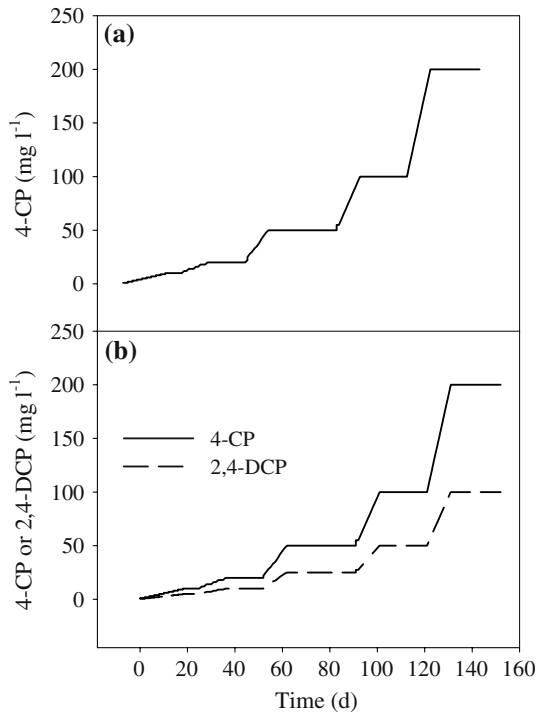

Fig. 1 Experimental design of instantaneously fed SBR experiments showing changes in feed chlorophenol concentrations of SBR4 (a) and SBRM (b)

added to feed of the reactors to represent readily degradable potion of chlorophenol containing industrial wastewaters. The composition of mineral medium was similar to that in our previous study (Sahinkaya and Dilek 2006). The N/COD ratio varied between 12 and 5.7% depending on the feed chlorophenols concentrations. The nitrogen concentration in the feed was always higher than the requirement (N/COD ratio should be at least 5%) not to create suboptimal conditions for biomass growth.

The degree of removal via adsorption was followed extracting 4-CP and 2,4-DCP on biomass with 0.1 M NaOH and adsorption of chlorophenols on biomass were not detected throughout the reactors operation.

Batch experiments

Batch experiments were conducted in 500 ml erlenmeyer flasks stoppered with cotton plugs. The working liquid volume was 250 ml. All experiments were carried out in an orbital shaking incubator set at 200 rpm and 26 ± 1°C. Biomass samples taken from SBRM and SBR4 were washed two times before the experiments. Then, cultures were resuspended in distilled water and parallel batch reactors were seeded to have biomass concentration around 200 mg MLVSS l^{-1}. Reactors were supplemented with 4-CP at varying concentrations as sole carbon and energy sources in addition to inorganic components of the feed medium. As a nitrogen source, 183 mg l^{-1} NH_4CI was added and the N/COD ratio was higher than 10% in all batch assays.

Reactors not receiving biomass were also operated at the same conditions to monitor the removal of 4-CP via volatilization and no evaporation loss was detected.

During the experiments, samples were drawn from each flask at pre-determined time intervals to monitor pH and the concentrations of 4-CP. MLVSS concentrations were also determined at the start and end of the reactors operation.

Isolation of pure cultures

In the isolation of bacteria, the procedure given by Wang et al. (2000) was used. Mixed culture samples, obtained from SBRs, were diluted to have maximum 30 colonies on solidified agar medium. The growth medium used in SBRs was solidified and used during the isolation of pure strains. Solidified agar medium supplemented with mixture of 50 mg l^{-1} 4-CP and 25 mg l^{-1} 2,4-DCP. Streaked agar plates were incubated at 30°C. After development of colonies, single colonies were selected and streaked to new agar plates. This procedure was repeated at least five times in order to ensure the purity of culture. API 20 NE identification kits were used to define the isolated cultures.

Analytical techniques

5-Chloro–2-hydroxymuconic semialdehyde concentration, the *-meta* cleavage product of 4-chlorocatechol, was monitored by measuring absorbance at 380 nm (Farrell and Quilty 1999). MLVSS and chloride concentrations (titrimetric method) were determined according to Standard Methods (APHA 1995). COD measurements were carried out using Hach COD vials (HACH Water Analysis Handbook 1992).

A high-performance liquid chromatography (HPLC) equipped with a Nucleosil C18 column (4.6×250 mm^2), and a SPD-10Avp UV-VIS detector set at 280 nm was used for chlorophenols measurements (detection limit was around 0.05 mg l^{-1}).

Results

Performance of SBR4 and SBRM at varying feed chlorophenols concentrations

Metabolization of 4-CP results in production of 4-chlorocatechol and -meta cleavage of 4-chlorocatechol causes the formation of CHMS, metabolization of which leads to the production of pyruvic acid and chloroacetic acid. Chloroacetic acid may then be dehalogenated to form glycolate, which may be utilized along with pyruvic acid in TCA cycle (Farrel and Quilty 1999). During the operation of the reactors with an inlet concentration of 10 mg l^{-1} 4-CP, despite complete disappearance of chlorophenols, a yellowish

color was observed in the effluent of reactors on Day 21. The behavior was attributed to the generation of an intermediate that, based on absorbance measurement, was assumed to be CHMS (Farrel and Quilty 1999). The observation of high quantities of metabolite at the beginning of the acclimation was also reported by Moreno-Andrade and Buitron (2004). A gradual decrease of the intermediate concentration was observed, which was attributed to the progressive acclimation of the microbial population (Fig. 2); complete removal of chlorophenols and CHMS was observed throughout the reactors operation.

The change in observed yield coefficient (Y) and COD removal efficiencies at different feed 4-CP concentrations are shown in Fig. 3. The average COD removal efficiency in the absence of chlorophenol was 94% and no adverse effect of chlorophenols addition on COD removal efficiency was detected. The observation of low-COD concentrations in the effluent of the reactors is an indication of complete chlorophenol removal. Additionally, chloride ion measurements indicated an average 101 ± 7% chlorophenols removal at the end of the cycle. The Y value in the absence of chlorophenol was observed to be 0.425 ± 0.008 mg MLVSS (mg COD)$^{-1}$, and it decreased with increasing initial chlorophenols concentrations in SBRM. The trend was different in the case of SBR4 as the addition of 4-CP caused a sharp decrease from 0.43 in the absence of 4-CP to an average value of 0.235 ± 0.007 mg MLVSS (mg COD)$^{-1}$ in the presence of 4-CP.

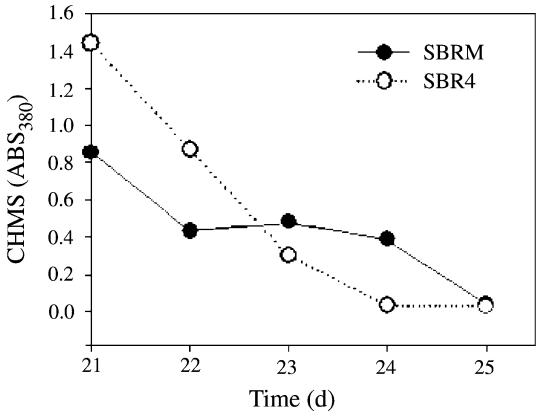

Fig. 2 5-Chloro-2-hydroxymuconic semialdehyde (*CHMS*) concentrations in the effluent of reactors fed with 10 mg l^{-1} 4-CP (SBR4) and mixture of 10 mg l^{-1} 4-CP and 5 mg l^{-1} 2,4-DCP (SBRM). CHMS concentration was measured as absorbance at 380 nm (ABS_{380})

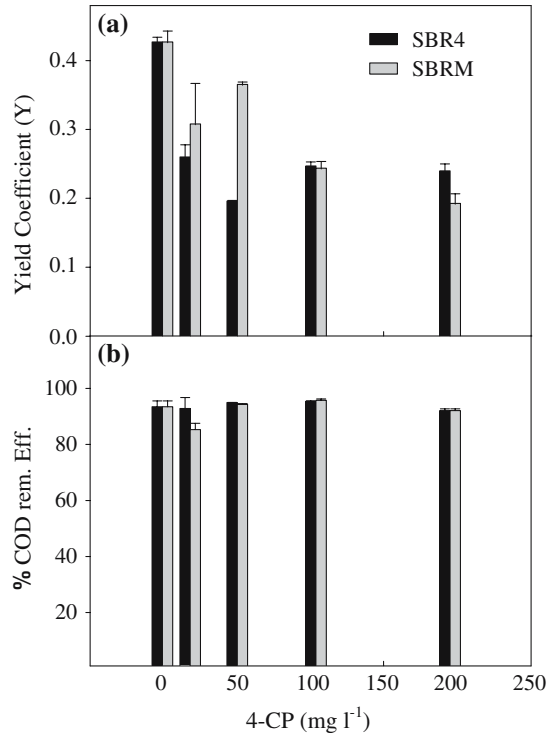

Fig. 3 Change of Y values and COD removal efficiencies with respect to feed 4-CP concentrations in SBRs

Kinetics of chlorophenols degradation

The feed concentration of chlorophenols was increased stepwise to acclimatize biomass. When the highest chlorophenols concentrations were reached in the SBR4 (200 mg l^{-1} 4-CP) and SBRM (200 mg l^{-1} 4-CP and 100 mg l^{-1} 2,4-DCP), biomass samples were drawn in order to conduct batch assays. Batch experiment results revealed that Haldane substrate inhibition model could be used to predict the specific 4-CP degradation rates (Fig. 4). It was assumed that only specialist biomass are responsible for chlorophenols degradation and the fraction of specialist biomass was estimated as the contribution of chlorophenols to feed on COD basis. Therefore, competent biomass concentration in the community will increase as the feed chlorophenol

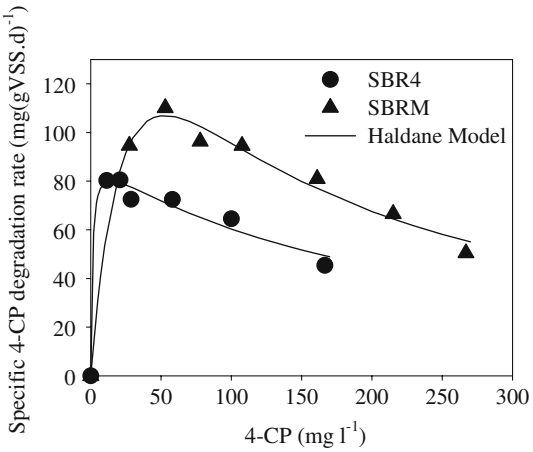

Fig. 4 Dependence of specific 4-CP degradation rate on initial 4-CP concentration in batch experiments seeded with culture obtained from SBR4 or SBRM (data points represent experimental data, while, smooth curves are optimized fit of Haldane equation)

concentrations are increased (Ellis et al. 1996; Magbanua et al. 1998; Hu et al. 2005a, b). Based on these assumptions, the following equation was used to describe 4-CP degradation kinetics in the absence of 2,4-DCP;

$$\frac{\mathrm{d}(S)}{\mathrm{d}(t)} = -\frac{q_{\mathrm{m}} S X a}{K_{\mathrm{s}} + S + (S^2/K_i)}, \tag{1}$$

where, q_{m} is the maximum specific 4-CP degradation rate (mg 4-CP (g $\mathrm{MLVSS_s}$ h)$^{-1}$), K_{s} and K_i are half saturation and self-inhibition constants, respectively, X is total biomass concentration (g MLVSS l^{-1}), and lastly a is the COD fraction of chlorophenols in the feed of SBRs. In the calculation of specific 4-CP degradation rates, competent biomass concentration ($\mathrm{MLVSS_s}$) was considered. Hence, the observed equation for 4-CP degradation in SBR4 (Fig. 4) was;

$$\frac{\mathrm{d}(S)}{\mathrm{d}(t)} = -\frac{92 S X a}{1.104 + S + (S^2/194.4)}. \tag{2}$$

The results of SBRM experiments, which will be discussed later, showed that micro-organisms biodegrade firstly the more biodegradable compound (2,4-DCP) and when this becomes limiting then they use 4-CP as substrate. Similar result was also reported by Quan et al. (2005). Therefore, the proposed model for 4-CP degradation in SBRM is;

$$\frac{\mathrm{d}(S)}{\mathrm{d}(t)} = -\frac{q_{\mathrm{m}} S X a}{\left(1 + \frac{I}{K_{ii}}\right) K_{\mathrm{s}} + S + (S^2/K_i)}, \tag{3}$$

where, I is the 2,4-DCP concentration, K_{ii} is 2,4-DCP inhibition coefficient on 4-CP degradation, and a is the 4-CP and 2,4-DCP fraction in the feed on COD basis. It should be noted that in the determination competent biomass fraction having ability to degrade 4-CP, total fraction of 4-CP and 2,4-DCP in the feed was taken into consideration as culture having ability to degrade one type of chlorophenol may also have ability to degrade another structurally similar chlorophenol. Although such a generalization cannot be done, in our case the validity of the assumption has been proved in our previous studies (Sahinkaya and Dilek 2002, 2005). Also, the observed competitive inhibition claims that the same enzymes are responsible for the degradation of both chlorophenols, therefore, it is reasonable to assume that the same community is responsible for the degradation of 4-CP and 2,4-DCP. The q_{m}, K_{s}, and K_i values in Eq. 3 were determined in batch experiments in the absence of 2,4-DCP (Fig. 4) and K_{ii} value was obtained by fitting of experimental results of SBRM when it received 200 mg l^{-1} 4-CP and 100 mg l^{-1} 2,4-DCP. The best fitting value of K_{ii}, which is competitive inhibition coefficient of 2,4-DCP on 4-CP degradation, was observed to be 0.17 mg l^{-1}. The relatively small value of K_{ii} indicates a high-toxic effect of 2,4-DCP on 4-CP degradation. It was observed that simply a zero order degradation model was good enough to predict 2,4-DCP concentrations in SBRM and the resulting equation is;

$$\frac{\mathrm{d}(S)}{\mathrm{d}(t)} = -\frac{248.49 S X a}{(1 + (I_0 - kt)/0.17)34.98 + S + (S^2/79.74)}. \tag{4}$$

The observed equations were solved with the help of POLYMATH 4.02 assuming that the biomass concentration was constant during the

reaction time as the initial biomass concentration is very high and the average difference between initial and the final value was around 10%.

In order to evaluate the efficacy of using competent biomass in the degradation models, 4-CP concentration profile in SBR4 when it received 50 mg l^{-1} was estimated assuming specialists (Model 1) or generalists (all biomass) (Model 2) was responsible for the degradation (Fig. 5). Similar to observation of Ellis et al. (1996), specialist biomass gave much better fit to the experimental data. Ellis and Eliosov (2004) reported that the feed COD fraction gave better approximation of competent biomass fraction when the test compound's fraction in feed was high ((1%). In our study, the fractions of chlorophenols in SBR4 and SBRM varied as 10–45% and 12–53% in respective order, therefore, the accurate estimation of competent biomass fraction using the feed COD contribution is more likely due to high fraction of chlorophenols in the feed.

The experimental results of SBR4 obtained when it received 100 and 200 mg l^{-1} of 4-CP were depicted in Fig. 6. Complete 4-CP and high-COD removals were observed and the developed model fitted reasonably well to the experimental data for both conditions (Fig. 6a). During the removal of 4-CP, CHMS concentration in the medium increased simultaneously and the highest concentration was reached when 4-CP was completely removed (Fig. 6a, c). After complete removal of 4-CP from the medium the concentration of CHMS decreased sharply and it was completely removed.

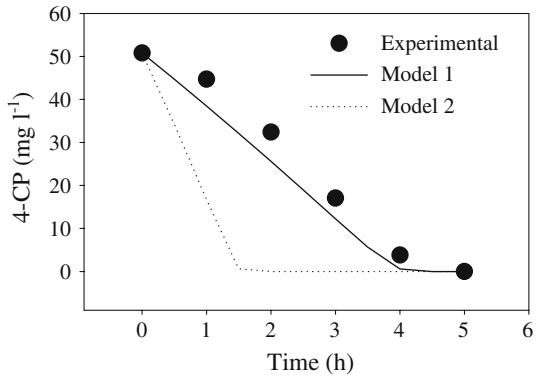

Fig. 5 The prediction of 4-CP concentration profile in SBR4 considering competent (*Model 1*) or total biomass concentrations (*Model 2*)

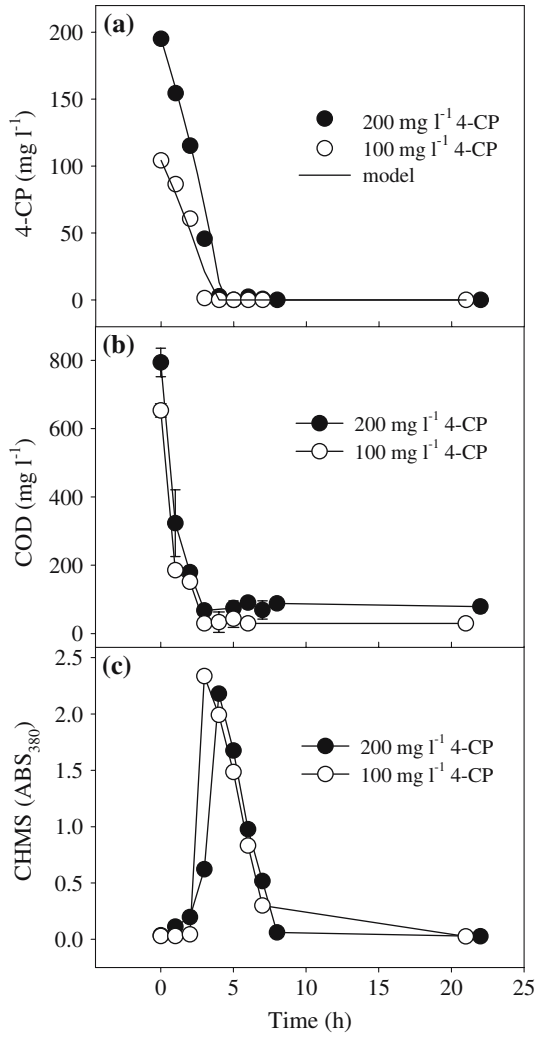

Fig. 6 4-CP concentrations and Haldane model fit (**a**), COD (**b**), and CHMS (**c**) concentrations in one cycle of SBR4 receiving 100 or 200 mg l^{-1} 4-CP

In the case of 4-CP and 2,4-DCP mixture, a competitive type inhibition of 2,4-DCP on 4-CP degradation can be clearly seen from the analyses of Fig. 7. Almost complete removal of 2,4-DCP was achieved within 4 and 6 h (Fig. 7a) and 4-CP degradation was almost negligible up to this point when are all together in the same batch. The competitive inhibitory effect of 2,4-DCP on 4-CP degradation can also be clearly seen from Fig. 7c, which depicts normalized concentrations for SBRM receiving 200 mg l^{-1} 4-CP and 100 mg l^{-1} 2,4-DCP. Zero order degradation model was

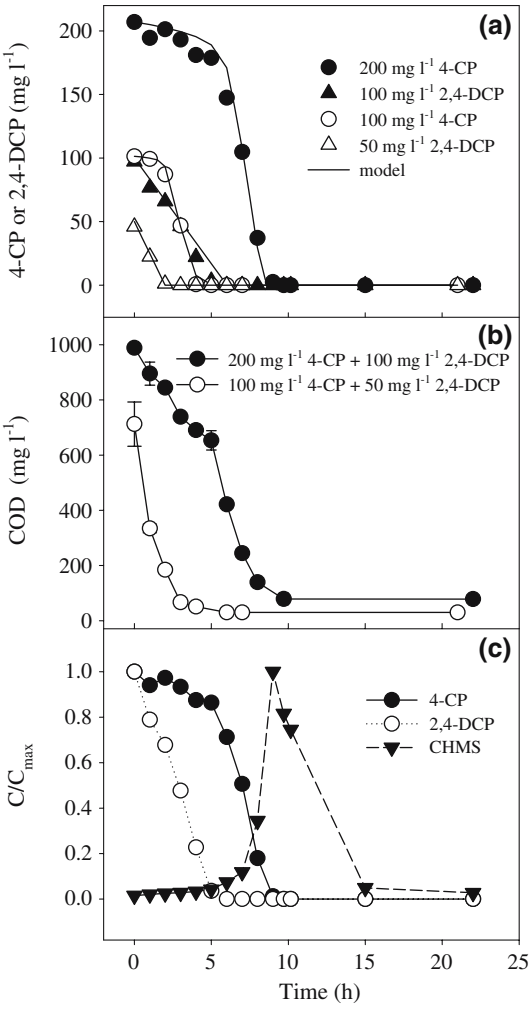

Fig. 7 Chlorophenol concentrations and model fit (**a**), COD concentrations (**b**), and the normalized concentrations of 4-CP, 2,4-DCP, and CHMS (**c**) in one cycle of SBRM receiving 100 mg l^{-1} 4-CP and 50 mg l^{-1} 2,4-DCP or 200 mg l^{-1} 4-CP and 100 mg l^{-1} 2,4-DCP

good enough to predict time course variation of 2,4-DCP (Fig. 7a) and degradation rates (k) were 12.5 and 16.6 1 h^{-1} for initial concentrations of 50 and 100 mg l^{-1}, respectively. After complete degradation of 2,4-DCP, there remained no inhibitory effect and 4-CP degradation started immediately and was completed within the next 5 h (Fig. 7a). The developed model also fitted reasonably well to the 4-CP measurements.

The average specific degradation rates on the basis of total biomass concentrations (SDR) are depicted in Fig. 8. The 4-CP SDR values in SBRM

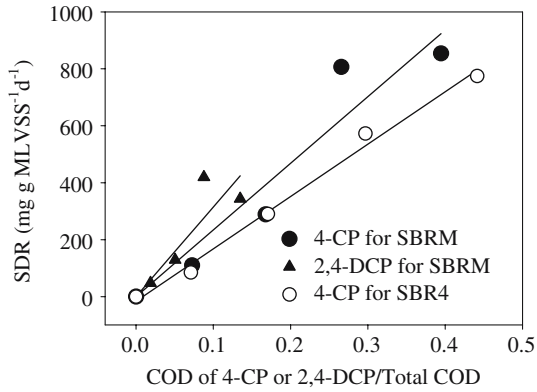

Fig. 8 Variation of specific degradation rates (*SDRs*) in SBRs with the fraction of chlorophenols in the feed on COD basis

were calculated after complete removal of 2,4-DCP as negligible removal occurred in the presence of 2,4-DCP due to competitive inhibition.

Discussion

Although, the degradation of CHMS was achieved after acclimation of cultures, it has been widely reported as being a dead-end metabolite, especially for pure cultures (Sae'z and Rittmann 1993; Hill et al. 1996). Therefore, mixed cultures are particularly important when the emphasis is placed on complete mineralization of toxic organics to CO_2. In both SBRs, CHMS concentration increased simultaneously with the removal of 4-CP and the highest CHMS concentration was reached when 4-CP was just completely removed (Figs. 5a–c, 6c). The calculated peak CHMS concentrations using the extinction coefficient given by Farrel and Quilty (1999) was only within 3–10% of its calculated stoichiometric amounts (the case if all 4-CP had been converted to CHMS) in both reactors throughout the operation. This conclusion can also be drawn from the COD measurements as COD concentrations when CHMS concentrations were at their maximum were close to those observed at the end of the cycles due to the low concentrations of CHMS. Hence, it can be concluded that simultaneous 4-CP and CHMS removal occurred in the reactors although CHMS removal rate was

slightly lower, which caused a slight accumulation in the medium.

In 4-CP degradation, the first step is the attack of phenol hydroxylase to 4-CP, which results in 4-Chlorocatechol production. In 2,4-DCP case, the first step is the production of 3,5-Dichlorocatechol with the use of 2,4-DCP hydroxylase (Radjendirane et al. 1991). It was reported that phenol hydroxylase has a broad substrate specificity and it can catalyze the turnover of 2,4-DCP (Hollender et al. 1997). It is also known that 2,4-DCP hydroxylase has ability to convert 4-CP to 4-Chlorocatechol (Beadle and Smith 1982). Therefore, the use of the same enzymes for the initiation of 4-CP and 2,4-DCP degradation led to observation of competitive inhibition.

The relation between the observed kinetic parameters and the reactor's feed concentrations should be known to predict the fate of the SOC of interest at different operational conditions. Many researchers claim that only a specific population of microbial community is responsible for the degradation of particular compounds and the fraction of specialists in the community may be approximated as the fraction of COD contributed to the feed by that compound (Ellis et al. 1996; Magbanua et al. 1998; Hu et al. 2005a, b). Magbanua et al. (1998) reported that generally the influent COD fraction underestimated the competent biomass fraction by a factor of about two, which may be due to the convergence of degradation pathways for different SOCs. Contrary to this finding, Hu et al. (2005b) reported that the competent fraction for 4-CP (0.01–0.03%) in SBR receiving multicomponent feed was two order magnitudes lower than the contribution of 4-CP to influent COD (2.0%). Although it seems that the use of substrate specific MPN method is better approach compared to use of COD fraction, this method may also produce results with high coefficient of variations (69–130%) due to long-term variations in the microbial community of bioreactor (Magbanua et al. 1998). Therefore, the use of COD fraction to roughly approximate competent biomass fraction is more realistic than assuming all biomass is responsible for SOC degradation (Magbanua et al. 1998).

The SDRs of chlorophenols increased linearly with increasing chlorophenols fraction in the feed (Fig. 8). The reason of this observation is increase in the fraction of chlorophenol degrading specialized biomass in the community as specialized biomass grows only on chlorophenols. A better linear relationship was observed for 4-CP in the absence of 2,4-DCP (SBR4). The possible reason of this observation is complex substrate interaction in multiple substrate conditions (SBRM). It was also observed that the 4-CP SDRs in SBRM were higher compared to those in SBR4. The presence of 2,4-DCP may increase the amount of enzymes utilized in 4-CP breakdown as the same enzymes are responsible for the initiation of 4-CP and 2,4-DCP degradation. Although SDRs of 4-CP showed great variation depending on feed 4-CP concentrations and 2,4-DCP presence, those calculated on the basis of competent biomass concentrations (SDR_c) were relatively constant in both reactors (1,643.62 ± 318.28 and 1,604.5 ± 483.4 mg $MLVSS_c$ l^{-1} day^{-1} for SBR4 and SBRM, respectively) and did not show any correlation with feed 4-CP concentrations. Similar to SDR_c, the isolated dominant species in both reactors were observed to be alike as *Pseudomonas stutzeri* and *Pseudomonas vesicularis* in SBR4, and *P. stutzeri* and *Pseudomonas sp.* in SBRM were the dominant species.

Although the specific degradation rate of chlorophenols calculated based on competent biomass concentration (SDR_c) is constant, the specific degradation rate calculated on the basis of total biomass concentration (SDR) shows variation depending on the chlorophenol concentrations in the feed due to change in the fraction of specialist in the community. Therefore, the dependence of SDR on SDR_c can be shown as;

$$SDR = aSDR_c. \quad (5)$$

Using the average SDR_c values given above (1,643.62 ± 318.28 and 1,604.5 ± 483.4 mg $MLVSS_c$ l^{-1} day^{-1} for SBR4 and SBRM, respectively), SDR values were calculated using Eq. 5 and the results were compared with the measured values in Fig. 9. The calculated values are in good

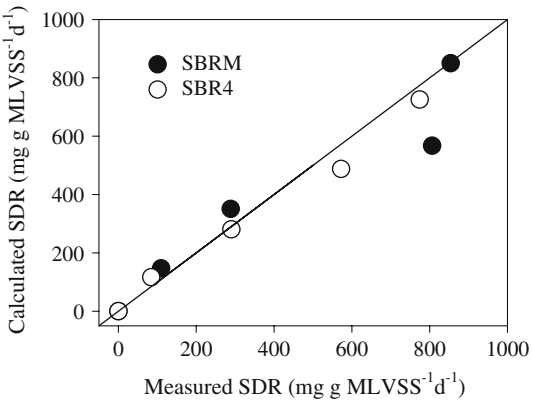

Fig. 9 Measured and calculated specific 4-CP degradation rates (*SDRs*) for SBRs

agreement with the experimental data. Therefore, it can be concluded that the competent biomass fraction can be approximated as contribution of chlorophenols to the feed on COD basis.

As the results showed that SDRs of chlorophenols depends on feed concentrations, the kinetic equation observed at a specific feed condition cannot directly be used to predict the reactor performance at variable feed concentrations (Fig. 5). Therefore, in those cases, competent biomass concentration should be considered as SDR value based on competent biomass concentration (SDR_c) was relatively constant and did not depend on feed concentration.

Conclusions

Degradation kinetics of 4-CP in the presence (SBRM) and absence of 2,4-DCP (SBR4) were investigated in long-term operated SBRs with varying feed chlorophenols concentrations. The following conclusions can be drawn from this work:

(1) The complete degradation of chlorophenols was observed for all the studied conditions up to 200 mg l^{-1} 4-CP and 100 mg l^{-1} 2,4 DCP concentration in the feed.
(2) Although CHMS accumulation was observed during the degradation of 4-CP in both reactors, the peak concentrations of CHMS were only within 3–10% of its stoichiometrically calculated value, which indicated simultaneous removal of 4-CP and CHMS.
(3) The presence of 2,4 DCP affected 4-CP degradation which started only after disappearance of 2,4 DCP.
(4) In the kinetic studies, it was assumed that only specialist biomass are responsible for chlorophenols degradation and the fraction of specialist biomass was estimated equal to the feed contribution of chlorophenols on COD basis. The developed models based on this assumption well fitted the experimental data for both SBRM and SBR4.
(5) The SDRs of chlorophenols increased with increasing feed chlorophenols concentrations due to increase in competent biomass fraction. SDRs calculated on the basis of competent biomass concentrations (SDR_c) were relatively constant in both reactors and did not show any correlation with feed 4-CP concentrations.
(6) Similar microbial species were observed to be dominant in the reactors.

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