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Journal of Hazardous Materials B137 (2006) 282–287

Journal of Hazardous Materials

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Effect of biogenic substrate concentration on 4-chlorophenol degradation kinetics in sequencing batch reactors with instantaneous feed

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Received 24 October 2005; received in revised form 31 January 2006; accepted 31 January 2006
Available online 13 March 2006

Abstract

Two sequencing batch reactors (SBRs) instantaneously fed with 200 mg/l 4-chlorophenol (4-CP) were operated at different feed peptone concentrations to investigate the effect of biogenic substrate (peptone) concentrations on reactor performance, yield coefficient (Y) and 4-CP degradation kinetics. One of the reactors was operated at 10 days of sludge retention time (SRT) and the other was operated at 20 days of SRT. High chemical oxygen demand (COD) removal efficiencies (90–95%) and complete 4-CP removals (detection limit was 0.05 mg/l) were observed even in the absence of peptone. Accumulation of 5-chloro-2-hydroxymuconic semialdehyde (CHMS), meta cleavage product of 4-CP, was observed, which was completely removed at the end of the reactor cycle. It was concluded that decreasing peptone concentrations did not affect 4-CP degradation profiles and Haldane equation can be satisfactorily used to predict time course variation of 4-CP concentrations. It was assumed that specialists (competent biomass) are only responsible for 4-CP degradation and its concentration was constant although peptone concentration in the feed was varied, as competent biomass grows on 4-CP only. Model developed using this assumption well tracked the experimental data. The kinetic coefficients obtained for the reactor operated at 10 days of SRT were also valid for the reactor operated at 20 days of SRT although higher degradation rates were observed due to higher steady state biomass concentrations.

Keywords: 4-Chlorophenol; Biogenic substrate; Degradation kinetic; Competent biomass

1. Introduction

A wastewater treatment plant generally receives influent with a mixture of recalcitrant synthetic organic chemicals (SOCs) and biogenic substrates. This means that SOCs and biogenic compounds often coexist in many wastewater reactors. Interactions among these multiple substrates are complex, partially due to the toxicity, competition for enzymes and cofactors [1]. Therefore, it would be interesting to evaluate how biogenic substrate concentration affects the removal efficiency and kinetics of SOCs. Many researchers claim that a specific competent biomass fraction was responsible for the degradation of a specific compound, which is equal to the fraction of chemical oxygen demand (COD) contributed to the feed by that compound [1–4]. This means that the presence of biogenic substrate does not guarantee the enhanced biodegradation of SOCs [1]. For example, Kulkarni and Chaudhari [5] reported that the degradation rate of *p*-nitrophenol decreased with the addition of glucose. In another study, Hu et al. [1] reported that at standard oxygen conditions 4-chlorophenol (4-CP) degradation rate decreased with the supplementation of biogenic substrate, whereas, 2,4-dichlorophenol (2,4-DCP) degradation rate increased in the presence of biogenic substrate. In the same study, it was also reported that at elevated oxygen conditions, although biogenic substrate addition did not affect 4-CP degradation rate, it caused increase in degradation rate of 2,4-DCP. These results showed that different chemicals might give different response to the presence of biogenic substrate depending on assay conditions.

Previously, we have investigated the effect of biogenic substrate availability on 2,4-DCP [6] and 4-CP [7] degradation for short term experiments. Also, we have investigated the effect of biogenic substrate concentration on the performance of sequencing batch reactor (SBR) (filling time was 8 h) fed with mixture of 4-CP and 2,4-DCP [8]. However, no detailed

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doi:10.1016/j.jhazmat.2006.01.069

kinetic study has been provided. Therefore, in the present study, 4-CP was selected as a model compound and its biodegradation kinetics was extensively evaluated at various biogenic substrate concentrations using long term operated instantaneously fed SBRs at 10 and 20 days of sludge retention times (SRTs).

2. Materials and methods

2.1. SBR experiments

A 2.5 l glass vessel with the 2 l of working volume was used as SBR. Primary settling tank effluent of Greater Municipality of Ankara Domestic Wastewater Treatment Plant was used as initial inocula. First of all, the reactor with 10 days of SRT (SBR10) was fed with peptone only (400 mg/l) for 2 weeks and then 4-CP was fed to the reactor at low concentration and the concentration was gradually increased to 200 mg/l within around 5 months (Fig. 1). During the acclimation of culture to chlorophenols, peptone concentration was kept constant at 400 mg/l. After acclimation period, peptone concentration was decreased gradually, and then, the reactor was fed with 4-CP as a sole carbon source (Fig. 1). When SBR10 received 200 mg/l 4-CP and 200 mg/l peptone, another SBR with 20 days of SRT (SBR20) was started up using the wasted sludge from SBR10. The peptone concentration in SBR20 was also decreased similar to SBR10. 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 desired SRT values. 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 hydraulic retention time (HRT) in both reactors. Reactors were housed in a water-bath in which temperature was kept at $26 \pm 0.5\,^{\circ}C$ and dissolved oxygen concentration in the reactors was kept at least 3 mg/l using air pumps.

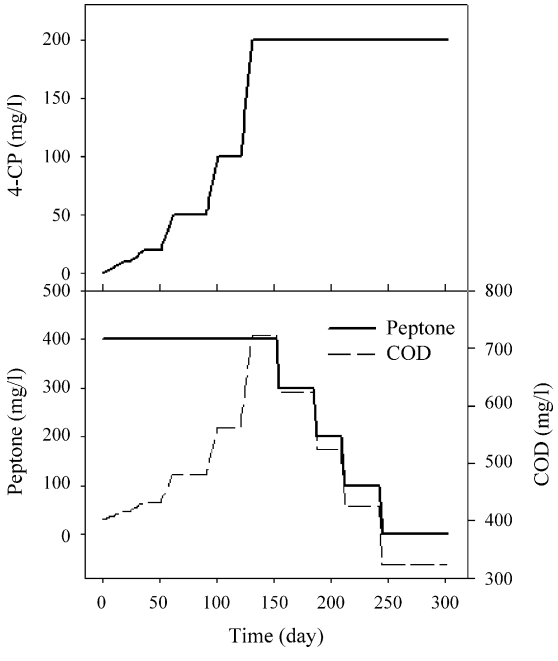

Fig. 1. Feed 4-CP, peptone and COD concentrations in SBR10.

When the pre-determined concentration of peptone was reached, reactors were allowed at least two SRT to reach steady state conditions, which was also checked measuring the COD and mixed liquor volatile suspended solids (MLVSS) at the end of three successive cycles. After observation of steady state-conditions, samples were drawn for the determination of 4-CP, COD, 5-chloro-2-hydroxymuconic semialdehyde (CHMS) and MLVSS at different time intervals.

The composition of the synthetic wastewater used in the experiments contained 400–0 mg/l peptone (1 mg peptone ~ 1 mg COD); 200 mg/l 4-CP (1 mg 4-CP ~ 1.62 mg COD); 30 mg/l NaCI; 44.6 mg/l $MgSO_4$; 400 mg/l K_2HPO_4; 200 mg/l KH_2PO_4; 0–212 mg/l NH_4CI; 3.7 mg/l $MgCl_2{\cdot}6H_2O$, $CaCl_2{\cdot}2H_2O$ and $FeCl_2{\cdot}2H_2O$; 0.057 mg/l $MnSO_4$; 0.046 mg/l $ZnSO_4$; 0.049 mg/l $CoSO_4$; 0.076 mg/l $CuSO_4$. Tap water was used in the preparation of feed solution throughout the reactors operation. The total nitrogen (N) content of proteose-peptone (Oxoid), used as a biogenic substrate, was around 12%. As peptone concentration was decreased in the feed solution, the excluded amount of N was supplemented with increased concentration of NH_4CI not to allow culture to be N limited. The initial pH of the reactors was around 7.2 ± 0.2, which decreased to around 6.5–7 due to production of HCI during 4-CP degradation and nitrification of NH_4^+.

Also, 4-CP adsorbed on wasted sludge was extracted using 0.1N NaOH, and removal via adsorption was not detected throughout the reactors operation.

2.2. 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 $26 \pm 1\,^{\circ}C$. Biomass samples taken from SBR10 receiving 200 mg/l 4-CP and 400 mg/l peptone was washed two times with distilled water in order to remove organics adsorbed on biomass. Then, culture was resuspended in distilled water and parallel batch reactors were seeded to have biomass concentration of around 200 mg/l. Reactors were supplemented with inorganic components of the feed medium and varying concentrations of 4-CP as a sole carbon source. The ratio 4-CP to biomass in batch reactors was ranged between 0.05 and 0.94 on COD basis. The same amount of nitrogen (48 mg/l N) in the excluded proteose-peptone (Oxoid) was added to batch reactors as NH_4CI.

Reactor not receiving biomass was also operated as a control at the same conditions to follow the removal of chlorophenols via volatilization and no evaporational loss of 4-CP was detected.

During experiments, a 5 ml sample was filtered through a 0.45-μm filter and subjected to 4-CP analyses. MLVSS concentrations were measured at the start and end of the reactor operation. Also, variations of pH with incubation time were followed.

2.3. Isolation of pure cultures

In the isolation of bacteria, the procedure given by Wang et al. [9] was used. The synthetic wastewater supplemented with 50 mg/l 4-CP was solidified and used in the isolation of pure strains. Mixed culture samples, obtained from SBR10, were diluted to have maximum 30 colonies on solidified agar medium incubated at 30 °C. Single colonies were selected and streaked on new agar plates and 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.

2.4. Analytical techniques

CHMS concentration, the meta cleavage product of 4-chlorocatechol, was followed by measuring OD at 380 nm [10] at which CHMS gives maximum absorbance. MLVSS and chloride concentrations (titrimetric method) were determined according to standard methods [11]. COD measurements were carried out using Hach COD vials according to the EPA approved reactor digestion method [12]. In this method, after 2 h digestion, COD values of samples were directly read using Hach Spectrophotometer (Model No. 45600-02, Cole Parmer Instrument Co., USA).

A high performance liquid chromatography (HPLC) method was used to determine the 4-CP concentration. The HPLC (Shimadzu, LC-10AT) used was equipped with a Nucleosil C18 column (4.6 mm × 250 mm), LC-10Atvp solvent delivery module, an SC/L0Avp system controller and a SPD-10Avp UV–vis detector set at 280 nm. Retention time of 4-CP was 7.5 min. Solvent used in the analyses was methanol (60%), pure water (38%) and acetic acid (2%) at the flow rate of 0.75 ml/min. The sample injection volume was 20 μl and the minimum detection limit was 0.05 mg/l.

All the experiments and measurements were done in duplicate and arithmetic averages were taken throughout the data analysis and calculations. Coefficient of variations (CV) for COD and MLVSS measurements was less than 10%, whereas it was less than 5% for 4-CP measurements.

3. Results and discussion

3.1. Effect of biogenic substrate concentration on the performance of SBR10

Complete 4-CP removal with no significant variation in the degradation patterns and high COD removals (90–95%) were observed for all studied conditions (Fig. 2). The effluent COD concentration was about 28 mg/l in the absence of 4-CP and it changed between 30 and 50 mg/l in the presence of 4-CP with varying peptone concentrations. Low effluent COD concentrations were the first evidence of complete 4-CP mineralization, which was supported by HPLC analyses. Also, released chloride ion concentrations indicated 101 ± 7% 4-CP mineralization.

After complete elimination of peptone from the feed, 400 mg/l peptone was readded to the feed of the reactor. In Fig. 2(b), data corresponding to 400 mg/l peptone 1 was

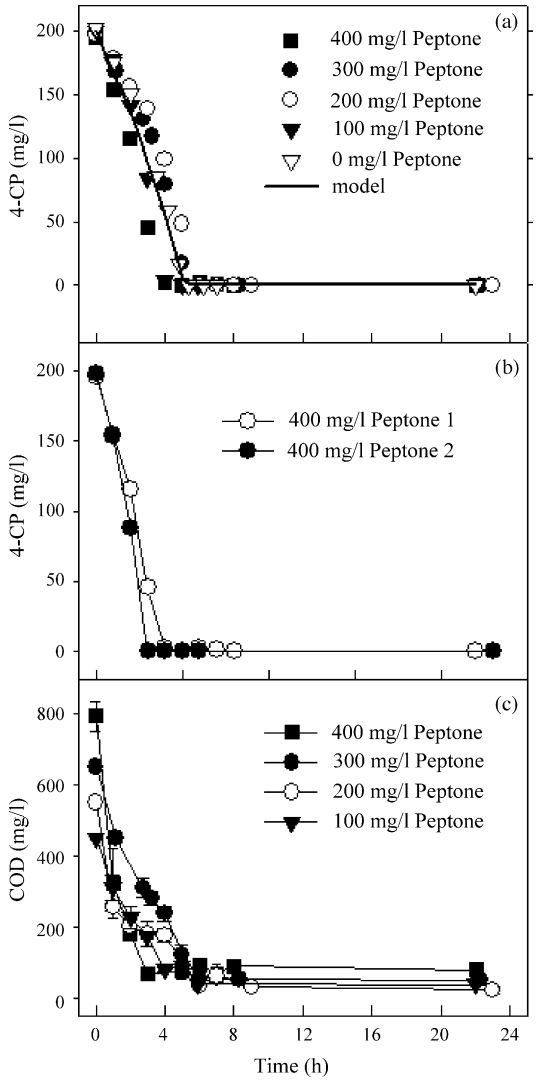

Fig. 2. Time course 4-CP and COD variations at different feed peptone concentrations in SBR10.

observed at the first peptone addition, whereas, data belonging to 400 mg/l peptone 2 was observed when peptone was readded after its complete elimination. Variation in the 4-CP degradation patterns was not significant (Fig. 2(b)) and the observed small change can be attributed to the natural variability of culture over time. Similarly, Ellis et al. [3] reported that biodegradation kinetics might show variation (standard deviation of ±50%) even in long-term operated steady state reactor due to natural variability of culture. In another study, Kaewpipat and Grady [13] observed significant difference over time in microbial communities of even two identically operated activated sludge reactors. Similar to those findings, isolation and identification studies in SBR10 revealed change in dominant species during the operation of the reactor. *Pseudomonas vesicularis* and *Pseudomonas stutzeri* were dominant species when the reactor fed with 400 mg/l peptone, whereas, *Stenotrophomonas maltophilia* and *Pseudomonas fluorescens* were the dominant species when peptone was completely eliminated from the feed, i.e., chlorophenols were the sole carbon sources.

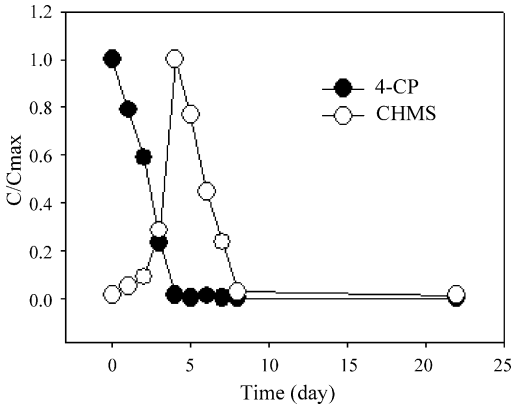

Fig. 3. Time course variations of normalized 4-CP and CHMS in SBR10 receiving 400 mg/l peptone.

Metabolization of 4-CP results in production of 4-chlorocatechol and meta cleavage of which causes the formation of CHMS. The metabolization of CHMS 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 [10]. During the operation of the reactors a yellowish color was observed, which was removed at the end of the cycle. The intermediate gave the maximum absorbance at 380 nm and have the all characteristics of CHMS [10]. During the degradation of 4-CP, the concentration of CHMS increased concomitantly, which reached its maximum when 4-CP was just completely removed from the medium (Fig. 3). After reaching peak value, its concentration decreased and complete removal of the intermediate has been observed. Similar patterns have also been observed for other peptone concentrations and SBR20 (data not shown). Using the extinction coefficient of CHMS [10], its highest concentration was calculated between 7 and 10 mg/l for all studied conditions. Therefore, CHMS accumulation was only 2.5–3.6% of its theoretical value assuming complete transformation of 4-CP to CHMS. The measured COD values were also in good agreement with this observation as the COD concentration when CHMS reached its maximum was almost equal to the effluent COD value due to the low concentration of CHMS. Therefore, simultaneous removal of 4-CP and CHMS occurred in the reactor although CHMS removal rate was slightly lower, which caused a slight accumulation in the medium.

Almost identical Y values (0.220 ± 0.009 (CV = 4.25%) mg MLVSS/mg COD) were observed when peptone concentration was between 400 and 100 mg/l and it slightly decreased (0.154 ± 0.005 mg MLVSS/mg COD) with the complete elimination of peptone from the feed (Fig. 4(a)). The average Y value was 0.207 ± 0.03 (CV = 14.49%) mg MLVSS/mg COD for whole set of experiments. Although biomass concentration decreased with decreasing peptone concentration, degradation rates (DRs) of 4-CP did not correlate with peptone concentration (Fig. 4(b)) and the average value was 40.53 ± 7.9 mg/l h (CV = 19.5%). Unlike to DRs, specific 4-CP degradation rates (SDRs) increased when peptone concentration was below 200 mg/l and the highest value was reached in the absence of peptone.

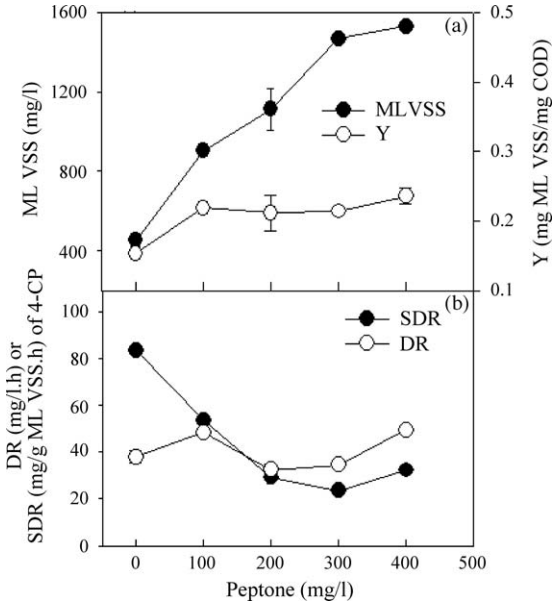

Fig. 4. Effect of feed peptone concentration on steady state performance of SBR10.

Many researchers claim [1–4,14] that only a specific population of microbial community is responsible for the degradation of particular compounds, especially if degradation requires unique pathways and the fraction of specialists (competent biomass) in the community may be assumed equal to the COD fraction of SOC of interest in the feed (e.g., synthetic organic chemicals (SOCs)). If the assumption is correct, the 4-CP degrading biomass will grow on 4-CP only [1–4,14]. Therefore, in our study, the concentration of specialist biomass might remain constant as 4-CP concentration in the feed was kept constant. If only competent biomass is responsible for 4-CP degradation, degradation rates (DRs) of 4-CP should not change with feed peptone concentration, whereas, SDRs should increase as the fraction of competent biomass increased in the community with decreasing peptone concentrations. Our observations (Fig. 4) partially agreed with this hypothesis and 4-CP degradation patterns were very similar at different peptone concentrations (Fig. 2(a) and (b)).

3.2. *Effect of biogenic substrate concentration on the performance of SBR20*

In order to understand the effect of SRT on 4-CP degradation kinetics, another reactor with 20 days of SRT (SBR20) was operated parallel to the SBR10. Similarly, complete 4-CP and high COD removals were observed even in the absence of peptone. Similar to the results observed for SBR10, peptone did affect 4-CP degradation profile in SBR 20 (Fig. 5).

The effect of peptone concentration on the performance of SBR20 was also summarized in Fig. 6. MLVSS concentrations decreased with decreasing peptone concentration in the feed. SDR of 4-CP was observed to be almost constant in the presence of peptone and it significantly increased with the complete exclusion of peptone from the feed. Similar to SBR10, almost identical

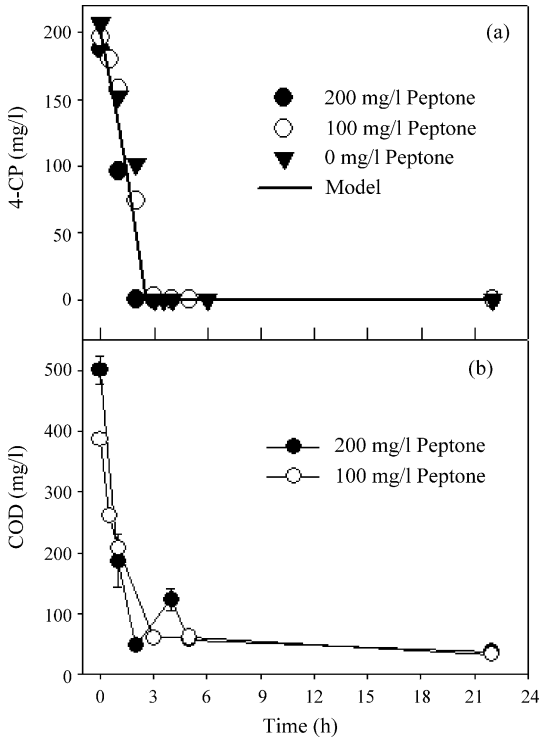

Fig. 5. Time course 4-CP and COD variations at different feed peptone concentrations in SBR20.

Y values (0.263 ± 0.009 (CV = 3.4%) mg MLVSS/mg COD) were observed when the feed included peptone and the Y decreased to a value of 0.175 ± 0.005 mg MLVSS/mg COD with the complete elimination of peptone. The average Y value for whole set of experiments was observed to be 0.234 ± 0.04 (CV = 18%) mg MLVSS/mg COD. Although slightly lower values were observed in SBR10, the Y values were quite similar for both reactors (Figs. 4 and 6).

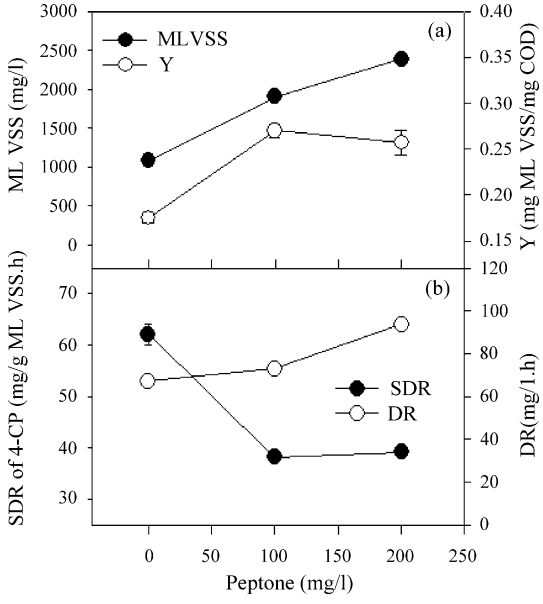

Fig. 6. Effect of feed peptone concentration on steady state performance of SBR20.

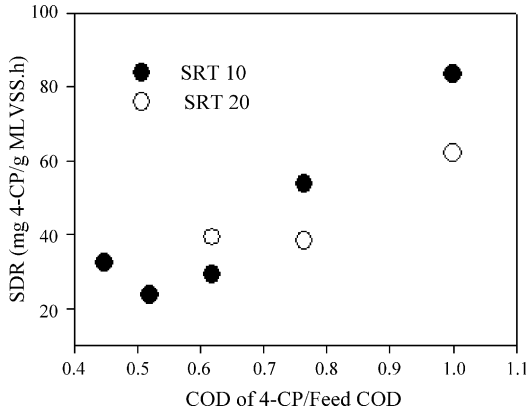

Fig. 7. SDRs of 4-CP at varying 4-CP fractions in the feed on COD basis.

The volumetric removal rate of 4-CP in SBR 20 was around two times higher compared to SBR 10. It is known that a high steady state biomass concentration in the treatment system usually exhibits high volumetric conversion capacity and the flexibility to the fluctuated loading rates [15].

Also, 4-CP SDRs for SBR10 and SBR20 showed an increasing trend with decreasing peptone concentration or increasing 4-CP fraction in the feed (Fig. 7).

Although we observed that biogenic substrate did not affect aerobic 4-CP degradation, it promoted anaerobic pentachlorophenol (PCP) degradation [16] as biogenic substrate was used as electron source in reductive dechlorination process.

3.3. Kinetics of 4-CP degradation

Results of batch experiments revealed that degradation rates decreased at high 4-CP concentrations and Haldane substrate inhibition model could be used to predict degradation rates (Fig. 8). In the calculation of SDRs, competent biomass concentration was only considered and the fraction of competent biomass in the community was assumed to be equal to the fraction of 4-CP in the feed on COD basis. Hence, the following equation was used:

$$\frac{d(S)}{d(t)} = -\frac{q_m S X a}{K_s + S + S^2/K_i} \quad (1)$$

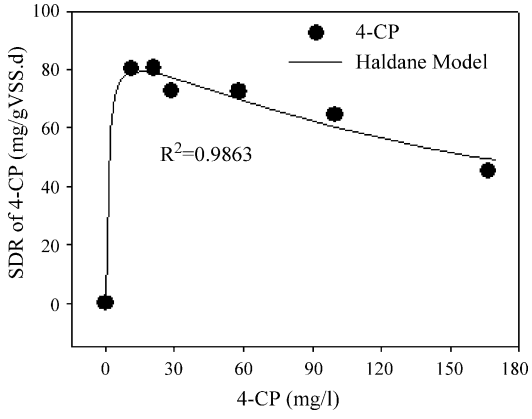

Fig. 8. SDR_c values at varying initial 4-CP concentrations in batch reactors.

Table 1
Average competent biomass concentrations and DRs in SBR 10 and SBR 20

Reactor	Competent biomass (mg MLVSSc/l)	DR (mg/l h)
SBR10	655.76 ± 117.27	40.53 ± 7.9
SBR20	1339 ± 183	77.91 ± 14

where q_m is maximum specific 4-CP degradation rate (mg 4-CP/g MLVSSc h), K_s and K_i are half saturation and self-inhibition constants, respectively, X is total biomass concentration (g MLVSS/l) and lastly a is the COD fraction of 4-CP in the feed of the SBR.

For batch experiments sludge taken from SBR10 when it received 200 mg/l 4-CP and 400 mg/l peptone, therefore, the value of a was 0.446. Experimental data was fitted to the Haldane equation using the nonlinear least squares technique with the help of MATLAB 6.5. Hence, the 4-CP degradation kinetics in batch assays (Fig. 8) was observed to be

$$\frac{d(S)}{d(t)} = -\frac{92SXa}{1.104 + S + S^2/194.4} \quad (2)$$

The average X_c (or Xa) values in SBRs (Table 1) were used in Eq. (2) and the predicted 4-CP degradation profiles gave good fit to the experimental data (Figs. 2 and 5). Using the average DRs and competent biomass concentrations (Table 1), the SDR values on the basis of competent biomass (SDR_c) were calculated as 58.11 and 61.8 mg/g h for SBR20 and SBR10, respectively. It can be concluded that although DR value observed in SBR 20 was around two times higher, SDR_c values of SBR 10 and 20 are quite similar. Therefore, the observed higher degradation rate value for SBR 20 was only due to observed higher competent biomass concentration.

4. Conclusions

The effect of biogenic substrate concentration on 4-CP degradation kinetics were extensively investigated in instantaneously fed SBRs operated at 10 and 20 days of SRTs. High COD removal efficiencies and complete chlorophenol removals were observed even in the absence of peptone. Decreasing peptone concentration did not cause significant change in chlorophenol degradation patterns. The reason of this observation was that a fraction of biomass grown on 4-CP only (competent biomass) was responsible for 4-CP degradation. Model developed based on this assumption gave good fit to the experimental data.

A wastewater treatment plant generally receives influent with a mixture of SOCs and biogenic substrates. Therefore, the results of the study can be used to design a bioremediation system as well as to optimize operational conditions.

Acknowledgement

The authors wish to express their gratitude to the Middle East Technical University Research Fund which supported this work (project no. BAP-2002-03-11-01).

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