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EFFECTS OF 4-CHLOROPHENOL LOADINGS ON ACCLIMATION OF BIOMASS WITH OPTIMIZED FIXED TIME SEQUENCING BATCH REACTOR

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ABSTRACT

Chlorinated phenols in many industrial effluents are usually difficult to be removed by conventional biological treatment processes. Performance of the aerobic sequencing batch reactor treating 4-chlorophenol containing wastewater at different loadings rates from 0.0075 to 1.2 g4CP/L.d was evaluated. The sequencing batch reactor was operated with fill, react, settle and decant phases in the order of 10:370:90:10 min, respectively, for a cycle time of 8 h at 10 days solid retention time and 16 h hydraulic retention time in the stable period. The effects of 4-chlorophenol loadings on the 4-chlorophenol and chemical oxygen demand removal percents, yield coefficient (Y), biomass variation and sludge volume index were investigated. High chemical oxygen demand removal efficiencies (95±3.5%) and approximately complete 4-chlorophenol removal (>99%) were observed even in the absence of growth substrate. The degradation of 4-chlorophenol led to formation of 5-chloro-2-hydroxymuconic semialdehyde, which was more oxidized, indicating complete disappearance of 4-chlorophenol via meta-cleavage pathway. A compact sludge with excellent settleability (sludge volume index=47±6.1 mL/g) developed during entire acclimation period. High removal efficiencies with sequencing batch reactor 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 pathways for treating xenobiotics containing wastewater.

Key words: Sequencing batch reactor, 4-chlorophenol, biodegradation, organic loading, mixed culture, xenobiotic

INTRODUCTION

Chlorinated phenols are xenobiotic contaminants that occur during numerous anthropogenic activities and become released into the environment (Vidal and Diez, 2003). The most important sources of these compounds can be traced either to paper manufactures or fungicide and herbicide producers (Annachatre and Gheewala, 1996). Additionally, wastewater from industries such as polymeric resin production, oil refining, petrochemicals and coking plants also contain chlorophenols (Quan *et al*., 2003).

Because of the toxicity of chlorophenols and their persistence in the environment, techniques for their removal are urgently needed (Bollag *et al*., 2003).

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Among various methods developed for removal of chlorophenols, physico-chemical methods such as adsorption and ion exchange are usually used to concentrate the chlorinated phenols on the solid phase which require further mineralization by chemical and biological oxidation. Chemical oxidation methods are fast, but may result in formation of undesirable by-products and also may require expensive chemical agents.

Biodegradation of chlorophenols is more specific and relatively inexpensive (Eker and Kargi, 2006). Anaerobic and aerobic treatment processes were developed by many researchers for removal of chlorophenol from wastewater (Armenante *et al*., 1999; Atuanya *et al*., 2000; Buitron *et al*., 2001; Bali and Sengul, 2003; Zilouei *et al*., 2006).

The recalcitrance of chlorophenols results from the carbon–halogen bond, which is cleaved with great difficulty and the stability of their aromatic structure, resulting in their accumulation in nature. However, a number of bacteria have been shown capable of the aerobic degradation of mono-chlorophenols (Farrel and Quilty, 2002a). Most of the investigations on biodegradation of chlorophenols focused on suspended pure culture studies using different bacteria and fungi (Hill *et al*., 1996; Kim and Hao, 1999; Westerberg *et al*., 2000; Farrel and Quilty, 2002b; Zouari *et al*., 2002; Wang *et al*., 2003). Pre-adaptation of the activated sludge cultures to the chlorophenols was reported to improve the rate and the extent of biodegradation of those compounds (Bali and Sengul, 2002; Sahinkaya and Dilek, 2002).

It is also important to point out that capabilities of biomass in degrading biorefractory compounds are determined by the induction of the synthesis of the enzymes able to develop specific metabolic pathways; this process is favored by dynamic conditions that are typical of periodic systems. As a consequence, a promising alternative to completely stirred tank reactors (CSTR) in treating mixed or industrial wastewater are discontinuous reactors such as sequencing batch reactors (SBRs) that allow both dynamic conditions and optimal substrate concentration. Furthermore, these reactors are characterized by a large spectrum of operating conditions (easily obtainable on time scale) and high operation flexibility (Tomei *et al*., 2004).

In the limited number of studies with mixed cultures chlorophenols were used as the sole carbon and energy source and the activated sludge culture were acclimated (Kargi *et al*., 2005a; Sahinkaya and Dilek, 2007).

The objectives of the paper were to investigate the performance of optimized fixed time sequencing batch reactor (SBR) in the biodegradation of 4-chlorophenol (4CP) at high concentration and to evaluate the effects of 4CP loading rate on important operational parameters.

MATERIALS AND METHODS

Experimental set-up

Two laboratory-scale aerobic SBR reactors consisted of a 12 L cylindrical reactor made of plexiglas with an internal diameter of 17 cm (working volume of 9 L). Phase duration and operating condition of a typical SBR working cycle are shown in Table 1. Each cycle lasted 8 h. The volume exchange in each cycle was 4.5 L (exchange ratio 0.5). The peristaltic pump, mixer, blower and solenoid valve were controlled by a PLC time controller (Omron, Japan). The sketch of SBR system was shown in Fig. 1.

Table 1: Detail of working cycle in the SBR system

Phase	Fill	React	settle	Decant
Time (min)	10	370	90	10
Feed pump	on	off	off	off
Blower	off	on	off	off
Mixer	on	on	off	off
Decant valve	off	off	off	on
Condition	anoxic	aerobic	anoxic	anoxic

Experiments

A stock solution of 4CP (Merck chemical co., Germany) dissolved in 0.02 N NaOH, was used to adjust different concentration of 4CP in reactors. A fresh solution was prepared every day. The main reactor, inoculated with the activated sludge, has been fed with 4CP and growth medium. Also, 4CP control reactor (including 4CP and growth medium, but not biomass) was operated under the same conditions to follow 4CP removal via volatilization. During experiments, the reactor was sampled at predetermined time intervals and analyzed immediately for COD, 4CP and 5-chloro-2-hydroxy muconic semialdehyde (CHMS). MLVSS measurement was also carried out at the beginning and the growth phase to calculate yield coefficient (Y) according to Saez and Rittman (1993). First order constant (k) values were calculated for each working cycle according to formula $\ln(C/C_i)=-kt$, where C is the substrate concentration at time t and C_i is the initial substrate concentration (Chan and Lim, 2007). Sludge retention time (SRT) was adjusted to 10 days by removing 1/10 of the sludge at the end of each operation cycle before settling the sludge.

Wastewater composition

Synthetic wastewater was prepared with ordinary tap water and 4CP and glucose as sources of carbon and energy. Nutrients with following

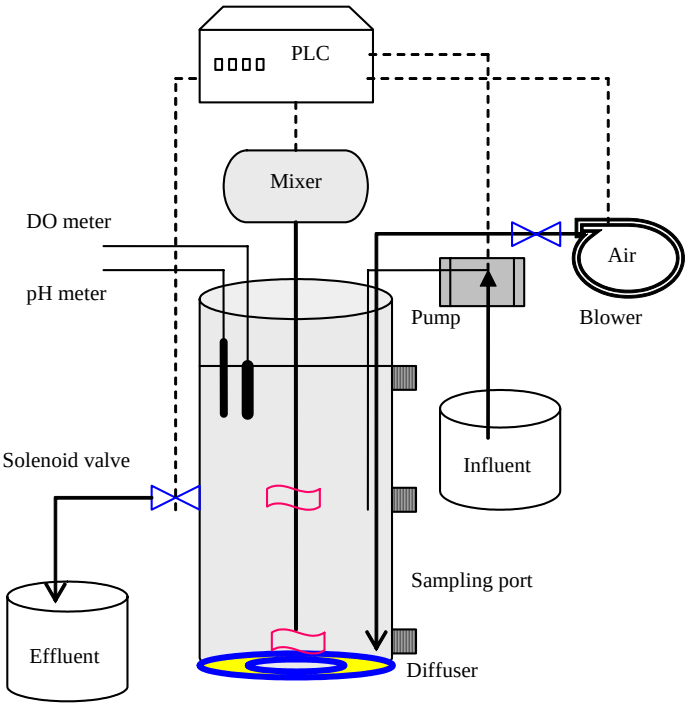

Fig. 1: Diagram of pilot scale of SBR

composition were used: K_2HPO_4 58 mg/L, KH_2PO_4 25 mg/L, $MgSO_4.7H_2O$ 75 mg/L, $CaCl_2.2H_2O$ 50 mg/L and trace solution 1 mL/L. The composition of the trace solution was: $FeCl_3.6H_2O$ 1.5 g/L, H_3BO_3 0.15 g/L, $CuSO_4.5H_2O$ 0.03 g/L, KI 0.03 g/L, $MnCl_2.4H_2O$ 0.12 g/L, $Na_2MoO_4.2H_2O$ 0.06 g/L, $ZnSO_4.7H_2O$ 0.12 g/L, $CoCl_2.6H_2O$ 0.15 g/L (Beun *et al*., 1999). COD content of the feed wastewater was kept constant at 1000±30 mg/L in the first stage of experiments while 4CP content was changed between 0 and 600 mg/L. Once 4CP increased up to 800 mg/L as sole carbon and energy source, COD content of feed raised close to 1300 mg/L (1 mg4CP/L=1.62 mgCOD/L).

Seed culture

The activated sludge culture obtained from aeration unit of Isfahan municipal wastewater treatment plant was used as the seed sludge.

Analysis

The 4CP concentration was determined using 4-aminoantipyrine method as in APHA (1995). Detection limit of this method for determination of 4CP is 0.1 mg/L. CHMS concentration, the meta-cleavage intermediate product of 4CP biodegradation, was followed by measuring absorption at 380 nm with Spectronic 20D spectrophotometer (Farrel and Quilty, 1999). COD, TSS, VSS and sludge volume index (SVI) were determined according to APHA (1995). Prior to 4CP, COD and CHMS analyses, sample were centrifuged and supernatant were used.

RESULTS

Start-up and acclimation

Start-up with the glucose as growth substrate was quite rapid and high COD removal up to 97% was achieved at two steps COD increment from 500 to 1000 mg/L. After two weeks stable operating conditions with biomass 3000 mg/L and SRT=10 days have been obtained; the effluent COD was in the range of 20-30 mg/L (data not shown). This indicated that the municipal seed culture was able to oxidize glucose as growth substrate easily.

In the next step of study biomass was acclimated

to 4CP and the feed 4CP organic loading rate (OLR) has been gradually increased from 0.0075 to 0.9 g4CP/L.d (5 to 600 mg/L) while total COD was maintained at 1000 mg/L by a decrease of glucose. The detail of acclimation is shown in Fig. 2.

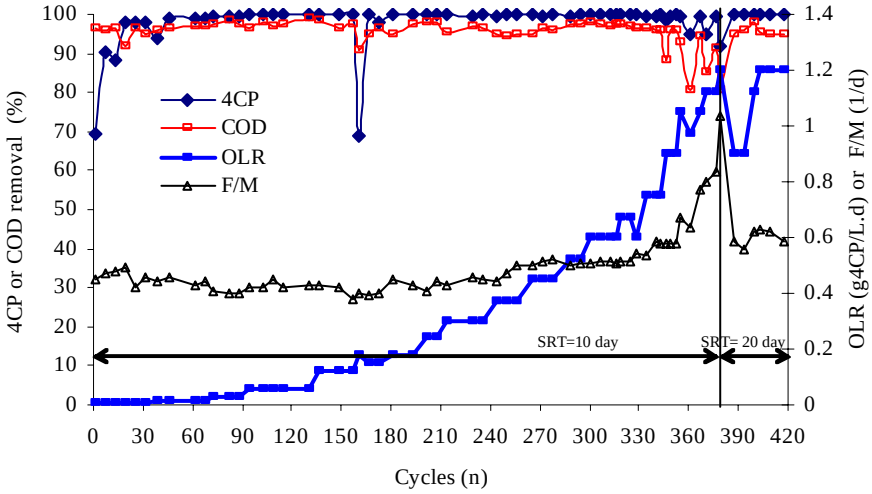

Fig. 2: Detail of acclimatization procedure to increasing 4CP loading rates

In order to ensure effective biomass acclimatization, biomass gradually was exposed to the 4CP loadings and each step increase has given only when stable operating conditions in terms of 4CP removal were obtained. At the beginning (0.0075 g4CP/L.d) 30 working cycles were required to obtain 4CP residual concentration in the effluent around detection limit, while for the subsequent steps, 6 to 9 cycles were sufficient. Also the variation of F/M ratio during reactor operation was shown in Fig. 2.

The F/M ratio was in the range of 0.4 to 0.55 (mgCOD/mgMLVSS.d) during steady state conditions of reactor operation at 0.0075 to 0.9 g4CP/L.d and showing a relatively lower microorganism to substrate ratio, while the F/M ratio at OLR up to 1.2 g4CP/L.d was increased to 1.03 (mgCOD/mgMLVSS.d) at SRT 10 days, which indicate process inhibition due to high substrate loading containing toxic compound and decrease of biomass. When SRT=20 days was applied the F/M ratio sharply decreased to optimum range in spite of high 4CP organic loading rate, and high removal efficiencies in term of 4CP and COD were recorded. It is important to point out that the effluent COD observed in all the 4CP biodegradation was in the same range of the effluent COD in the absence of 4CP; thus also in this case it is possible to assume that it is due to biomass lyses products. The residual COD and 4CP and $4CP_{COD}$/TCOD ratio are reported in Fig. 3.

CHMS in the reactor operation

Metabolization of 4CP results in production of this yellowish intermediate. During the operation of the reactor with feed OLR of 0.0075 to 0.03 g4CP/L.d (5 to 20 mg4CP/L), despite complete disappearance of 4CP, a yellowish color was observed in the effluent of reactor on cycles 88. A gradual decrease of the CHMS concentration was observed, which was attributed to the progressive acclimation of the microbial population; complete removal of 4CP and CHMS was observed throughout the reactor operation. The comparisons of CHMS variation at different 4CP concentrations are shown in Fig. 4.

Solid variations and sludge settleability

Mixed liquor volatile suspended solids were monitored throughout the study to assess the viability of the suspended biomass during SBR operation with TSS of effluents (Fig. 5). The MLVSS was in the range of 2000 to 3500 mg/L during rector operation at 0.0075 to 1.2 g4CP/L.d. With increase in organic loading rate, the MLVSS content slowly decreased, indicating the inhibitory nature of the effluent feed on biomass growth.

Very little wash out of the reactor solids occurred in the operation period (23±10 mg/L). Sludge volume index (SVI) is one of the most important parameters to evaluate the settleability of sludge in any aerobic suspended growth system. The SVI was good (47±6.1 mL/g) during acclimation of biomass. The variation of SVI also is present in Fig. 5.

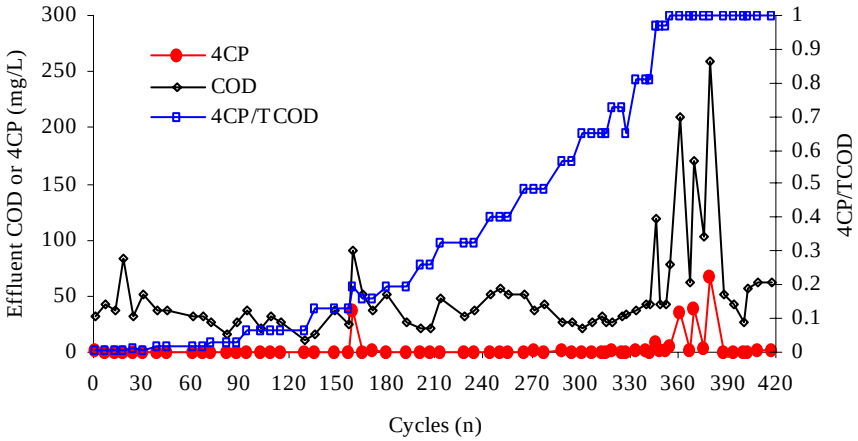

Fig. 3: Trend of 4CP/TCOD ratio and effluent 4CP and COD

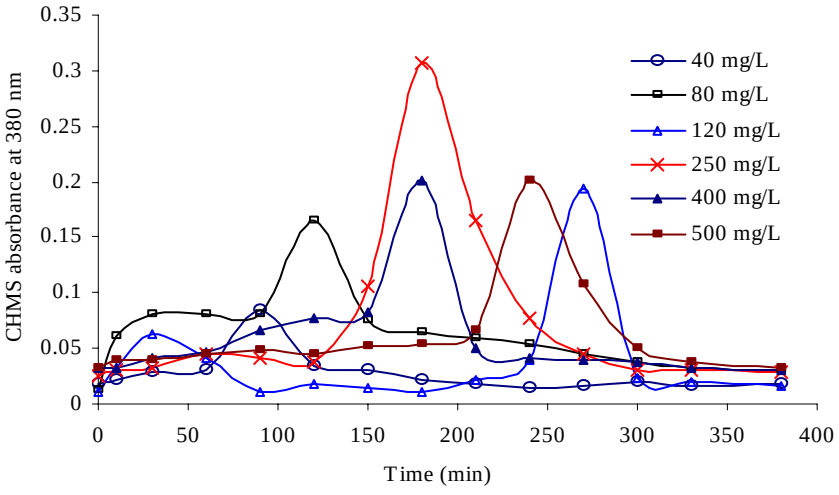

Fig. 4: CHMS variation at different 4CP concentration

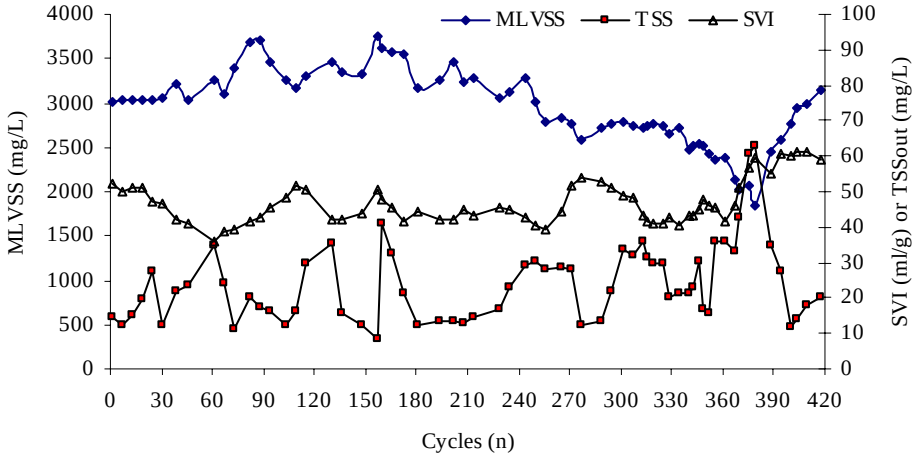

Fig. 5: Variation of solids and sludge settleability during acclimation period

First order-constant

The substrate concentration was represented the 4CP concentration. During the reaction period, the value of the first order-constant (k) was evaluated at all operation period for acclimation at different OLR (Fig. 6). The value of k was computed from the slope of the linear part of ln (substrate variation) versus time.

Biomass yield coefficient

The changes in observed yield coefficient (Y) trend at different feed 4CP organic loading rates are shown in Fig. 7. The Y in the absence of 4CP was observed to be 0.48±0.02 mgMLVSS/mgCOD, and it decreased with increment of initial 4CP loadings in the range of from 0.0075 to 1.2 g4CP/L.d to an average value of 0.19±0.06 mgMLVSS/mgCOD in the acclimation period.

Abiotic loss of 4CP

Volatilization tests were carried out with batches at 50, 100, 200 and 400 mg4CP/L concentrations in the control reactor. The data in Fig. 8 shows that there is little volatilization of 4CP in the reactor (1-3 %W/W) in the aeration phase. The degree of removal via adsorption was followed extracting 4CP on biomass with 0.1 N NaOH. Results showed that the contribution of adsorption on sludge was negligible for 4CP removal. This confirms that the main pathway for 4CP removal in the biological system was wholly through biodegradation.

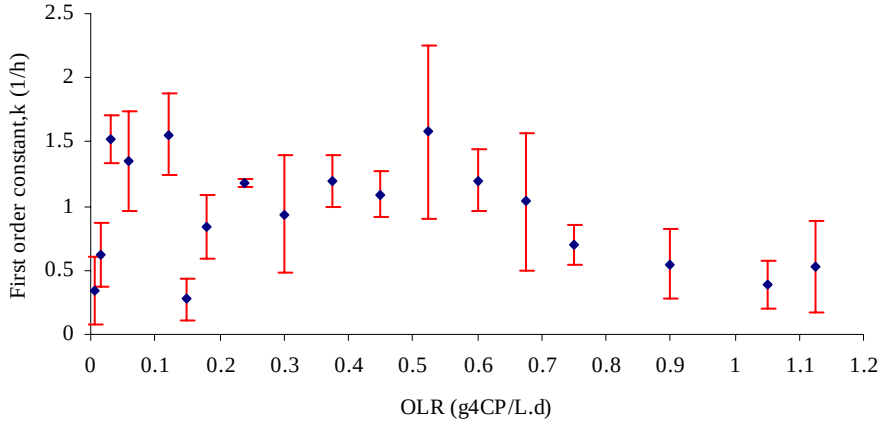

Fig. 6: Variation of k value to increasing 4CP loadings rates

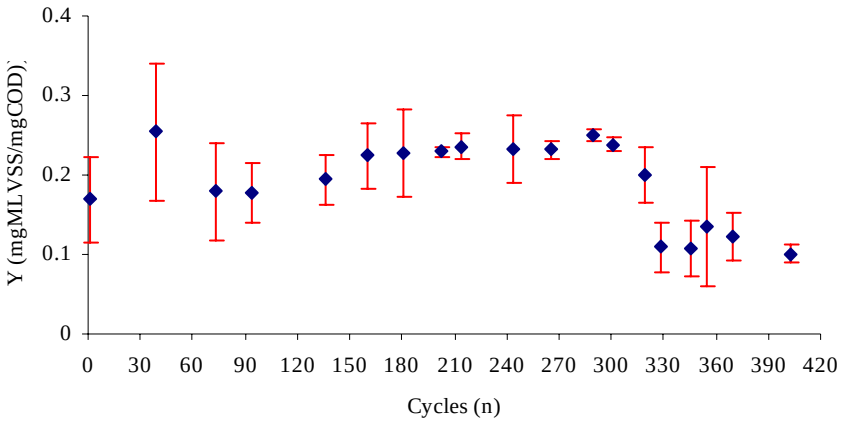

Fig. 7: Effect of 4CP concentration on the Y value during acclimation period

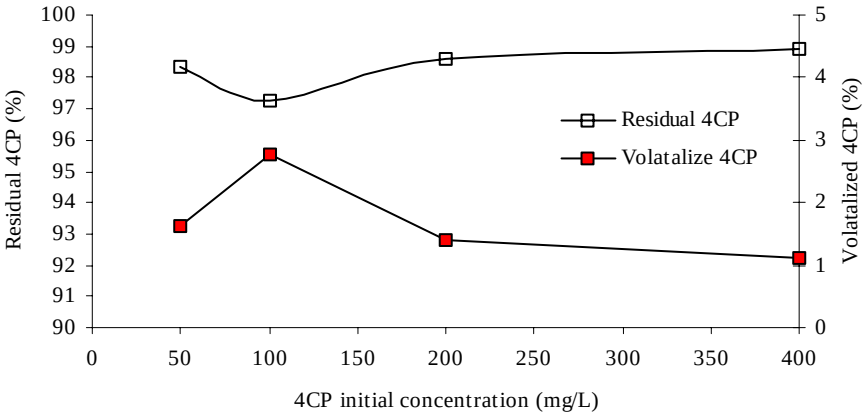

Fig. 8: Volatilization during aeration at different initial 4CP concentrations

DISCUSSION

The reactor acclimation to 4CP up to 1.2 g4CP/ L.d (800 mg/L) lasted for five months (>420 cycles) with optimized fixed time cycle duration of 8 h. Complete biodegradation of 4CP easily obtained in the present and absent of growth substrate. High removal efficiency and effluent 4CP concentration lower than 0.1 mg/L were observed for the whole of experimental period excluding some cases which reactor had required more time or biomass content for complete removal (Figs. 2-3). This behavior in the early of reactor operation can be explained considering that when the biomass in the system was at the first time in contact with the xenobiotic compound, the development of new metabolic pathways for its biodegradation was more complex and time consuming than the adaptation to a loading increase (Tomei *et al*., 2003). In the other study, Sahinkaya and Dilek (2007) worked with SBR system with 24 h cycle time treating 4CP containing wastewater in the present of peptone at SRT of 10 days. Acclimated sludge is obtained within five months to ultimately to 200 mg4CP/L. While in present study, at the same time high 4CP concentration was acclimated to biomass. This provides further support to the earlier supposition that when the conventional fixed time strategy

(cycle 24 h) was applied, a complete degradation of the 4CP (>99%) was observed in the first 8 h. For this case, the microorganisms were subjected to 16h of aeration without substrate. It has been observed that the starvation period had a negative influence on the activity of the acclimated community (Moreno and Buitron, 2004; Buitron and Moreno, 2004).

In 4CP degradation, the first step is the attack of phenol hydroxylase to 4CP, which results in 4-chlorocatechol production and then CHMS formation (Farrel and Quilty, 1999). Although, the degradation of CHMS was achieved after acclimation of biomass, it has been widely reported as being a dead-end metabolite especially for pure culture (Saez and Rittman, 1993; Farrel and Quilty, 1999). Therefore, mixed sludge is important when the emphasis is placed on complete mineralization of toxic compounds to CO_2. The main advantage achieved by microbial consortium formed by activated sludge is the interaction between all species present in flocs and its microbial diversity (Moreno and Buitron, 2004). In all of experiments, CHMS was increased simultaneously with the removal of 4CP and highest CHMS concentration was reached when 4CP was just disappeared (Fig. 4). It becomes visible within 0.5 to 2 h specified with yellow color accumulation and was removed at the end of reaction time. When complete removal of 4CP was not possible, this yellow color remained in the reactor.

Low TSS wash out in acclimation time indicate the good acclimation of SBR treating such toxic wastewater (Fig. 5). Also sludge volume index (SVI) variation at different 4CP loadings rates is shown that the SVI slightly increased with increasing initial 4CP loadings rates because of toxic and detrimental effects of high 4CP loadings on sludge settleability. The same results were reported by Kargi *et al*., 2005b. However, the SVI showed high settleability properties and was in the range of 37 to 61 mL/g. The good sludge settleability could be explained by the feast and famine conditions prevailing in the reactor which favored floc forming organisms (Cheisa and Irvine, 1985).

The changes in the first order constant (k) value provide a quantitative estimate of the inhibitory effects of 4CP on the bioactivity. The inhibitory effect seemed to be more pronounced with the increase in the influent 4CP loadings as shown by decreasing k value when the influent 4CP concentration was increased from 0.5 to 1.2 g4CP/L.d (Fig. 6). Buitron and Moreno (2004) have been reported that constants variation is faster at the beginning of the acclimation, and as acclimation time grows the constants tend to toward uniformity. This evaluation can be well approximated by a first order dynamics on the acclimation time.

As seen from Fig. 7, the biomass yield coefficient (Y) decreased with increasing 4CP loadings for the acclimated cultures. The range of Y value was observed to be from 0.1 to 0.26 mgMLVSS/mgCOD. The reason of decrease in Y can be attributed to the fact that 4CP is not good substrate for biomass and it has a strong inhibitory effect on the biomass growth. This result confirms the finding of the other studies. Rutgers *et al*., (1997) reported that the growth yield coefficients on chlorinated phenols are lower than those of heterotrophic growth on non-chlorinated compounds. They studied growth yield coefficient on various chlorophenols in chemostat culture and reported the growth yield coefficient within the range of 0.255-.11 molC/molC. Similarly, Sahinkaya and Dilek (2007) observed that the Y value in the absence of chlorophenol was 0.425±0.008 mgMLVSS/mgCOD and decrease to an average value of 0.235±.007 mgMLVSS/mgCOD when 4CP concentration increased finally to 200 mg/L (0.18 g4CP/L.d). It is pertinent to mention that the sludge growth yield represents the energy converting efficiency of energy flow from catabolism to anabolism. Chlorinated phenols could dissipate energy produced from catabolism, therefore significantly reduced the sludge yield (Chen *et al*., 2006).

The present study indicates that this type of reactor with acclimated sludge to target refractory compound has high potential in treating industrial wastewater. Also these results suggest that operating SBR with optimized cycle time and phase durations might facilitate the formation of sludge with good settleability and retain comparable removal of 4-chlorophenol and total COD. Hence, the practice of optimized cycling

should be considered in wastewater treatment systems whenever possible.

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