

COD, *para*-chlorophenol and toxicity removal from *para*-chlorophenol containing synthetic wastewater in an activated sludge unit

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Abstract

Chlorinated phenolic compounds present in some chemical industry wastewaters cause severe toxic effects on the organisms and often are resistant to biological degradation. Synthetic wastewater containing different concentrations of *para*-chlorophenol (4-chlorophenol, 4-CP) was biologically treated in an activated sludge unit for COD, 4-CP and toxicity removal. Effects of feed 4-CP concentration on COD, 4-CP, toxicity removals and on sludge volume index were investigated at a constant sludge age of 20 days and hydraulic residence time (HRT) of 25 h. Resazurin method based on dehydrogenase activity was used for determination of the toxicity of the feed and effluent wastewater. COD and 4-CP removals were not affected by the presence of 4-CP in the wastewater up to feed 4-CP concentration of 925 mg l⁻¹ because of almost complete degradation of 4-CP yielding lower than 50 mg l⁻¹ 4-CP in the aeration tank. Percent COD, 4-CP and toxicity removals decreased and the effluent COD, 4-CP and toxicity levels increased with further increases in the feed 4-CP concentrations above 925 mg l⁻¹ because of inhibitory concentrations of 4-CP in the reactor. Biomass concentration in the aeration tank decreased and the sludge volume index (SVI) increased with feed 4-CP concentrations above 925 mg l⁻¹ resulting in lower COD and 4-CP removal rates. The rates of COD and 4-CP removals indicated substrate (4-CP) inhibition for the feed 4-CP concentrations above 925 mg l⁻¹ corresponding to the reactor 4-CP of above 200 mg l⁻¹. The system should be operated at the feed 4-CP concentrations of less than 900 mg l⁻¹ (4-CP_R < 200 mg l⁻¹) in order to obtain high rates and extents of COD, 4-CP and toxicity removals at a sludge age of 20 days and HRT of 25 h.

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1. Introduction

Biological treatment of some industrial wastewaters, such as pulp and paper, pesticides and plastics industry effluents results in low treatment efficiencies, because of toxic effects of chlorophenol compounds present in such effluents. Because high levels of toxicity caused by chlorophenols, toxicity removal should also be considered in the biological treatment of such industrial effluents along with chemical oxygen demand (COD) and chlorophenol removals.

Among different physical, chemical and biological methods used in removal of chlorophenols from wastewater, physical methods, such as adsorption and ion exchange are usually used to concentrate chlorophenols on solid phases. Adsorbed organic

compounds require further treatment by chemical and/or biological oxidation for complete mineralization [1–3]. Chemical oxidation methods are fast, but expensive which may result in formation of undesirable by products. Biodegradation of chlorophenols by aerobic or anaerobic treatment is more specific and relatively inexpensive [2–5]. Most of the investigations on biodegradation of chlorophenols focused on suspended pure culture studies using different bacteria and fungi [6–16]. Usually, a carbohydrate substrate was used as the primary metabolite and the chlorophenols were the cometabolite in biodegradation of chlorophenols [9–10,14]. Limited number of studies was reported on biological treatment of chlorophenol containing wastewaters using activated sludge cultures by batch or fed-batch operations [5,17]. Pre-adaptation of the activated sludge cultures to chlorophenols was reported to improve the rate and the extent of biodegradation of those compounds [5,17]. Recent investigations on biodegradation of chlorophenols focused on the use of immobilized cells or biofilm reactors [18–22]. Biofilm

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reactors are more resistant to high concentrations of chlorophenols, because of high biomass concentrations and diffusion barriers within the biofilm for the toxic compounds. However, it is difficult to control some parameters, such as the biofilm thickness, dissolved oxygen concentration, pH, and redox potential in biofilm reactors due to heterogenous nature of such reactors. Suspended culture systems offer major advantages, such as better control and operation as compared to the biofilm reactors and may yield high removal efficiencies for COD, chlorophenols and toxicity if operated with high sludge recycle at high sludge ages.

The position and the number of chlorine groups on the aromatic ring have a profound effect on biodegradability of chlorophenols. Usually biodegradability decreases and toxicity level increases with increasing number of chlorine groups [4]. Different biological tests were used for toxicity assessment of individual chemicals or complex effluents [23–26]. One of the toxicity assessment method used is ‘resazurin assay’ which is relatively simple, inexpensive and rapid method for assessment of the toxicity of chemical compounds and water samples [23–25]. Basic principle of the method is the measurement of percent inhibition on dehydrogenase activity of bacteria in the presence of toxic compounds. Toxicity values obtained with the resazurin assay are comparable to those obtained with the more commonly used biological methods such as *Daphnia magna*, and Microtox TM [26].

There are no systematic studies in literature on biological treatment of 4-CP containing wastewaters in an activated sludge unit over a large range of feed 4-CP concentrations for COD, 4-CP and toxicity removals. Therefore, the major objective of this study is to investigate the performance of an activated sludge unit treating *para*-chlorophenol (4-CP) containing synthetic wastewater for a large range of feed 4-CP contents between 0 and 1400 mg l⁻¹. Effects of the feed and the reactor 4-CP concentrations on the rate and extent of COD, 4-CP and toxicity removals, and also on sludge settling characteristics (sludge volume index, SVI) were investigated systematically. Toxicity levels of the feed and effluent wastewaters were determined and toxicity removals were quantified by using the resazurin reduction method.

2. Materials and methods

2.1. Experimental system

The laboratory scale activated sludge unit used throughout the study consisted of an aeration tank of volume 7.61 and a sludge settling tank of 1.61, made of stainless steel.

The aeration and sludge settling tanks were separated by an inclined plate with holes which allowed passage of the wastewater from the aeration to the settling tank through the holes. The inclined plate had a 3 cm gap at the bottom which allowed the passage of the settled sludge from the settling to the aeration tank. Aeration tank was vigorously aerated by using an air pump and several porous difusors. Synthetic wastewater was kept in a deep refrigerator at 4 °C in order to avoid any decomposition and was fed to the aeration tank with a desired flow rate by a peristaltic pump. The effluent was removed from the top of the settling tank by gravitational flow. Temperature, pH

and dissolved oxygen (DO) concentrations in the aeration tanks were measured twice a day and were adjusted to desired levels. Temperature, pH and DO levels throughout the study were $T=25 \pm 2$ °C, pH 7.5 ± 0.5 and $DO=2 \pm 0.5$ mg l⁻¹, respectively.

2.2. Wastewater composition

The synthetic wastewater used throughout the study was composed of diluted molasses, urea, KH₂PO₄ and MgSO₄ resulting in COD/N/P=100/8/1.5. Typical composition of the feed wastewater was COD₀=2500 ± 200 mg l⁻¹, total nitrogen=200 ± 20 mg l⁻¹, PO₄-P=38 ± 2 mg l⁻¹, MgSO₄=50 mg l⁻¹, 100 mg l⁻¹ CaCl₂ and desired concentration of 4-CP. COD content of the feed wastewater was kept constant at 2500 ± 200 mg l⁻¹ while 4-CP content was varied between 0 and 1400 mg l⁻¹ which contributed to the COD content of the wastewater by a factor of 1.62 (i.e. 1 g 4-CP=1.62 g COD). pH of the feed wastewater was nearly 6.9. 4-CP was dissolved in hot water at 50 °C before adding to the synthetic wastewater.

2.3. Organisms

The activated sludge culture obtained from PAK MAYA Bakers Yeast Company wastewater treatment plant in Izmir was used as the seed culture. The activated sludge culture was grown in the aeration tank using the same synthetic wastewater in the presence of 50 mg l⁻¹ 4-CP for several weeks for adaptation of the culture to 4-CP before starting the continuous operation.

2.4. Experimental procedure

Experiments were started batch wise. About 71 of the synthetic wastewater containing 50 mg l⁻¹ 4-CP was placed in the aeration and was inoculated with 11 of the pre-adapted activated sludge culture. The system was operated batch-wise for 3 days to obtain a dense culture of the activated sludge before starting the continuous operation. Feed wastewater was fed to the reactor with a flow rate of 7.3 l d⁻¹ and removed with the same rate to yield an HRT of 25 h. Temperature, pH and DO were approximately $T=25 \pm 2$ °C, pH 7.5 ± 0.5 , $DO=2 \pm 0.5$ mg l⁻¹, respectively throughout the experiments. pH in the aeration tank increased to nearly pH 8 during the experiments because of ammonia released from urea biodegradation. pH was controlled around 7.5 by manual addition of dilute (0.1 M) sulfuric acid several times a day. Sludge age (sludge retention time, SRT) and hydraulic residence times (HRT) were kept constant throughout the study at SRT=20 d and HRT=25 h, respectively. Five percent (0.38 l) of the sludge was removed from the aeration tank everyday to adjust the sludge age to 20 days. Every experiment was conducted until the system reached the steady-state yielding the same COD and 4-CP contents in the effluent for the last 3 days. Average time elapsed for each experiment was about 10–15 days. The samples collected from the feed and effluent wastewater at the steady-state were analyzed for COD, 4-CP contents and the toxicity levels after

centrifugation. A baseline experiment with 4-CP-free wastewater was performed under the same experimental conditions.

2.5. Analytical methods

Samples were withdrawn everyday for analysis and centrifuged at 8000 rpm (7000 g) for 20 min to remove biomass from the liquid phase. Clear supernatants were analyzed for COD and 4-CP contents. 4-Aminoantipyrene colorimetric method developed for determination of phenol and derivatives in form of phenol index was used for 4-CP analysis as specified in the standard methods [27]. Chemical oxygen demand (COD) was determined using the closed reflux method according to the standard methods [27]. Biomass concentrations were determined by filtering the samples through 0.45 μm milipore filter and drying in an oven at 105 °C until constant weight. The samples were analyzed in triplicates with less than 3% S.D. from the average.

Resazurin reduction method was used to determine the toxicity of the feed and effluent wastewater [24–25]. The test organisms (washed activated sludge) to be subjected to the toxic feed and effluent wastewater were cultivated on nutrient broth before using for determination of the toxicity of wastewater samples. The test cultures were transferred every 2 days to new medium to keep the sludge age constant during the course of toxicity experiments. In the presence of active bacterial culture with dehydrogenase enzyme activity, resazurin changes color from blue to pink forming the reduced compound resorufin. Inactive bacteria does not cause any change on resazurin color and remain blue. Therefore, the color of the resazurin solution is an indicator of bacterial activity. A visible spectrometer was used to determine the color change at a wavelength of 610 nm.

3. Results and discussion

Sixteen different experiments were performed with different feed 4-CP concentrations between 0 and 1400 mg l^{-1} while keeping the sludge age (SRT) at 20 days, hydraulic residence time (HRT) at 25 h and the feed COD at $2500 \pm 200 \text{ mg l}^{-1}$. Fig. 1 depicts variation of percent COD removal and the effluent COD content with the feed 4-CP concentration. Percent COD removals were above 90% for the feed 4-CP concentrations below 925 mg l^{-1} which decreased rather sharply when the feed

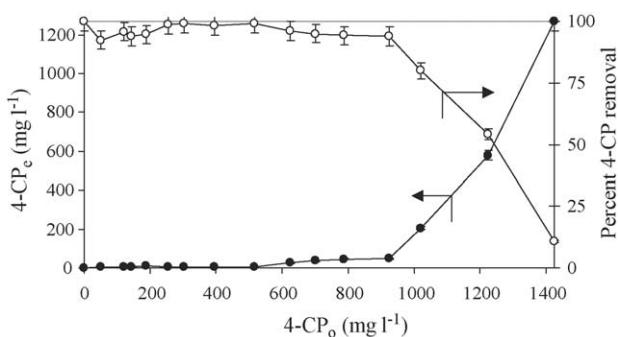


Fig. 1. Variations of the effluent 4-CP and percent 4-CP removal with the feed 4-CP content ($\text{COD}_0 = 2500 \pm 200 \text{ mg l}^{-1}$, HRT = 25 h, SRT = 20 d).

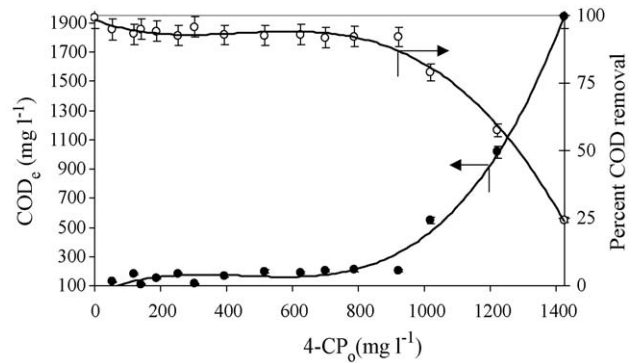


Fig. 2. Variations of the effluent COD and percent COD removal with the feed 4-CP content ($\text{COD}_0 = 2500 \pm 200 \text{ mg l}^{-1}$, HRT = 25 h, SRT = 20 d).

4-CP was greater than 925 mg l^{-1} and reached 24% when the feed 4-CP was 1425 mg l^{-1} . The effluent COD also increased with the increasing feed 4-CP greater than 925 mg l^{-1} , because of inhibitory effects of high 4-CP contents on the organisms. The effluent COD was nearly 1940 mg l^{-1} ($\text{COD}_0 = 2560 \text{ mg l}^{-1}$) with a feed 4-CP concentration of 1425 mg l^{-1} . Apparently, steady-state 4-CP concentrations were lower than 50 mg l^{-1} when the feed 4-CP was smaller than 925 mg l^{-1} resulting in negligible inhibition and therefore more than 90% COD removal.

Fig. 2 depicts variations of effluent 4-CP contents and percent 4-CP removals with the feed 4-CP concentrations. Percent 4-CP removal and the effluent 4-CP contents did not change considerably yielding above 94% 4-CP removal for the feed 4-CP concentrations below 925 mg l^{-1} . Due to inhibitory effects of high steady-state 4-CP concentrations on the organisms, feed 4-CP contents above 925 mg l^{-1} caused severe drops in percent 4-CP removals yielding high effluent 4-CP concentrations. Percent 4-CP removal was almost 100% up to feed 4-CP content of 500 mg l^{-1} , which decreased slightly yielding 4-CP removals between 94 and 98% when the feed 4-CP content was lower than 925 mg l^{-1} . Further increases in the feed 4-CP resulted in sharp decreases in 4-CP removals yielding nearly 11% removal with an effluent 4-CP content of 1270 mg l^{-1} when the feed 4-CP content was 1425 mg l^{-1} .

Variation of effluent wastewater toxicity levels with the feed 4-CP content showed a similar trend as those of the COD and 4-CP removals. Fig. 3 shows variation of percent toxicity removal and the toxicity levels of the effluent with the feed 4-CP content. Almost complete toxicity removals were observed when the feed 4-CP content was lower than 925 mg l^{-1} yielding reactor 4-CP levels below 50 mg l^{-1} . However, percent toxicity removals decreased and the effluent toxicity levels increased sharply for the feed 4-CP contents above 925 mg l^{-1} , due to high levels of intact 4-CP present in the aeration tank. Percent effluent toxicity was nearly zero for the feed 4-CP concentrations below 925 mg l^{-1} , which increased to nearly 49% for the feed 4-CP of 1225 mg l^{-1} and further to 71% with a feed 4-CP content of 1425 mg l^{-1} . Feed 4-CP contents below 925 mg l^{-1} resulted in 4-CP levels lower than 50 mg l^{-1} in the aeration tank yielding effluents with almost zero toxicity.

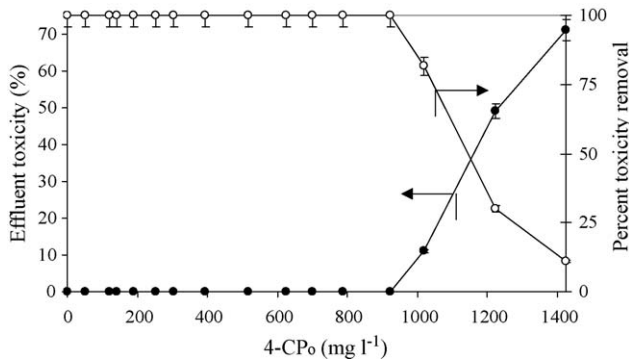


Fig. 3. Variations of the effluent toxicity and percent toxicity removal with the feed 4-CP content ($COD_0 = 2500 \pm 200 \text{ mg l}^{-1}$, $HRT = 25 \text{ h}$, $SRT = 20 \text{ d}$).

Para-chlorophenol (4-CP) concentration in the feed wastewater also affected the biomass concentration in the aeration tank at steady-state and the sludge volume index (SVI). Fig. 4 shows variations of aeration tank biomass concentration and the sludge volume index with the feed 4-CP content. Biomass concentration for the feed 4-CP of 120 mg l^{-1} ($4-CP_R = 5 \text{ mg l}^{-1}$) was 9400 mg l^{-1} which dropped to 7200 mg l^{-1} for the feed 4-CP content of 625 mg l^{-1} ($4-CP_R = 25 \text{ mg l}^{-1}$) and further to 1000 mg l^{-1} with a feed 4-CP of 1425 mg l^{-1} ($4-CP_R = 1270 \text{ mg l}^{-1}$). Biomass concentrations decreased with increasing feed and therefore steady-state reactor 4-CP contents due to high maintenance requirements of the organisms at inhibitory 4-CP concentrations. As a result of low biomass concentrations at high 4-CP contents, low COD, 4-CP and toxicity removals were obtained. The sludge volume index (SVI) values also increased with increasing feed and reactor 4-CP contents because of low biomass concentrations at high feed and reactor 4-CP contents. The SVI values were below 100 ml g^{-1} for the feed 4-CP contents below 925 mg l^{-1} indicating good settling sludge. Further increases in the feed 4-CP content above 925 mg l^{-1} resulted in SVI values above 120 ml g^{-1} indicating low quality sludge with undesirable settling characteristics. SVI values of nearly 125 and 280 ml g^{-1} were obtained for the feed 4-CP contents of 1020 and 1425 mg l^{-1} corresponding aeration tank (reactor) 4-CP levels of 205 and 1270 mg l^{-1} . Apparently feed 4-CP contents above 925 mg l^{-1} resulted in high 4-CP con-

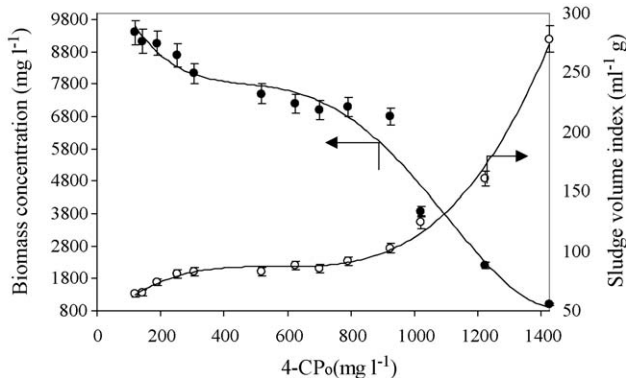


Fig. 4. Variation of steady-state biomass concentration and the sludge volume index (SVI) with the feed 4-CP content ($COD_0 = 2500 \pm 200 \text{ mg l}^{-1}$, $HRT = 25 \text{ h}$, $SRT = 20 \text{ d}$).

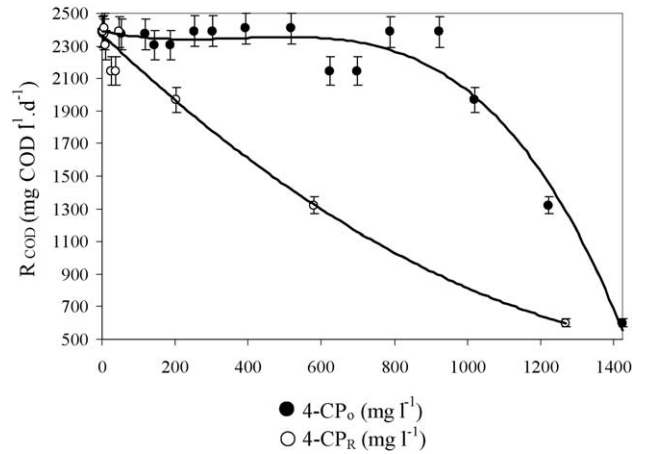


Fig. 5. Variation of volumetric rate of COD removal with the feed and reactor (effluent) 4-CP contents ($COD_0 = 2500 \pm 200 \text{ mg l}^{-1}$, $HRT = 25 \text{ h}$, $SRT = 20 \text{ d}$).

tents in the aeration tank causing inhibition and therefore low biomass concentrations and high SVI values (bulking sludge). Feed 4-CP concentrations should be below 925 mg l^{-1} in order to obtain good settling sludge and high biomass concentrations in the aeration tank yielding effective COD, DCP and toxicity removals.

The volumetric rate of COD removal ($R_{COD} = Q(S_0 - S)/V$) also varied with the feed and therefore, with the steady-state 4-CP concentrations in the reactor as a result of inhibitory effects of 4-CP on the organisms. Volumetric removal rates of COD (R_{COD} , $\text{mg COD l}^{-1} \text{ d}^{-1}$) were plotted against the feed and the steady-state aeration tank (reactor) 4-CP contents in Fig. 5. Volumetric rate of COD removal did not change significantly with the feed 4-CP content when the feed 4-CP was lower than 925 mg l^{-1} because of almost complete biodegradation of 4-CP. Further increases in the feed 4-CP above 925 mg l^{-1} yielded significantly reduced COD removal rates. The reactor 4-CP contents were lower than 5 mg l^{-1} for the feed 4-CP levels below 500 mg l^{-1} which slowly increased to 50 mg l^{-1} when the feed 4-CP was 925 mg l^{-1} yielding negligible 4-CP inhibition on COD removal and biomass growth. Further increases in the feed 4-CP above 925 mg l^{-1} yielded high 4-CP levels (above 200 mg l^{-1}) in the aeration tank and therefore low COD removal rates due to 4-CP inhibition. COD removal rate was nearly $2380 \text{ mg COD l}^{-1} \text{ d}^{-1}$ when the feed and the aeration tank 4-CP contents were lower than 925 and 50 mg l^{-1} , respectively. The rate decreased to nearly $600 \text{ mg COD l}^{-1} \text{ d}^{-1}$ for the feed and aeration tank 4-CP contents of 1425 and 1270 mg l^{-1} , respectively. The rate of COD removal decreased steadily with the increasing aeration tank (reactor) 4-CP concentration indicating severe inhibition effects of 4-CP on the organisms at high 4-CP concentrations.

Fig. 6 depicts variation of 4-CP removal ($R_{CP} = Q(CP_0 - CP_R)/V$) rate with the feed and the steady-state aeration tank (reactor and also the effluent) 4-CP contents. Since the reactor 4-CP contents were lower than 50 mg l^{-1} , when the feed 4-CP concentrations were below 925 mg l^{-1} , the volumetric rate of 4-CP removal increased with increasing feed and the reactor 4-CP contents because of substrate (4-CP)

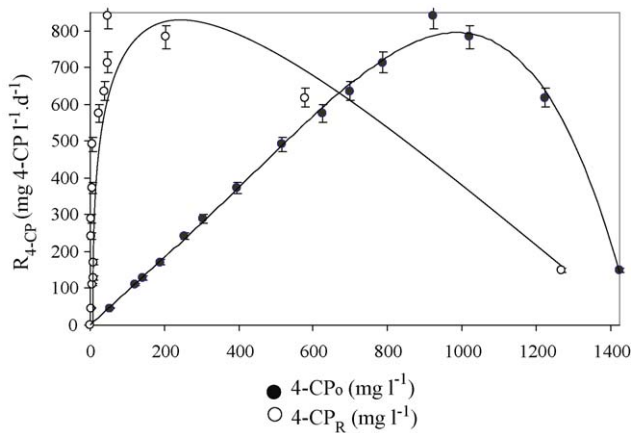


Fig. 6. Variation of volumetric rate of 4-CP removal with the feed and reactor (effluent) 4-CP contents ($COD_0 = 2500 \pm 200 \text{ mg l}^{-1}$, $HRT = 25 \text{ h}$, $SRT = 20 \text{ d}$).

limitations. 4-CP was used by the organisms as a co-substrate with no significant inhibition when the reactor 4-CP concentrations were below 50 mg l^{-1} ($4-CP_R < 925 \text{ mg l}^{-1}$). However, the rate of 4-CP removal decreased with the feed 4-CP contents above 925 mg l^{-1} since the reactor 4-CP concentrations increased to inhibitory levels of above 200 mg l^{-1} . The rate of 4-CP removal decreased from $840 \text{ mg 4-CP l}^{-1} \text{ d}^{-1}$ at $4-CP_R = 48 \text{ mg l}^{-1}$ ($4-CP_0 = 925 \text{ mg l}^{-1}$) to $150 \text{ mg 4-CP l}^{-1} \text{ d}^{-1}$ at $4-CP_R = 1270 \text{ mg l}^{-1}$ ($4-CP_0 = 1425 \text{ mg l}^{-1}$). Apparently, when 4-CP concentrations were below 200 mg l^{-1} in the aeration tank, 4-CP removal was limited by the low 4-CP concentrations. However, when the reactor 4-CP content was above 200 mg l^{-1} , the rate decreased considerably due to severe inhibitions caused by high 4-CP contents. 4-CP content of the feed wastewater should be below 925 mg l^{-1} yielding the aeration tank 4-CP contents of lower than 50 mg l^{-1} under the specified conditions, in order to obtain high rates of COD and DCP removals.

Fig. 7 depicts variation of specific 4-CP removal rate ($\text{mg 4-CP/mg biomass d}$) with the feed and the steady-state aeration tank (also the effluent) 4-CP contents. Since the reactor 4-CP contents were lower than 250 mg/l , when the feed $4-CP_0$ concentrations were below 1223 mg/l , the specific rate of 4-CP

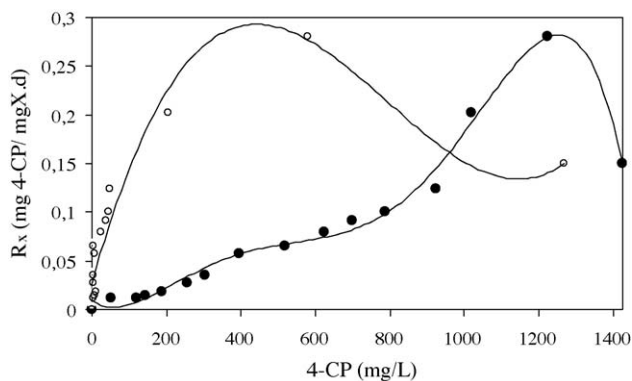


Fig. 7. Variations of specific 4-CP removal rate with the feed and reactor (effluent) 4-CP contents ($COD_0 = 2500 \pm 200 \text{ mg/l}$, $HRT = 25 \text{ h}$, $SRT = 20 \text{ d}$). (●) Feed 4-CP, (○) reactor 4-CP.

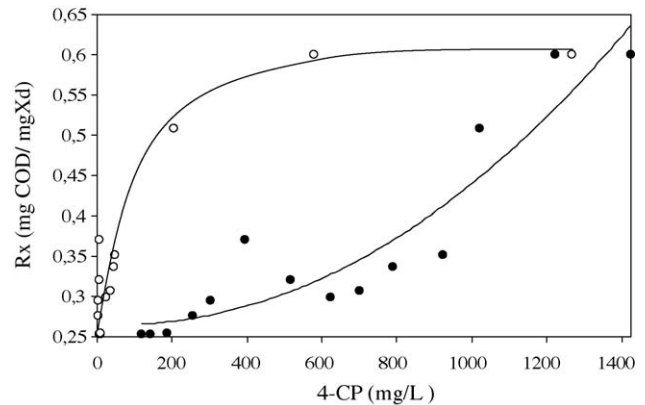


Fig. 8. Variation of specific COD removal rate with the feed and reactor (effluent) 4-CP contents ($COD_0 = 2500 \pm 200 \text{ mg/l}$, $HRT = 25 \text{ h}$, $SRT = 20 \text{ d}$). (●) Feed 4-CP; (○) reactor 4-CP.

removal increased with increasing feed and the reactor 4-CP contents because of substrate (4-CP) limitations. At high feed $4-CP_0$ ($>1200 \text{ mg/l}$) or the reactor $4-CP_R$ ($>500 \text{ mg/l}$) contents, the specific rate of 4-CP removal decreased because of 4-CP inhibition on the organisms.

Fig. 8 shows variation of specific COD removal rate with the feed and the steady-state aeration tank (also the effluent) COD contents. Biomass concentration decreased with the increasing feed 4-CP contents above 923 mg/l since the reactor 4-CP concentrations increased to inhibitory levels of above 200 mg/l . As a result of low biomass concentrations at high feed $4-CP_0$ contents, higher specific COD removal rates were obtained. Variations of specific COD removal rates with the reactor 4-CP content indicated a typical saturation (Monod) kinetics. At low 4-CP contents the COD removal rate increased with increasing reactor 4-CP content indicating no inhibition, but substrate limitation. However, the rate leveled off at high reactor 4-CP contents due to no substrate limitations, but saturation.

As compared to the literature studies on 4-CP degradation [5,13,17], our system was capable of tolerating much higher concentrations of feed 4-CP (upto 925 mg l^{-1}) which is thought to be because of the presence of high concentrations of well adapted bacterial culture in the aeration tank. The IC_{50} value of 4-CP for the activated sludge organisms was found to be $500 \text{ mg 4-CP l}^{-1}$ in our toxicity studies. Therefore, 4-CP inhibitions would be negligible when the steady-state 4-CP levels were below 200 mg l^{-1} as we determined in this study.

4. Conclusions

Synthetic wastewater containing different concentrations of *para*-chlorophenol (4-CP) was subjected to biological treatment in a laboratory scale activated sludge unit. Feed 4-CP content was varied between 0 and 1425 mg l^{-1} while the sludge age, hydraulic residence time (HRT) and the feed COD contents were constant at 20 days, 25 h and $2500 \pm 200 \text{ mg COD l}^{-1}$, respectively throughout the operation. Percent COD and 4-CP removals were above 90% for the feed 4-CP concentrations below 925 mg l^{-1} because of low 4-CP and high biomass concentrations in the reactor. Further increases in the feed 4-CP

above 925 mg l^{-1} resulted in sharp decreases in COD and 4-CP removals because of high 4-CP and low biomass concentrations in the reactor. Apparently, the steady-state aeration tank 4-CP levels were high enough ($>200 \text{ mg l}^{-1}$) to cause inhibitions on microbial growth, as well as on COD and 4-CP oxidations at high feed 4-CP levels above 925 mg l^{-1} . Percent COD and 4-CP removals decreased from 92 and 94% at the feed 4-CP of 925 mg l^{-1} to 24 and 11%, respectively with a feed 4-CP content of 1425 mg l^{-1} . Percent toxicity removal from the feed wastewater also showed a similar trend. Almost complete toxicity removals were achieved when the feed 4-CP was lower than 925 mg l^{-1} yielding the reactor 4-CP levels of lower than 50 mg l^{-1} . Percent toxicity removal decreased to 11% when the feed 4-CP was 1425 mg l^{-1} yielding the reactor 4-CP content of 1270 mg l^{-1} .

Steady-state biomass concentration in the aeration tank also decreased considerably due to high maintenance requirements of the organisms at inhibitory 4-CP concentrations. Low biomass concentrations at high feed 4-CP contents resulted in low COD, 4-CP and toxicity removals. Sludge volume index (SVI) values increased with increasing feed 4-CP contents and reached a level of 280 ml g^{-1} at the feed 4-CP of 1425 mg l^{-1} due to low biomass concentrations at high feed 4-CP contents. Feed 4-CP contents of above 1425 mg l^{-1} yielded the reactor 4-CP contents above 200 mg l^{-1} which was inhibitory for the organisms resulting in low biomass concentrations and therefore low percent COD, 4-CP and toxicity removals and also low sludge settling characteristics (i.e. high sludge volume index values). The system should be operated for the feed 4-CP contents below 925 mg l^{-1} or the reactor 4-CP of below 200 mg l^{-1} in order to obtain effective COD, 4-CP and toxicity removals with good settling sludge.

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