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# Efficacy of *Aspergillus* sp. for Degradation of Chlorpyrifos in Batch and Continuous Aerated Packed Bed Bioreactors

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**Abstract** Aerobic biodegradation of chlorpyrifos (CP) by *Aspergillus* sp. was investigated in batch and continuous packed bed bioreactors. The optimal process parameters for achieving the maximum removal efficiency (RE), determined using a batch bioreactor packed with polyurethane foam pieces, were inoculum level: 2.5 mg(wet weight)$mL^{-1}$, pH 7.0, temperature 28 °C, DO 5.8 mg $L^{-1}$, and CP concentration 300 mg $L^{-1}$. The continuous packed bed bioreactor was operated at flow rates ranging from 10 to 40 mL $h^{-1}$ while keeping other parameters at their optimal level. Steady-state CP removal efficiencies greater than 85 % were obtained up to the inlet loading of 180 mg $L^{-1}$ $d^{-1}$. The continuous bioreactor behaved as a plug flow unit and was able to stabilize quickly after perturbation in the inlet loading.

**Keywords** Bioreactor · Chlorpyrifos · Polyurethane foam · Inlet loading rate · Removal efficiency

## Introduction

The organophosphate chlorpyrifos (CP), a broad-spectrum insecticide, is a risk to human and other biological components of aquatic and terrestrial ecosystems. The conventional chemical and physical methods for the removal of CP are not only time-consuming and uneconomical but also result in the formation of secondary pollutants. Thus, a dependable and economical method for the detoxification of CP present in soil, water, etc. is required. Fungal species have been successfully used for degrading toxic organic compounds in biofilters [1–3]. Fungi are quite resistant to changes in temperature, pH, humidity, etc. In addition, fungal hyphae facilitate adsorption of xenobiotic compounds through increased mass transfer [4, 5]. Application of fungi also improves the partitioning of hydrophobic compounds between liquid and cellular system. Their ability to adapt quickly to the operating conditions of the bioreactor

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is also of substantial advantage [6]. It has already been reported that phylogenetically distinct fungal communities are capable of degrading CP and its degradation product, 3,5,6-trichloro-2-pyridinol (TCP), through their co-metabolic activities or by using them as carbon source [7–10].

In our earlier work, we have presented results on the biodegradation of CP by *Pseudomonas* sp. Iso 1, isolated from CP-contaminated agricultural soil [11]. Fungal species were also isolated along with the bacterial species and used for degrading CP in shake flasks with high percent degradation [12]. Encouraged by these results and other inherent advantages of fungi, it was planned to use fungal isolate for the biodegradation of CP and compare its efficacy vis-a-vis the bacterial isolate. In this paper, we report the kinetic constants of CP degradation as well as the optimum operating conditions for the maximum biodegradation of CP in an aerated batch packed bed bioreactor using the fungal isolate *Aspergillus* sp. F1. The performance of a continuous aerated packed bed bioreactor has also been investigated using the optimized values of process parameters obtained from the batch studies.

## Materials and Methods

### Selection of Fungus and Inoculum Preparation

The fungal isolate used in the batch and continuous experiments was *Aspergillus* sp. F1 isolated from the CP-contaminated agricultural soil and characterized by ITS gene [12]. A stock culture of the enriched fungal isolate used in all experiments was prepared by growing it on potato dextrose agar (PDA) plates using mineral salt medium (MSM) at 28 °C. A known amount of the fungal mycelia of the isolate (F1) suspended in 10 mL of MSM to give a concentration of 2.5 g(wet weight)$L^{-1}$ and kept in separate culture tubes [13] was used as the inoculum in the bioreactor packed with polyurethane cubes (1 $cm^3$).

### Batch and Continuous Bioreactors

Biodegradation studies were carried out in a batch packed bed bioreactor for evaluating the kinetic constants and optimizing the process parameters. The optimum values of parameters (DO, CP concentration, temperature, pH, and inoculum level) thus obtained were used for operating the continuous packed bed bioreactor to maximize the percent removal of CP. The batch and continuous packed bed bioreactors were similar to those used earlier [11]. Effects of pH, temperature, DO, and inoculum levels were investigated in the batch mode to obtain the optimum values keeping the CP concentration fixed at 150 mg $L^{-1}$. Effect of substrate (CP) concentration was then studied at the optimum values of inoculum level, pH, and temperature and varying the CP concentration from 100 to 400 mg $L^{-1}$. All measurements were made in triplicate and mean values were used.

For extracting the residual CP and accumulated TCP in the bioreactor, a known volume of the culture broth was treated with equal volume of chloroform. The extracts thus obtained were first dried over anhydrous $Na_2SO_4$ and then evaporated in a centrifugal evaporator at room temperature. The residual organic matter was redissolved in equal volume of methanol and analyzed using high-performance liquid chromatography (HPLC 600 E, Waters Co. Milford, USA) coupled to a Photo Diode Array Detector (Waters 2998). Water Spherisorb® 5.0-μm ODS2 S (id 4.6 mm, length 250 mm) column was used for separation. A mixture of methanol and water (85:15, *v*/*v*) at a flow rate of 1.0 mL $min^{-1}$ was used as the mobile phase [14]. An aliquot (10 μL) was injected into the chromatographic column and peak areas were used to

quantify the chlorpyrifos and TCP. The sensitivity of the system was of the order of 1.2 nm (optical resolution) and ±1.0 nm (wavelength accuracy).

Performance of the continuous packed bed bioreactor under optimized conditions and at various inlet loading rates ($S_iQ/V$=10–40 mL $h^{-1}$) was evaluated in terms of percent removal efficiency (RE) and elimination capacity (EC) defined by

$$\text{Percent Removal Efficiency (RE)} = \frac{S_i - S_e}{S_i} \times 100 \quad (1)$$

and

$$\text{Elimination Capacity (EC)} = \frac{Q(S_i - S_e)}{V} \quad (2)$$

where $S_i$ and $S_e$ are the inlet and outlet concentrations of CP, $Q$ is the volumetric flow rate of feed, and $V$ is the working volume of the bioreactor.

### Statistical Analyses

One-way analysis of variance (ANOVA) was used for determining the effects of DO, inoculum level, pH, and temperature on CP removal using SPSS 16.0 statistical package.

## Results and Discussion

### Batch Biodegradation

The effects of inoculum level, pH, temperature, DO, and CP concentration on the percentage removal efficiency were evaluated using an aerated packed bed batch bioreactor. These experiments also helped in optimizing various parameters. The inoculum level in the range of 1–4 mg(wet weight)$mL^{-1}$ was used to evaluate its effect on the percent degradation of CP. The percent degradation increased rapidly as the inoculum level increased from 1 to 2.5 mg $mL^{-1}$; thereafter, it tended towards a constant value of 92 %.

The pH range of 5.0–9.0 was used to examine its effect on the percent removal of CP. The percent degradation of chlorpyrifos was quite high in the acidic pH range and it increased with an increase in pH. The maximum percent removal of 91 % was obtained at pH 7.0. Effect of temperature on the percent removal of CP was investigated in the temperature range of 20–40 °C. The percent RE increased up to 90.3 % at 28 °C and thereafter started decreasing. The dissolved oxygen (DO) range of 3.2–7.0 mg $L^{-1}$ was used to study its effect on the percent degradation of CP. The percent removal increased rapidly to 92.3 % up to the DO level of 5.8 mg $L^{-1}$ and then it tended towards a constant value due to oxygen saturation [15]. The optimum values of DO (5.8 mg $L^{-1}$), inoculum level (2.5 mg $mL^{-1}$ wet weight), pH (7.0), and temperature (28 °C) thus obtained were used in subsequent experiments. From the ANOVA, it was seen that DO, inoculum level, pH, and temperature had a significant effect ($P$<0.05) on the removal of CP.

The effect of initial concentration on the percent removal of CP was studied by varying the CP concentration from 100 to 400 mg $L^{-1}$ with an increment of 50 mg $L^{-1}$ under the optimal conditions of inoculum, DO, pH, and temperature. The % removal of CP was more than 90 % up to the concentration of 300 mg $L^{-1}$ and thereafter it decreased rapidly indicating the possibility of substrate inhibition. Residual chlorpyrifos is considered to be critical as CP

can remain for a longer period in the system depending on its initial concentration and biodegradation rate causing inhibitory effect on the microbial population [16–18].

The kinetic parameters for chlorpyrifos biodegradation under the optimized conditions were calculated using the Michaelis–Menten kinetics model-based relation.

$$\frac{1}{t}\ln\frac{S_0}{S_i} = \left(\frac{S_0 - S}{t}\right)\left(\frac{1}{K_s}\right) + \frac{V_{\max}}{K_s} \tag{3}$$

Here $S_o$ and $S_i$ are the CP concentrations in the beginning ($t$=0) and at any time $t$, $K_s$ is the substrate constant, and $V_{\max}$ is the maximum reaction rate.

The plot of $\frac{1}{t}\ln\frac{S_o}{S}$ against $\frac{S_o - S}{t}$ shown in Fig. 1 was used to evaluate the values of $K_s$, $V_{\max}$, and $V_{\max}/K_s$ that were found to be 263 mg $L^{-1}$, 92.07 mg $L^{-1}$ $day^{-1}$, and 0.3501 ($day^{-1}$), respectively. A high value of $V_{\max}/K_s$ indicates the high substrate-degrading ability of the microbial species. The value of $V_{\max}/K_s$ obtained under the optimized conditions is better than the value (0.238 $d^{-1}$) reported earlier [12] for non-optimized conditions. Thus, by using the optimized levels of the process parameters, the substrate uptake can be increased significantly.

## Biodegradation in Continuous Bioreactor

The performance of continuous packed bed bioreactor for the CP degradation was studied at varying flow rates (i.e., the CP loading rates) under the optimum process conditions obtained through the batch experiments. During the period of experiments, the ambient temperature remained fairly constant at 25±2 °C; hence, no temperature control was used. These results are shown in Fig. 2. The rate of accumulation of TCP is also shown in this plot. Initially, the bioreactor was operated at a low flow rate of 10 mL $h^{-1}$ to facilitate proper fungal growth and establish the steady-state condition. The steady state was achieved on the fifth day of operation which was evident from the almost constant removal efficiency (89.6 %). On the tenth day, the flow rate was increased to 15 mL $h^{-1}$. After a sharp initial dip observed on the 11th day, the performance of bioreactor recovered quickly and became almost constant at 89 % on the 14th day. On the 16th and 22nd days, the flow rate was again increased to 20 and 25 mL $h^{-1}$,

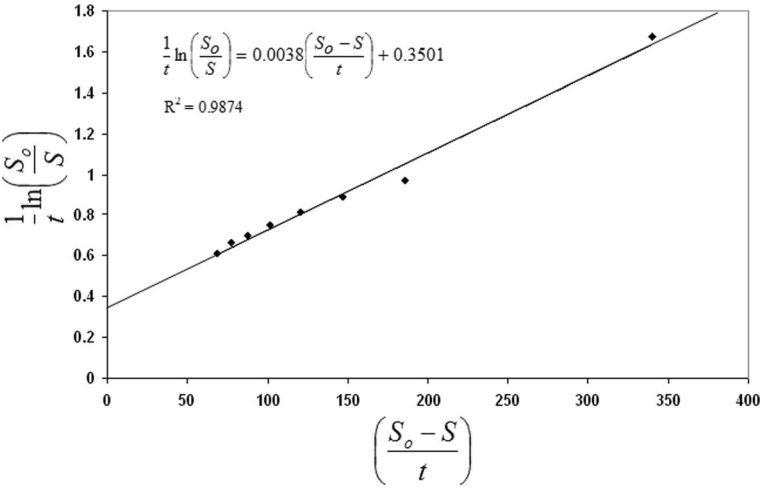


**Fig. 1** Plot of $\left(\frac{1}{t}\ln\frac{S_o}{S}\right)$ vs $\left(\frac{S_o - S}{t}\right)$

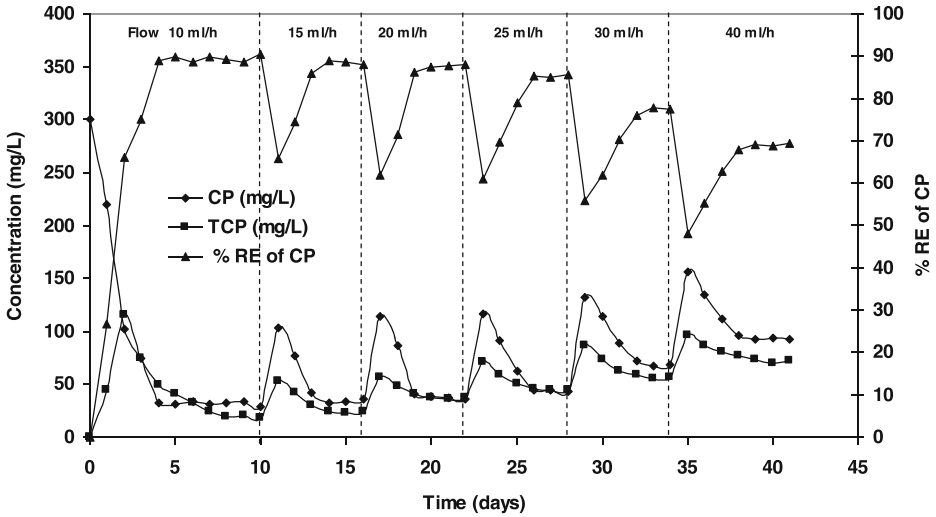


**Fig. 2** Bioreactor performance with change in gas flow rate and inlet CP concentration

respectively, and once again a sharp decrease followed by quick recovery in the bioreactor performance was observed at both the flow rates. On the 28th day, the flow rate was increased to 30 mL $h^{-1}$, and after a sharp dip, the removal efficiency stabilized at 77.6 %. At the flow rate of 40 mL $h^{-1}$, the steady-state RE value decreased sharply and stabilized at around 68 %. It was also seen that in the beginning, the production of TCP increased up to 120 mg $L^{-1}$ and then dropped down slowly and stabilized at around 19 mg $L^{-1}$ on the eighth day of operation. These steady-state values then increased slowly with an increase in the loading rate and reached to 72 mg $L^{-1}$ at a flow rate of 40 mL $h^{-1}$. The initial higher accumulation level of TCP is due to the slow acclimation of fungal species with respect to TCP. After the initial acclimation, the fungal species started degrading TCP also. The buildup of TCP (19–72 mg $L^{-1}$) in the bioreactor at the steady state is due to the higher amount of CP being applied through increased loadings.

Figure 3 shows the variation of elimination capacity, removal efficiency, and TCP production (mg $L^{-1}$ $d^{-1}$) with respect to the inlet loading rate of CP. For practical operations, the inlet loading rate at which the mechanism in the bioreactor changes from the mass transfer to bioreaction-controlled zone, as indicated by the point of intersection of removal efficiency versus elimination capacity curves, may be taken as the approximate limit of the operating range of the bioreactor. The optimum operating range is found between 180 and 250 mg $L^{-1}$ $d^{-1}$ and is lower than that for bacterial species.

## Bioreactor Behavior

From the experiments performed at various flow rates, it is seen that with increment in the flow rate, the substrate concentration increases rapidly to a maximum value and then it decreases gradually and becomes constant (Fig. 2). Taking the maximum substrate concentration after every flow increment as $S_i$ and its subsequent values at various time intervals till the next flow increment as $S_e$, the plots between substrate concentrations $S_e$ versus time $t$ for various loading

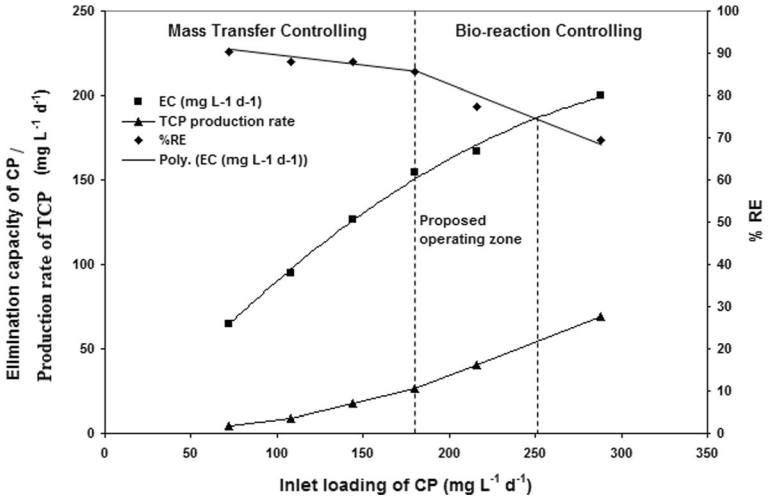


**Fig. 3** Influence of inlet CP load on the removal efficiency and elimination capacity of the CP and TCP accumulation

rates as shown in Fig. 4 indicate that the packed bioreactor behaves as a batch unit and the variation of substrate concentration can be expressed by the equation

$$S_e = S_i e^{k_1 t} \tag{4}$$

The values of $k_1$ evaluated from the regression analysis of the experimental data at each loading rate and the corresponding $R^2$ values are also shown in Fig. 4. In each case, the above equation gives a very good fit indicating the batch mode behavior of the bioreactor. It is seen that the values of $k_1$ decrease as the loading rate increases. At low loading rates ($Q$=15 and 20 mg $mL^{-1}$ $h^{-1}$), there is only a slight decrease in $k_1$ values while it is larger at higher loading rates ($Q$=25–40 mg $mL^{-1}$ $h^{-1}$) (Fig. 4).

The plug flow behavior of a continuous packed bioreactor is expressed in terms of equation

$$\frac{S_e}{S_i} = e^{-k\theta} \tag{5}$$

where $S_i$ is the inlet substrate concentration, $S_e$ is the exit substrate concentration, $k$ is the first order rate constant, $\theta$ is the hydraulic retention time=$V/Q$, $V$ is the volume of the reactor, and $Q$ is the volumetric flow rate. A plot of $\frac{S_i}{S_e}$ vs $\theta$ is shown in Fig. 5 for the entire range of flow rate covered in the continuous experiments. The least squares analysis of data gave

$$\frac{S_e}{S_i} = e^{-0.0214\theta} \tag{6}$$

with $R^2$=0.983.

## Comparison of the Performance of Continuous Bioreactors Loaded with Bacterial and Fungal Species

In order to compare the CP degradation efficacy of bacterial and fungal species in continuous packed bed bioreactors, the results reported earlier for bacterial isolate (*Pseudomonas* Iso 1)

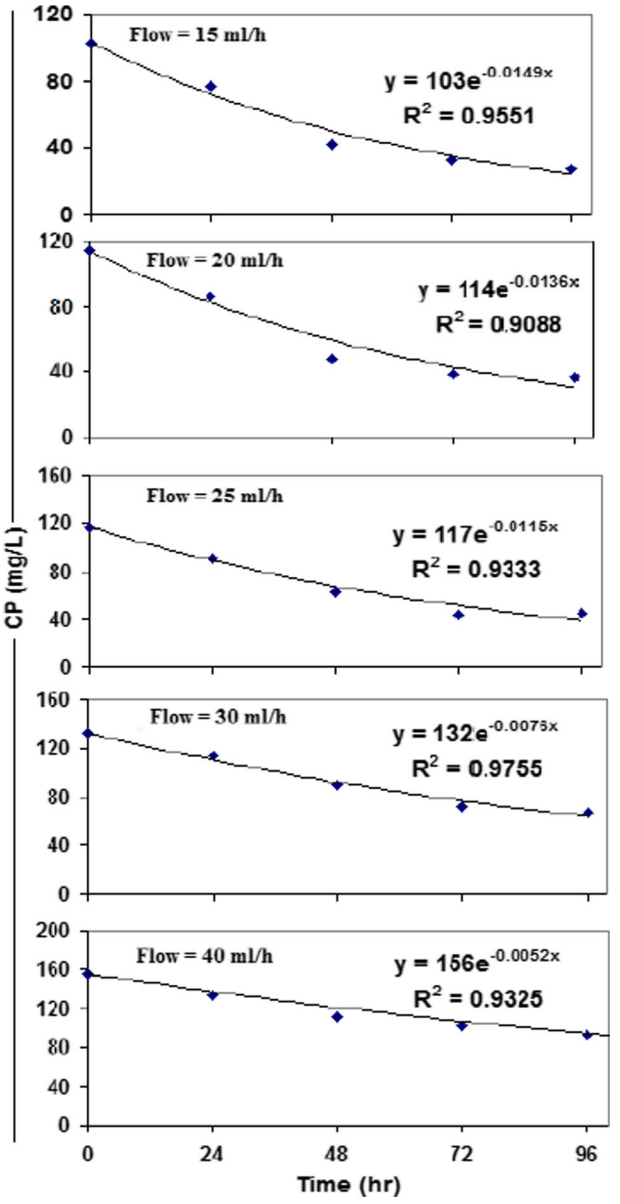


**Fig. 4** Effect of loading rate on the performance of continuous packed bed bioreactor

[11] are compared with the present results for the fungal isolate (*Aspergillus* sp.) in Table 1. It is seen that the removal efficiency (RE) and the elimination capacity (EC) are higher for the bacterial isolate than the fungal. The values of $k$ and $k_1$ for the bacterial isolate are also larger than those for the fungal isolate. It is also interesting to see that in both the cases, the order of magnitude of $V_{max}/K_s$ obtained through batch reactor studies are similar to those of $k$ and $k_1$ obtained through continuous bioreactor studies. The bacterial species are found to be more efficient in biodegrading CP than the fungal species. The lower degradation capacity of the fungal species may be attributed to the increased mass transfer resistance due to aggregation of

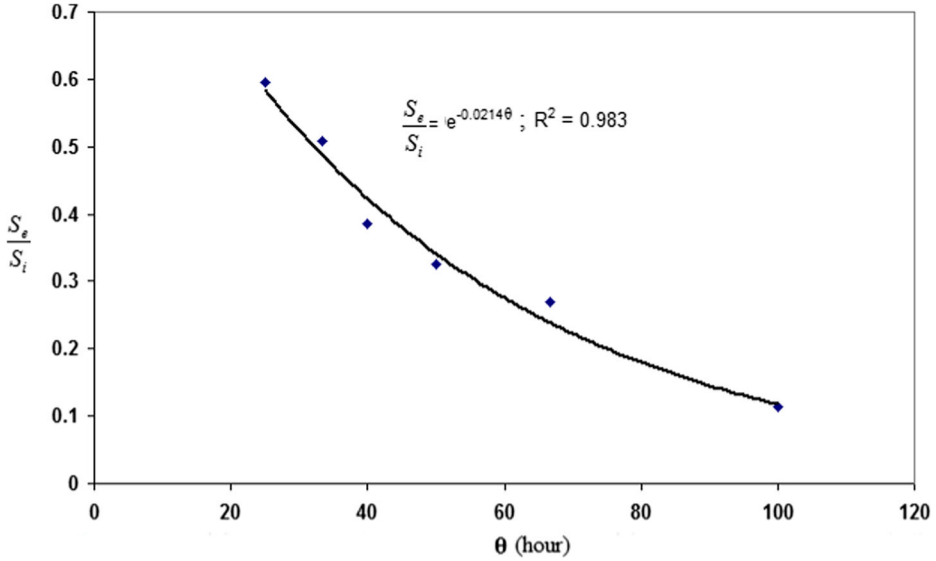


**Fig. 5** Variation of the rates of exit to inlet concentration of substrate ($\frac{S_e}{S_i}$) with hydraulic retention time ($\theta$) in the bioreactor

fungal cells [19, 20]. Further, the bacterial species also have plasmid-mediated catabolic genes such as *opd/mpd* [21, 22] which are responsible for the enhanced CP degradation. These genes are completely absent in the fungal species.

**Table 1** Comparison between performance of bacterium and fungus in bioreactor

| S. no. | Parameters | Bacterium (*Pseudomonas* sp.) | Fungus (*Aspergillus* sp.) |
|---|---|---|---|
| 1 | Kinetic parameters | | |
| | $K_s$ (mg $L^{-1}$) | 270.3 | 263.0 |
| | $V_{max}$ (mg $L^{-1}$ $d^{-1}$) | 50.78 | 92.07 |
| | $V_{max}/K_s$ ($h^{-1}$) | 0.00783 | 0.0146 |
| 2 | Optimal process parameters | | |
| | Inoculum level | $300\times10^6$ CFU $mL^{-1}$ | 2.5 mg(wet weight)$mL^{-1}$ |
| | pH | 7.5 | 7.0 |
| | Temperature | 37 °C | 28 °C |
| | Dissolve oxygen (mg $L^{-1}$) | 5.5 | 5.8 |
| | CP concentration (mg $L^{-1}$) | 500 | 300 |
| 3 | Removal efficiency (%) | 91 at an inlet load of 300 mg $L^{-1}$ $d^{-1}$ | 89 at an inlet load of 180 mg $L^{-1}$ $d^{-1}$ |
| 4 | Elimination capacity | 289.8 mg $L^{-1}$ $d^{-1}$ at an inlet load of 480 mg $L^{-1}$ $d^{-1}$ | 199.7 mg $L^{-1}$ $d^{-1}$ at an inlet load of 288 mg $L^{-1}$ $d^{-1}$ |
| 5 | $k_1$ ($h^{-1}$) at flow rate (15–40 mL $h^{-1}$) | 0.0191–0.0065 | 0.015–0.005 |
| 6 | $k$ ($h^{-1}$) | 0.027 | 0.021 |
| 7 | Duration of operation (days) | 42 | 45 |

## Conclusions

Based on the above results, it is possible to conclude that the *Aspergillus* sp. is quite efficient in the biodegradation of CP. The operating range for continuous bioreactor is found to be in the range of 180 to 250 mg $L^{-1}$ $d^{-1}$. The TCP production has increased constantly and might have played a role in the lowering of the removal efficiency. The continuous packed bed bioreactor is quite stable and regains its performance quickly after the perturbation in the flow rate. The bacterial species are better CP degraders than the fungal species.

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