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Removal of chlorophenol using a batch and airlift inner loop bioreactor using *Aspergillus fumigatus*

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ABSTRACT

In the current study, elimination of chlorophenol by the *Aspergillus fumigatus* was investigated in an airlift inner loop bioreactor (ALBR). The effect of empty bed residence time (EBRT), concentration of peptone and initial chlorophenol concentration on the removal efficiency of chlorophenol was examined. Bioreactor displayed a high elimination rate of 16.59 mg/(L h) at the experimental conditions of 40 h EBRT, 150 mg/L initial chlorophenol concentration, and 0.2 g/L peptone. The removal efficiency was around 50% at 25 h EBRT. The experimental outcomes displayed that chlorophenol and removal efficiency of the bioreactor were higher at the lower concentration of peptone. Also, the *Aspergillus fumigatus* used chlorophenol as a carbon source, as demonstrated by the growing biomass concentration at fixed peptone concentration. The ALBR restored to the original operation after the transient condition (shutdown, restart, and shock load) and the constant reactor performance was maintained. The biological treatment of chlorophenol is one of the promising treatment techniques as it is relatively less expensive and results in degradation of contaminants.

Keywords: Chlorophenol; Airlift bioreactor; Biodegradation; Wastewater; *Aspergillus fumigatus*

1. Introduction

Recent years have witnessed a rapid increase in industrial activities to meet growing consumer needs. Several unused raw materials and undesirable by-products from industrial processes often tend to stay in the environment thereby causing detrimental impacts on ecosystem [1–3]. The contaminants often find their ways to enter into the food chain and adversely affect health of flora and fauna [4,5]. Chlorophenols (CPs) constitute a class of environmental contaminants that are highly persistent and toxic. They are mainly originated from a variety of sources such as industrial wastes, weed killers and degradation of chlorinated hydrocarbons [6]. CPs are recalcitrant and undergo accumulation in biomass and cause harmful effects on health. Chlorophenolic compounds have been confirmed to cause genetical change, heart disease, immune based disease, and might cause loss of life [7–9]. Various environmental remediation approaches have been investigated for removal of aqueous contaminants including chlorophenols such as adsorption, ion-exchange, solvent extraction and membrane technologies [10,11]. However, most of these methods have limitations owing to their huge

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cost of operation, generation of lethal sub products and less energy productivity. These treatments usually convert the toxic substance from one phase to another. On the other hand, the treatment using biological approach is a potential alternative with promising removal efficiency and comprehensive mineralization of the CPs. The biodegradable and energy effective action offers a sustainable solution [9,12].

Many researchers have suggested a variety of microbes that consume CPs as a carbon and energy source such as *Chlorella vulgaris*, *Coenochloris pyrenoidosa*, *Candida tropicalis*, *Pseudomonas putida*, *Burkholderia* sp., and *Kocuria rhizophila* [13–15]. Biodegradation of CPs in several bioreactor schemes in low oxygen or no oxygen situations has been studied. Bioreactors like packed bed reactors [16,17] and up-flow anaerobic sludge blanket reactors have been investigated regarding their efficacy in contaminant removal [18]. Aerobic biodegradation is frequently chosen for minor CPs like as mono and di-CPs and occasionally tri-CPs. Though, for the handling of complex CPs such as 3,4 and 5-CPs, an anaerobiotic biodegradation is preferred [12].

Wastewater treatment plant normally covers the combination of diverse artificial organic compounds (AOCs) along with biological substrates. Commonly recommended plants are unable to adequately manage stubborn compounds, and they typically exit without being entirely treated. Process development and optimization can aid in the treatment of resistant substances [19]. This necessitates knowledge of biomass acclimation, the contact among biomaterials substrate and recalcitrant chemicals and reactor operational requirements. For higher performance and efficiency of the bioreactors, several variables including space time, inlet loading rate and the existence of other minor carbon and nitrogen sources must be evaluated and improved [20].

Airlift inner loop bioreactor (ALBR) is a promising option with immovable parts or mixer, thus being cost-effective. In ALBR, forced gas is cast-off for interior blending and airing [21]. The difficulties related to flooding in packed bed reactor can be avoided in ALBR [22]. The biological removal or modified phosphorus from municipal wastewater was performed by one-stage phoredox reactor with hydraulic up-flow [23]. The removal of 4-nitrophenol was performed using alginate-multiwall carbon nanotube [24]. The wastewater was treated in the small-scale integrated fixed-bed activated sludge [25].

A few works on biodegradation of CP and other compounds have been reported by means of ALBR [22]. For immobilisation of pure culture *Achromobacter* sp., they used a ceramic honeycomb-like building put within the riser. For the elimination of 2,4,6-trichlorophenol, a packed bed bioreactor prepared with a net draught tube riser (PB-ALBR) was built [26]. For the degradation of CP in this investigation, a basic ALBR was used without any microbe immobilisation [27]. Because there is no agitator for mixing and no carrier for immobilising microorganisms, the bioreactor is simple to use and operate. The main aim of the research work was to use *Aspergillus fumigatus* to continuously biodegrade CP in an ALBR loop bioreactor. The impact of numerous aspects such as empty bed residence time (EBRT), peptone content, and initial substrate concentration on ALBR removal effectiveness was investigated during continuous operation.

2. Materials and methods

2.1. Reagents

Analytical grade chlorophenol (98% purity) was provided by HiMedia. A standard solution of 4-chlorophenol was produced in 0.02 M NaOH, and the pH was adjusted to 7 with orthophosphoric acid of 1 M. The other mineral compounds used in the tests were of analytical grade and procured from Merck, India. For high-performance liquid chromatography (HPLC) analysis, HiMedia, India provided HPLC grade reagents.

2.2. Microorganisms

The MTCC No. 343, *Aspergillus fumigatus* was obtained from Institute of Microbial Technology (IMTECH), Chandigarh, to evaluate its capability to reduce CP.

2.3. Preparation of growth medium

The growth medium for *Aspergillus fumigatus* was Czapek Yeast Extract Agar (CYEA). CYEA medium consist of 10 mL of Czapek concentrate, 1.0 g of K_2HPO_4, 5.0 g of yeast extract, 30 g of glucose, 15 g of agar, 1 L of distilled water. The Czapek concentrate is made by $NaNO_3$ – 30.0 g, KCl – 5.0 g, $MgSO_4{\cdot}7H_2O$ – 5.0 g, $FeSO_4{\cdot}7H_2O$ – 0.1 g, 1 L of distilled water, 100 mL Czapek concentrate can be kept without disinfection. The precipitate of $Fe(OH)_3$ can be re-suspended by shaking well before use.

2.4. Technique for preservation of cells

The microbes of *Aspergillus fumigatus* were preserved in the medium of agar. The slants were made with agar-agar. New culture was shifted to 100 mL of liquid media. It was prepared by CYEA without agar to cultivate the microbes on a big scale. The media was allowed to grow for 7 d. Liquid media of 100 mL was shifted into a 0.5 L vessel and these cultures were used for other experiments. For all the items, the media was autoclaved under 121°C at 1.1 kgf/cm^2 gauge pressure for 15 min. Strict measures were followed while adding and relocating the culture.

2.5. Preparation of CP solution

The stock solution comprising CP was ready by diluting the standard solution to the chosen concentrations. The CP concentrations were mixed in the array of 50–500 mg/L. Standard solution of aqueous CP was organized by dissolving the particular amount (1 g) of CP in distilled water.

2.6. Bioreactor medium

The bioreactor was nourished with the mineral solution medium comprising CP as a carbon and energy source. The composition of the mineral solution medium is shown in Table 1. The minor nutrients and CP served as supplement to the medium and were sterilized by sieve sterilizing. The pH of the solution was adjusted to 7.35 ± 0.1 by means of 1 M H_3PO_4 and 1 M NaOH. For this bioreactor study, the standard was equipped by means of the distilled water.

Table 1
Composition of synthetic wastewater

Nutrients	Concentration (g/L)
Diazanium sulfate	0.2
Potassium dihydrogen phosphate	1
Sodium hydrogen phosphate	1
Magnesium sulfate heptahydrate	1
Yeast extract	0.04
Chlorophenol	50–150 × 10^{-3}
pH	7–8

2.7. Airlift bioreactor

ALBR was fabricated using acyrilic glass as reported in literature [22]. A 4 LPM air flow rate was used during the studies. The experiments were performed at 32°C. The effects of peptone concentration, CP concentration and EBRT on removal performance were investigated.

2.8. Experimental conditions

The present investigation was focused on continuous removal of CP for 120 d, divided in four phases. During each phase, the experiment was conducted for about 30 d by varying the EBRT. After every 10 d the experimental conditions were modified by changing the initial CP concentration and peptone concentration as given in Table 2. Finally, the performance of the reactor was investigated by shut down and shock load of the reactor.

2.9. Analytical methods

Biomass concentration was determined by measuring optical density at 600 nm by UV-Visible spectrophotometer (Elico BL-200, India). The residual concentration of CP was determined by HPLC system (JASCO, US) coupled with MD-2015 photodiode array detector and 2080 plus isocratic pump. The sample of 1 mL volume was centrifuged at 10,000 rpm for 10 min and supernatant was filtered through 0.22 μm filter before analysis. The column used was Agilent TC-C18 (25 mm 94.6 mm). The sample was eluted at a flow rate of 0.75 mL/min with mobile phase consisting of methanol:water:acetic acid (80:19:1%). The detection wave length was 280 nm. The CP degradation (%) was calculated by analyzing the area under the curve.

3. Results and discussion

The ALBR was run in steady state for 120 d, in which the influence of various factors such as EBRT, concentration of peptone, and inlet loading rate on CP elimination were investigated and recorded in Table 2. During continuous operation, the bioreactor demonstrated good CP removal efficiency. This acclimatisation period lasted for 10 d, following which the operation was run in a continuous manner.

3.1. Effect of initial CP Concentrations

By altering the EBRT of 25–40 h and peptone concentration (0.2–1 g/L) and progressively rising CP concentration, the consequence of inlet CP concentration on the removal efficiency of the bioreactor was investigated. When the starting CP content was augmented from 20 to 150 mg/L, the bioreactor showed better than 99% removal efficiency. Fig. 1 depicts the influence of inlet substrate concentration on biodegradation. The CP concentration was rapidly increased after 100 mg/L to test the bioreactor's shock load steadiness. Even when subjected to sudden loads, the bioreactor maintained its steadiness and removal efficiency. Complete mineralization was seen at lower CP concentrations, with no evidence of the intermediate. The bacteria were able to use CP as only carbon source, according to these findings [21]. Another researcher reported that the significant point is that 2,4-DCP at slightly elevated concentrations (>20 mg/L) within the reactor caused a strong competitive inhibition on 4-CP degradation [19].

Table 2
Experimental conditions for the removal of chlorophenol using ALBR

Stages	Days	EBRT (h)	Peptone concentration (g/L)	Initial chlorophenol concentration (mg/L)
I	0–10		0.2	50
II	11–20	25	0.5	100
III	21–30		1.0	150
IV	31–40		0.2	50
V	41–50	30	0.5	100
VI	51–60		1.0	150
VII	61–70		0.2	50
VIII	71–80	35	0.5	100
IX	81–90		1.0	150
X	91–100		0.2	50
XI	101–110	40	0.5	100
XII	111–120		1.0	150

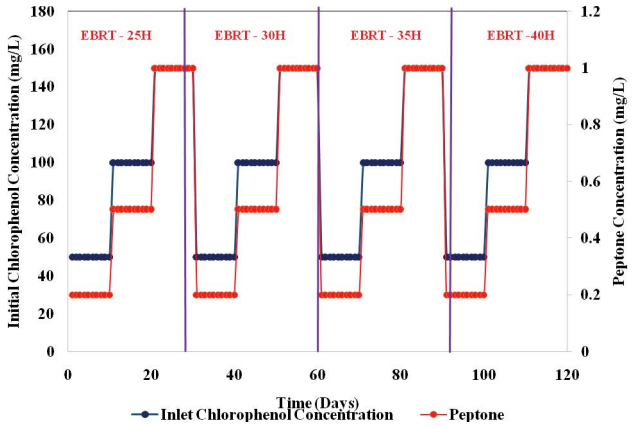

Fig. 1. Inlet conditions of chlorophenol and peptone in ALBR using *Aspergillus fumigatus*.

3.2. Peptone effect

The influence of peptone on CP exclusion in ALBR was investigated under diverse operational circumstances. The ALBR was started with 0.2 g/L peptone and 25 h EBRT at 50 mg/L CP concentration. Fig. 2 shows the influence of peptone on the removal of CP by ALBR. The bioreactor removed 88.6% of the initial CP in the influent. Furthermore, when the initial CP content was increased from 100 to 150 mg/L, the removal efficiency rose to nearly 95% after 25 h of EBRT. When using EBRT for 25 h, the removal efficiency climbed to roughly 90.6% and was stabilised using 1 g/L peptone. The peptone concentration in the influent was around 0.2 g/L from the 30th day of the process, with an initial CP concentration of 50 mg/L and 30 h increase in EBRT. In the incidence of low peptone concentration, the removal efficiency of the bioreactor increased from 90.6% to 94% at steady-state. The concentration of peptone in the inflow was augmented to 0.5 g/L on the 30th day of the process. At this time, the EBRT was set to 30 h, and the CP concentration in the inflowing was steadily raised. The feed concentration of chlorophenol to the bioreactor was 150 mg/L. The performance of bioreactor significantly augmented as the concentration of peptone was increased from 0.2 to 1 g/L. The bioreactor was able to eliminate CP in the feed with more than 94% removal efficiency for 150 mg/L feed concentration.

The peptone concentration in the inflow was about 0.2 g/L from the 60th day of the process, with an initial CP concentration of 50 mg/L and a 35 h increase in EBRT (Fig. 2). In the occurrence of low concentration of peptone, the bioreactor's removal efficiency increased from 94% to 97.3% at steady state. The peptone concentration in the inflowing was increased to 0.2 g/L on the 60th day of the operation. At this time, the EBRT was set to 35 h and the CP concentration in the inflow was steadily raised. CP was removed at a rate of 150 mg/L in the ALBR. In the occurrence of 0.2 to 1.0 g/L peptone, the bioreactor performance improved dramatically. The bioreactor was able to eliminate up to 150 mg/L of CP from the influent with a removal efficiency of above 97.3%. Increasing the EBRT from 5 h to 40 h yielded similar effects. At an EBRT of 40 h, the maximal elimination of 100% was achieved. Lower peptone concentrations resulted in a considerable rise in biomass concentration in the medium. The mixed consortium can use CP as a carbon source with wide-ranging mineralization.

The occurrence of a greater concentration of peptone inhibits the *Aspergillus fumigatus* from degrading CP. The removal efficiency rose at low peptone concentrations, though minimum level of peptone was essential to sustain the operation of the system. This is because the peptone was used as a nitrogen source for the *Aspergillus fumigatus* during the acclimation stage. Furthermore, it was observed that microbial growth increased when peptone concentrations decreased and CP concentrations increased. The observation could be plausibly attributed to the utilization of chlorophenol by *Aspergillus fumigatus* as a source of carbon. The effect of peptone on the degree of CP elimination by *Aspergillus fumigatus* is depicted in Fig. 2. The CP have been reported to degrade in the occurrence of additional carbon and nitrogen sources like as glucose, dextrose, peptone, and yeast extract in various studies. The breakdown of CPs increased in the occurrence of secondary carbon and nitrogen sources [18,19,28].

There are several reports on the degradation of chlorophenols in the presence of other carbon and nitrogen sources such as glucose, dextrose, peptone, and yeast extract. In the presence of secondary carbon source, an increase in the degradation of chlorophenols CP and 2,4-CP [19], phenol and chlorophenols [29], pentachlorophenol and glucose [28], phenol and chlorophenols [29], 4-chlorophenol and glucose [30] was observed. However, there are others reports that discussed the opposing outcomes that sometimes the degradation of higher chlorophenols were inhibited [14,15]. More systematic studies are needed in this regard to investigate the complex mechanisms involved in degradation of chlorophenols and related compounds.

3.3. Effect of EBRT

For 120 d, the outcome of EBRT on the elimination of CP by ALBR was investigated. The starting concentrations of peptone and CP were around 0.2–1 g/L and 50–150 mg/L, respectively. Fig. 3 depicts the change in CP biodegradation at various EBRTs. There was no dramatic variation in removal efficiency when the EBRT was gradually raised from 25 to 30 h. At 40 h of EBRT, the bioreactor had removed more than 100% of the CP. Fig. 4 displays the outcome of varying EBRT on the volumetric elimination rate of CP. To restore the biodegradation capacity of reactor, the EBRT was gradually increased to 25, 30, and 40 h. It takes some time for the bioreactor to regain its full degrading efficiency. There was a modest increase in biodegradation rate after 25 h of EBRT. When EBRT was raised to 30 h, the biodegradation rate eventually increased to 66% at steady state. With the passage of time and a rise in EBRT, the bioreactor was able to restore its biodegradation capability. Fig. 4 shows the link among volumetric loading rate and volumetric removal rate. The removal rate rose exponentially with the loading rate up to 16.67 mg/(L h), with a volumetric removal rate of 16.59 mg/(L h) being reported. Conversely, when the loading rate has risen to 22.25 mg/(L h), the volumetric removal rate was drastically reduced, as seen in Fig.

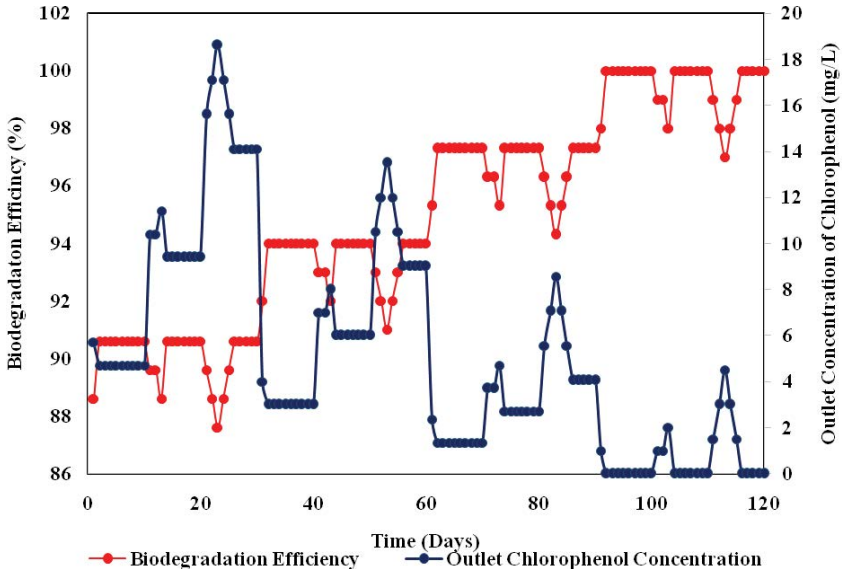

Fig. 2. Biodegradation efficiency and outlet chlorophenol concentration with time.

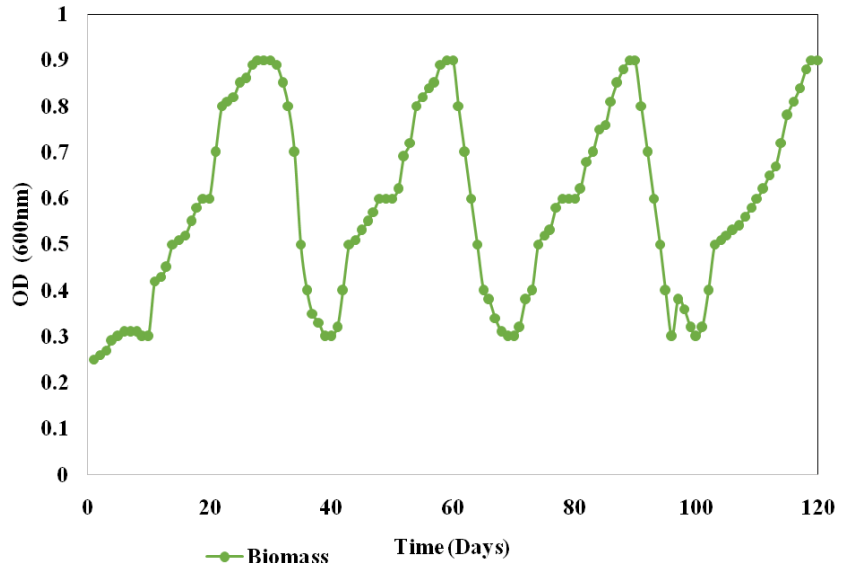

Fig. 3. Change in microbial concentration with time measured with optical density.

4. The EBRT should be maintained at 24 h and the loading rate at 16.67 mg/(L h) to achieve a peak or better than 98% removal rate. In another investigation, it was discovered that lowering EBRT in upflow anaerobic sludge blanket (UASB) reactor reduced the breakdown of 4-CP. At 16, 12, 8, and 6 h, the UASB found 90.1%, 88.3%, 84.6%, and 83% degradation of 4-CP, respectively [31]. Table 3 indicates the behaviour of individual bioreactors for the removal of CPs, with the number of research findings indicating that 4-CP could be removed at a loading rate of less than 200 mg/L. The bioreactor obtained 99.8% removal for a higher loading rate of 400 mg/(L d) in the current study. The efficacy of ALBR in removing 4-CP is outstanding, and it may be effectively used to infected water [22,26]. Hydraulic residence times above 15 h resulted in more than 90% COD and complete 4-CP and toxicity removals along with well settling sludge [31]. In another work, it was concluded that degradation of 4-CP was decreased with a decrease in EBRT in UASB. The elimination of 4-CP up to 800 mg/L of loading rate with 90% efficiency [30]. Similar studies regarding the removal of chlorophenol using a mixed microbial consortium in ALBR achieved the 99.8% removal, however transient conditions such as shock load and shut down were not examined there [22]. In the present study, the bioreactor had achieved 99% removal for higher loading rate of 150 mg/(L d).

3.4. Response to shock loads on the ALBR

Bioreactors are commonly predictable to handle both steady state and transient state influent loads successfully.

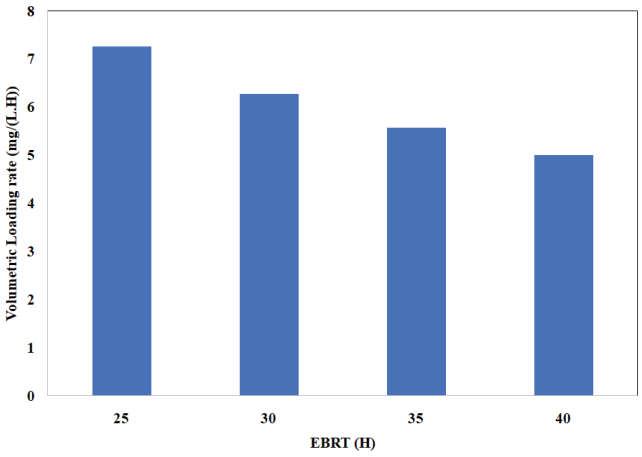

Fig. 4. Effect of residence time rate on the volumetric removal rate of chlorophenol (50 mg/L) in the ALBR with 0.2 g/L of peptone.

Procedures like as ALBR shutdown for machine-driven maintenances, care or shut down for a limited day (through vacations or holiday interruptions), and occasionally for an extended period commonly should not be evaded. Throughout such provisional breaks of no impurity loading, the microbes in the ALBR are exposed to lack of nutrient supply. When the bioreactor process is restarted and carbon source chlorophenol is fed to the reactor, the microbial growth response is dependent on numerous factors such as microbial population and movement, period of the starvation, existing state of the packing material and the involved biomass, and the supply of CP.

From 120th day onward the ALBR was provisionally locked down for a period of 24 d. Through this period, no CP was provided to the ALBR. To avoid anaerobic circumstances of the microbial arrangement, air EBRT of 2 h was maintained to the ALBR. From the day 145, later getting additional nutrient stream, the ALBR was resumed with the initial CP concentration of 0.02 g/m^3 a gas flow rate of 0.12 g/m^3, and a corresponding EBRT of 2.47 min. The outlet concentration of CP in ALBR was measured daily. On the first day (145th day) of the operation the biodegradation efficiency of 40% as shown in Fig. 5. After 7 d of restart the ALBR reaches the maximum biodegradation efficiency as seen. On the 155th day of operation the initial CP and EBRT was increased to 100 mg/L and 30 h respectively. On the 156th day of the operation the biodegrading efficiency of 90% shown in Fig. 6. After 7 d of restart the ALBR reaches the maximum biodegradation efficiency as seen in Fig. 6.

The restoration of system performance after being subjected to 14 d of malnourishment during the transient operation showed the capability of biomass to restore to normal state. The outcome from this work confirmed that the ALBR was proficient of enduring comparatively long term malnourishment with speedy regaining to full performance when pollutant load restarted. The biodegradation efficiency was originally lesser for few hours after resumption. Then, the biodegradation competence progressively increased and continued to exhibit high efficiency for 5 d. The microbes in the ALBR displayed good restorative action. This work is significant regarding the use the ALBR for industrial scenario where the inflow load fluctuates on a daily basis. The effect of inlet loads on the biodegradation efficiency of the ALBR is an important aspect of consideration.

In the current work, the constancy of the ALBR is evaluated by exposing the column to sudden loading situations for a period of 45 d for CP immediately after the shut down and restart days of ALBR. The procedure for shock loading conditions is taken from the literature [31] and the days of operation of shock loading is fixed based on their normal days of operation proportionately. The operational settings for shock loading process for CP removal are stated. It can be displayed from Fig. 6 that through the first 10 d (Normal Load I), when initial concentration is kept in the range of 50 mg/L at a EBRT of 50 h, the removal efficiency is found in the range of 91%. For the CP initial loading rate of 4.08 g/(m^3 h), the corresponding elimination capacity found is in the range of 3.5 g/(m^3 h).

The higher standards of removal efficiency and elimination capacity found show that when the ALBR is adapted with the microbial culture, it can express actual higher removal rates for lesser toxin concentration. For the following 10 d (Shock Load-I), the EBRT is decreased in the range of 50–25 h and the removal efficiency found is in the series of 80% which is immobile display comparatively well removal efficiency. The inlet CP load is in the range of 20 g/(m^3 h) and the elimination capacity calculated in this phase is ranging from 12 g/(m^3 h). For next 10 d of operation (Normal Load-II), when inlet concentration is maintained in the range of 50 mg/L at EBRT of 50 h,

Table 3
Comparative summary of chlorophenol removal using various reactors

Reactor	Initial concentration (mg/L)	Retention time (h)	Inlet loading rate (mg/(L d))	Removal efficiency (%)	References
Fluidized bed reactor	99.13	24.4	97.5	98.7	[27]
Packed bed reactor	20	2.78	172	100	[16]
Continuous stirred tank reactor	20	4.72	115	100	[28]
Up-flow anaerobic sludge blanket	40	12	80	88.3	[30]
Air lift bioreactor	400	24	400	99.8	[21]
Air lift bioreactor	150	50	600	99	This study

the removal efficiency is obtained in the range of 91% in Fig. 6. For the CP inlet loading rate of 4.08 g/(m^3 h), the corresponding elimination capacity obtained is in the range of 3.5 g/(m^3 h) for ALBR. For the next 10 d (Shock Load-II), the EBRT is maintained at 50 h and the inlet concentration increased from 50 to 150 mg/L, the removal efficiency found is in the series of 80% which is immobile viewing moderately better removal efficiency. Another study reported the dynamic behaviour of the bio filter was tested at different process conditions through vigorous short, medium and long-term shock loads and the stability of the biomass within the reactor was apparent from the fast response of the bio filter to recuperate and handle intermittent shutdown and restart operations. The researchers reported that the bio filter responded well to the sudden changes and a higher removal efficiency of 79% was achieved with an inlet xylene concentration of 5 g/m^3[32].

Future studies should investigate integrated approaches for removal of chlorophenols and combining with portable water quality monitoring devices for in situ detection [33,34]. Lab-on-a-chip microfluidic technology offers a promising option in this regard [35,36]. Further, the possibilities of recovering the valuable substances from contaminated aqueous streams should be explored to deliver sustainable technological solutions [37].

4. Conclusion

Utilizing *Aspergillus fumigatus* as a single strain, an airlift bioreactor was used to assess the elimination of CP in

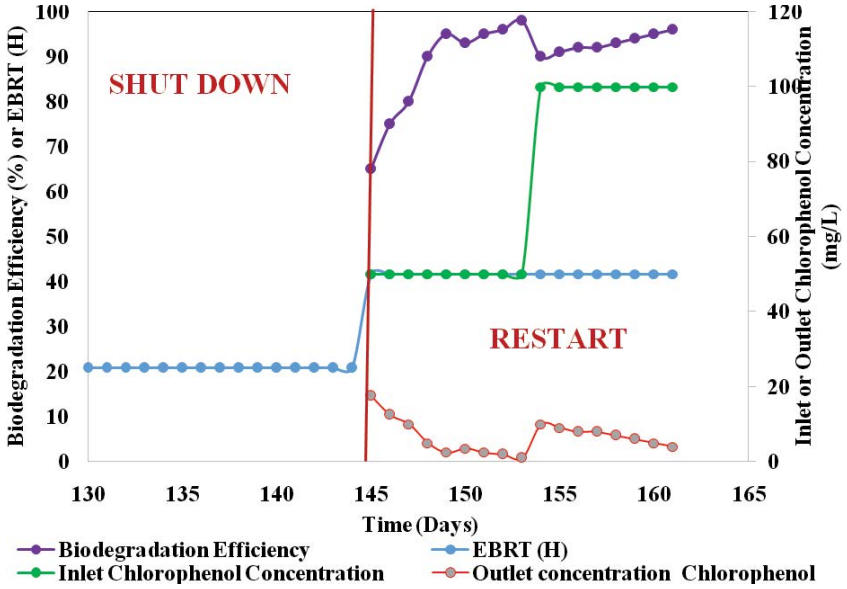

Fig. 5. Effect of shutdown period on the removal efficiency of chlorophenol using ALBR.

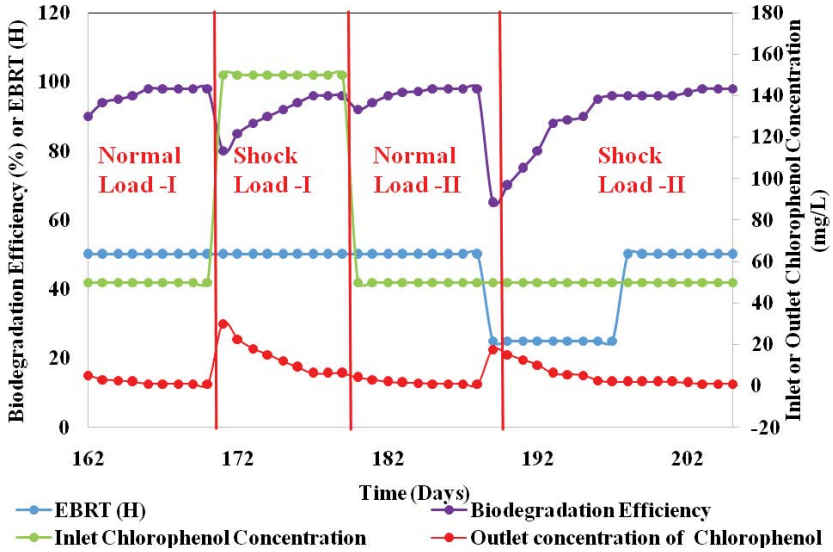

Fig. 6. Effect of shock load on the removal efficiency of chlorophenol using ALBR.

synthetic wastewater. The findings revealed that EBRT, initial CP concentration, and peptone concentration have significant impact on CP elimination efficiency. During functioning, the bioreactor had an outstanding removal efficiency for CP, with an elimination rate of higher than 99%. The optimal EBRT for the bioreactor was discovered to be 40 h, and anything less resulted in microbial saturation. The result of peptone demonstrated that a lower peptone levels increased percentage removal; however, some peptone is required to sustain removal of CP. At 40 h of EBRT and 0.2 g/L peptone, the maximal initial substrate concentration of 150 mg/L CP was efficiently eliminated up to 99%. The ALBR responded quickly to the transient state (shutdown, restart, and shock load) and maintained its stability. The pure strain's durability and efficiency under various working circumstances indicate its suitability for in situ bioremediation.

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