Chapter 6: Simultaneous removal of *p*-cresol and methylene blue dye through Upward-Flow Packed Bed Biofilm Reactor (UFPBBR): Kinetics, Phytotoxicity and Bacterial toxicity assessment

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6.1. Introduction

The expansion of industrial activities has led to a significant increase in the volume of wastewater containing various toxic chemicals. The pollutants such as PAHs (polycyclic aromatic hydrocarbon), BTEX (Benzene, Toluene, Ethylbenzene and Xylene), dyes, heavy metals, phenolic compounds, and pesticides are generally found in industrial wastewater (Swain et al., 2020). The food, textile, apparel, fine chemicals, and bulk chemicals sectors all employ phenolic compounds and dyestuffs in significant amounts as raw materials, intermediate compounds, and finished goods (Abu-Nada et al., 2021). Phenol and its derivatives are the most prevalent organic contaminants, and even in low quantities, their presence influences the use of water resources (Alimohammadi et al., 2022). *p*-Cresol (4-Methylphenol), a phenolic compound with the formula $CH_3C_6H_5O$ and water solubility of more than 2.15 g L^{-1} at 25 °C, has been designated as a persistent priority hazardous chemical (Jaafari et al., 2018). It exerts harmful effects on the eyes, skin, and respiratory tract and produces nervous system depressive disorders. It may also adversely impact the cardiovascular system, lungs, kidneys, and liver, presenting a severe environmental hazard (Mahdavianpour et al., 2018b). Similarly, methylene blue dye (MBD) is widely used in industries (e.g., textile, paper, petrochemicals, etc.) (Bharti et al., 2019). MBD has the capability of inducing carcinogenesis as well as genetic changes. The by-products of the degradation of dyes in water provide a substantial risk to the environment (Bharti et al., 2019).

Due to the complicated chemical structure and toxicity of *p*-cresol and dyes, it is essential to eliminate these pollutants from industrial effluents before their discharge into the environment. The degradation of cresols and MBD has been reported using several physical and chemical approaches. These processes include ozonation, flocculation, coagulation, solvent extraction, adsorption, chemical oxidation, and membrane process (Bharti et al., 2019, Jaiswal et al., 2023). However, the drawback of such techniques is that they consume huge energy, uneconomical, and produce metabolites that may be more toxic than the original pollutants (Jaiswal et al., 2023, Kuc et al., 2022). On the other hand, biodegradation is a cost-effective and environmentally benign technique that ensures that the contaminants are almost completely degraded without generating potentially toxic by-products (Bharti et al., 2019, Kuc et al., 2022).

Usually, bioremediation is carried out through two modes: (i) free cells and (ii) immobilized cells processes. The free cell system is associated with applying microorganisms as suspended cells, whereas immobilized cells refer to the attached microorganism onto porous support media. The free microbes have several disadvantages, such as losing microorganisms, toxic effects from high ambient concentrations, and unpredictable microbial growth rates (Sonwani et al., 2019). As a result, immobilization technology is required to overcome these disadvantages (Bao et al., 2021). Since the microorganisms are immobilized on the carrier, it provides a high removal efficiency of pollutants by preventing direct contact of microorganisms with the pollutant, chemical stability, and high biomass development in the face of adverse environmental circumstances (Maurya et al., 2022). Many researchers have observed that immobilizing the microbial cells in a suitable matrix can enhance the ability of microbial species to degrade organic pollutants during the bioremediation process (Shahabivand et al., 2022). Several carriers such as activated carbon (AC), sugarcane bagasse (SB), low-density polyethylene (LDPE), calcium alginate (CA), polyacrylamide, polyurethane foam (PUF) and polypropylene (PP) have been used to immobilize the microorganisms (Aurya et al., 2022, Sonwani et al., 2019). However, studies reported that PUF has excellent chemical resistance, high porosity, and stability, making it a superior carrier for immobilization (Majul et al., 2022). From the perspective of process engineering, PBBRs have numerous benefits, including high yield operation, convenience in scaling up, potential automation of separation processes leading to high degrees of purification, ability to treat large volumes of wastewater continuously by the specific number of immobilized cells, and the ability to reuse biomass (Hassan et al., 2019). The other benefit of PBBRs is the growth of micro-niches with varying oxygen concentrations inside the pore of carrier material and within the layers of the biofilm (Iliuta and Iliuta, 2022). Many reports of immobilized cells in PBBRs successfully employed to biodegrade various types of pollutants such as pesticides, mono and polycyclic aromatic hydrocarbons, dyes, pharmaceuticals, etc. (Sonwani et al., 2019, Zhou and Nemati, 2018). However, very few research articles are available on the simultaneous removal of *p*-cresol and methylene blue dye.

This study investigates the simultaneous biodegradation of *p*-cresol and MBD from wastewater using UFPBBR. Furthermore, attempts have been made to optimize the contact time, pH, and sodium acetate (SA) dose for maximum removal of *p*-cresol and MBD from wastewater. Kinetic study was performed to estimate the specific growth rate of microbes under different conditions.

The performance of the Upward-Flow Packed Bed Biofilm Reactor (UFPBBR) was analysed in terms of % removal of *p*-cresol and MBD at different flow rates. The phytotoxicity and bio-toxicity were also investigated.

6.2. Materials and methods

6.2.1. Composition of mineral salt medium

In this present study, *p*-cresol (99%) and MSM (Mineral salt medium) were prepared as per the methods given in **Chapter 3 (Section 3.2.1)** in detail. The stock solutions of *p*-cresol (1000 mg L^{-1}) and MBD (1000 mg L^{-1}) were prepared to obtain the various concentrations of *p*-cresol and MBD. The synthetic wastewater was prepared by adding *p*-cresol and MBD into MSM using the stock solutions. NaOH (0.1 M) and HCl (0.1 M) were used to adjust the pH of the synthetic wastewater.

6.2.2. Isolation of bacterial strain for effective biodegradation of *p*-cresol

The isolation of bacterial strain and their evaluation for effective biodegradation of *p*-cresol followed the methodology given in **Chapter 3 (Section 3.2.2)** in detail.

6.2.3. Upward flow-packed bed biofilm reactor setup

A UFPBBR was constructed using borosilicate glass. The reactor had a height of 65 cm and an outer diameter of 5.8 cm, while the inner diameter was 5.5 cm, with a working capacity of 1.54 L. Polyurethane foam (PUF) (1 cm × 1 cm × 1 cm) with porosity 0.60 was used as the support material to immobilize the bacterial consortium (*Serratia marcescens* strain HL 1 and *Ochrobactrum intermedium* VrB9), owing to strong resistance to water adsorption and high porosity (Moghaddam et al., 2019). Initially, the column was operated in batch mode (free cell) along with MSM as a nutrient source as well as *p*-cresol (100 mg L^{-1}- 400 mg L^{-1}) and MBD (50 mg L^{-1}-125 mg L^{-1}) separately as a carbon source for the bacterial consortium under aerobic conditions to observe the specific growth. To develop biofilm onto PUF, UFPBBR was filled with *p*-cresol (100 mg L^{-1}) and MBD (50 mg L^{-1}) for 15 days running the operation (Sonwani et al., 2019). After successful biofilm formation, all optimization and performance were evaluated. The constant volumetric air flow rate (~1 L min^{-1}) was maintained using a rotameter (Flow Point, India). Dissolved oxygen within the reactor and pH were measured using a DO meter (HD

2109.1; Delta OHM; Italy) and pH meter (HD 2305.0; Delta OHM; Italy). The experiments were conducted under closed laboratory conditions (30±2 °C). A peristaltic pump (ELECTROlAB, PP- 50 V) was used to supply wastewater to the reactor.

Table 6.1. Specification and operating condition of UFPBBR

Parameters	**Operating conditions**
Inlet flow rate	10 to 50 (mL min^{-1})
p-cresol and Methylene blue dye concentration	100 mg L^{-1} and 50 mg L^{-1}
HRT	4.3 to 0.85 (hr)
Pollutant loading rate	302.4 to 1512 (mg L^{-1} day $^{-1}$)
pH	7.0 ± 0.5
Dissolve oxygen	5 ± 1 (mg L^{-1})
Air flow rate	1 L min^{-1}

6.2.4. Microbial-specific growth kinetic

The bacterial growth kinetics at different concentrations of *p*-cresol and MBD were analysed as per the details given in **Chapter 3 (Section 3.2.5)**.

6.2.5. Analytical methods

Bacterial consortium concentration, residual MBD concentration, COD were analyzed by UV/VIS spectrophotometer (Perkin Elmer lambda 25 UV/VIS spectrophotometer) by taking optical density (OD) at 600 nm (Bera et al., 2019b). The residual concentration of *p*-cresol was analyzed as per the details given in **Chapter 3 (Section 3.2.4)**. Scanning electron microscopy (SEM, (Nova SEM 450, FEI company, USA (S.E.A.) Pvt. Ltd. Singapore) was used to evaluate the morphological characteristics of PUF. The bioluminescence intensities of the treated, untreated, and control samples were measured using a Horiba Fluorescence spectrophotometer (Model No.: PTI Quanta master TM 8000 series). The COD and TOC of the samples evaluated using the methods provided in **Chapter 5 (Section 5.2.3)**.

6.2.6. Biodegradation kinetic study

The general equation for the biodegradation of pollutants is given by

$$\frac{dC}{dt} = -k_p \times C_p^n \qquad (6.1)$$

where, k_p, C_p and n are the biodegradation rate constant for the pollutant, the concentration of the pollutant at any time t = t, and n order of reaction, respectively.

Integrating **Eq. (6.1)** for n = 0 and 1.

C_0 - C_t = k_{p0}× t……………………………………………………………………..(6.2)

ln ($\frac{C_0}{C_t}$) = k_{p1}× t……………………………………………………………...…………(6.3)

Where, k_{p0}, k_{p1} and C_0 are the biodegradation zero-order, first-order rate constant, and initial pollutant concentration, respectively.

6.2.7. Performance evaluation of upward flow packed bed biofilm reactor

After optimizing the process parameters, the performance of the UFPBBR was evaluated based on the percentage removal efficiency (RE), elimination capacity of the pollutant (ECp), and inlet loading rate of the pollutant (ILRp) by varying the inlet flow rate from 10 to 50 mL min^{-1}, using **Eq 6.4, 6.5,** and **6.5,** respectively.

% Removal efficiency (RE) = $\frac{C_{in}-C_{out}}{C_{in}} \times 100$ ………………………………………………...(6.4)

Elimination capacity (EC$_p$) (mg L^{-1}d^{-1}) = $\frac{C_{in}-C_{out}}{V} \times Q$…………………………….....……(6.5)

Inlet loading rate (ILR$_p$) (mg L^{-1} d^{-1}) = $C_{in} \times \frac{Q}{V}$……………………………………..……..(6.6)

where C_{in} and C_{out} denote the inlet and outlet concentration of the pollutant (*p*-cresol and MBD) (mg L^{-1}), respectively. In addition, Q represents the feed flow rate (mL min^{-1}), and V denotes the working volume of the bioreactor (L).

6.2.8. Phytotoxicity and bacterial toxicity assessment

Phytotoxicity of *Vigna radiata L.* (i.e., Mung seeds) were analysed as per the details given in **Chapter 3 (Section 3.2.8)**. The bacterial toxicity of treated, untreated, and controlled sample were analysed as per the details given in **Chapter 3 (Section 3.3.6**)

6.3. Results and discussion

6.3.1. Specific growth of bacterial consortium under the influence of *p*-cresol and methylene blue dye

The specific growth rate was determined by plotting a semi-logarithmic plot **(Eq. (3.3))** between biomass growths vs. culture period. **Fig. 6.1.** illustrates the change in bacterial consortium-specific growth with different concentrations of *p*-cresol (100 mg L^{-1} to 400 mg L^{-1}) and MBD (25 mg L^{-1} to 125 mg L^{-1}). Maximum specific growth rates for *p*-cresol (100 mg L^{-1}) and MBD (50 mg L^{-1}) were found at 0.21±0.0083 h^{-1} and 0.07±0.00284 h^{-1}, respectively. However, further increase in the concentration of *p*-cresol (100 mg L^{-1} to 400 mg L^{-1}) and MBD (50 mg L^{-1} to 125 mg L^{-1}) resulted in a continuous decrease in specific growth rate **(Fig. 6.1.).** The decrease in specific growth rate may be due to the toxic effect of substrate concentrations. So, for further studies, the most effective concentrations for simultaneous biodegradation of *p*-cresol and MBD were 100 mg L^{-1} and 50 mg L^{-1}, respectively.

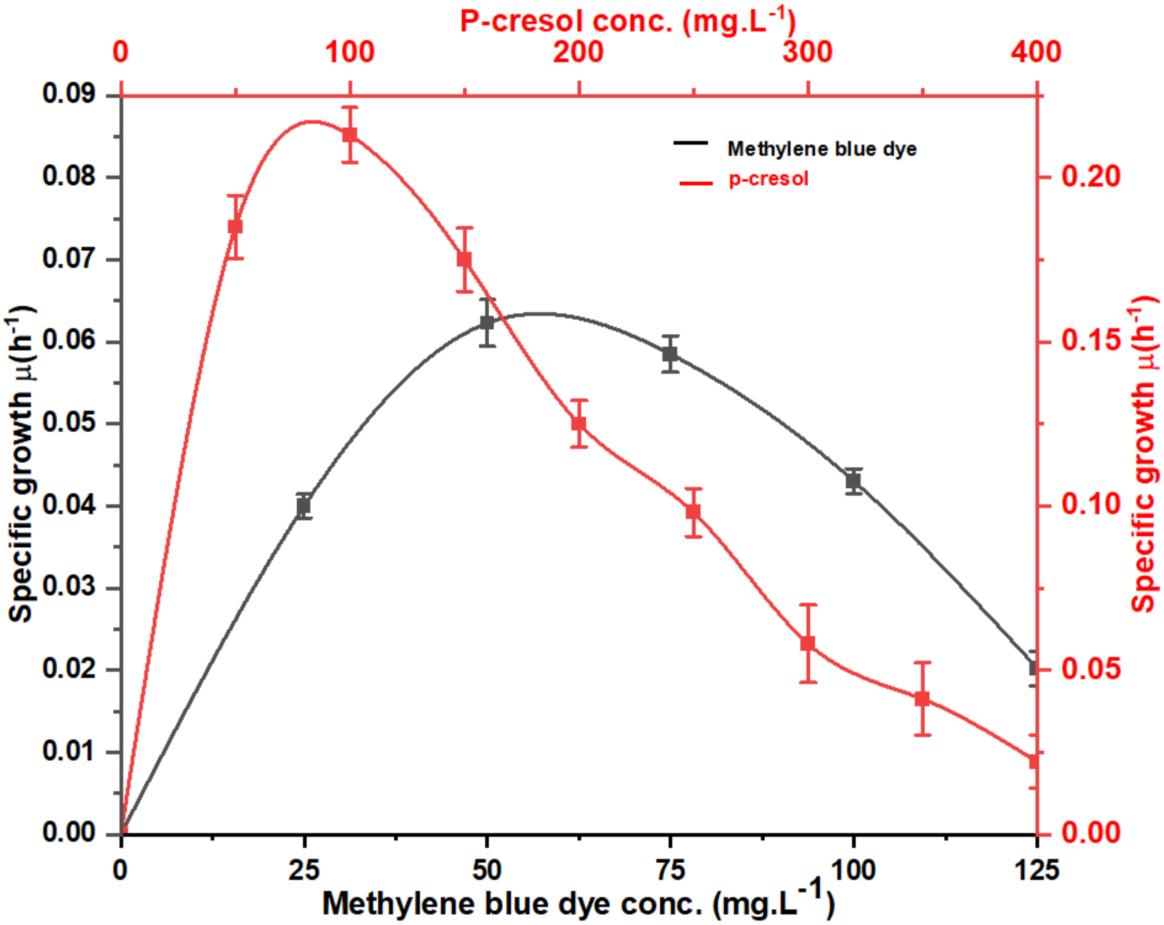


Fig. 6.1. Impact of *p*-cresol and MBA concentrations on the specific growth of the bacterial consortium

6.3.2. Morphology analysis of carrier

The bacterial consortium was initially introduced into a UFPBBR containing PUF carriers during the bioreactor process. The bioreactor was operated in a batch mode for 15 days, allowing the formation of biofilms on the carriers (Sonwani et al., 2021). Biofilm development in the media is influenced by various process variables, including pH, temperature, the availability of nutrients, and the concentration of substrate (Kureel et al., 2017). The morphology of PUF was examined using SEM before and after the bacterial consortium was immobilized. Before immobilization, the surface of the PUF contained numerous small pores that facilitated biofilm growth **(Fig. 6.2.).** Derakhshan et al. (2018) reported that microorganisms secrete and release extracellular polymers, significantly forming a robust biofilm capable of withstanding hydraulic pressure. The morphology of the carriers indicates the presence of biofilm formation on each UFPBBR carrier. These biofilm-formed carriers were then utilized to investigate *p*-cresol and MBD biodegradation from wastewater.

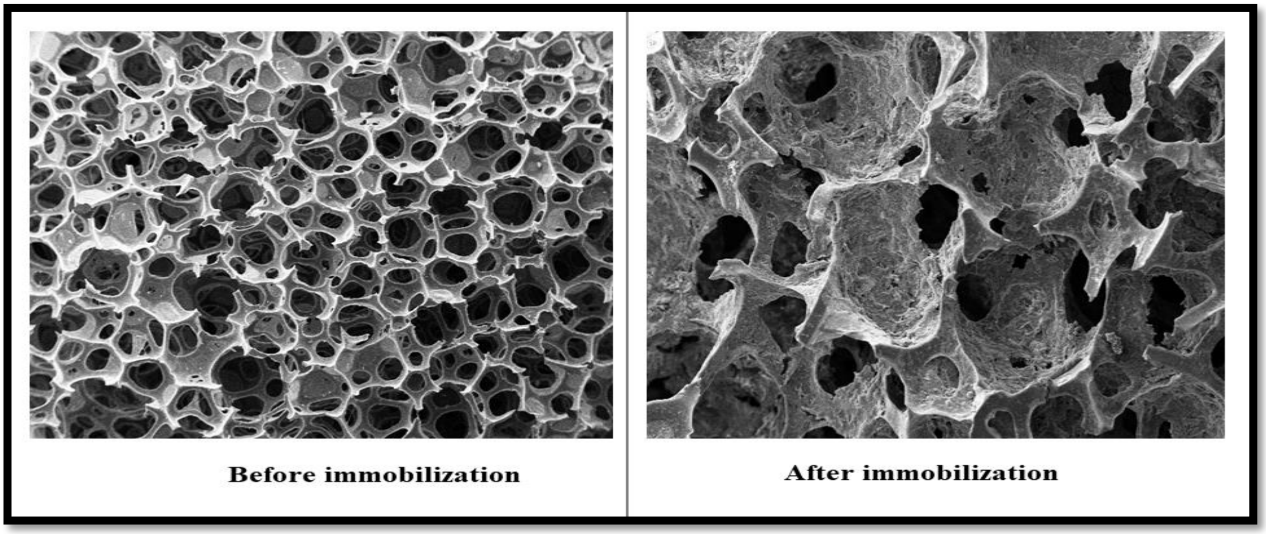


Fig. 6.2. SEM image of PUF before and after immobilization of bacterial consortium

6.3.3. Effect of contact time for simultaneous biodegradation of *p*-cresol and methylene blue dye

In the biodegradation process, the contact time of pollutants to immobilized cells is vital in determining treatment efficiency. Short contact time leads to insufficient interaction between microbial biomass and substrate (i.e., *p*-cresol and MBD). However, prolonged contact times can lead to the accumulation of toxic metabolites, excess biomass, and the inhibition of microbial activity, which can reduce the efficiency of the treatment. So, for simultaneous removal of *p*-cresol and MBD, contact time (1-7 days) was optimized at pH 7.0 and temperature 30±2.0 °C. **Fig. 6.3.** illustrates the effect of contact time on the degradation of *p*-cresol and MBD. It was found that removal efficiency continuously increases with the processing time of up to 5 days. The maximum removal efficiencies of *p*-cresol (100 mg L^{-1}) and MBD (50 mg L^{-1}) were 90.43±2.14% and 80.23±1.63% for 5 days of operation. After 5 to 7 days, no significant changes were observed in removing *p*-cresol and MBD. The reason might be that the rate of growth of bacterial cells is the same as the rate of death of bacterial cells. So, the optimal value for contact time was 5 days.

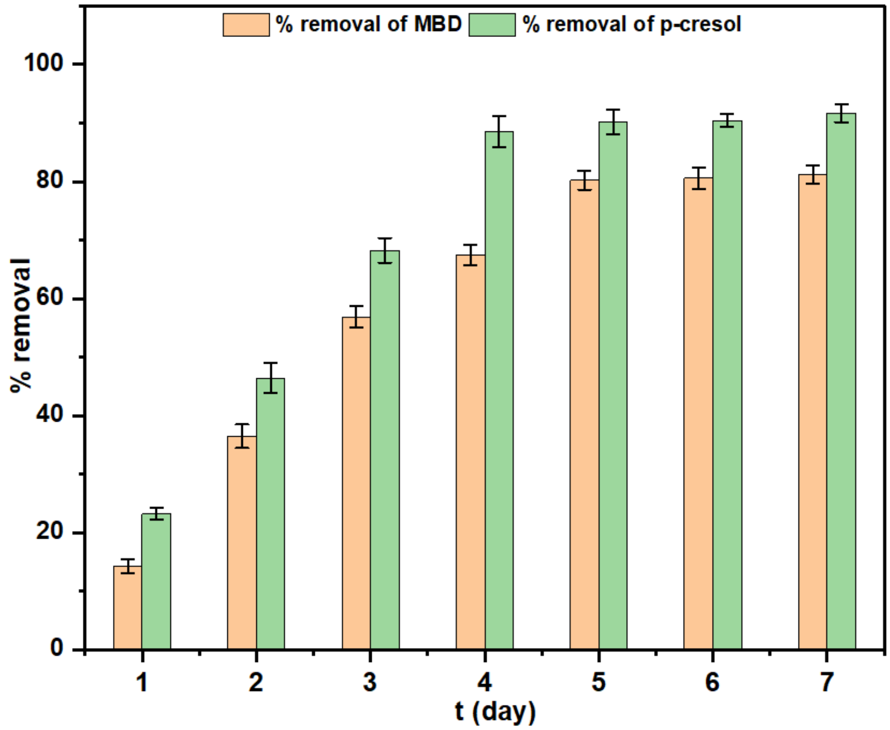


Fig. 6.3. Effect of contact time for biodegradation of *p*-cresol (100 mg L^{-1}) and methylene blue dye (MBD) (50 mg L^{-1})

6.3.4. Effect of pH on simultaneous biodegradation of *p*-cresol and methylene blue dye

Maintaining a specific pH range is essential for the survival of microbes and optimal functioning. It is reported that highly acidic or alkaline conditions can adversely affect the bio-activities of microorganisms (Huang et al., 2022). The effect of pH on the removal of *p*-cresol and MBD is illustrated in **Fig. 6.4.** Maximum removal efficiencies of *p*-cresol and MBD were found to be 89.85±1.26% and 80.73±1.83%, respectively at neutral medium (~ pH 7.0). However, removal efficiencies of both pollutants were continuously decreased in extremely acidic and alkaline mediums. The loss in % removal efficiency (for *p*-cresol and MBD) may be due to disrupting microbial communities, or enzymes secreted by microbial communities may get denatured, lose their catalytic activity, or become inert in an acidic or basic environment (Yaashikaa et al., 2022). Therefore, neutral medium was identified as the most favorable condition for bacterial consortium growth. Singh et al. (2022a) used *Serratia Marcescens* for *p*-cresol removal and found maximum removal efficiency at pH 7. Kishor et al. (2021) used *Bacillus albus* MW407057 to biodegrade the methylene blue dye and found pH 7 was optimum for the maximum degradation of methylene blue from wastewater.

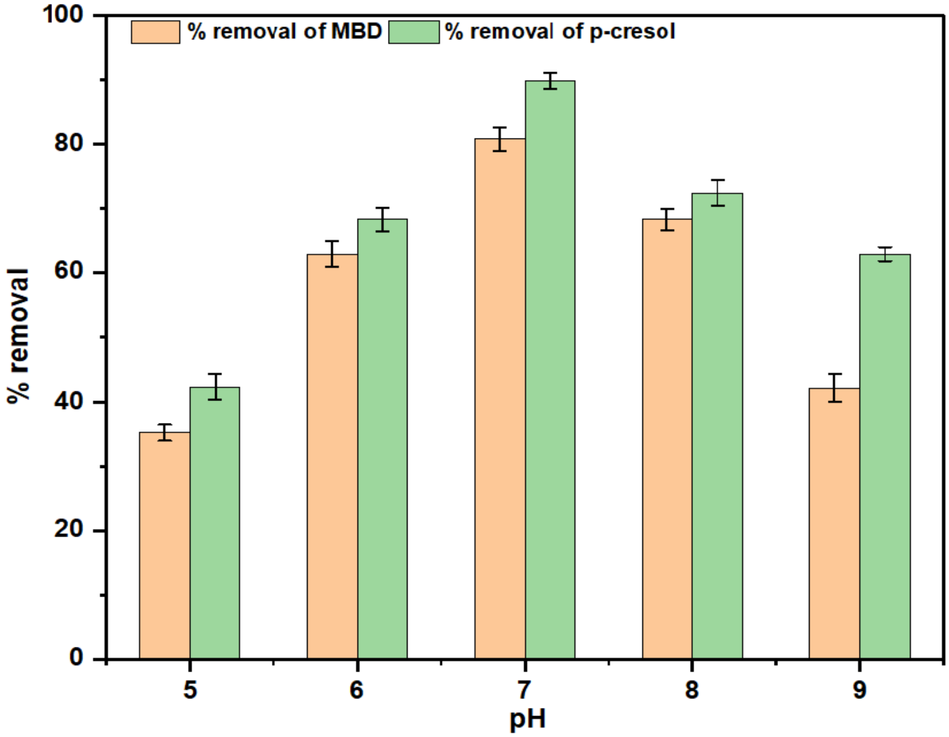


Fig. 6.4. Effect of pH on simultaneous biodegradation of *p*-cresol and methylene blue dye (MBD) (contact time 5 days)

6.3.5. Effect of sodium acetate as co-substrate on simultaneous biodegradation of *p*-cresol and methylene blue dye

The co-substrate serves as an additional carbon source and helps enhance the degradation of hazardous pollutants. Sodium acetate (SA) was used as a co-metabolic substrate to investigate the effect of its doses (g/100 mL) on the biodegradation of *p*-cresol and MBD. The impact of SA doses (0, 0.1, 0.2, 0.3, & 0.4 g/100 mL) on the simultaneous biodegradation of *p*-cresol and MBD is illustrated in **Fig. 6.5.** The removal efficiencies were increased from 89.85±1.26% to 96.69±2.13% for *p*-cresol and 80.73±1.83% to 92.0±1.58% for MBD with increasing the amount of SA from 0 to 0.3 g/100 mL. The same effect was observed in case COD (68.35±1.21% to

76.23±2.14%) and TOC (71.25±1.72% to 80.28±1.24%) removals **(Appendix 6 (a)).** However, with further increases in SA dose (0.3 g/100 mL to 0.4 g/100 mL), a minimal rise in removals of *p*-cresol and MBD observed. The increase in removal efficiency of *p*-cresol and MBD in the presence of SA may be due to SA serving as an easily accessible carbon source, stimulating the growth and metabolic activity of bacteria that decompose *p*-cresol and MBD. In addition to the faster bacterial growth, co-metabolism is another potential factor that might contribute to the improved biodegradation of *p*-cresol and MBD when additional carbon sources are added (Liang et al., 2019). So, 0.4 g/100 mL SA was considered the optimal dose for simultaneous biodegradation of *p*-cresol and MBD from wastewater.

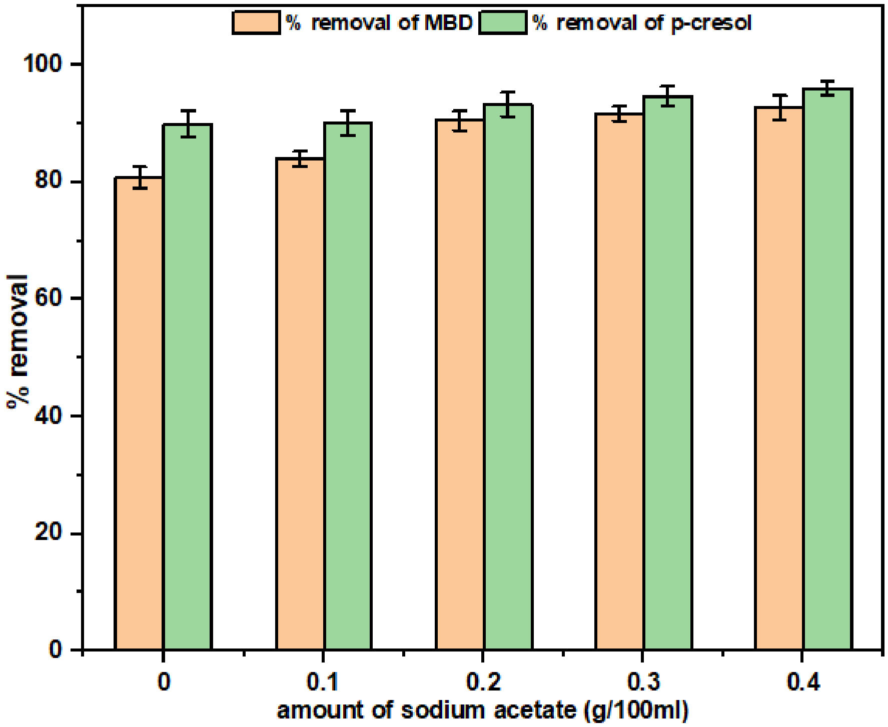


Fig. 6.5. Effect of sodium acetate on simultaneous % removal of *p*-cresol and methylene blue dye

6.3.6. Biodegradation kinetics for simultaneous removal of *p*-cresol and methylene blue dye

Biodegradation kinetics of simultaneous removal of *p*-cresol (100 mg L^{-1}) and MBD (50 mg L^{-1}) was analyzed on optimized conditions. Biodegradation kinetics followed by *p*-cresol and MBD is illustrated in **Fig. (6.6.)** after five days of incubation time. The rate constant values for zero and first-order were obtained using **Eq. (6.2)** and **Eq. (6.3).** Zero and first-order biodegradation kinetic for *p*-cresol and MBD were represented in **Fig. 6.6. (a, b)** and **Fig. 6.6. (c, d),** respectively. A first-order rate equation. was found to be the suitable model to represent the *p*-cresol (R^2 = 0.99, root mean square error = 0.08, residual chi square = 0.01) and MBD (R^2 = 0.93, root mean square error = 0.21, residual chi-square = 0.04) biodegradation kinetics, with rate constant (k_{p1}) of 0.55 day^{-1}, 0.50 day^{-1}, respectively.

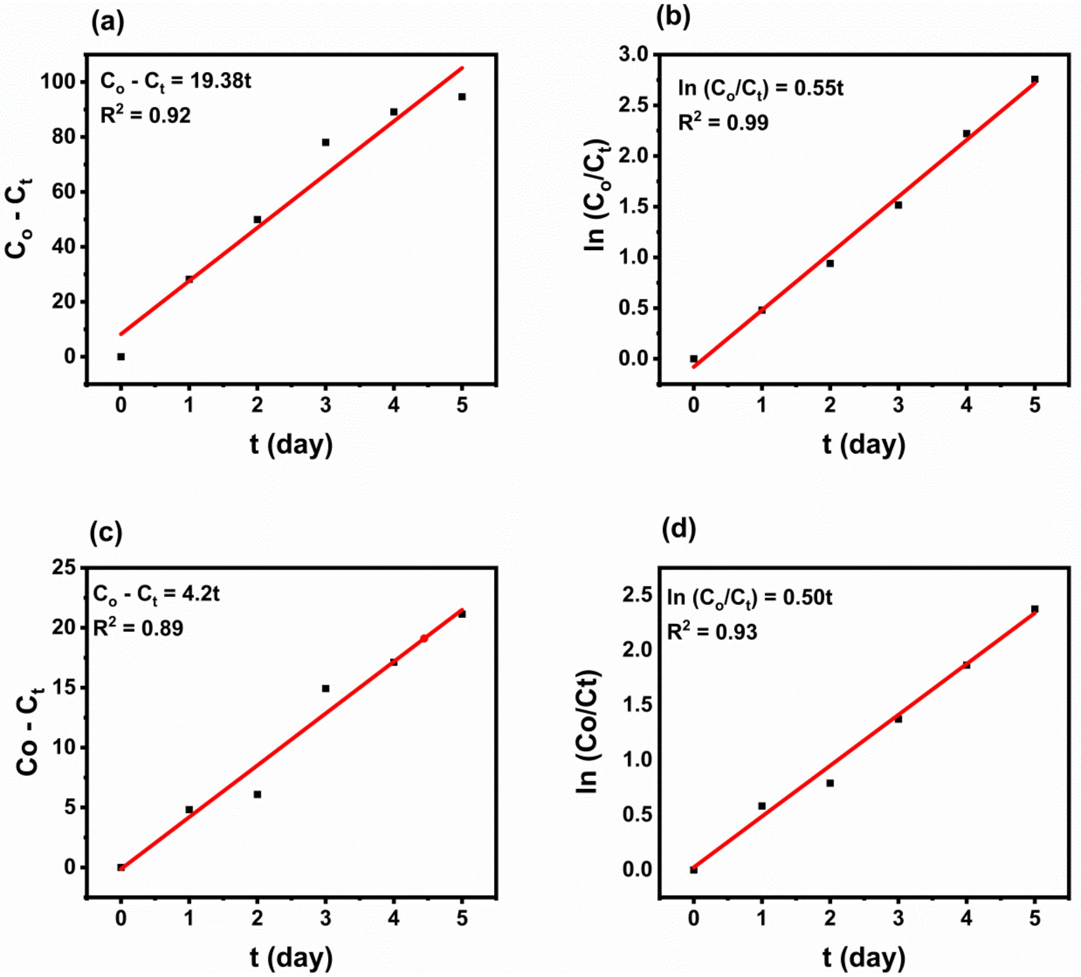


Fig. 6.6. Biodegradation kinetics for *p*-cresol (a) zero-order (b) first-order and biodegradation kinetics for methylene blue dye (c) zero-order and (d) first-order

Based on k_{p1} for *p*-cresol and MBD, the biodegradation rate of *p*-cresol was faster than MBD. It may be due to the complex structure of MBD than *p*-cresol. Panigrahy et al. (2020) used *Pseudomonas cetronellolis* NS1 isolated from cokeoven wastewater in biodegradation *p*-cresol. They found that *p*-cresol biodegradation followed first-order kinetic with a rate constant of 0.117 h^{-1} and a coefficient of R^2 = 0.94. Wang et al. (2022) immobilized *Phanerochaete chrysosporium* in calcium-alginate to remove bisphenol and found that biodegradation follows pseudo-first-order biodegradation kinetics. Mohamed et al. (2019) used *Trametes hirsute* for the simultaneous removal of methylene blue dye (25 mg L^{-1}) and phenol (10 mg L^{-1}) and found that the first-order kinetics followed for both pollutants.

6.3.7. Performance assessment of upward flow packed bed biofilm reactor

The performance of UFPBBR was analyzed in terms of %RE, $EC_{p,}$ and ILR_p of *p*-cresol and MBD at different influent flow rates between 10 mL min^{-1} to 50 mL min^{-1} under optimized conditions (*p*-cresol = 100 mg L^{-1}, MBD= 50 mg L^{-1}, pH 7.0±0.5, and SA= 0.3 g/100 mL) **(Fig. 6.7. (a, b)).**

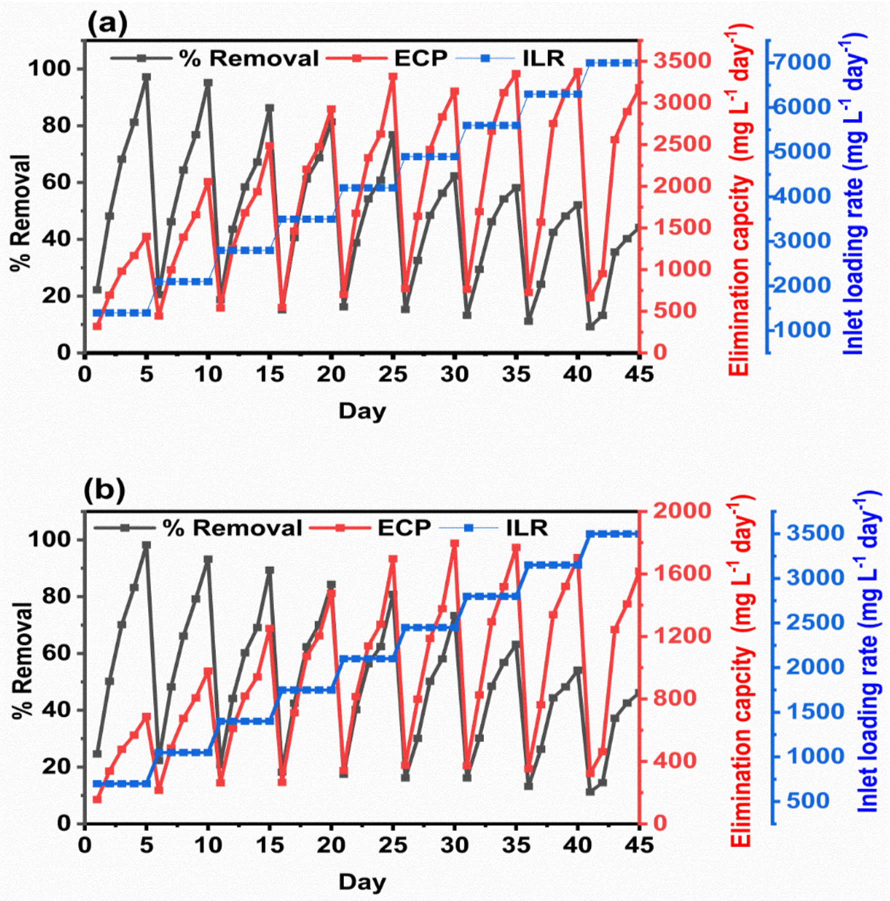


Fig. 6.7. Performance assessment of upward flow packed bed biofilm reactor (a) for *p*-cresol and (b) for MBD

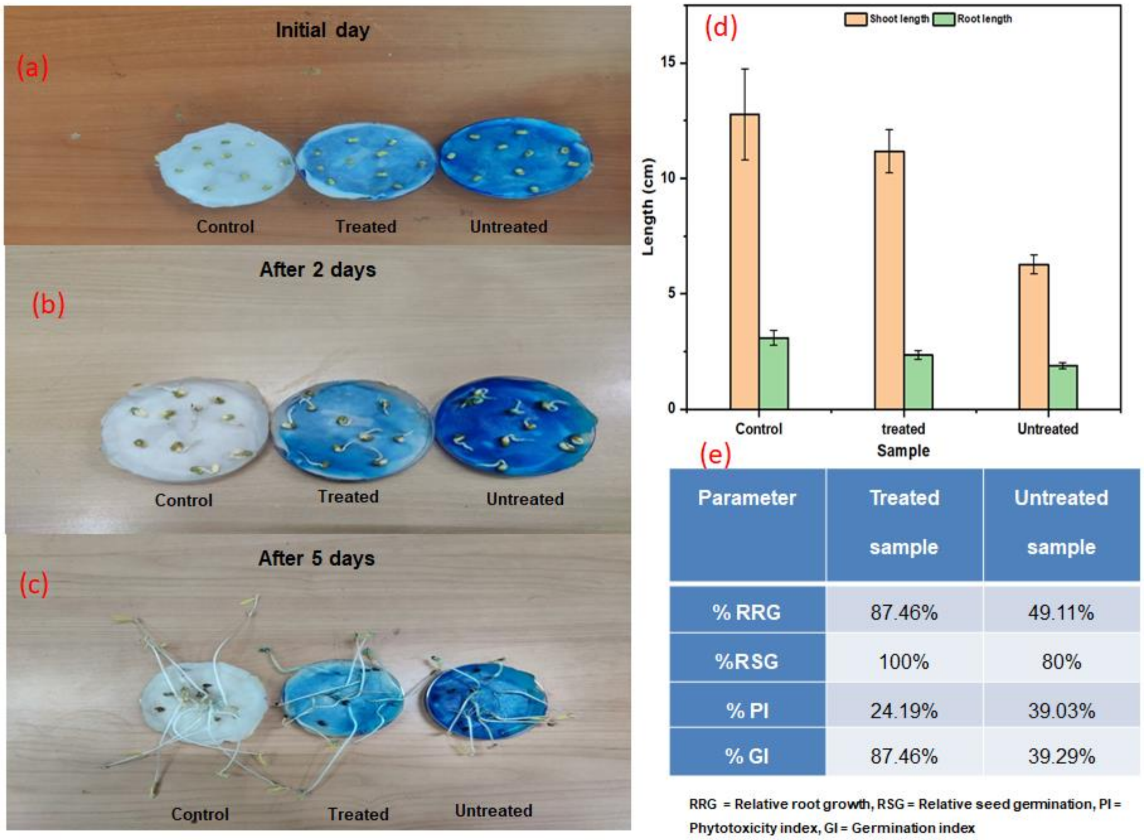
Each cycle was operational for 5 days. The UFPBBR was performed in the first cycle at a constant flow rate of 10 mL min^{-1} (HRT (4.3 h)). At this operating condition at the end of the fifth day, the maximum % RE and EC_p were found to be 97.23 %, 1400.112 mg L^{-1} day $^{-1}$ for *p*-

cresol and 98.41%, 687.61 for MBD, respectively. Further, on the sixth day, the flow rate increased from 10 mL min^{-1} to 15 mL min^{-1} (HRT (4.3 h to 2.86 h)), and the resultant slight decrease in % RE was observed for *p*-cresol 95.12 % (with EC_p 2056.968 mg L^{-1} day $^{-1}$) and 97.53% for MBD (with EC_p 978.915 mg L^{-1} day $^{-1}$) at the end of 10th day. However, UFPPBR became stable, and the maximum % RE of 76.85% and 82.62% at the end of 25 days corresponding ECs were evaluated to be 3319.92 and 1697.85 mg $L^{-1}d^{-1}$ *p*-cresol and MBD respectively. Nevertheless, the efficiency of the ECp was enhanced as the flow rate increased because substrate availability within the bioreactor increased. Subsequently, with a further increase in flow rate from 30 mL min^{-1} to 50 mL min^{-1}, the % RE decreases more rapidly from 76.85% to 44.78% and 80.41 to 46.14% for *p*-cresol and MBD, respectively, at the end of 45 days.

The removal efficiency decreased with an increased pollutant flow rate because of a shorter contact period between the immobilized cell and the pollutants, limiting the mass transfer rate between pollutants to biofilm. Also, the increased flow rates may decrease the amount of nutrients available by sweeping them out faster, which reduces microbial enzyme activity. This may suppress microbial activity and decrease the biodegradation rate (Zhou and Nemati, 2018). Sometimes, it is observed that high flow rate also influences the biodegradation pathway of pollutants. Shorter contact time results in incomplete degradation into harmless by-products. This can result in the accumulation of intermediate or by-product compounds that may be more persistent or even more toxic than the handling pollutant (Kim et al., 2008). These by-products may change the optimal environment's pH, reducing the pollutants' biodegradation (Logeshwaran et al., 2018). So, for effective biodegradation of pollutants, a suitable retention time is required for the surface diffusion of the pollutants and complete degradation by microorganisms (Lal Maurya et al., 2022b).

6.3.8. Phytotoxicity assessment

Seed germination and seed growth were estimated based on 2 and 5 days of observation, respectively, as illustrated in **Fig. 6.8. (a, b)** and **Fig. 6.8. (c).** The root length and shoot length of the *Vigna radiata L.* was illustrated in **(Fig 6.8. (d)).** When the seeds were soaked in distilled water (i.e., control sample), 100% germination was obtained with root and shoot lengths of 3.1±.31 cm and 12.77±1.97 cm, respectively. However, when seeds germinated in untreated samples, germination of 80%, root length of 1.89±0.13 cm, and shoot length of 6.27±0.41 cm were observed. In the case of the treated sample, seed germination of 100%, root length of 2.35±0.18 cm, and shoot length of 11.17±0.93 cm was recorded. The untreated sample showed lower seed germination rates than the treated wastewater **Fig. 6.8. (e).** The observed results indicate that bacterial consortia, isolated from activated sludge, can degrade the *p*-cresol and MBD and make it less toxic. The PI of the treated sample (24.19) was lower than the untreated sample (39.03). A higher PI (%) value could result in various adverse effects, such as stunted growth, reduced yield, chlorosis (yellowing of leaves), necrosis (tissue death), or even plant death. As per outcomes, the treated wastewater was suitable for growing *Vigna radiata* seed.



Parameter	Treated sample	Untreated sample
% RRG	87.46%	49.11%
%RSG	100%	80%
% PI	24.19%	39.03%
% GI	87.46%	39.29%

RRG = Relative root growth, RSG = Relative seed germination, PI = Phytotoxicity index, GI = Germination index

Fig. 6.8. Phytotoxicity assessment of *Vigna radiata L.* seed germination on (a) 0th day, (b) 2nd day (c) growth and (d) comparative study of root and shoot length, (e) % Relative root growth, % Relative seed germination, Phytotoxicity index, and Germination index

6.3.9. Bacterial toxicity assessment

The bioluminescence intensity of *Pseudomonas fluorescence (P.f.)* was used to evaluate the bacterial toxicity of untreated and treated samples. For bioluminescence measurement, 1 mL of *P. f.* was mixed with 10 mL of control, untreated, and treated samples. After introducing *P. f.*, acute and chronic toxicity were assessed at 1 and 24 hours, respectively **Fig 6.9.** The bioluminescence intensity of *P.f.* at 504 nm for the untreated sample (0.53×10^6) was lower than the controlled sample (2.18×10^6) after acute exposure **Fig. 6.9.(a).** However, the bioluminescence intensity of the treated sample increased to 1.71×10^6, which was close to the value of the control sample. This suggests a significant reduction in acute toxicity for the treated sample. The bioluminescence intensity of the treated sample (1.71×10^6) demonstrated an improvement in comparison to the untreated sample (0.44×10^6) after chronic exposure **Fig. 6.9.(b).**

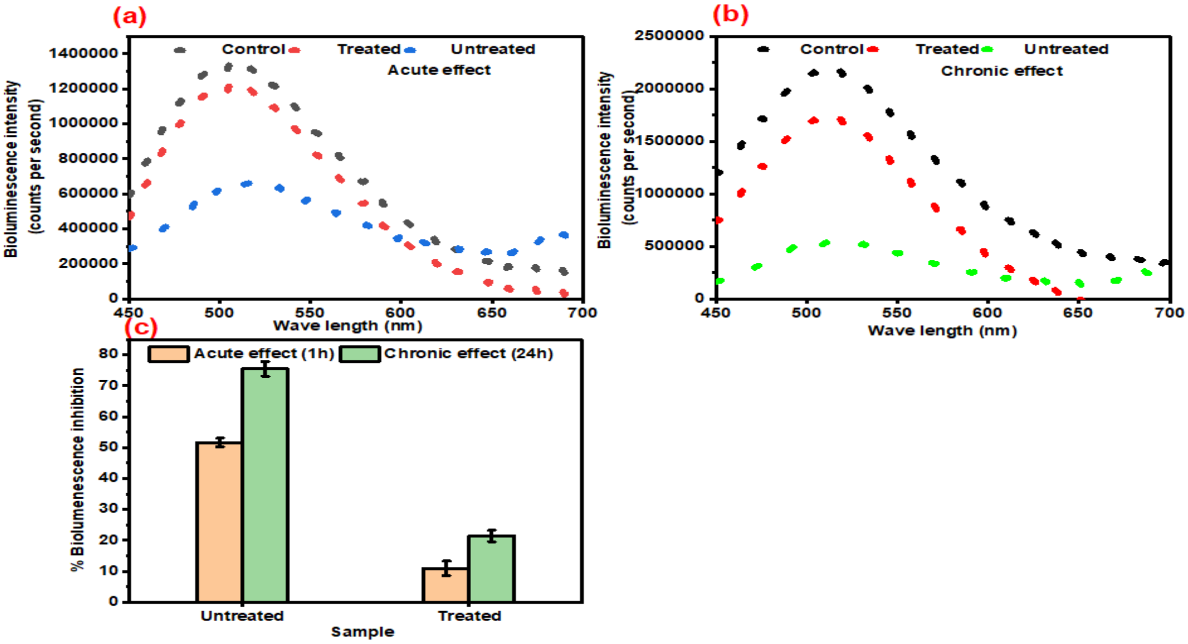


Fig. 6. 9. Impact of acute (a), chronic exposure (b) and % bioluminescence inhibition (acute and chronic exposure) (c) on photoluminescence bacteria (*P.f.*) for control, untreated and treated sample

This improvement can be attributed to the enhanced biodegradation of toxic by-products and improved mineralization. Bioluminescence inhibition at 504 nm was calculated for both treated and untreated samples **Fig. 6.9.(c).** The treated sample showed only 10.92% bioluminescence inhibition for acute exposure, whereas the untreated sample showed 51.64% inhibition. However, in the case of chronic exposure, the bioluminescence inhibition was 10.92% for the treated sample and 75.42% for the untreated sample. These results indicate a higher mortality rate of bacteria in the untreated sample than in the treated sample for acute and chronic exposure.

6.4. Practical applications

The food, textile, apparel, fine and bulk chemicals industries extensively utilize phenolic compounds and dyestuffs at various stages, from raw materials to final products. These substances, although persistent and harmful to the environment, are crucial components. Due to their potential to cause mutations and cancer, both the USEPA and European nations have designated them as priority pollutants. Therefore, eliminating these substances is crucial to safeguard the environment and human well-being. Among the available options, biological methods stand out as they offer a cost-effective approach with a substantial capacity to remove phenolic compounds and dyes from wastewater. Given these points, it's evident that the current study holds practical significance.

6.5. Conclusions

UFPBBR was used for the simultaneous removal of *p*-cresol and MBD from wastewater. The maximum removals of *p*-cresol and MBD were obtained to be 91.69±2.13% and 95±1.58% under optimized conditions (i.e., contact time of 5 days, pH of 7, and SA of 0.3 g/100 mL). First-order biodegradation kinetics showed better predictability than zero-order kinetics for the simultaneous removal of *p*-cresol and MBD. The maximum % RE and EC_p were found to be 97.23 %, 1400.112 mg L^{-1} day $^{-1}$ for *p*-cresol and 98.41%, 687.61 for MBD, respectively. Phytotoxicity and bacterial toxicity assessment confirmed the detoxification of *p*-cresol and MBD solution. The potential of the UFPBBR can be expanded to remove various toxic contaminants effectively.