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Intensified biodegradation of Congo red dye by mixed culture in a sequential bioreactor: Kinetics and phytotoxicity studies

Ravi Kumar Sonwani[1,2]*, Diwakar Patel[3], Alankriti Singh[2], Ram Sharan Singh[1] & BirendraNath Rai[1]

[1]Department of Chemical Engineering & Technology, Indian Institute of Technology (BHU), Varanasi-221 005, Uttar Pradesh, India
[2]Department of Chemical Engineering; [3]Department of Chemistry, Indian Institute of Petroleum and Energy (IIPE), Visakhapatnam-530 003, Andhra Pradesh, India

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Although textile industries lead the economic sector in several countries, the pollution that they cause, particularly to water bodies, is a serious environmental concern. Dyes and their metabolites are harmful to human and aquatic life due to their toxic, mutagenic, and carcinogenic potential. In this study, we have made an effort to treat the complex Congo red (CR) dye-containing wastewater in an anaerobic-aerobic sequential bioreactor. The impact of CR dye concentration (20-100 mg/L) on the performance of sequential bioreactor was examined. The CR dye removal efficiencies (REs) of 97, 88.1, 75.9, 68.7, and 42.56% were obtained at 20, 40, 60, 80 and 100 mg/L of CR dye, respectively. Similarly, the overall Chemical oxygen demand (COD) RE of 90% was also obtained. It was found that the CR dye and COD REs were high at low concentrations of CR dye. The obtained experimental data was fitted with growth and substrate inhibition models. A phytotoxic study indicated that the Chickpea (*Cicer arietinum* L.) grown in treated wastewater showed better germination (93%) than untreated wastewater (66%).

Keywords: Aquatic pollution, Azo dye, Chemical oxygen demand, Chickpea, *Cicer arietinum*, Dye removal efficiency, Sequential bioreactor, Textile industry, Wastewater

Synthetic dyes are extensively used in pharmaceutical, textile, cosmetic, leather and plastic industries[1,2]. The dye-loaded wastewater discharge from these industries is a considerable concern worldwide. This issue needs more focus in developing countries like India, where more than 1.5 million litre of dye-loaded wastewater is discharged per day[3]. Annually, over 280,000 tons of dyes are reported to be released into the aquatic bodies[1]. The wastewater from the textile processing industries is highly coloured with high Chemical oxygen demand (COD) and Biochemical oxygen demand (BOD)[4,5]. Azo dye is the class of synthetic dye that is most commonly used in industries due to easy availability and cost-effectiveness, and it is shared about 60-70% of the total dye used[2]. The presence of dyes in the aquatic environment is not only deteriorating natural photosynthesis but also reveals adverse effects on aquatic life[6]. Azo dyes and their metabolites are harmful to living beings due to their toxic, mutagenic, and carcinogenic potential[2,7]. Congo Red (CR) is an azo dye with two azo groups, and it is extensively applied in the textile industry. CR can cause a potential health hazard to humankind[8]. Therefore, an economical and sustainable technique is required for the remediation of dye.

Physicochemical methods including electrochemical oxidation, adsorption, ozonation, etc. have been widely tested for wastewater which contains dyes[5,6]. However, these methods have some drawbacks as they are associated with high cost, operational difficulties, and sludge disposal issues[8]. In contrast, the biological degradation of azo dye is a promising technique as it is an eco-friendly, efficient, and economical approach[9]. In the last few decades, the microorganisms belonging to fungi, bacteria, and algae have been examined to treat the various kinds of dyes[10]. The bacterial cultures are proved to be most favourable over the fungi or yeast culture due to their fast growth rate, low amount of secondary waste generation, and capability to adjust any complex conditions[11]. The bacterial species isolated and cultured from soil, wastewater, and animal excrement are successfully used for the biodegradation of dye in different forms either pure or consortium[12]. Compared

*Correspondence:
Phone: +91 9454643826 (Mob.)
E-Mail: raviks.rs.che16@itbhu.ac.in

to pure bacterial culture, the mixed culture could degrade the pollutants more efficiently.

Aerobic and anaerobic type of processes can be used to degrade the azo dye in a single stage. However, the complete mineralization of complex azo dye could be limited under aerobic or anaerobic processes, individually[13]. The anaerobic process supports the degradation of azo dyes and produces the toxic aromatic amine, whereas complete mineralization of aromatic amine requires the aerobic process[14]. This suggested that the anaerobic-aerobic sequential process is more reasonable for the treatment of azo dyes[15]. In the sequential bioreactor, the biodegradation of azo dye by microorganisms is first started with reductive cleavage of azo bond under anaerobic conditions, which generates aromatic amine intermediates. These intermediates could not be degraded under anaerobic conditions. Therefore, the effluent from anaerobic treatment was subjected to aerobic treatment for the biodegradation of aromatic amine intermediates[16]. The biodegradation of dye in an anoxic-aerobic bioreactor was examined by García-Martínez *et al.*[17], and they found that the sequential bioreactor was effective for the complete biodegradation of dye. In another study, an anaerobic-aerobic sequential bioreactor was developed to treat the wastewater containing azo dye[18]. In the last few decades, the anaerobic-aerobic sequential bioreactors have received enormous attention to treat a wide range of pollutants from wastewater.

This work investigates the applicability of an anaerobic-aerobic sequential bioreactor for biodegradation of wastewater containing Congo red (CR) dye. The effect of inlet concentration on the performance of sequential bioreactor was also investigated. Further, the biodegradation kinetics of CR dye was evaluated using growth and inhibition models.

Materials and Methods

Wastewater

The CR dye was obtained from Sigma-Aldrich, India. The chemicals for the synthetic wastewater preparation were procured from Merck and High media, India. Mineral salt medium of the following compositions (g/L): NaH_2PO_4 (2.5), KH_2PO_4 (1.8), $MgSO_4$ (0.2), $FeCl_3$ (0.01), $MnSO_4.H_2O$ (0.2), yeast extract (2.0), peptone (1.0), and glucose (1.0) was used[11]. The pH of the synthetic wastewater was adjusted using 0.1N NaOH and 0.1N H_2SO_4. The synthetic wastewater was prepared by adding CR dye into mineral salt medium and used for the biodegradation study.

Source of bacterial species for dye degradation

Dye-contaminated soil and wastewater samples were collected from the nearby site of textile industries located at Bhadohi, UP, India. These samples were used for preparation of bacterial consortium. Initially, soil (20 g) and dye-contaminated wastewater (10 mL) were supplemented in mineral salt medium containing CR red dye and incubated at 37°C and 120 rpm for 10 days. This step was repeated with continuously increasing CR dye. The potential acclimatized bacterial culture was used as an inoculum source in the anaerobic-aerobic sequential bioreactor to degrade the CR dye.

Sequencing bioreactor

The schematic diagram of a laboratory-scale set-up is presented in Fig. 1. Anaerobic (R1) and aerobic (R2) bioreactors were made of polyvinyl chloride with a total capacity of 45 and 30 L, respectively. A provision was given in the aerobic bioreactor to supply the air through sparger using an air pump (KNF, Labport, Germany). An agitator was provided to uniformly mix the sample with the help of a mechanical stirrer (IKA, Eurostar 40). The synthetic wastewater was kept in a feed tank (200 L), which was supplied to the bioreactor by a peristaltic pump (Watson Marlow 323E/D). The bioreactor was operated at a laboratory temperature of 31±3°C. Initially, the wastewater was supplied into an anaerobic bioreactor (R1). The biodegraded sample of the anaerobic bioreactor (R1) was transferred into an aerobic bioreactor (R2) for further dye degradation. The samples were taken from anaerobic and aerobic

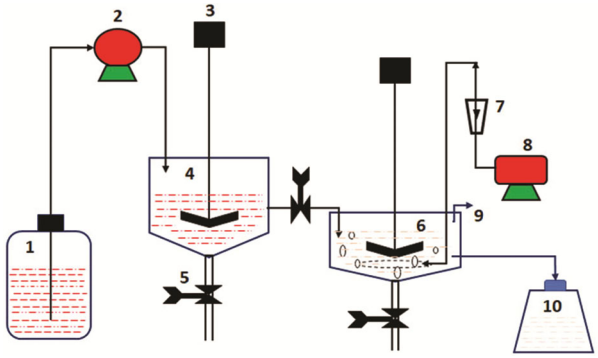

Fig. 1 — Schematic diagram of experimental set-up: 1- Feed tank; 2- Peristaltic pump; 3- Stirrer; 4- Anaerobic bioreactor; 5- Flow control valve; 6- Aerobic bioreactor; 7- Rotameter; 8- Air pump; 9- Gas outlet; and 10- Effluent tank

bioreactors at regular intervals for analysis of remaining COD and CR dye. The removal efficiency (RE) of CR dye and COD were calculated by the following equations:

Dye removal efficiency (RE) =

$$\frac{C_{in}-C_{out}}{C_{in}} \times 100 \quad \text{Eq. (1)}$$

COD removal efficiency (RE) =

$$\frac{COD_{in}-COD_{out}}{COD_{in}} \times 100 \quad \text{Eq. (2)}$$

where C_{in} is the inlet concentration of CR dye (mg/L) and C_{out} is the outlet concentration of CR dye (mg/L). Similarly, COD_{in} represents the inlet COD of the wastewater sample (mg/L) and COD_{out} represents the outlet COD of the wastewater sample (mg/L).

Analytical methods

COD of the samples was evaluated using standard protocols[19,20]. The concentration of CR dye was measured by a UV-vis spectrophotometer (ELICO SL-2010). Prior to COD and CR dye analysis, the supernatant and pellets were separated by centrifuge (5000 rpm for 10 min). The supernatant was used for COD and dye analysis, and bacterial cell mass was used to evaluate the growth kinetics. pH and DO were analyzed by respective probes of pH and DO meters.

Determination of kinetic parameters

The biodegradation kinetics of dye are necessary to design an economical and efficient process[21]. Generally, non-inhibitory and inhibitory type models have been broadly examined in the biodegradation kinetics study of pollutants[4]. Monod equation is a type of non-inhibitory model and is typically used for the microbial growth and substrate unitization study[22]. The general form of the model is given by Eq. (1).

$$\mu = \frac{1}{X}\frac{dX}{dt} = \frac{\mu_{max}C}{Ks+C} \quad \text{Eq. (3)}$$

where μ, X, and C are the specific growth rate of cell mass (1/h), mass of microbial cell (mg), and concentration of CR dye (mg/L), respectively. μ_{max} and Ks represent the maximum specific growth rate of cell mass (1/h) and half-velocity constant (mg/L), respectively. At the high loading rate of pollutants, the Monod model does not provide satisfactory results due to substrate inhibition. The growth of microorganisms under substrate inhibition could be examined by Andrews-Haldane model (Eq. 2).

$$\mu = \frac{\mu_{max}\,C}{Ks+C+\frac{C^2}{K_i}} \quad \text{Eq. (4)}$$

where Ki is the constant of CR dye inhibition (mg/L). The set of batch experiments was performed at different concentrations of the CR dye (20-100 mg/L) in the Erlenmeyer flask (250 mL) to determine the kinetic parameters.

Phytotoxicity assay

The wastewater containing dye reveals adverse effects on humans in terms of toxicity. The toxicity includes the germination of plant seeds in the presence of pollutants and subsequently measures the seed germination rate. The plant species, namely *Vigna radiata*, *Lepidium sativum*, *Chlorella vulgaris*, etc., have been employed[12]. In this work, *Cicer arietinum* L. seeds were used for the toxicity analysis. The Petri plates (14× 90 mm) were taken for the seed germination test. The details of the experimental set-up are well reported elsewhere[11]. Plates were kept at 25 ± 2.0°C for 15 days. The initial concentration of CR dye in wastewater was kept 20 mg/L. The seeds germination was recorded after 2 days, and the length of root (cm) and shoot (cm) were estimated after fifteen days. The percent of seeds germination was calculated by the following equation:

$$Germination\ (\%) = \frac{No.of\ seeds\ germinated}{No.of\ seeds\ sowed} \times 100 \quad \text{Eq. (5)}$$

Results and Discussion

Removal of dye in an anaerobic-aerobic sequential bioreactor

Biodegradation of CR dye was carried out in an anaerobic-aerobic sequential bioreactor at laboratory condition (31± 3.0°C). Initially, the synthetic wastewater (20 mg/L of CR dye) was added into an anaerobic bioreactor. The acclimatized bacterial consortium was inoculated (10 mL) into the anaerobic bioreactor to initiate the biodegradation process. The performance of an anaerobic-aerobic sequential bioreactor is presented in Fig. 2. Initially, the dye RE was low, and this may occur due to the lag phase (Fig. 2A). The dye RE was improved with process time and extended to 82.7% in 192 h. Furthermore, the effluent from the anaerobic bioreactor was transferred into the aerobic bioreactor for the complete degradation of remaining CR dye and its intermediates. The aerobic bioreactor helped in the dye removal and its intermediates released from anaerobic treatment. The RE was further improved with process time in an aerobic bioreactor. The maximum RE was found to be 97.5% in an anaerobic-aerobic sequential bioreactor in 336 h. The increase in the dye RE with process time was due to micro-organisms attain sufficient time to acclimatize to the environment[23-25].

Figure 2B denotes the effect of CR dye (20-100 mg/L) on RE. It was observed that the RE

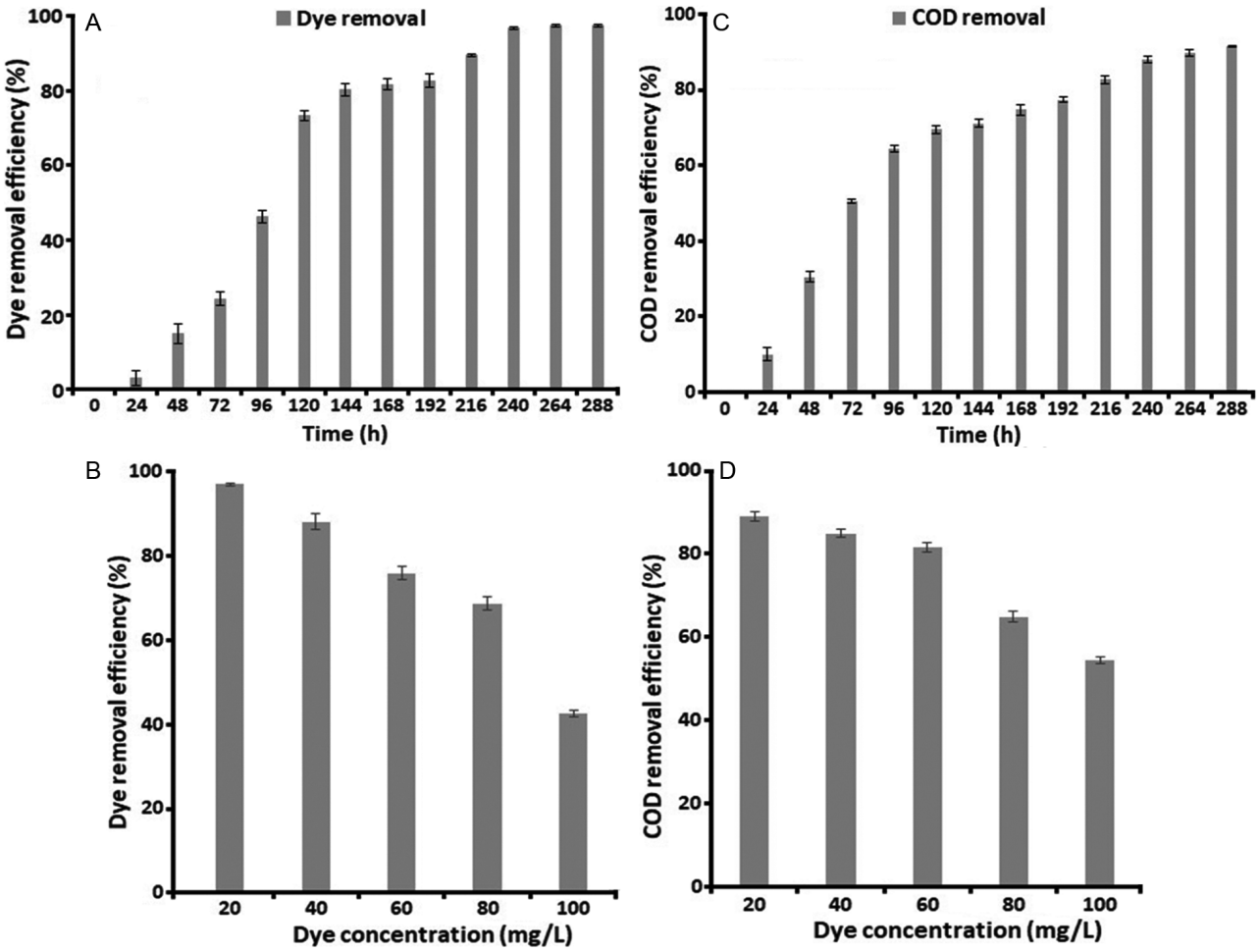

Fig. 2 — Performance of an anaerobic-aerobic sequential bioreactor: Effects of (A & C) process time; and (B & D) concentration on dye and COD removal efficiency, respectively.

reduced with increasing CR concentration, and this was probably due to less biomass formation at a high concentration of dye. The dye REs of 97, 88.1, 75.9, 68.7 and 42.56% were obtained at 20, 40, 60, 80 and 100 mg/L of CR dye, respectively. The low efficiency of the process at high concentration was probably because of CR dye inhibition[26]. Gopinath *et al*.[27] have examined the biodegradation of CR dye in an integrated bioreactor, and they found that the RE was maximum at lower concentration. In another study, Talha *et al*.[26] used *Brevibacillus parabrevis* to treat the CR dye in a bioreactor, and they found that the removal efficiency was enhanced with decreasing concentration of CR or *vice-versa*.

Removal of COD in anaerobic-aerobic sequential bioreactor

The overall performance of bioreactor was evaluated in terms of COD RE. The COD RE with respect to process time is shown in Fig. 2C. It was observed that the overall COD RE was improved with process time, and the maximum COD RE was obtained at the end of the process. The overall COD REs of 30.1, 72.1, 84.8, and 91.8% were achieved at 48, 144, 240 and 288 h, respectively. The increase in the COD RE with time confirmed that the anaerobic-aerobic sequential bioreactor was effective for the biodegradation of synthetic wastewater. Gopinath *et al*.[27] have evaluated the CR dye removal efficiency of an integrated bioreactor, and they found that the COD RE was increased with time, which may be because of microbes get sufficient time to acclimatize.

Figure 2D presents the COD RE with respect to different concentrations. Initially, at low concentration, COD RE was high. Furthermore, with increasing CR dye, the COD RE was gradually decreased and finally reached to minimum at the highest concentration of dye. The highest COD RE of 89.1% was found at 50 mg/L of CR dye, whereas the lowest COD RE of 54.47% was attained at 100 mg/L of CR dye. The

loading of pollutant impeded the growth of microorganisms and corresponding reduced the performance of the treatment system[28]. The higher concentration of dye caused substrate inhibition which reduced the growth of microorganisms[29]. The COD RE of textile effluent was sharply declined with increasing the initial dye concentration in an up-flow anaerobic sludge bioreactor[19].

Kinetic study of Congo red dye degradation

Several inhibitory and non-inhibitory models have been employed for the biokinetics study. Monod model under the non-inhibitory and Andrew-Haldane model under substrate inhibitory shows the better correlation coefficient. Figure 3 shows the relationship between specific growth rate (μ) *vs*. CR dye concentration fitted with Monod and Andrew-Haldane models. The value of μ was increased with CR dye up to 60 mg/L, and after that, it was subsequently declined. The obtained kinetic parameters are given in Table 1. μ_{max} and *Ks* were estimated to be 0.07 (1/h) and 9.87 (mg/L), respectively using Monod model. Similarly, μ_{max},*Ks*, and *Ki* were found to be 0.138 (1/h), 23.9 (mg/L), and 62.3 (mg/L), respectively using Andrew-Haldane model. The fitted model with an obtained kinetic constant is given by the following equations:

$$\mu = \frac{0.07\,C}{9.87+C} \qquad \text{Eq. (6)}$$

$$\mu = \frac{0.138\,C}{23.9+C+C^2/62.3} \qquad \text{Eq. (7)}$$

The value of *Ki* (62.3) denoted the concentration at which substrate inhibition was started and above this concentration, the microbial activity was adversely affected, and impeded the biodegradation rate. Gopinath *et al.*[27] have evaluated CR dye degradation at various concentration of 100-1000 mg/L, and used different models to analyze the inhibition kinetics. They reported that the values of μ_{max}, *Ks*, and *Ki* were found as 0.398 (1/h), 181.5 (mg/L), and 766.6 (mg/L), respectively by Haldane model. Talha *et al.*[26] have examined the growth kinetics with different concentrations of CR dye using Monod model, and they reported that the value of μ_{max} and *Ks* as 0.019 (1/h) and 39.44 (mg/L), respectively. The obtained values of kinetic constant are different from previous studies because of different process conditions, microorganisms, and wastewater compositions.

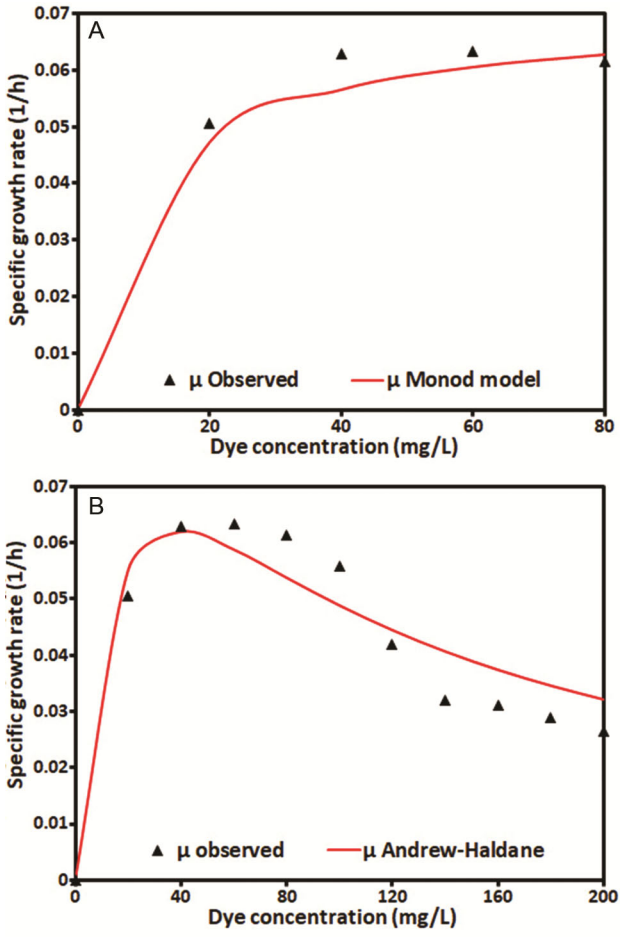

Fig. 3 — Experimental data of specific growth rate *vs*. Congo red dye concentration fitted with (A) Monod and (B) Andrew-Haldane model.

Analysis of samples

The removal of dyes by bacteria could be due to biodegradation and adsorption mechanism. During biodegradation, the cell biomass does not become colored, while in adsorption, the original biomass appeared colored because of adsorption of dye molecules[30]. UV-visible spectra (380-650 nm) of the untreated and treated samples are represented in Fig. 4. The disappearance of the peak of treated CR dye at 498 nm indicates the 'near complete' removal of CR dye. After degradation, the biomass almost remained colourless, which suggested the decolonization of CR dye was probably due to the biodegradation.

Table 1 — Kinetic parameters determined using Monod and Andrew-Haldane models

Model	Max specific growth rate, μ_{max} (1/h)	Half-velocity const., *Ks* (mg/L)	Inhib. const., *Ki* (mg/L)	Corr. coeff. (R^2)
Monod	0.070	9.87	-	0.96
Andrews-Haldane	0.138	23.9	62.3	0.93

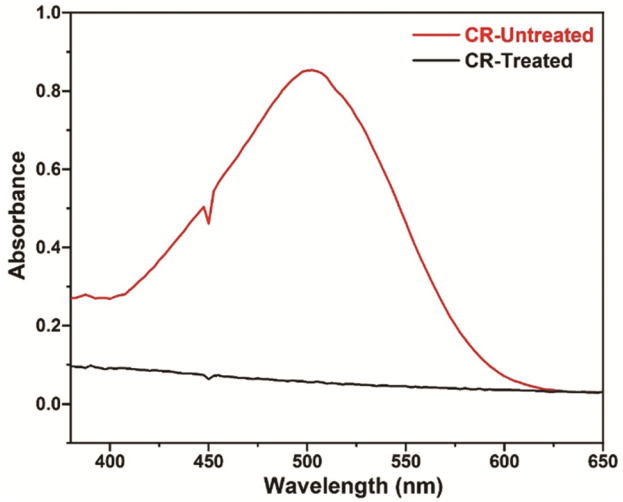

Fig. 4 — UV-Visible spectra of untreated and treated Congo red dye using bacterial consortium.

Table 2 —Phytotoxic analysis of treated/untreated wastewater on *Cicer arietinum* seeds

Parameters	Distilled water	Wastewater	
		Untreated (Control)	Treated
Germination (%)	93	66	93
Root length (cm)	3.72±0.12	1.34±0.18	3.45±0.21
Shoot length (cm)	3.82±0.19	0.66±0.15	3.53±0.17

Phytotoxicity study

The chicpea *Cicer arietinum* seeds were selected to examine the impact of treated and untreated CR dye wastewater in Phytotoxicity study. The growth of *C. arietinum* seeds in distilled, untreated and treated water is represented in Table 2. The seeds grown in distilled water (control) showed 93% germination, 3.72 ± 0.12 cm of root, and 3.82 ± 0.19 cm of shoot, whereas seeds grown in treated CR dye (50 mg/L) wastewater represented 93% of seeds germination, 3.45 ± 0.12 cm of root, and 3.53 ± 0.17 cm of shoot length (Table 2). It was found that when seed was exposed to treated CR dye solution revealed more germination (93%) as compared to untreated dye wastewater (66%). Also, the length of root (2.57 times) and shoot (5.34 times) were higher in the case of treated CR dye wastewater than untreated CR dye wastewater. The improved growth of seeds with treated CR dye wastewater supported that it can be used for plant growth[31].

Conclusion

The isolated bacterial consortium was examined to degrade the CR dye in an anaerobic-aerobic sequential bioreactor. The influence of process variables like dye concentration and process time with respect to the CR dye removal was analyzed. The performance of the bioreactor was high at a low inlet loading rate or vice versa. The growth and inhibition kinetics were evaluated using Monod and Andrews-Haldane models. The growth of bacterial species inhibited above 60 mg/L of CR dye. The phytotoxicity study indicated that the treated sample improved the chickpea (*Cicer arietinum* L.) seeds germination than untreated samples. Overall, an anaerobic-aerobic sequential approach could be suitable for treating wastewater containing dyes. Sequential systems can be further explored to treat the other pollutants.

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Conflict of interest

Authors declare no competing interests.

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