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Research Article

Treatment of Mixed Azo Dyes in an Aerobic Sequential Batch Reactor and Toxicity Assessment Using *Vigna radiata*

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Azo dyes are the most widely used dyes in the textile industry due to their stability, but their redundancy to degradation is of significant concern, particularly to aquatic ecosystems. In the present study, a lab-scale aerobic sequential batch reactor (SBR) was operated to analyze the degradation of mixed reactive azo dyes at a concentration of 100–1000 mg/L. The chemical oxygen demand (COD) removal increased from 34% to 61.15% and then dropped to 21.16% at the highest used concentration. The biochemical oxygen demand (BOD) removal decreased from 63% to 55.55% to 28.14% with an increasing dye concentration. The biosorption experiment and dried activated sludge (DAS) successfully removed about 0.300 mg of dyes by absorption within 2 hours. A toxicity assessment was carried out by employing a phytotoxicity test on *Vigna radiata*. The percentage of germination was used to detect the toxic effects of untreated dye-containing wastewater on plant growth. The treated wastewater showed 100% germination compared to 70% in untreated wastewater containing 100 mg/L mixed dyes, confirming the treatment's efficacy.

1. Introduction

Synthetic dyes used most frequently in textile industries are significant contributors to the causes of pollution. The participation of textile industries as polluters of water bodies has witnessed an alarming increase in the past few decades [1]. Azo dyes are the most often used and have the distinctive azo (–N=N-) group. These highly stable dyes find their applications in several other industries apart from textiles, such as cosmetics, paper, food, and leather [2]. The color affects the penetration of sunlight and thus aquatic plants [3], and the high nitrogen content often leads to eutrophication [4]. The consequences associated with an ineffective treatment of textile wastewater involve several risks. More than 25% of amine-based dyes are carcinogens, and several are carcinogenic to humans [5]. Hence, effluents containing these xenobiotic and recalcitrant dyes cannot be degraded by conventional wastewater treatment methods [6]. The removal option necessitates the development of an appropriate and effective method for containing the pollution induced by these dyes.

The conventional methods of treating wastewater include physical, chemical, and biological processes [7, 8]. Physical and chemical treatments are not efficient in degrading the azo dye-contaminated effluent, and they also produce secondary waste products that would require further treatment [9]. Biological treatment involves several microorganisms that accumulate and degrade dyes and chemicals present in wastewater [10]. This natural and environmentally friendly method is surprisingly effective and has several advantages over the other methods. It is cost-effective, and the number of secondary pollutants produced is far lesser than other treatment methods [11].

The biological treatment system includes the activated sludge process (ASP), a widely used procedure in wastewater treatment [12]. Aerobic, facultative, and anaerobic microorganisms can carry out the ASP under appropriate oxic/anoxic conditions. The azo dye is degraded by a consortium when it acts as an electron acceptor, causing the reduction of the azo bond and resulting in aromatic amines under anaerobic conditions [12]. The aerobic process involves the degradation of pollutants by flocculating biomass in an aeration tank.

The sequencing batch reactor operates in time, and it is a fill-and-draw type of activated sludge process. An SBR can perform equalization, neutralization, biological treatment, and secondary clarification in a single tank [13]. There are numerous studies done to find a suitable process for reusing textile wastewater. For the possibility of reusing the textile effluent, bioreactor studies are done [14]. Bioreactors are used for the effective biomass production in biological treatment methods that can degrade the dyes present in wastewater. Aerobic degradation of dyes has been used to facilitate the conventional microbes to increase the degradation ability, while the anaerobic method used for the degradation of dyes may result in aromatic amines and a few hazardous byproducts.

There have been various studies on the treatment of textile wastewater using various anaerobic reactors. Sequential batch reactor has proved to be a good option as it is dependent on aerobic microbes and time consumption for the treatment method is less, and with the addition of the activated sludge process, it makes it a more viable option for the treatment of textile effluents. There have been reports on the treatment of individual dyes in the bioreactor, but in a real-time textile effluent, there is always a mixture of dyes present that contaminate the environment. The primary goal of this research is to design a suitable reactor system for the effective degradation of mixed dye. The novelty of the work would be the usage of the indigenous activated sludge microbes acclimatized to alkaline pH to degrade the mixed dyes in the designed sequential batch reactor.

A batch study was carried out to assess the consortium's degradative effectiveness in the sludge. The study proposes utilizing a sequencing batch reactor to treat synthetic textile wastewater (including reactive mixed dyes, such as reactive red, reactive black, and reactive brown). The scale-up study examines their operating parameters including COD, BOD, hardness, chloride, TDS, pH, and temperature.

2. Materials and Methods

2.1. Preparation of Synthetic Wastewater. The composition of synthetic wastewater (0.5 g/L) includes potassium dihydrogen phosphate (0.5 g/L), magnesium sulfate (0.1 g/L), ferric chloride (0.025 g/L), ammonium chloride (1 g/L), calcium chloride (0.01 g/L), yeast extract (0.025 g/L), and lactose (0.15 g/L) [15]. The pH was increased to 8.5. After sterilizing, the dyes were membrane-filtered and added to the prepared synthetic wastewater. Each dye, reactive red (RR), reactive brown (RBr), and reactive black (RB) [16], was applied at various concentrations of 100 mg/L, 500 mg/L, and 1000 mg/L, respectively. The dyes were bought at India Mart. Merck Chemicals were purchased and utilized in the production of synthetic wastewater.

2.2. Batch Studies for Decolorization of Mixed Dyes. About 200 ml of synthetic wastewater was added to the mixed dyes, and 100 ml of the collected activated sludge was combined and kept for incubation for enrichment in an orbital shaker for five days at 30°C. A control containing distilled water instead of activated sludge was also incubated. Every 24 hours, samples were withdrawn from the conical flask and centrifuged at 8000 rpm for 10 minutes. The degradation of mixed azo dyes was measured using a UV-visible spectrophotometer and HPLC, and the percentage of decolorization for mixed dyes was studied: 518 nm for RR, 440 nm for RBr, and 614 nm for RB.

2.3. Operational Setup. Activated sludge was recovered from the aeration tank of the Koyambedu Sewage Treatment Plant (STP) in Chennai after five days. A 14 L bioreactor constructed of an acrylic tank with a height of 30 cm and a breath of 24 cm was developed for the activated sludge process. Figure 1 shows the SBR setup for the treatment of textile wastewater. A porous air diffuser was added to the bottom for aeration, and a stirrer was utilized at 150 pm for homologous mixing. Each SBR cycle featured five phases: FILL (30 minutes), REACT (22 hours), SETTLE (1 hour), DRAW (30 minutes), and IDLE (30 minutes). The temperature was kept at room temperature throughout the process (37°C). A working volume of 8 L was used, with 4 L of activated sludge and 4 L of synthetic wastewater, with dye concentrations of 100 mg/L, 500 mg/L, and 1000 mg/L, respectively. Equal ratios of the individual dyes were taken for each concentration. The reactor was dye-free for three days to allow the sludge to acclimate to the synthetic wastewater. During the DRAW phase, the SBR effluent was collected and chemical analyses were performed using standard methods. A UV-vis spectrophotometer was used to measure the deterioration of reactive red, reactive black, and reactive brown at the respective nm. The concentrations of the synthetic dyes were increased once each concentration was completed. COD removal, BOD removal, MLSS, and MLVSS were all determined on a regular basis.

2.4. Analyses. Standard procedures were used to estimate COD, BOD, TDS, hardness, and chloride for the investigation of the physicochemical parameters [17]. Throughout the treatment, the pH was monitored using a pH meter and maintained at 8.5. A thermometer was used to measure the temperature variations.

2.5. HPLC Analysis. A 0.45 m membrane filter was used to filter the wastewater from the reactor containing the degraded dyes. The filtrates were then extracted with an

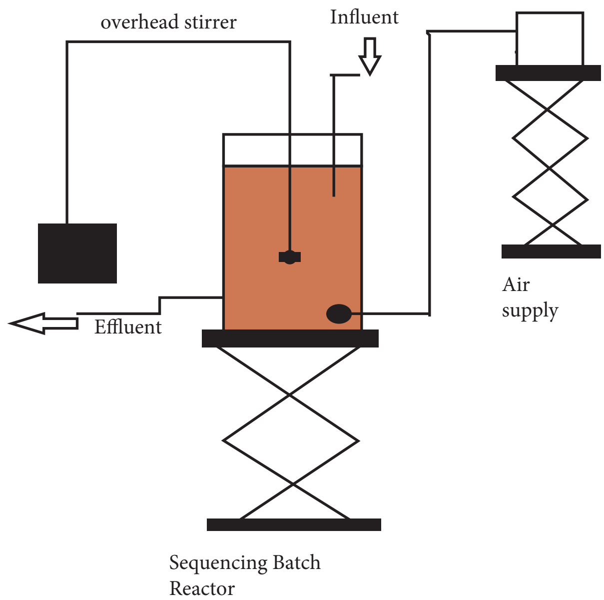


Figure 1: SBR setup for the treatment of textile wastewater.

equivalent amount of ethyl acetate and flash-evaporated in a temperature-controlled water bath (50°C) in a rotating vacuum evaporator. The residues were diluted in 2 mL of HPLC grade methanol and analyzed using HPLC. A Shimadzu prominence binary gradient HPLC system was the HPLC model. The extracted samples were analyzed using HPLC with a mobile phase of 100% methanol [18].

2.6. Biosorption Studies on Treated Wastewater. To further decolorize the SBR effluent, a batch study employing dried activated sludge was performed. Activated sludge from the aeration tank of the Nesavakam sewage treatment facility in Chennai was centrifuged, and the pellet was completely washed with distilled water. The sludge was distributed in a sterile Petri plate and placed in a hot air oven at 60°C overnight. The dried activated sludge (DAS) was carefully scraped from the Petri plate and ground to a fine powder with a mortar and pestle. It was then sieved through a 105 μm diameter mesh and kept in a dry container before being used as a biosorbent in the experiment [19].

The experiment was carried out with 100 mg/L of treated wastewater and with 0.5 g of dry activated sludge. A UV-visible spectrophotometer was used to examine the initial absorbance, and samples were collected at 20, 40, 60, 80, 100, and 120 minutes; they were centrifuged at 10,000 rpm for 10 minutes, and the absorbance was measured spectrophotometrically. The control was 100 mL of treated wastewater without dried activated sludge

$$qe = \frac{(Co - Ce)V}{W}, \quad (1)$$

where qe is the amount of dye adsorbed by the biomass, Co is the initial concentration of dye, Ce is the final concentration of dye, V is a volume of dye solution, and W is the weight of the biomass.

2.7. Phytotoxicity Study. The phytotoxicity study was conducted at room temperature with *Vigna radiata* (green gram). Three separate pots containing 20 green gram seedlings were irrigated with 10 ml of distilled water as a control, untreated synthetic wastewater, and treated synthetic wastewater [20]. The germination% was estimated using the following formula:

$$\text{germination percentage} = \frac{\text{no. of seeds sprouted} \times 100}{\text{no. of seeds sown}}. \quad (2)$$

3. Results and Discussion

3.1. Decolorization of Mixed Dyes. Batch studies were conducted to examine the removal of mixed reactive dye by activated sludge microorganisms [21]. In five days, the mixed reactive dye removal of each of the individual dyes was up to 40% of the mixed dye, 39% of the RR, 56% of the RB, and 42% of the RBR in the aerobic condition at room temperature and at a pH of 8.5 at 150 rpm (Figure 2). It could be noted that the individual dye RR has been utilized in higher percentage compared to the other two dyes used in the study. The results of the study also show that individual dye degradation was more efficient than mixed dyes. The reason could be the mixed dyes in the wastewater could have more toxicity for the microbes compared to the individual dyes and have complex structures compared to the individual dyes.

3.2. Sequential Batch Reactor System with 100 mg/L of Mixed Reactive Dyes. The SBR system was initially loaded with 100 mg/L of the mixed reactive dyes. The pH was maintained at 8.5. On the first day of seeding, the COD concentration in the synthetic wastewater was 320 mg/L, which decreased to

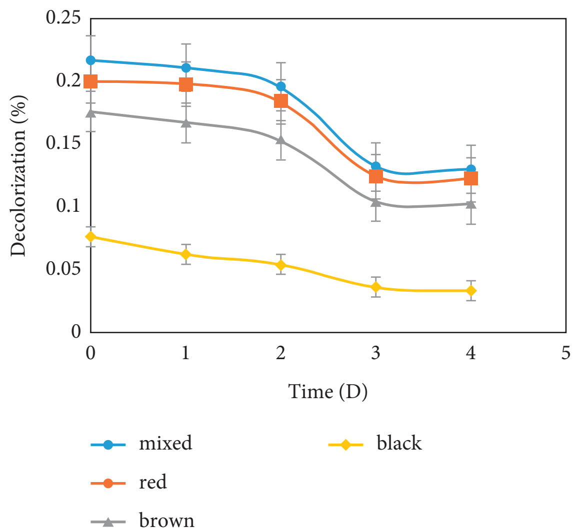


Figure 2: Decolorization of 100 mg/L of mixed reactive dyes and individual dyes in batch study.

110 mg/L after 10 SBR operating days. On the second day, the COD concentration in synthetic wastewater was half that of the first, at around 160 mg/L. The treatment demonstrated the active microbial community degradation of mixed dyes existing in synthetic wastewater, and the COD reduction in the figure gradually decreased until the tenth day. The microorganisms in the activated sludge could not decrease the COD after the tenth day, and it remained constant. The COD level at the end of the operation was 110 mg/L, implying that 34.375% of the COD was removed in 10 days. The bacterial consortia present in the activated sludge might lower COD levels on the tenth day. BOD levels in the effluent also suggested the use of mixing colors. Initially, the BOD concentration in the wastewater was around 900 mg/L; only one-fourth of this concentration was decreased to 570 mg/L on the second day. The percentage of BOD rapidly decreased to 480 mg/L on the tenth day and demonstrated a 63% BOD elimination, whereas the percentage of BOD increased to 660 mg/L on the third day and 810 mg/L on the fifth day, indicating competition among microbes to survive in the wastewater [22].

TDS (total dissolved salts) were originally 600 mg/L and eventually lowered to 400 mg/L. TDS dropped precipitously on day two and then increased to 700 mg/L. TDS did not decrease after day six. The chloride content of the synthetic wastewater was 400 mg/L, with 350 mg/L at the completion of the SBR treatment. The initial concentrations of mixed liquor suspended solids and mixed liquor volatile suspended solids were 2000 mg/L and 1000 mg/L, respectively, but increased to 6000 mg/L and 3000 mg/L at the end of the process, indicating an increase in the microbial population in the activated sludge by using the dyes as a carbon source in the wastewater. There was a significant increase from days four to six when the MLSS and MLVSS were 8700 mg/L and 4400 mg/L, respectively, indicating that microbial growth was rapid [23]. Following that, the population decreased toward the conclusion of the studies, which might be attributed to a decrease in dye concentration used by the microbes in the activated sludge. The hardness of the water was determined to be 700 mg/L, indicating significant hardness. On the tenth day following treatment, 700 mg/L of hardness was reduced to 300 mg/L of hardness (Table 1).

3.3. Sequential Batch Reactor System with 500 mg/L of Mixed Reactive Dyes.

The SBR was initially loaded with 4000 mg/L of COD, which was reduced to 1554 mg/L at the end of the SBR operation by the microbes present in the activated sludge. The COD reduced to 2400 mg/L on the third day, but increased to 3440 mg/L on the fifth day, indicating that the dye concentration is too high for the microbes in the activated sludge to degrade. On the sixth day, the COD concentration decreased from 3440 mg/L to 1600 mg/L, indicating that the activated sludge had acclimatized to the wastewater with 500 mg/L of dye concentration and was, therefore, able to reduce the COD level. The COD concentration increased on day eight to 2640 mg/L and then declined on the ninth and tenth days to 1900 mg/L and 1554 mg/L, respectively. As a result, 61.15% of COD removal was achieved at the end of the operation in SBR. The system maintained a COD removal at 61.15%, demonstrating that the microorganisms were able to degrade the dyes and other refractory substances in the seeded wastewater [24].

The MLSS and MLVSS levels in the planted sludge were initially 1000 mg/L and 500 mg/L, respectively. On the second day, MLSS and MLVSS increased to 2700 mg/L and 1300 mg/L, respectively. It gradually increased, indicating that the microorganisms acclimatized, and thus the population of microbes in the reactor increased until the seventh day when it was 7400 mg/L and 3500 mg/L, respectively, after which the microbes lost their ability to absorb the dye in the synthetic wastewater, which is the carbon source. As a result, there was no increase in population after the seventh day. The MLSS and MLVSS were 6600 mg/L and 3300 mg/L at the completion of the SBR operation, respectively.

Initially, the synthetic wastewater utilized in dye concentration investigations at 500 mg/L comprised 900 mg/L of BOD. The BOD concentration increased to 1290 mg/L on the second day and again to 1620 mg/L on the third day, indicating that the dye concentration was too high for the microorganisms in the reactor. They were unable to decrease the BOD level until the third day when it decreased to 600 mg/L, demonstrating how the microbes in the activated sludge were trying to acclimate to the high concentration of dye. The BOD level increased to 780 mg/L on the fifth day, after which there was no increase but a progressive reduction until the end of the operation, indicating that the bacteria in the sludge acclimatized to the dye concentration of 500 mg/L. BOD was 400 mg/L on the tenth day; hence, the overall percentage of BOD removal observed in SBR was 55.55%. TDS was initially 900 mg/L and was decreased to 650 mg/L [25]. As a result, TDS was reduced by 27% in SBR. On the first day, the hardness of the water was 1000 mg/L, which was considered to be the maximum hardness. The hardness of the water increased to 1500 mg/L. The hardness of the water steadily decreased after the fourth day, reaching 400 mg/L at

Table 1: Characteristics of the influent and effluent in SBR with wastewater containing 100 mg/L of mixed dyes.

Parameters	Influent (mg/L)	SBR effluent (mg/L)
COD	320	110
BOD	900	480
Chloride	600	400
TDS	400	350
Hardness	700	300

Table 2: Characteristics of the influent and effluent in SBR with wastewater containing 500 mg/L of mixed dyes.

Parameters	Influent (mg/L)	SBR effluent (mg/L)
COD	4000	1554
BOD	900	400
Chloride	900	650
TDS	1300	760
Hardness	1000	400

the end of the process. The chloride content of the synthetic wastewater seeded into the reactor was 1300 mg/L, which subsequently dropped to 760 mg/L after 10 days of SBR operation (Table 2). As a result, the acclimatized microbes in the sludge removed 41.53% of the chloride.

3.4. Sequential Batch Reactor System with 1000 mg/L of Mixed Reactive Dyes. The pH ranged from 8.5 to 8 in 1000 mg/L trials, but it was maintained at eight until the completion of the operation at room temperature. The influent for the 1000 mg/L trials had a high concentration of COD of 6000 mg/L. Every day, the COD concentration decreased gradually; however, because of the 1000 mg/L research, the microorganisms were unable to adjust to the high COD concentration. COD fell progressively to 5742 mg/L on the sixth day, then rapidly to 4700 mg/L on the eighth day, before declining to 4730 mg/L on the ninth day. It remained constant for a few more days, indicating that the microbe had lost its capacity to decrease COD. In 10 days of SBR operation, the quantity of COD removed was 21.16%.

The BOD concentration in the influent was 1670 mg/L, which gradually decreased to 1200 mg/L at the end of the process, indicating that the bacteria in activated sludge tried to acclimate to the synthetic wastewater with a high concentration of dye, but after the tenth day, they lost the ability to degrade the dyes, and the BOD and COD remained constant. The amount of BOD was lowered to 28.14%. TDS was 900 mg/L and reduced to 700 mg/L on day five and remained constant until day seven when it decreased to 654 mg/L and reached 600 mg/L on day 10. In 10 days, 33.33% of TDS was removed.

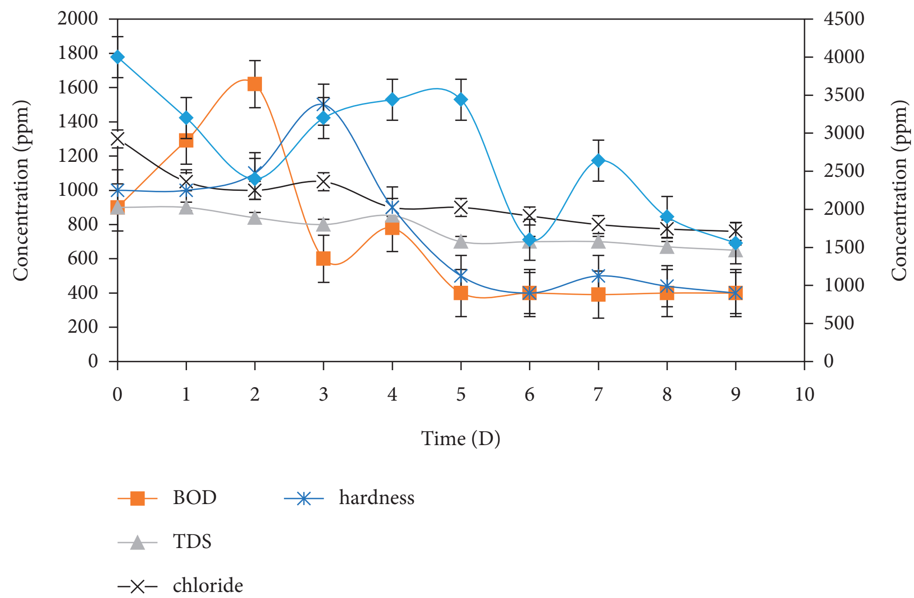
On the day of seeding, the MLSS and MLVSS were 3000 mg/L and 1500 mg/L, respectively. Since the dye concentration in the 1000 ppm tests was high, the sludge seeded to the SBR included a large population of microorganisms for effective degradation. After two days, the bacteria gradually acclimatized to the synthetic wastewater and increased to 5300 mg/L of MLSS and 2600 mg/L of MLVSS on the sixth day. After the carbon supply in the synthetic wastewater decreased after the sixth day, the microbe population began to decline and reached 4000 mg/L of MLSS and 2000 mg/L of MLVSS at the conclusion of the SBR operation. The chloride concentration started at 1700 mg/L and decreased to 1400 mg/L on the third day. After the third day, the concentration decreased drastically, reaching 1016 mg/L on the fourth day. On the tenth day, it was 833 mg/L. Chloride elimination was 51% after 10 days of operation. Because it was 1000 mg/L, the hardness of the water was extremely high, i.e., 1400 mg/L, which reduced to 900 mg/L on the fourth day and 551 mg/L on day 10, achieving a 60% hardness reduction in SBR (Table 3 and Figure 3) Physicochemical analyses for synthetic wastewater in SBR contain (i) 100 mg/L, (ii) 500 mg/L, and (iii) 1000 mg/L (Figure 4). The pH, MLSS, and MLVSS for synthetic wastewater in SBR contain (i) 100 mg/L, (ii) 500 mg/L, and (iii) 1000 mg/L.

The results obtained at a concentration of 100 mg/L were comparable to those reported by Shaw et al. (2002) [26], who discovered that COD removal occurred at a rate of 70–80%. Tantak and Chaudhari (2006) [27] recorded 81.95, 85.57, and 77.83% COD in aerobic SBR after Fentons' oxidation. El-Gohary and Tawfik (2009) [28, 29] reported 78% COD removal and 68.9% BOD removal in aerobic SBR after coagulation-flocculation at 6.35 h HRT. As a result, chemical pretreatments can be used to improve the COD efficiency, color removal, and BOD removal in an anaerobic sequential batch reactor.

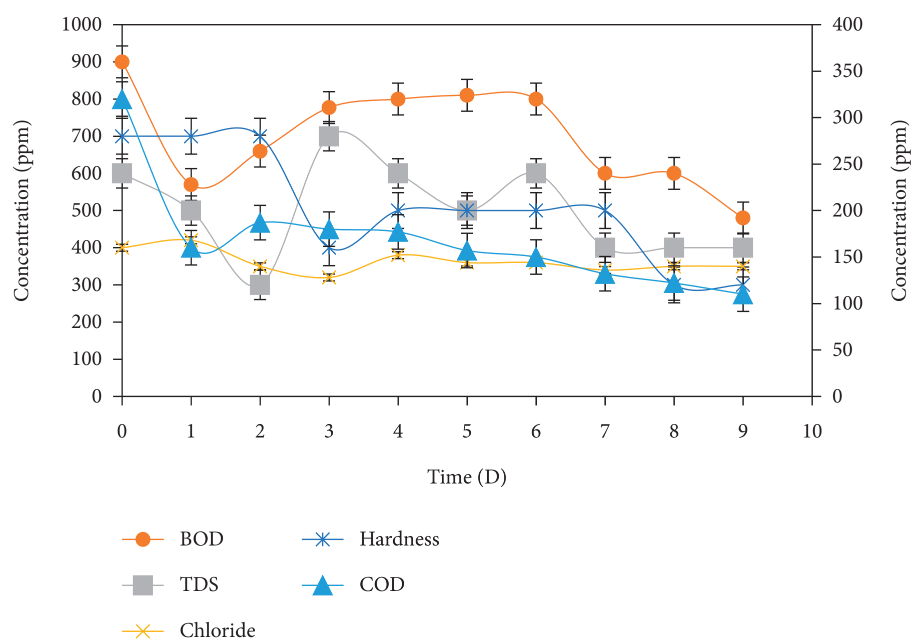
3.5. HPLC Analysis. An HPLC system equipped with UV-vis at 369 nm was used to examine the degradation of mixed reactive dyes in synthetic wastewater. HPLC analysis revealed a number of degraded products. When the retention time of the initial day wastewater sample peak was compared to that of the wastewater sample after treatment, it revealed damaged products. Figure 5(a) depicts the elution profile of the initial day wastewater with 100 mg/L of mixed dyes, which displayed a high peak at 2.625 minutes and other peaks at 3.093, 3.998, and 4.614 minutes. After 10 days of treatment in SBR, the elution profile of SBR-treated wastewater revealed a decreased peak at 2.814 minutes, 3.312 and 4.354 minutes, indicating that the peak at 2.652 minutes observed in the opening day elution profile was reduced and shifted to 2.814 minutes (as shown in Figure 5(b)). Peaks were found in the elution profile of the SBR influent at 2.638 and 3.296 retention times for 500 mg/L concentration, as illustrated in Figure 5(a). Following wastewater treatment in the SBR, the retention time was shifted to 3.252 and 4.081, and the height of the peaks was reduced, indicating that a few molecules were degraded during the treatment, as shown in Figure 6(b). The elution profile of untreated wastewater, i.e., the influent to SBR, revealed peaks at 1.850, 2.646, 32.896, 3.295, and 4.120 minute retention times in 1000 mg/L tests, as shown in Figure 7(a). The wastewater sample showed a change in retention time and reduced peaks after SBR treatment. Peaks were identified at 2.644 and 4.887 retention times,

Table 3: Characteristics of the influent and effluent in SBR with wastewater containing 1000 mg/L of mixed dyes.

Parameters	Influent (mg/L)	SBR effluent (mg/L)
COD	6000	4730
BOD	1670	1200
Chloride	900	600
TDS	1700	833
Hardness	1400	557

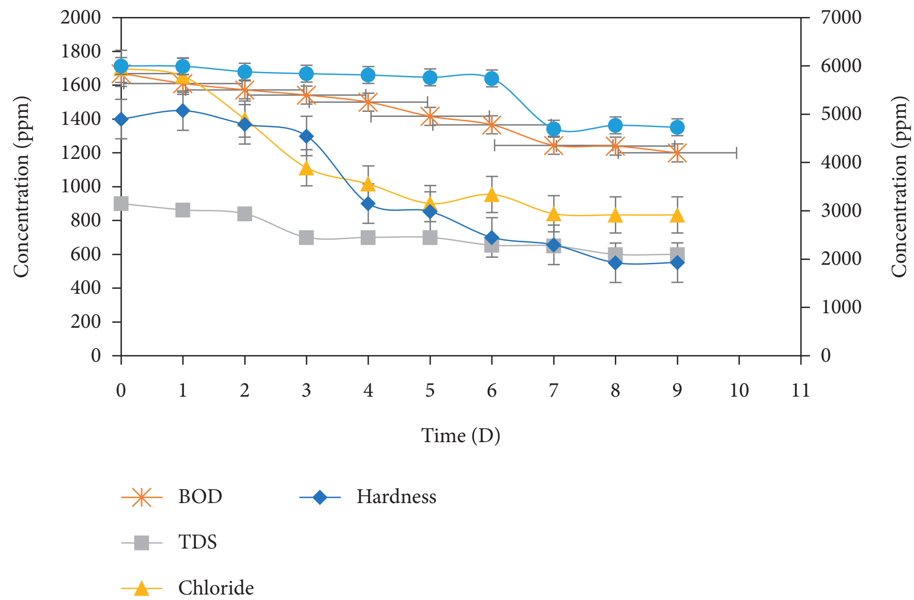


(a)



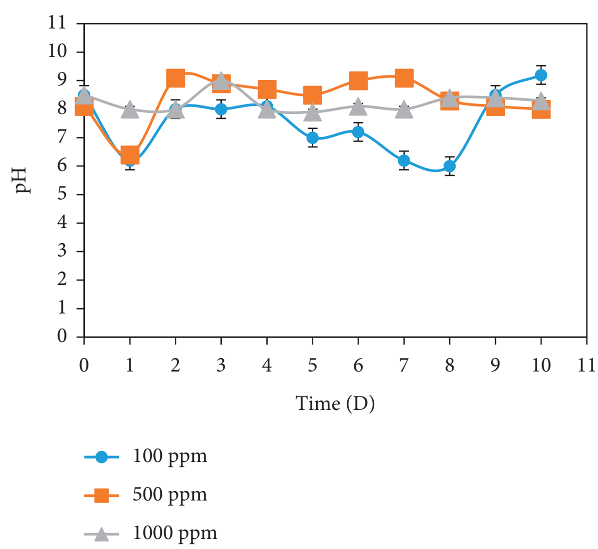
(b)

Figure 3: Continued.

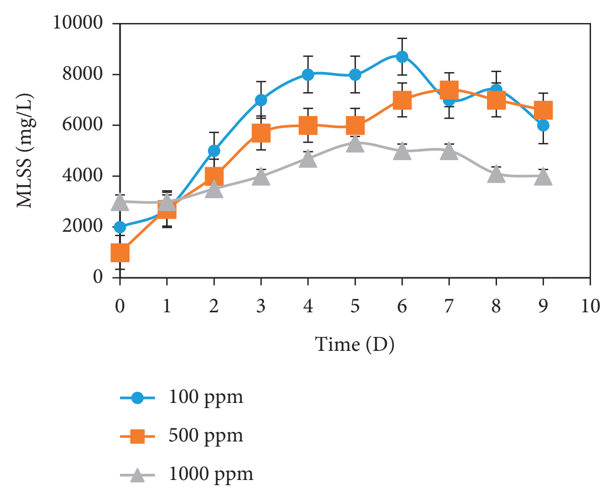


(c)

Figure 3: Physicochemical analyses for synthetic wastewater in SBR containing (a) 100 mg/L, (b) 500 mg/L, and (c) 1000 mg/L.

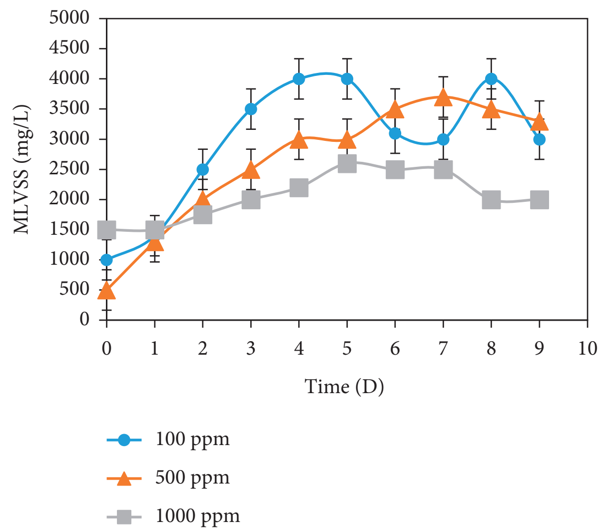


(a)



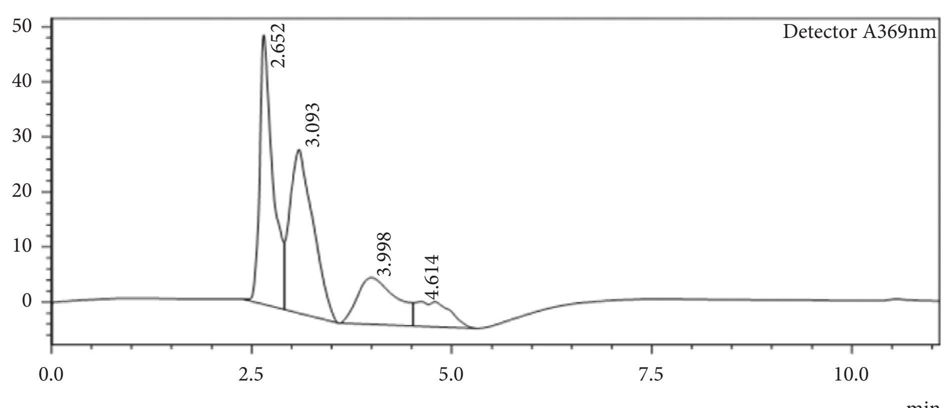
(b)

Figure 4: Continued.

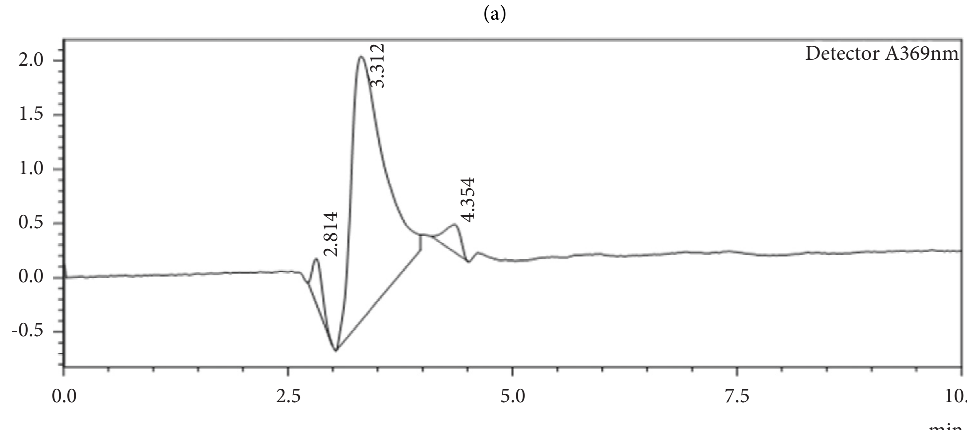


(c)

Figure 4: The pH, MLSS, and MLVSS for synthetic wastewater in SBR containing (a) 100 mg/L, (b) 500 mg/L, and (c) 1000 mg/L.



(a)



(b)

Figure 5: HPLC analysis of influent and effluent of SBR operated with 100 mg/L of mixed dyes.

indicating that degradation occurred during the SBR treatment, as shown in Figure 7(b). The peaks eluted in the synthetic wastewater at different concentrations proved that the dyes were broken down with the aromatic ring structure, which is depicted well in HPLC analysis. The degraded compounds with the retention time have been reported as

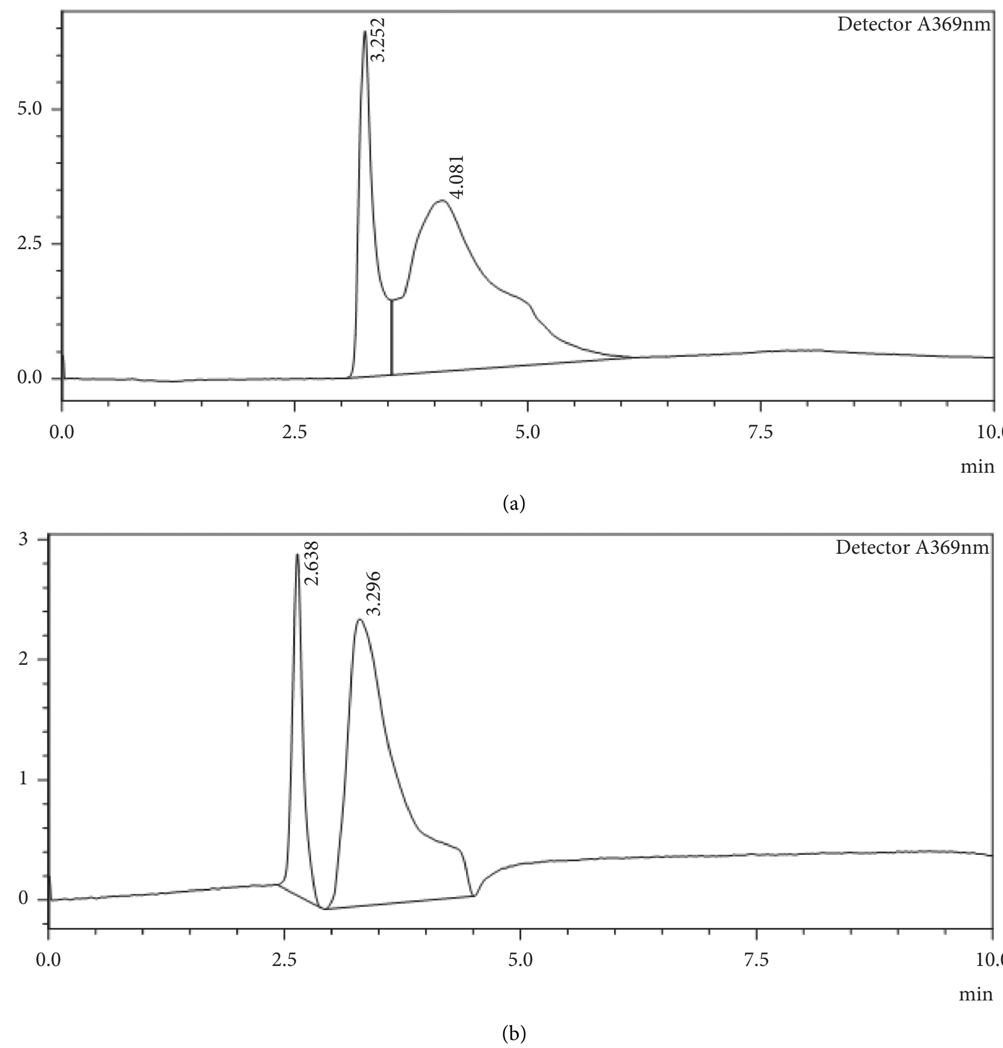


Figure 6: HPLC analysis of influent and effluent of SBR operated with 500 mg/L of mixed dyes.

synthetic individual dyes in the literature [16, 30, 31]. This analysis proves that the mixed textile dyes could be degraded well with the SBR employed in the current study.

3.6. Biosorption Studies. The biosorption of colors by the prepared adsorbent was detected in the standard dye solution and is used to treat the wastewater that the SBR and MBBR treated to increase the decolorization%. As a result, the dried activated sludge's capacity to absorb dyes present in treated wastewater was tested in 100 mg/L. At 20 minutes, the RR adsorbed amount was 0.07 mg/g, 0.011 mg/g of RB, and 0.015 mg/g of RBR. Adsorption gradually increased to 60 minutes, following which there was fast adsorption seen at 80 minutes (Figure 8). At 80 minutes, 0.286 mg/g, 0.118 mg/g, and 0.254 mg/g of RR, RB, and RBR, respectively, were adsorbed, and adsorption increased gradually until two hours of adsorption % dropped. From the results, it could be noted that each individual dye showed a different biosorption by the microbes and hence it reflected on the degradation of the individual and mixed dyes.

3.7. Phytotoxicity Studies. Green gram germination was seen in distilled water and treated wastewater in phytotoxicity studies. Untreated wastewater demonstrated lower germination and root and shoot length than control and treated wastewater in all three concentrations, 100, 500, and 1000 mg/L. The amount of toxicity in wastewater has been reduced following treatment. Phytotoxicity testing was carried out for seven days. Germination occurred in 100 mg/L of treated wastewater and the control. The germination percentage in untreated wastewater was only 70%. The chemicals and dyes inhibited plant growth because they were not degraded in untreated wastewater. Germination was reported to be 70% in untreated wastewater. The root and shoot lengths of untreated wastewater were much shorter than those of control and treated wastewater, demonstrating that the wastewater had been treated and the dyes had been degraded in treated wastewater. Because the dyes were not degraded in untreated wastewater, the chemicals and dyes impeded plant development [16, 20, 30].

Germination percentages for both control and treated wastewater were 100% in 500 mg/L experiments. Because the

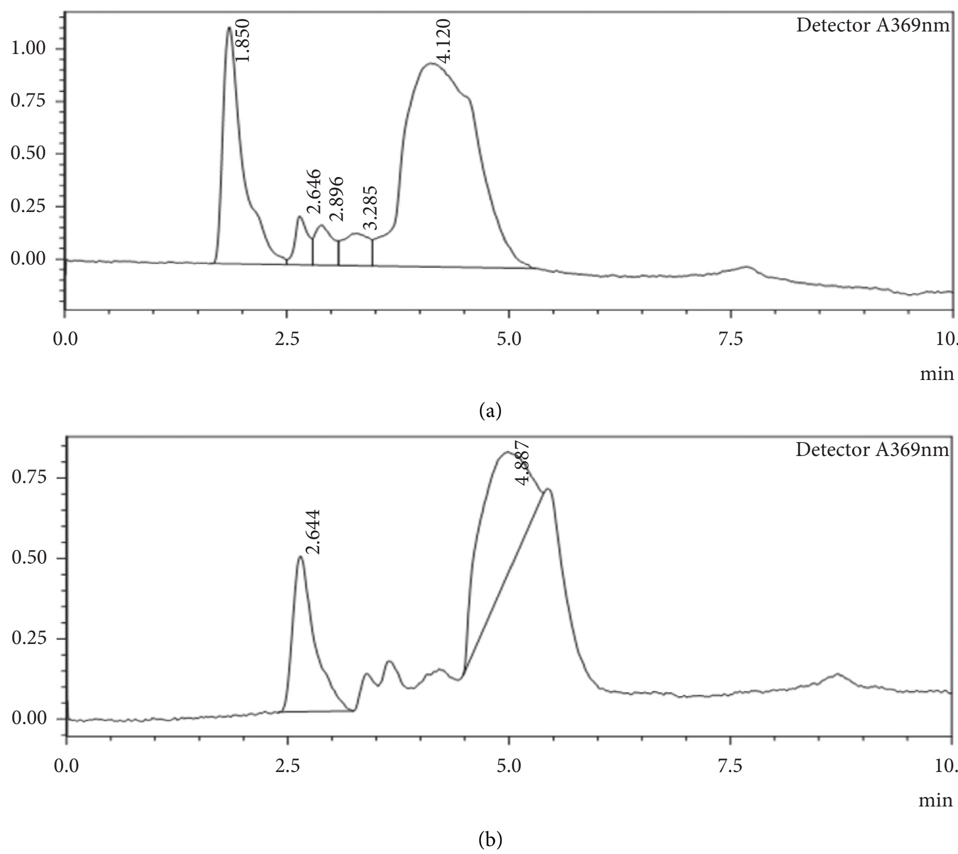


Figure 7: HPLC analysis of influent and effluent of SBR operated with 1000 mg/L of mixed dyes.

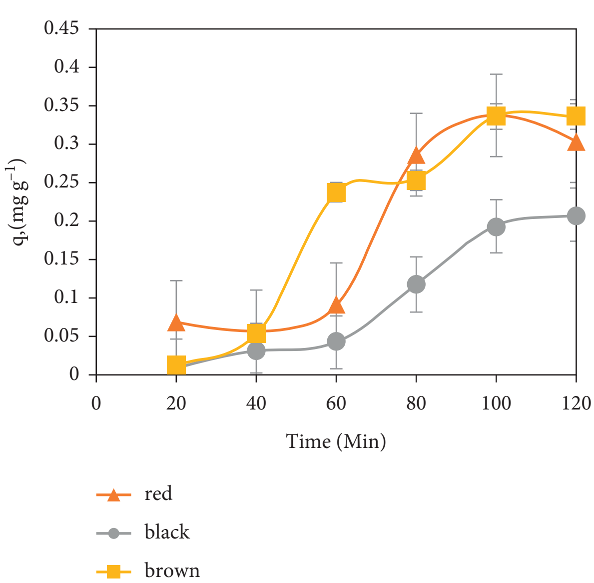


Figure 8: Biosorption of dyes in treated wastewater using DAS.

dye concentration was so high (500 mg/L), only 60% of the seeds germinated in untreated wastewater. Control and treated wastewater were able to boost plant growth at 1000 mg/L concentrations. For both control and treated wastewater, 100% germination was found. Only 60% of the plants germinated in untreated wastewater; this was due to the dye concentration being very high in 1000 mg/L of untreated wastewater, which impeded plant development. The sprouts seeded in treated wastewater had greater root and shoot lengths than the control, with a root length of

Table 4: Phytotoxicity study using green gram with wastewater containing (a) 100 mg/L of mixed dyes, (b) 500 mg/L of mixed dyes, and (c) 1000 mg/L of mixed dyes.

(i) Concentration of mixed dyes (100 mg/L)	*Root length (cm)*	*Shoot length (cm)*	*Germination percentage*
Distilled water	3.28	19.25	100%
Untreated wastewater	2	12.94	70%
Treated wastewater	6.6	16.2	100%
(ii) Concentration of mixed dyes (500 mg/L)	*Root length (cm)*	*Shoot length (cm)*	*Germination percentage*
Distilled water	3.2	16.13	100%
Untreated wastewater	1.22	7.7	60%
Treated wastewater	3.96	18.28	100%
(iii) Concentration of mixed dyes (1000 mg/L)	*Root length (cm)*	*Shoot length (cm)*	*Germination percentage*
Distilled water	2.26	13.76	100 %
Untreated wastewater	1.66	11.33	60 %
Treated wastewater	2.77	17.23	100 %

3.96 cm and a shoot length of 18.28 cm; this might be attributed to the presence of activated sludge, which acts as a fertilizer for the plants.

The phytotoxicity test with *Vigna radiata* revealed that high dye concentrations could impair plant growth. The results were compatible with Karthikeyan and Kanchana (2014) [20]. The development of plants in untreated wastewater was less than that of plants in treated wastewater. In 500 mg/L studies, the treated wastewater plants had greater root and shoot lengths than the control; this might be attributed to the fertilizing quality of the activated sludge employed in wastewater treatment (Table 4).

4. Conclusion

The synthetic wastewater containing mixed textile colors was treated with an SBR in the current study. SBR treatment with activated sludge produced effective results and has the ability to remove mixed reactive azo dyes from wastewater. The research may be extended to include a comparison of the performance of SBR and a moving med biofilm reactor (MBBR) in different mixed dye concentrations The decolorization technique employs dried activated sludge that has been chemically activated and is used to treat wastewater that has been treated in an SBR reactor. The sludge produced by the process can be utilized for remediation by an alkaliphilic bacterial consortium. The phytotoxicity tests in *Vigna radiata* revealed outstanding plant growth with a high dye concentration. As a consequence, the data revealed that plant development differed between treated and untreated wastewaters, with treated wastewater having longer root and shoot lengths than the control. Shrubs and other phytoremediation plants, on the other hand, can be utilized to entirely remove the textile dye.

Data Availability

Data used to support this study are available from the corresponding author upon request.

Disclosure

A preprint has previously been published with the following details "Akshaya Vidhya T, et al., (2019), Treatment of Mixed Azo Dyes in an Aerobic Sequential Batch Reactor and Toxicity Assessment using *Vigna radiata*, bioRixv, doi: https://doi.org/10.1101/680249."

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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