Biodecolorization of Azo Dye Mixture (Remazol Brilliant Violet 5r and Reactive Red 120) by Indigenous Bacterial Consortium Obtained From Dye Contaminated Soil

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Abstract

Discharge of the untreated wastewater containing dyestuff into the surrounding aquatic environment is of significant environmental concern. These dying effluents not only change the color of water bodies but also has many unfavorable conditions and release toxic by-products, which are mutagenic, carcinogenic, and hazardous to different life forms. The present study investigated the biodegradation and removal of dye mixture (Remazol Brilliant violet 5R and Reactive Red 120) using a new bacterial consortium isolated from dye contaminated soil. Among the total 15 isolates screened, the two most efficient bacterial species (SS07 and SS09) were selected and identified as *Enterobacter cloacae* (MT573884) and *Achromobacter pulmonis* (MT573885) through biochemical assays and 16S rRNA gene sequencing. The removal efficiency of dye mixture by *Enterobacter cloacae* and *Achromobacter pulmonis* at an initial concentration of 100 mg L^{-1} was 82.78 and 84.96%, discretely. The bacterial consortium was developed using selected isolates, and the optimum conditions for the removal of dyes were investigated by studying the effects of pH, temperature, carbon and nitrogen sources, dye concentration, and inoculum size. The maximum decolorization efficiency was achieved at pH, 7; temperature, 37°C; dye concentration, 100 ppm; and initial inoculum concentration, 0.5 ml, respectively. Mannitol and Ammonium sulfate was identified as the most suitable carbon and nitrogen sources for better bacterial growth and decolorization. The maximum removal efficiency of 91.3% achieved at the optimal conditions after 72 h of incubation. Decolorization of azo dyestuff by the developed microbial consortia conforms to the zero-order reaction kinetics model. Consortia of *Enterobacter cloacae* and *Achromobacter pulmonis* was established as an effective decolorizer for the Remazol Brilliant violet 5R and Reactive Red 120 dye mixture with > 90% color removal.

1. Introduction

Rapid industrialization results in the discharge of a large amount of waste into the environment and creating more pollution. The majority of colored effluents released from textile, food, leather, dyestuff, and dyeing industries consist of various dyes [1]. The residual dyes released from these effluents introduce different organic and inorganic components in the natural water resources and land [2]. The release of textile wastewater into the surface water without any treatment can cause a rapid depletion of dissolved oxygen and lead to significant environmental damage. Further pollution can result from the intensive irrigation of agricultural lands and grassland contamination and pasture with water polluted through industrial effluents [3, 4]. The sunlight penetration into deeper layers of water bodies is significantly reduced, thereby disturbing photosynthetic activity, resulting in water quality deterioration and lowering the gas solubility. It causes acute toxic effects on aquatic flora and fauna. Most of the dyes released from wastewater, including their breakdown products, are toxic, carcinogenic, or mutagenic to humans and other life forms [5].

Azo dyes are the primary group of colorants broadly used in the textile industry for dyeing processes. These dyes are an environmental concern because of their color, bio recalcitrance nature, and potential toxicity to animals and humans. Azo dyes also exhibit a high level of resistance to degradation and deterioration, as their chemical structure contains aromatic compounds with one or more (-N = N-) azo groups [4, 6]. There are several physical and chemical techniques for dye removal from wastewater. Adsorption by activated carbon, peat, and silica gel is the most common and widely utilized technology in medium and small-scale industries. Advanced technologies such as membrane filtration, electrokinetic coagulation, flocculation, froth flotation, ion exchange, membrane filtration, ozonation, Fenton oxidation, and reverse osmosis are also used for the decolorization of dyes in wastewater [2, 7]. However, it is still challenging to treat the effluents using these conventional methods mainly because of high chemical oxygen demand, intense color, pH, and the excess amount of suspended solids, salts, sulfides, chlorine, and heavy metals [8, 9]. Moreover, the physical and chemical methods have the disadvantages of being highly expensive, coupled with the formation of a large amount of sludge and the emission of toxic substances [5, 10].

Recently, growing interest has been shown in the microbial treatment of azo dye contaminated wastewater. It is quite eco-friendly, inexpensive, produces lower amounts of sludge and fewer toxic metabolites, and has lower water consumption than many other alternatives. Diverse taxonomic groups of microbes such as bacteria, fungi, yeast, and algae have been reported for their ability to decolorize azo dyes [5]. Among the bioremediation techniques, wastewater treatment by using bacteria is emerging as one of the promising methods. Bacterial decolorization occurs due to the adsorption or biodegradation of dye molecules by microbial cells [11]. Enzymatic biotransformation initiates degradation and decolorization of azo dyes while using bacteria, and it involves the reductive cleavage of azo bonds with the azo reductase enzyme [9, 11]. Bacteria capable of dye decolorization, both in pure cultures or mixed cultures, have been reported. Microbial consortia utilization offers considerable advantages over using pure cultures in the degradation of synthetic dyes [12, 13].

On comparing the mixed culture with pure culture, the individual strains cannot degrade azo dyes completely. Biodegradation of azo dyes produces intermediate products such as aromatic amines, which vary in their resistance to biodegradation and can be mutagenic and carcinogenic. These intermediate compounds can limit the degrading bacterial growth and activity such that the treatment process becomes incompetent and unfeasible [8, 9]. In a microbial consortium, the individual strains may attack the dye molecule at different positions. They will also utilize metabolites produced by the co-existing strains for further reactions to occur. The degradation of aromatic amines will occur due to complementary organisms and makes the process more effective and efficient. As a result of synergistic metabolic activity, the biodegradation and mineralization by mixed microbial populations occur at a higher rate [10, 13]. Hence, microbial community structure and composition may influence azo dye removal efficiency since the different microbial taxa in the consortia may have different metabolic and ecological functions. Understanding the microbial community's structure and composition changes can offer critical data for optimizing the decolorization process [13]. Further maximum decolorization of the

dyes can be achieved by optimizing conditions such as incubation time, pH, temperature, dye concentration, and availability of suitable carbon or nitrogen sources [10].

Based on these considerations, the present study deals with the isolation and identification of bacterial strains from dye-contaminated soil and developing a bacterial consortium for efficient decolorization of a mixture of widely used textile azo dyes (Remazol Brilliant violet 5R and Reactive Red 120). In addition, the decolorization parameters had been optimized by the one-factor-at-a-time approach to attain maximum dye degradation by developed bacterial consortia.

2. Materials And Methods

2.1 Sample collection

The soil samples were collected from textile dye contaminated sites at the common effluent treatment plant (CETP), Tirupur, Tamilnadu, India. The samples were collected in sterile screw-capped vials and transported from sites to the laboratory as early as possible. Samples were stored at 4°C until further analysis.

2.2 Chemicals and Culture Medium

The textile azo dyes Remazol Brilliant violet 5R (RBV 5R) and Reactive Red 120 (RR 120) were purchased from SIGMA, India. The Minimal Salt Media (MSM) used for enrichment and decolorization was prepared by adding the following components: Na_2HPO_4 (12.8 g L^{-1}), KH_2PO_4 (3 g L^{-1}); NH_4Cl (1 g L^{-1}), NaCl (0.5 g L^{-1}), 0.05 M $MgSO_4$ (10 ml L^{-1}), 0.01 M $CaCl_2$ (10 ml L^{-1}) and 20% Glucose (30 ml L^{-1}) [14]. All the chemicals were of analytical grade and purchased from Hi-Media Laboratories, Mumbai. Furthermore, Nutrient broth and Nutrient agar media (Hi-Media Laboratories) was used for culture maintenance.

2.3 Isolation and screening of dye degrading bacteria

Soil samples were used for the isolation of dye degrading bacterial strains. An amount of 10 g of soil sample was aseptically inoculated into MSM medium amended with 100 mg L^{-1} of filter-sterilized RBV 5R and RR 120 in 250 mL Erlenmeyer flasks. Individual bacterial isolates were obtained from the enriched culture by plating on MSM agar medium containing 100 mg L^{-1} of individual dyes. The isolates which exhibited decolorization of the dye as a zone of clearance around them were picked and cultured for further experiments. The selected isolates were then purified by streaking on nutrient agar medium and further utilized for decolorization assay.

2.4 Decolourization assay

All decolorization assays were carried out in triplicates. A volume of 100 μL of precultured bacterial cultures was added to 10 mL of MSM containing 100 mg L^{-1} of RBV 5R, RR 120, and dye mixture (contains 100 mg L^{-1} of both RBV 5R and RR 120). The bio decolorization of individual and mixed dye by bacterial strains was observed for five days. Uninoculated MSM added with azo dyes was used as a control. The samples were withdrawn periodically to monitor the decolorization process, centrifuged at 10,000 rpm for 15 min, filtered through a syringe filter. Decolorization was measured using UV-Vis spectrophotometer at the corresponding λ_{max} of the dye (420 nm for BRV 5R, 535 nm for RR 120, and mixed dye) and was compared with the uninoculated control. The color removal efficiency of the bacterial cultures was determined using an Eq. (1):

$$\% \text{ of Decolourization} = ((A_0 - A_t) \times 100)/A_0 \quad \text{---------(1)}$$

Where, A_o = Initial absorbance; A_t = Final absorbance

The measurement of decolorization of dyes by bacteria was performed at an interval of 24 hours for five days, and the results were tabulated. Amongst the isolates, the two most efficient dye decolorizing bacterial strains were further taken for the compatibility analysis.

2.5 Compatibility analysis

Microbes involved in the consortia should not exhibit any antagonistic effect over other microbes in the consortia. The decolorization efficiency will be enhanced only when the organisms are compatible with each other. The compatibility analysis was done with the selected efficient strains. The test was performed as follows: two nutrient agar plates were taken, and each plate was bored with a well. The plates were smeared with one of the two selected cultures, and 10µl of the supernatant from another culture was added to the well and vice versa. The plates were then kept for incubation at 37˚C for 24 hours. The absence of any inhibition zone around the wells is considered as the strains were compatible. The test was repeated with permutations and combinations.

2.6 Identification of selected strains

The colony characteristics and microscopic morphology of the selected dye degrading bacterial strains were identified by using standard biochemical and microscopic techniques. Fresh cultures of the isolates were used to study colony characteristics, Gram's reaction, and cell morphology. The isolate's physiological and biochemical characteristics were evaluated by Voges–Proskauer, methyl red, indole, catalase, oxidase,

urease, citrate utilization, and H_2S production tests. The ability of the organisms to ferment several sugars, including glucose, lactose, sucrose, and mannitol were also performed. The study of colony characteristics and the cell morphology under microscope includes smear preparation, and Gram's staining was determined according to Bergey's Manual of Systemic Bacteriology [15].

Both strains were genetically identified by 16S rRNA sequencing after extracting the genomic DNA using the EXpure Microbial DNA isolation kit. The 16S region of the ribosomal rRNA gene was and amplified using the universal primers. The PCR amplification was done by initial denaturation at 94°C for 3 min followed by 30 cycles of 94°C for 30 sec, 60°C for 30 sec, 72°C for 60 sec, and a final extension at 72°C for 10 min. PCR purification was done by Montage PCR Clean-up kit (Millipore). The PCR product was sequenced using the universal primers. Using ABI PRISM® BigDyeTM Terminator Cycle Sequencing Kits with AmpliTaq® DNA polymerase (FS enzyme) (Applied Biosystems), sequencing was performed. The sequences were assembled and edited using Bioedit software and deposited in the NCBI database for accession numbers. The sequence generated from the automated sequencing of PCR amplified 16S ribosomal RNA was analyzed through the NCBI BLAST (http://www.ncbi.nlm.nih.gov) program to ascertain the possibility of a similar organism through alignment of homologous sequences and the required corresponding sequences that were downloaded. The evolutionary history was inferred using the Neighbor-joining method, which was performed on the Phylogeny.fr platform through online software: Muscle (v3.7), Gblocks (v0.91b), PhyML (v3.0 aLRT), and TreeDyn (v198.3) (Dereeper et al., 2010; Edgar, 2004). The sequences obtained in this study were deposited in the GenBank database.

2.7 Optimization of physio-chemical parameters

The optimum decolorization of azo dyes performed by adopting to standard One-Factor-At-A-Time (OFAT) approach, i.e., modifying one individual parameter while keeping the other parameters at a fixed level. The experiments were conducted with a consortium of SS07 and SS09 in MSM medium supplemented with 100 mg L^{-1} of mixed dye at various pH, temperature, carbon, and nitrogen sources. The medium was maintained at different pH (3, 5, 7, 9, and 11) by using 0.1M HCl and NaOH solution. The effect of temperature was studied by inoculating overnight culture and incubating in a shaker at 20°C, 25°C, 30°C, 37°C, and 40°C. Carbon sources (fructose, lactose, sucrose, starch, and mannitol) at a concentration of 1% were added individually as a supplement to MSM. Nitrogen sources, such as yeast extract, peptone, tryptone, ammonium sulfate, and urea, were added to MSM at a concentration of 0.5%. To find a bacterial consortium color removal efficiency at higher dye concentrations, MSM was amended with different concentrations (50, 100, 150, 200, and 250 mg L^{-1}) of azo dyes. A volume of 1 ml of the overnight culture was inoculated in the flasks and incubated for five days. The dye removal efficiency was observed using a UV-vis spectrophotometer after incubation periods of 24, 48, 72, and 120 h. In order to study the effect of inoculum concentration, the sterilized 100 ml MSM broth was amended with 100 mg L^{-1} of RBV 5R and RR 120. Different volumes (0.5, 1, 1.5, 2, and 2.5 ml) of overnight cultures were inoculated. The measurement of decolorization was performed at an interval of 24 hours for five days. A separate flask without culture in dye amended MSM was used as control.

2.8 FTIR analysis

The degradation of azo dyes was studied by analyzing the changes in Fourier-Transform Infrared Spectroscopy (FTIR) spectra of dye mixture before and after treatment. FTIR analysis was performed using SHIMADZU FTIR 8400S (Japan) in the mid-Infrared region of 400–4000 cm^{-1} with 16 scan speed. All samples were run in triplicate and the data presented are the average of the three measurements.

3. Results And Discussion

3.1 Isolation and selection of dye degrading bacterial isolates

Cultivable bacteria from the dye-contaminated soil sample were isolated using the MSM agar media plate containing azo dye using the spread plate technique. The plates were incubated at 37 ± 2˚C for 24 h, and colonies with distinct morphology and decolorization zone were selected and purified by regular subculturing. The early screening of microbial populations for the decolorization of azo dyes indicates 15 isolates designated as SS01 to SS15. The isolated strains were then subjected to quantitative screening for their ability to decolorize RBV 5R (100 mg L^{-1}) and RR 120 (100 mg L^{-1}) by inoculating in MSM broth for five days. Table 1 shows the decolorization of azo dyes (RBV 5R and RR 120) by individual isolates. Among the 15 isolates, 13 strains (SS01–SS13) decolorized more than 65% of the RBV 5R and all the strains decolorized above 78% of the RR 120 dye in 72 h. However, two unidentified strains designated as SS07 and SS09 were recognized as the most efficient decolorizer of both dyes. About 81 and 76 % of the RBV 5R and 90 and 88% of the RR 120 dye were decolorized after 72 h of incubation by SS07 and SS09, correspondingly. All the 15 isolates were further grown on MSM media amended with mixed dye. They showed the ability to decolorize dye mixture in 48 h, and the maximum decolorization was observed after 72 h of incubation at 37ºC. Similar to the decolorization of individual dyes, bacterial isolates labeled as SS07 and SS09 showed promising decolorization of 84.96 and 82.78% after 72 h (Table 1). At the same time, no other strain exhibited the potential to decolorize more than 80% of the dye mix even after incubation of 120 h. These two laboratory isolates were selected for further studies as it achieved maximum decolorization efficacy compared to all other strains.

Table 1
Percentage decolorization of Remazol Brilliant violet 5R and Reactive Red 120 by bacterial strains isolated from contaminated soil

Isolates Number	Percentage of Decolorization														
	Remazol Brilliant violet 5R (420 nm)					Reactive Red 120 (535 nm)					Mixed dye (535 nm)				
	Time (Hours)					Time (Hours)					Time (Hours)				
	24	48	72	96	120	24	48	72	96	120	24	48	72	96	120
SS01	10.53	42.86	71.43	66.67	52.39	9.52	40.48	80.95	78.57	71.43	13.04	39.13	76.43	72.08	67.39
SS02	4.76	38.1	71.43	61.9	57.14	11.9	45.24	85.71	80.95	73.81	10.86	41.3	78.6	73.91	71.74
SS03	4.76	38.1	66.67	57.14	52.38	14.29	47.62	83.33	80.95	76.19	10.87	39.13	78.6	76.09	73.91
SS04	4.76	42.86	71.43	57.14	47.62	11.9	42.86	85.71	83.33	78.57	8.69	41.3	76.43	78.26	73.91
SS05	14.29	38.1	66.67	61.9	38.1	7.14	47.62	83.33	80.95	76.19	15.21	39.13	78.6	77.43	71.74
SS06	4.76	33.33	66.67	57.14	38.1	4.76	33.33	78.57	71.43	64.29	8.69	23.91	63.04	62.6	63.91
SS07	14.29	28.57	80.95	57.14	42.86	16.67	50	90.47	80.95	78.57	15.21	45.65	84.96	80.43	73.91
SS08	10.53	47.62	66.67	66.67	52.39	14.29	47.62	85.71	83.33	76.19	13.04	39.13	78.26	76.09	69.56
SS09	10.53	33.33	76.19	52.39	52.39	14.29	50	88.09	83.33	76.19	4.35	23.91	82.78	71.74	76.09
SS10	4.76	38.1	66.67	57.14	52.39	11.9	45.24	80.95	83.33	73.81	10.87	41.3	77.6	73.91	66.09
SS11	14.29	42.86	61.9	57.14	47.62	7.14	40.48	78.57	71.43	64.29	15.21	36.96	72.43	73.91	68.26
SS12	4.76	47.62	61.9	57.14	52.39	14.29	45.24	85.71	78.57	76.19	6.52	36.96	78.26	76.09	73.91
SS13	4.76	47.62	66.67	71.43	47.62	11.9	47.62	83.33	78.57	71.43	4.35	30.43	54.35	65.22	71.74
SS14	4.76	19.05	42.86	47.62	47.62	4.76	30.95	78.57	71.43	69.05	10.87	41.3	71.73	72.6	66.09
SS15	10.53	19.05	33.33	38.1	47.62	14.29	35.71	80.95	83.33	73.81	13.04	45.65	78.6	78.26	71.74

3.2 Identification of dye-decolorizing bacteria

The two organisms which showed significant decolorization of the mixed dye were investigated for their morphological, cultural, physiological, and biochemical features. Results of biochemical experiments have represented in Table 2. Through Gram's staining, it was found that both the isolates were gram-negative straight rods. The isolate SS07 exhibited positive results for Voges-Proskauer, Citrate, Glucose, Lactose, Sucrose, Mannitol, and Catalase metabolism tests and negative results for Indole, Methyl red, Urease and Oxidase tests. The Indole production, Methyl red, Voges-Proskauer, and Urease tests produced negative results for the SS09 Bacterium. While, Citrate, Glucose, Catalase, and Oxidase test results were observed to be positive. The above results indicate that the isolates SS07 and SS09 closely related to *Enterobacter* and *Achromobacter* Genus, respectively.

Table 2
Biochemical results for selected bacterial isolates (SS07 and SS09)

Test	SS07	SS09
Gram's Staining	-	-
Shape	Straight rods	Straight rods
Indole Production	-	-
Methyl red	-	-
Voges-Proskauer	+	-
Citrate utilization	+	+
Glucose fermentation	+	+
Lactose fermentation	+	+/-
Sucrose fermentation	+	+/-
Mannitol fermentation	+	+/-
Urease	-	-
Catalase	+	+
Oxidase	-	+

Further, the 16S rDNA gene sequencing was also performed for molecular identification of the two bacterial isolates. The 16S rDNA sequences of bacterial isolates SS07 and SS09 were deposited to NCBI and Gene Bank (Accession no: MT573884, MT573885). BLAST similarity search showed that the bacterial isolate SS07 was similar to *Enterobacter* and SS09 was similar to *Achromobacter*. A phylogenetic tree constructed with similar sequences showed that isolates SS07 and SS09 belonged to *Enterobacter cloacae* and *Achromobacter pulmonis*, respectively (Fig. 1A and 1B).

3.3 Compatibility Testing

The compatibility of the bacterial strains is crucial for developing consortia that can degrade toxic dyes more efficiently. Both *Enterobacter cloacae* and *Achromobacter pulmonis* used in compatibility testing showed thriving and strong growth in the nutrient agar medium. Further, no reduction in the color removal percentage was observed during the decolorization of mixed azo dyes by the developed consortia. The above results suggest that the selected isolates were compatible with each other.

3.4 Optimization of physio-chemical parameters

3.4.1 Effect of pH and temperature

The pH of the culture medium plays a critical role in the optimal physiological performance and the transport of various nutrient components across the cell membrane [16]. The effect of pH was studied at different pH ranged between 3–11. Comparison of decolorization of mixed dye at various pH is presented in Fig. 2(A). The dye decolorization varies with pH, and the maximum decolorization was observed at pH 7 (87.54%), followed by pH 9 (80.43%). Figure 2(A) shows that a threefold surge in the color removal percentage can be achieved by increasing pH from 5.0 to 7.0. This indicates that neutral and basic pH values would be more favorable for decolorizing the azo dye. At the optimum pH, the microbial biomass surface becomes negatively charged, which enhances the binding of positively charged dye. Binding occurs through the electrostatic force of attraction, and it results in a considerable increase in color removal [12]. Below the optimum pH, the competency between the H^+ ions with dye cations, triggering a diminution in color removal efficiency. At alkaline pH, the azo bonds will be deprotonated to negatively charged compounds. It results in obstruction of azo dye decolorization. In acidic pH, the azo bond will be protonated, leading to decreased dye decolorization due to chemical structure changes [17, 18].

The growth and decolorizing ability of the consortium were largely dependent on the temperature. Since decolorization is a metabolic process, fluctuations in the temperature play a major role in treating dye effluent using bacteria [19]. Over a range of temperatures from 25 to 37ºC, the bacterial consortium color removal activity was found to be increased along with the temperature (Fig. 2B). Further increase in temperature beyond 37°C resulted in a marginal reduction in decolorization activity due to the loss of cell viability or thermal deactivation of decolorizing enzymes [20]. Earlier studies accentuated that the ideal temperature for the growth of bacterial consortia and dye decolorization was ranged between 25 to 37ºC [12]. Shah [19] reported that the optimal pH and temperature for the decolorization of Remazol Black B were 6 and 37ºC, respectively. Gunti et al. [21] achieved the complete decolorization of Acid orange 10 (200 mg L^{-1}) by *Bacillus subtilis* at pH 6 and 40º C of temperature. Similar observations

were conveyed on degrading Reactive Red 120 by *Bacillus cohnii* [22] and by a bacterial consortium of *Stenotrophomonas acidaminiphila, Pseudomonas putida, Pseudomonas fluorescence*, and *Bacillus cereus* [23].

3.4.2 Effect of Carbon and Nitrogen Sources

In order to enhance the decolorization performance of the bacterial consortium, additional carbon and nitrogen sources were supplied. As shown in Fig. 3A, the maximum decolorization of mixed dyes was observed as 86.09% with mannitol as a carbon source, while less decolorization was recorded with other supplements of carbon source. The decolorization was negligible in the presence of starch (36%) whereas moderate in the presence of fructose (59%), lactose (63%), and sucrose (74%). Nitrogen bases are vital media complements for NADH's renewal that acts as an electron donor to reduce azo dyes by microorganisms [24]. In our study, Ammonium Sulphate showed a maximum decolorization of 87.36%, followed by yeast extract (82.61%) after 72 h of incubation (Fig. 3B). Lalnunhlimi and Krishnaswamy [14] highlighted that the decolorization of mixed azo dyes (Direct Blue 151 and Direct Red 31) was improved while using yeast extract (0.5%) and sucrose (1%). In line with that, several studies [9, 25–27] have shown that the supplement of additional carbon and nitrogen sources can enhance the biodegradation of dyes by bacterial strains since the dyes are deficient in nutrient sources.

3.4.3 Effect of initial dye and Inoculum concentration

The ability of the bacterial consortium to decolorize the azo dyes at various concentrations (50, 100, 150, 200, and 250 mg L^{-1}) was investigated. Figure 4A illustrates a noticeable decrease in color removal due to an increase in dye's initial concentration. The dye removal was observed to be 91.65% at the concentration of 50 mg L^{-1}, which was slightly reduced to 91.3% at 100 mg L^{-1} of mixed dye. A further increase in dye concentration substantially reduced the color removal. This reduction may be due to the substrate inhibition effect and toxicity imposed by dye at higher concentrations [28, 29]. These observations were also supported by the recent findings of Martorell et al. [30] and Vargas-de la Cruz & Landa-Acuña [31]. Figure 4B showed the effects of inoculation size on decolorization of the dye and growth of the bacterial consortium. The result showed that at least 500 µl of inoculum was needed for the optimal decolorization performance within 72 h. When inoculation size further increased, both decolorization efficiency and strain growth were not increased. The results were consistent with those of some related researchers [30, 32, 33].

3.6 FTIR Spectral Analysis

The decolorization and biodegradation of azo dye were monitored by FTIR. A comparison between the FTIR spectrum of mixed dye before and after treatment with microbial consortia was given in Fig. 5. FTIR spectra of dye mix (Control) showed two peaks at 3348, 3379, and the broad peak at 2360 cm^{-1} are attributed to the N–H amino compound stretching. A strong, sharp peak at the wavelength of 1643 cm^{-1} is characteristic of double-bonded nitrogen groups such as azo (N = N) and imino (C = N) functional groups. Two specific peaks of wavelength 1080 and 1196 cm^{-1} are shown in the FTIR spectrum due to C-N (aliphatic amines) stretching and R-SO_3 (sulfur compounds). The change in adsorption position and appearance of new peaks was observed after treatment (Fig. 5). FTIR spectrum of azo dye mixture obtained after treatment with consortium showed a new peak at 2746 cm^{-1} for aldehyde stretching and two peaks at 1141 and 1211 for C–N stretching. Further reduction in peak intensity at 1080 cm^{-1} (belongs to sulfate ion) and strengthening the peak at 1365 cm^{-1} for C–N (Aromatic tertiary amine) were also observed. The significant difference in FTIR peak pattern of treated and untreated azo dyes signifies that microbial action led to biodegradation.

3.7 Kinetics study

In this present study, zero-, first-, second-order reaction kinetic models were applied to investigate the decolorization kinetics of mixed dye by developed bacterial consortia. The kinetic equation of zero-, first-, second-order reaction was expressed as below Eqs. (2)–(4):

$$[A]_t = [A]_0 - k_0 t \quad \text{——————(2)}$$

$$ln[A]_t = ln[A]_0 - k_1 t \quad \text{——————(3)}$$

$$1/[A]_t = 1/[A]_0 + k_2 t \quad \text{——————(4)}$$

Where $[A]_0$ and $[A]_t$ are the concentration of dye at the initial time (t = 0) and reaction time (t), respectively; t is the reaction time; k_0, $k_1$and k_2 is the apparent kinetic rate constant of zero-, first-, second-order reaction kinetics, respectively. For finding kinetic rate constant and correlation coefficient of zero-, first-, second-order kinetic models have plotted a graph of t versus $[A]_t$, t versus $ln[A]_t$, t versus $1/[A]_t$, respectively. It can be seen from Table 3, the value of the correlation coefficient ($R^2 > 0.95$) is closer to unity for the zero-order kinetic model than the first-, second-order kinetic models. Therefore, the results signified that mixed azo dyes decolorization followed the zero-order reaction kinetics well. The result is consistent with the previously reported work of Sudha et al. [34] and Tan et al. [35].

Table 3
The kinetic rate constant for the decolorization of mixed azo dyes

Dye Concentration (mg L^{-1})	Zero-order		First-order		Second-order	
	k_o (mg L^{-1} h^{-1})	R^2	k_1 (h^{-1})	R^2	k_2 (Lmg^{-1} h^{-1})	R^2
50	0.683	0.964	0.020	0.866	0.0044	0.790
100	1.404	0.960	0.020	0.863	0.0021	0.789
150	2.106	0.973	0.016	0.889	0.0009	0.811
200	2.717	0.952	0.015	0.857	0.0006	0.798
250	3.424	0.976	0.013	0.910	0.0003	0.840

4. Conclusion

The bacterial consortia strain developed in this study can decolorize about 91.3% of azo dye mixture (RBV 5R and RR 120) at 100 mg L^{-1}. At the start of the experiment, the constructed consortium reduced the concentration of azo dyes from 100 mg L^{-1} to 23 mg L^{-1} within 72 h. Further, the optimization of physicochemical conditions, including pH, temperature, carbon, and nitrogen source, effectually amplified the rate of decolorization. After using mannitol and ammonium sulfate as carbon and nitrogen sources and adjusting the pH to 7 and the temperature to 37°C, the bacterial consortia managed to decolorize 91.3% of the dye mix. The kinetic data indicate that the process was well described by zero-order kinetics. These investigations reveal the potential use of the developed bacterial consortia (*Enterobacter cloacae* and *Achromobacter pulmonis)* to treat dye effluent at an industrial scale efficiently. Further, field trials should be conducted to validate the efficiency of the developed consortia.

Declarations

Authors contributions

Rajendran R, Kiruthika S, Saranya P, and Arunkumar M were accountable for the conceptualization and design of the study. Kiruthika S and Saranya P worked in the acquisition of data through field and laboratory work. Arunkumar M interpreted the data and drafted the article. Rajendran R, Kiruthika S, and Vaishali C V revised it critically. All authors read and approved the final manuscript.

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Competing interests

The authors declare they have no competing interests.

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Figures

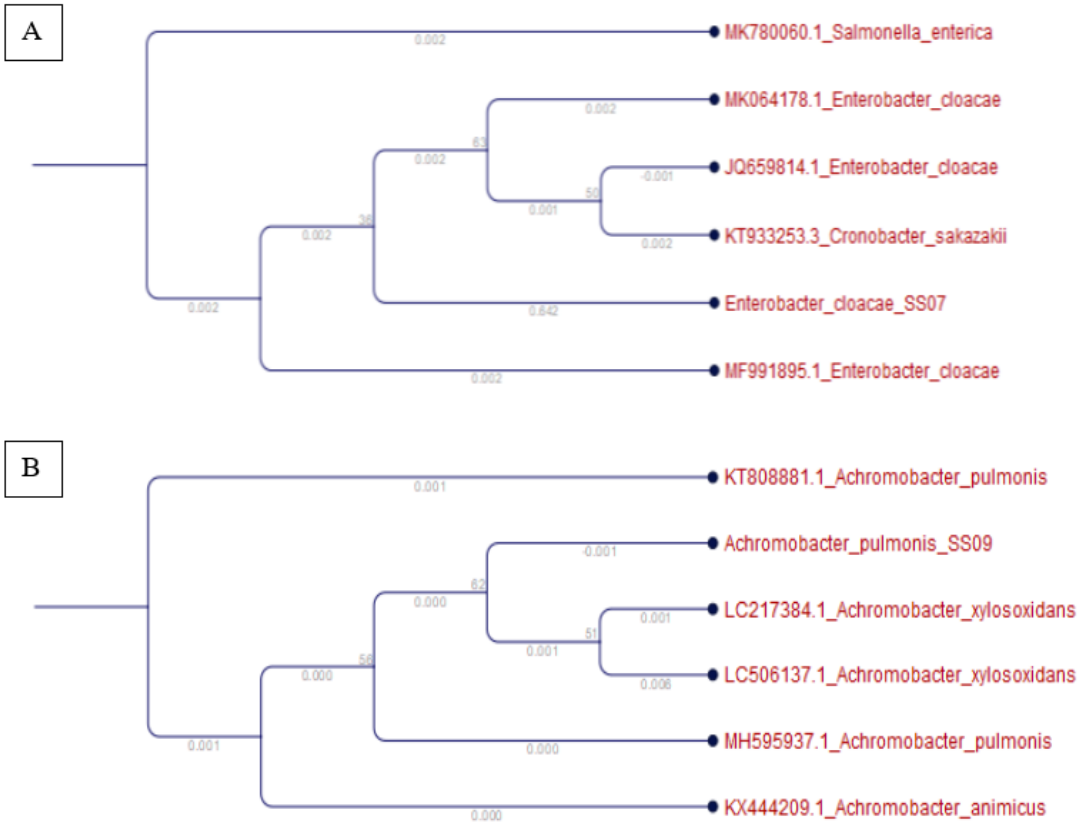

Figure 1

Phylogenetic relationship between the 16S rRNA sequence of the selected strains and the sequences from the database: A) SS07 B) SS09

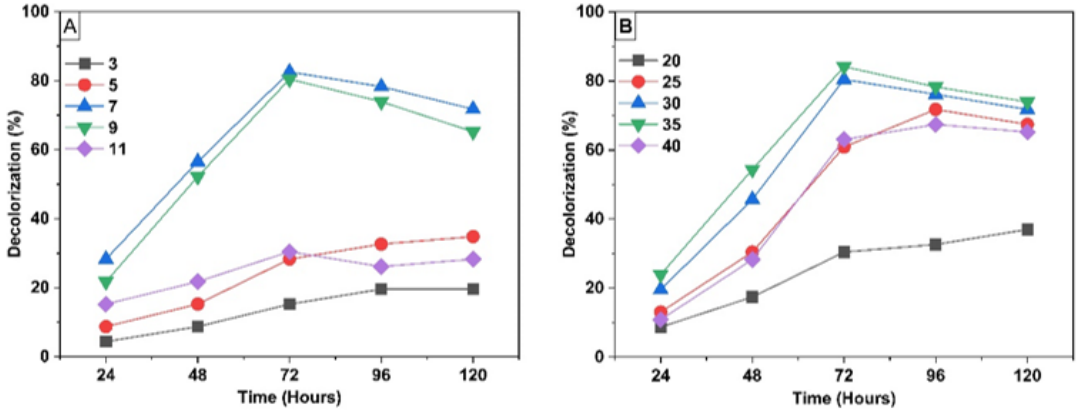

Figure 2

Effects of (A) pH and (B) temperature on decolorization of Azo dye mixture by developed consortia

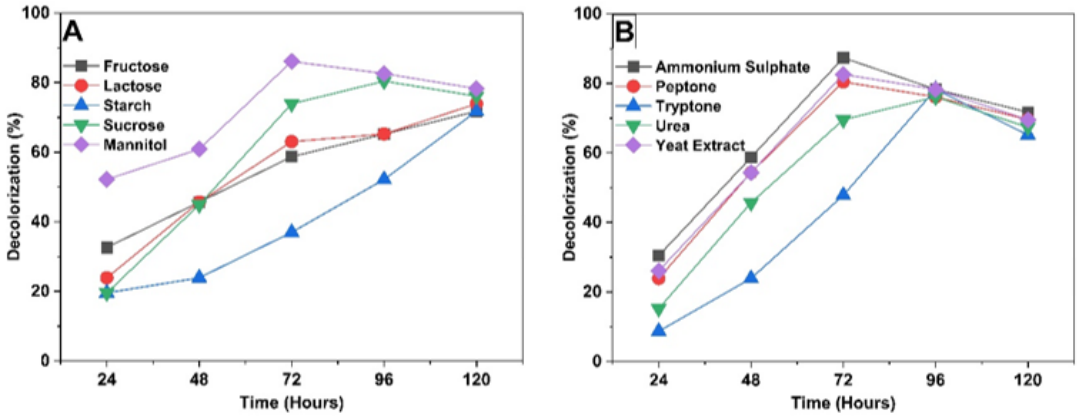

Figure 3

Effects of (A) carbon and (B) nitrogen sources on decolorization of Azo dye mixture by developed consortia

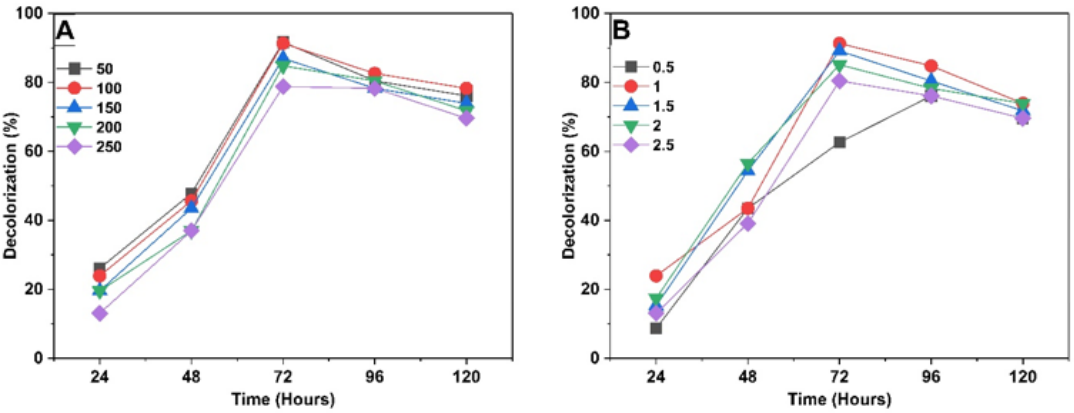

Figure 4

Effects of (A) dye concentration and (B) Inoculum size on decolorization of Azo dye mixture by developed consortia

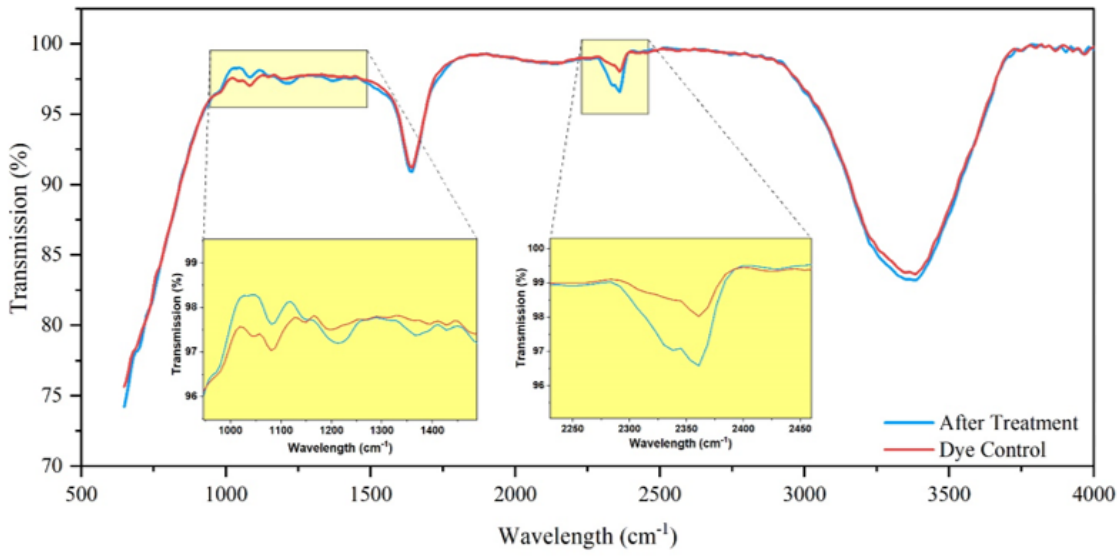

Figure 5

FTIR Spectra of azo dye mixture (before and after treatment)