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Eco-Friendly Biodegradation and Detoxification of Acid Red-88 textile dye by Textile Effluent Acclimatized Bacterial strain AR-1

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A B S T R A C T

Environmental bioengineering is continuously escalating its efforts in the biotic treatment of textile effluents, which is an ecologically friendly and affordable alternative to physico-chemical putrefaction processes. In the present study, effluent samples were collected from various textile and dyeing industries located in and around Tiruvannamalai, Tamilnadu, India and were exploited for the screening and isolation of bacterial strains that were capable of decolorizing the textile dye, Acid Red-88. Optimization of cultural conditions (Temperature, pH, Agitation speeds and Dye concentrations) were carried out to maximize the decolorization efficiency of AR-1 towards Acid Red-88. Decolorization efficiency was found to be optimized at 35°C, neutral pH, after 24 h of incubation. Static conditions proved to be effective in maximizing decolorization. Increase in dye concentration decreased decolorization efficiency of AR-1. Detoxification of Acid Red-88 textile dye by AR-1 was confirmed by Phytotoxicity studies on *Macrotyloma uniflorum.*

Introduction

Growing industrialization and urbanization consequences in the liberation of waste to the ecosystem, creating more pollution. The emancipation of noxious effluents from several textile industries adversely upsets the water resources, soil fertility, aquatic organisms and ecosystem integrity (Shyamala *et al*., 2014). Environmental pollution is the chief problem instigated by the anthropogenic activities where, human beings contaminate the biological system in numerous ways (Barathi *et al*., 2020a; Das *et al*., 2024). Clean water is an essential prerequisite for a human life as its accessibility has become a foremost problem nowadays due to increasing industrialization and population (Sharma *et al*., 2019; Bavani *et al*., 2021).

Dyes, decreases light absorption, and significantly upsets photosynthetic activity of aquatic life and may be lethal due to the presence of aromatics or heavy metals (Saratale *et al*., 2006; Hemapriya *et al*., 2010; Vijayanand and Hemapriya, 2013; Barathi *et al*., 2020b). Bioaccumulation of toxicants depends on the availability and persistence of the contaminants in water, food and

physico-chemical properties of the toxicants (Puvaneswari *et al*., 2006). Though there are many physical, chemical and biological treatment methods available, it is imperative to design a highly efficient with economically viable and environmentally benign method for the removal of the organic pollutants present in the industrial wastewater (Huang *et al*., 2019; Liu *et al*., 2019; Malathi *et al*., 2021a).

A wide range of methods has been developed for the removal of synthetic dyes from waters and wastewaters to decrease their impact on the environment (Malathi *et al*., 2021a). Employment of physical/chemical methods have innate drawbacks of being economically unfeasible, unable to remove the recalcitrant azo dyes and/or their organic metabolites completely, generating a significant amount of sludge that may cause secondary pollution problems (Hemapriya and Vijayanand, 2014; Vijayanand and Hemapriya, 2013).

Microbial decolorization of azo dyes has been of considerable interest since it is inexpensive, eco-friendly and produces a less amount of sludge (Kalyani *et al*., 2009; Saratale *et al*., 2009; Arulprakash *et al*., 2022). The effectiveness of microbial decolorization depends on the adaptability and the activity of selected microorganisms (Shyamala *et al*., 2014). The present study focuses on the eco-friendly biodegradation and detoxification of Acid Red-88 textile dye by newly isolated bacterial strain AR-1.

Materials and Methods

Sample Collection and Dye stuff used

The textile effluent samples were collected from both textile industries and dyeing units located in and around Tiruvannamalai District. Tamil Nadu, India. Textile effluent samples were in sterile polythene bags. Synthetic textile azo dye, Acid red 88 used for this study was procured from a local textile dyeing unit. Stock solution was prepared by dissolving 1 g of Acid red 88 in 100 ml distilled water. All chemicals used were of the highest purity available and of an analytical grade.

Enrichment and screening of bacterial strains decolorizing Acid Red 88

The textile effluent samples were serially diluted and plated onto the surface of Nutrient agar medium enriched with 50 ppm of Acid red 88. pH was adjusted to 7.0 before autoclaving and incubated at 37 °C for 5 days. Colonies surrounded by halo (decolorized) zones were picked and streaked on Nutrient agar medium containing dyestuff. The plates were re-incubated at 37 °C for 3 days to confirm their abilities to decolorize Acid Red 88. Morphologically distinct colonies of dye decolorizing bacteria were selected and restreaked several times to obtain pure cultures.

Decolorization assay

A loopful of the selected bacterial culture was inoculated in Erlenmeyer flask containing 100 ml of nutrient broth and incubated at 150 rpm at 37 °C for 24 h. Then, 1 ml of overnight broth culture of the bacterial strain was inoculated in 100 ml of nutrient broth containing 50 ppm of Acid Red 88 and reincubated at 37 °C till complete decolorization occurs. Suitable control without any bacterial culture was also run along with experimental flasks. 1.0 ml of sample was withdrawn every 12 h and centrifuged at 10,000 rpm for 15 min.

Decolorization extent was determined by measuring the absorbance of the culture supernatant at 520 nm using UV-visible spectrophotometer, according to Shyamala *et al*. (2014).

Decolorization efficiency (%) = Dye (i) – Dye (r) / Dye (i) X 100

Where, Dye (i) refers to the initial dye concentration, Dye (r) refers to the residual dye concentration. Decolorization experiments were performed in triplicates.

Optimizationd of culture conditions for biodecolorization of Acid Red 88

Effect of incubation time, temperature, pH, dye concentration and agitation rates

Optimization of culture conditions for decolorization of Acid red 88 was carried out by incubating the bacterial strain at different temperatures (20-50°C), different pH values of the medium (pH 4.0-10.0), various dye concentrations (200 -1000 ppm) and different agitation speeds (0-200 rpm) on dye decolorization extent of Acid red 88 by AR-1, was investigated.

Results and Discussion

Isolation and screening of bacterial strains decolorizing Acid Red 88

5 morphologically distinct bacterial isolates (AR-1 to AR-5) that was capable of decolorizing Acid Red 88 were isolated from different effluent samples. Among the above-mentioned isolates, AR-1 isolate was found to be the competent bacterial strain demonstrating maximum decolorization effectiveness (90 %) (Table 1), which was selected for the further studies. Similarly, many bacterial strains were reported to decolorize textile azo dyes (Hemapriya *et al*., 2010; Vijayanand *et al*., 2017, Barathi *et al*., 2020; Das *et al*., 2024).

Optimization of culture conditions for maximizing decolorizing ability of AR-1

Effect of Incubation Time

Dye decolorizing ability of AR-1 was reliant on on the bacterial growth. Decolorization progression started after 8 h and reached its optimum level within 24 h of incubation and thereafter gradually started to decline, due to the depletion of nutrients and accumulation of toxic metabolites (Fig 1). In disparity, decolorization of Methyl orange by *Bacillus* sp. was accomplished after 32 h of incubation (Shyamala *et al*., 2014). Similarly, Evan's Blue Decolorization by *E. coli* AKI-2 was achieved after 24 h of incubation (Aswinkumar *et al*., 2017).

Effect, of Temperature

Decolorization efficiency of AR-1 augmented with increase in incubation temperature, reaching highest levels between 30-40°C, with optimum being 35°C after 24 h of incubation. Similar results were reported by Das *et al*., (2024). Decolorization activity was substantially suppressed at temperatures more than 40°C. Interestingly, the Decolorization percentage was found to be reduced at temperatures below 30°C. Decolorization efficiency was significantly suppressed at temperatures more than 40°C, which might be due to the denaturation of the enzymes responsible for the decolorization (Bharathi *et al.,* 2020_b).

Effect of pH

Dye decolorization efficiency of the bacterial strain AR-1 was optimally exhibited at neutral pH (7.0). However, incubation at both acidic and alkaline pH slightly reduced the dye decolorization efficiency of the bacterial strain (Fig 3). Similarly, Pure cultures of *Proteus vulgaris* NCIM-2027 and *Micrococcus glutamicus* NCIM-2168 showed maximum decolorization efficiency at neutral pH (7.0) (Saratale *et al*., 2009). In contrast, optimal pH values for the decolorization of Acid orange by a halophilic bacterial consortium was found to be 8.0 (Vijayanand *et al*., 2017).

Fig.1 Effect of Incubation Time on the Decolorization of Acid Red-88 by AR-1

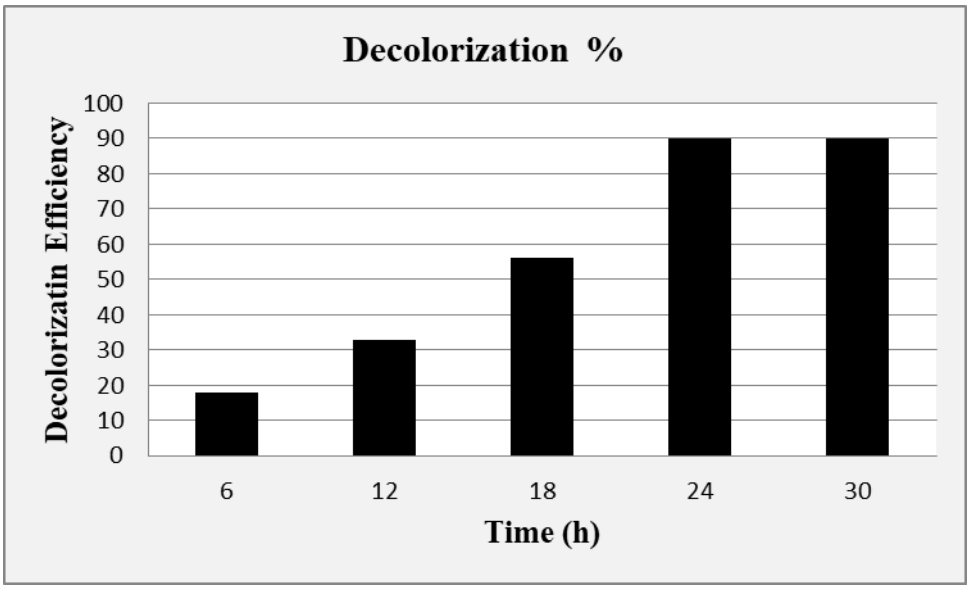


Fig 2 Effect of Incubation Temperature on the Decolorization of Acid Red-88 by AR-1

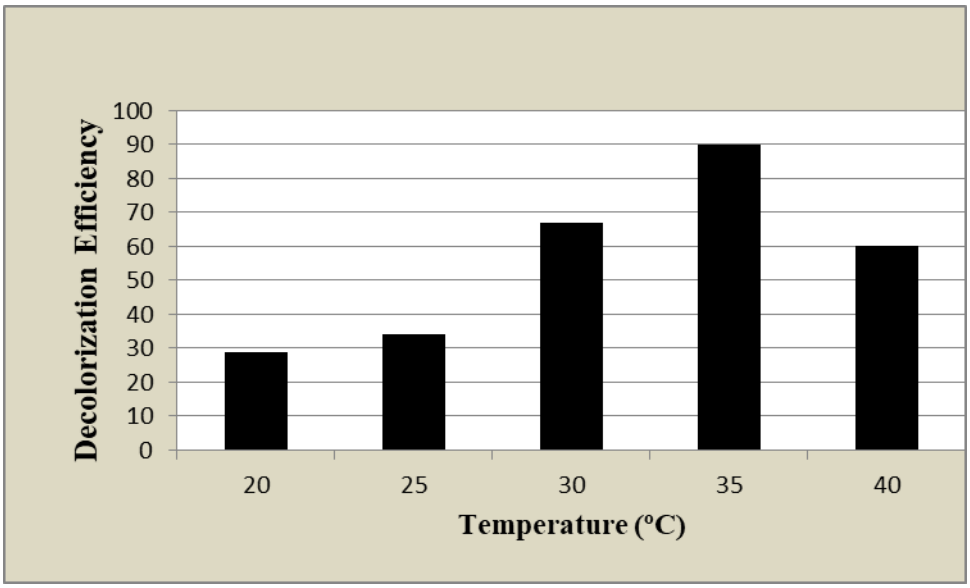


Fig 3 Effect of pH on the Decolorization of Acid Red-88 by AR-1

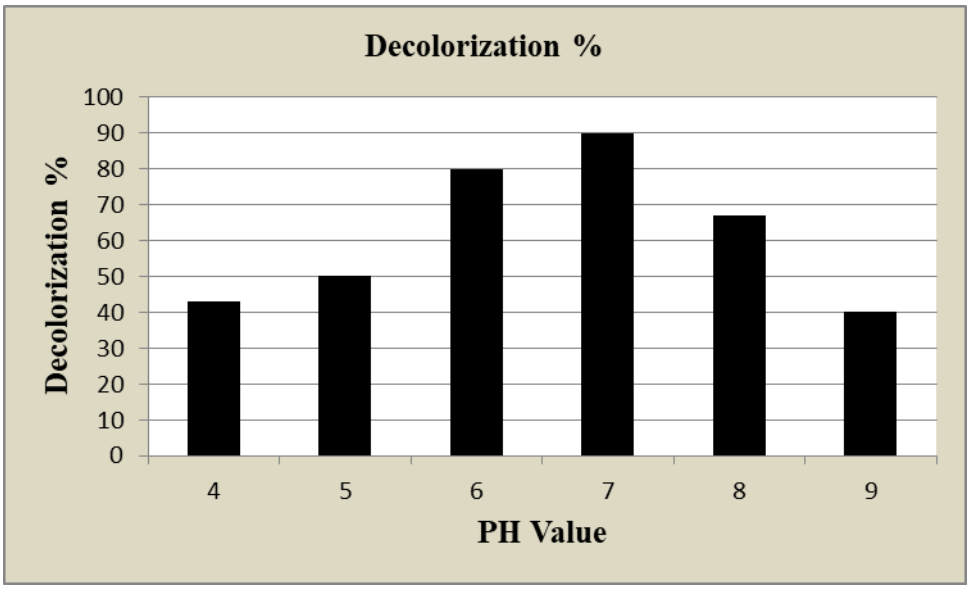


Table.1 Bacterial Strains Decolorizing Acid Red-88 under Aerobic Conditions

Sl. No	Isolates	Time taken for Maximum Decolorization	Decolorization Efficiency
1	AR-1	24 h	**90 %**
2	AR-2	24 h	78 %
3	AR-3	60 h	72 %
4	AR-4	60 h	76 %
5	AR-5	60 h	65 %

Fig.4 Effect of Dye Concentration on the Decolorization of Acid Red-88 by AR-1

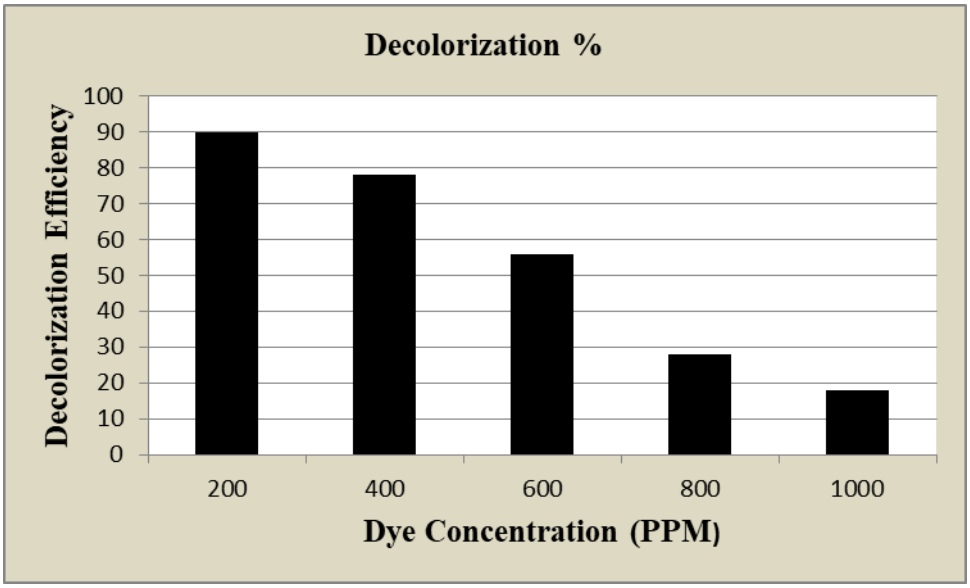
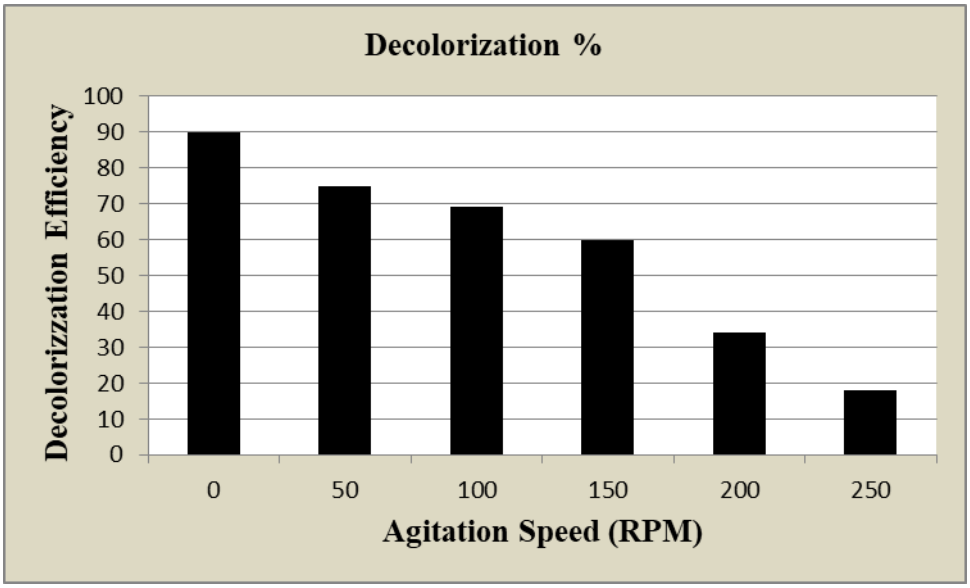


Fig.5 Effect of Agitation Speed on the Decolorization of Acid Red-88 by AR-1



Effect of Dye Concentration

Effect of different dye concentrations (200 - 1000 ppm) were studied on decolorization ability of AR-1. Fig 4 revealed that the decolorization rate gradually decreased with the increase in dye dosage. As the dye concentration increased, a deterioration in decolorization was attained. At high concentration (1000 ppm), decolorization capacity of the bacterial strain was significantly suppressed by Acid Red-88. Similar results were reported by Hemapriya *et al*., (2010) and Barathi *et al*., (2020). Decrease in the color removal effectiveness might be ascribed due to the toxicity of the dye to bacterial cells through inhibition of nucleic acid synthesis.

Effect of Agitation

The dye decolorization capacity of AR-1 was found to be greatly diminished with increases in agitation speeds. At 200 rpm, the decolorization ability of AR-1 was greatly

inhibited (Fig 5). Stationary conditions proved to be effective in maximizing decolorization percentage of AR-1. Azo dye decolorization by bacterial species is often initiated by enzymatic reduction mediated by azoreductase. Azo-reductase driven bacterial decolorization is normally inhibited in the presence of O_2 primarily due to the competition in the oxidation of the reduced group as the electron receptor.

Author Contributions

M. Thanigaimalai: Investigation, formal analysis, writing—original draft. R. Elaiyaraja: Validation, methodology, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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