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Sustainable Approach for the Bioremediation of Textile Azo Dye – Metanil Orange by Textile Effluent Adapted Bacterial Strain JHP-1

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A B S T R A C T

Environmental biotechnology is persistently expanding its efforts in the biological treatment of textile effluents, which is an eco-friendly and economically feasible alternate to physico-chemical decomposition processes. In the present study, effluent samples were collected from various textile and dyeing industries located in and around Kanchipuram, Tamil Nadu, India and were exploited for the screening and isolation of bacterial strains that were capable of decolorizing the textile dye, Metanil orange. Optimization of cultural conditions (Temperature, pH, Agitation speeds and Dye concentrations) were carried out to maximize the decolorization efficiency of JHP-1. 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 both decolorization efficiency of JHP-1. Glucose (carbon source) and yeast extract (nitrogen source) maximized the decolorization efficiency of JHP-1.

Introduction

Environmental pollution is the major problem instigated by the anthropogenic activities where, human beings pollute the biological system in numerous ways (Barathi *et al.,* 2020a).

Evolution of various types of industries contributes significant growth economically to the country on the other hand, these industries release ample of waste into the environment in the form of liquid and solid matter (Aswinkumar *et al.,* 2017). Clean water is an essential requirement for a human being as its availability has become a major problem nowadays due to increasing industrialization and population (Sharma *et al.,* 2019). Different types of chemicals released by the industries have heavily contaminated the waterbodies (Bavani *et al.,* 2021).

Amid various industries, leather processing and textile dyeing industries play a dynamic role in the discharging great amount of liquid waste (Vijayanand and Hemapriya, 2013). The waste discharged from these activities includes both organic and inorganic components, which are highly toxic, carcinogenic and mutagenic in nature. This industrial effluent bothers the

aquatic system which declines the prominence of the aquatic bodies (Hemapriya and Vijayanand, 2014). Owing to antagonistic environmental influences the effluents released from industries are given much importance during treatment process. Removal of waste from effluents is considered to be an essential process before letting out the effluent.

Common issues arouse due to the improper sewage treatment and its disposal into the environment includes soil and water pollution, diseases, obnoxious odour, fire hazards etc (Sarker *et al.,* 2013). Dyes may affect the photosynthetic activity in aquatic life because of reduced light penetration (Hemapriya *et al.,* 2010).

Government legislation is becoming more and more stringent, especially in the more developed/developing countries, regarding the removal of dyes from industrial effluents. Enforcement of this law will continue to ensure that textile and other dye utilizing industries treat their dye-containing effluent to the required standards (Robinson *et al.,* 2001).

Environmental biotechnology is constantly expanding its efforts in the biological treatment of colored textile effluents, which is an environmental friendly and low cost alternative to physico-chemical decomposition processes. Traditional wastewater treatment technologies have proven to be markedly ineffective for handling wastewater of synthetic textile dyes because of the chemical stability of these pollutants. Color is one of the most obvious indicators of water pollution and the discharge of highly colored synthetic dyes in textile effluents can be damaging to the receiving water bodies (Shyamala *et al.,* 2014).

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). 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). In view of the potential applications of biodecolorization processes in wastewater treatment, the present investigation emphasis on the eco-friendly approach for the bioremediation of Metanil orangea textile azo dye by a textile effluent adapted bacterial strain under aerobic conditions

Materials and Methods

Textile Azo Dye Used

Stock solution was prepared by dissolving 1 g of Metanil Orange in 100 ml distilled water. The dye solution was subjected for sterilization by membrane filtration, since dyes may be unstable to autoclaving. Reagents and chemicals used in this study were of the highest purity available and of an analytical grade.

Isolation and Screening of Bacterial Strains Decolorizing Metanil Orange

Textile effluent samples were serially diluted and spread over basal nutrient agar medium containing 50 ppm of Metanil Orange. pH was adjusted to 7.0 before autoclaving and incubated at 37°C for 5 days. Colonies surrounded by decolorized zones were selected and streaked on nutrient agar plates containing Metanil Orange (Hemapriya *et al.,* 2013).

The plates were re-incubated at 37°C for 3 days to confirm their abilities to decolorize Metanil Orange. Different colonies of dye decolorizing bacteria were picked and re- streaked several times to obtain pure cultures.

Decolorization Assay

1 ml of 24 h old culture of JHP-1 strain was inoculated in 100 ml of nutrient broth containing 50 ppm Metanil Orange and re-incubated at 37°C till complete decolorization occurs. Suitable control without any inoculum 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 590 nm using UV-visible spectrophotometer (Hitachi U 2800), according to Hemapriya *et al.,* (2010).

Decolorization efficiency (%) = Dye (i) - Dye (r) / Dye (i) × 100

Where, Dye (i) refers to initial dye concentration and Dye (r) refers to the residual dye concentration.

Optimization of various culture conditions for bacterial biomass and Metanil Orange decolorization

Influence of temperature, pH, agitation rates and dye concentrations

The influence of temperature, pH, agitation rates and dye concentration on dye decolorizing ability of JHP-1 strain was studied. This was carried out by incubating the bacterial strain at different temperatures (20-60°C), different pH values of the medium (pH 4.0-10.0), different agitation speeds (0-200 rpm) and various dye concentrations (200-1000 ppm). Bacterial biomass and decolorization percentage was measured at optimum growth (24 h).

Results and Discussion

Ever increasing demand for water and the dwindling supply has made the treatment and reuse of industrial effluents as an attractive option. Environmental biotechnology is persistently intensifying its efforts in the biological treatment of colored textile effluents, which is an environmental friendly and low cost alternative to physicochemical processes (Shyamala *et al.,* 2014).

Isolation and screening of bacterial strains decolorizing Metanil Orange

Six morphologically different bacterial isolates (JHP-1 to JHP-7) that was capable of decolorizing Metanil Orange were isolated from different effluent samples. Among the above mentioned isolates, JHP-1 isolate exhibited maximum decolorization efficiency (94 %) (Table 1), which was selected for the further studies. Similarly many bacterial strains were reported to decolorize textile azo dyes (Deng *et al.,* 2008; Hemapriya *et al.,* 2010; Vijayanand *et al.,* 2017).

Influence of Incubation time

Incubation time displayed a substantial role in optimizing both bacterial growth and dye decolorizing ability of JHP-1. The dye decolorization by the isolate was dependent on the bacterial growth. The bacterial cells started multiplying within 4 h and reached their maximum growth within 24 h and thereafter started to decline, due to the depletion of nutrients and accumulation of toxic metabolites (Fig. 1). In contrast, the maximum decolorization of Methyl orange by JHP-1 was achieved after 32 h of incubation (Shyamala *et al.,* 2014). Similar results were reported by Aswinkumar *et al.,* (2017).

Influence of Temperature

Fig. 2 revealed that JHP-1 strain showed maximum decolorizing activity and bacterial growth between 30-40°C, with optimum being 35°C after 24 h of incubation. However the bacterial biomass and dye decolorizing ability of the bacterial strain got gradually reduced when incubated at 30, 50 and 60°C. Decolorization efficiency of the JHP-1 was found to be suppressed at temperature below 30°C. Decolorization of Congo Red by Bacillus sp. VT-II was maximized at 40°C (Sawhney and Kumar, 2011). Decolorization activity was significantly suppressed at temperatures more than 40°C, which might be due to the loss of cell viability or denaturation of the enzymes responsible for the decolorization at elevated temperatures (Bharathi *et al.,* 2020_b).

Influence of pH

Dye decolorization efficiency of the bacterial strain JHP-1 was detected over a broad range of pH (5.0-9.0), with optimum decolorization being exhibited at neutral pH (7.0). However, incubation at both acidic and alkaline pH marginally bargained the dye decolorization efficiency of the bacterial strain JHP-1 (Fig. 3). 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).

Influence of Dye Concentration

The influence of diverse dye concentrations (200 - 1000 ppm) were investigated on decolorization ability of the bacterial strain JHP-1. Fig. 4 revealed that the decolorization rate progressively decreased with the increase in dye concentration. As the dye concentration increased, deterioration in color removal was achieved. At a concentration of 1000 ppm, decolorization % was significantly repressed. Similar result was reported by many researchers (Hemapriya *et al.,* 2010; Shyamala *et al.,* 2014; Barathi *et al.,* 2020_b). The toxicity of dye to bacterial cells might be attributed due to the inhibition of nucleic acid/ Protein synthesis.

Table.1 Bacterial Strains Decolorizing Metanil Orange under Aerobic Conditions

S. No.	Isolate	Decolorization Efficiency (%)
1.	JHP-1	94
2.	JHP-2	75
3.	JHP-3	48
4.	JHP-4	52
5.	JHP-5	60
6.	JHP-6	78
7.	JHP-7	80

Table.2 Effect of Carbon Sources on Decolorization Efficiency of JHP-1

S. No.	Carbon Source (gl^{-1})	Decolorization Efficiency (%)
1.	Glucose	94
2.	Maltose	65
3.	Sucrose	70
4.	Lactose	40
5.	Starch	68

Table.3 Effect of Nitrogen Sources on Decolorization Efficiency of JHP-1

S. No.	Carbon Source (gl^{-1})	Decolorization Efficiency (%)
1.	Peptone	94
2.	Yeast Extract	78
3.	Tryptone	68
4.	Beef Extract	58

Figure.1 Influence of Incubation Time on dye decolorization efficiency

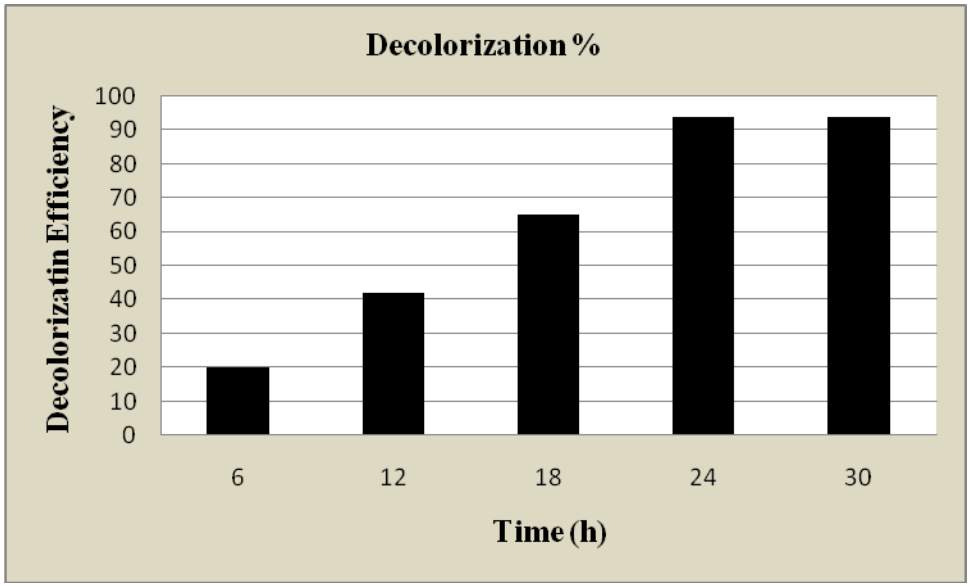


Figure.2 Influence of Temperature on dye decolorization efficiency

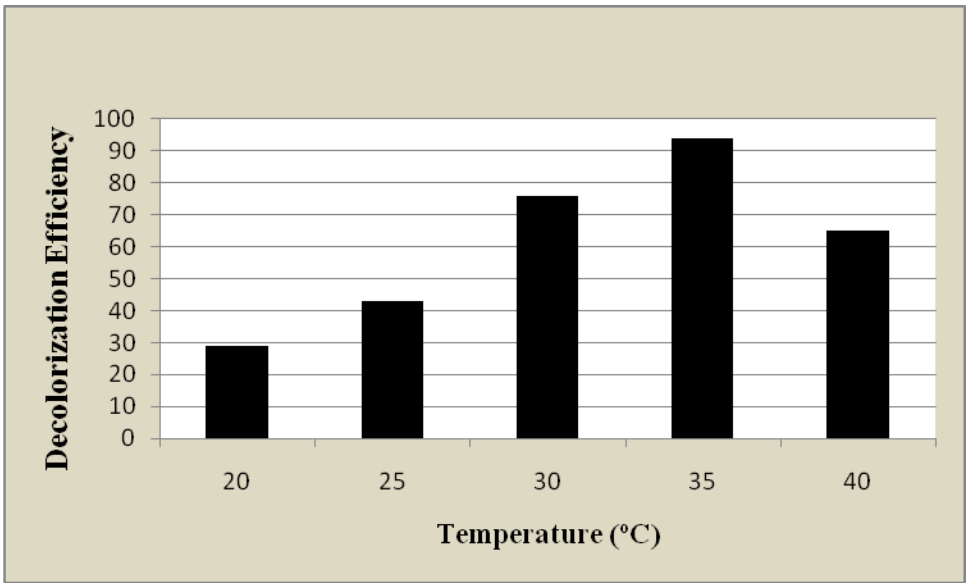


Figure.3 Influence of PH on dye decolorization efficiency

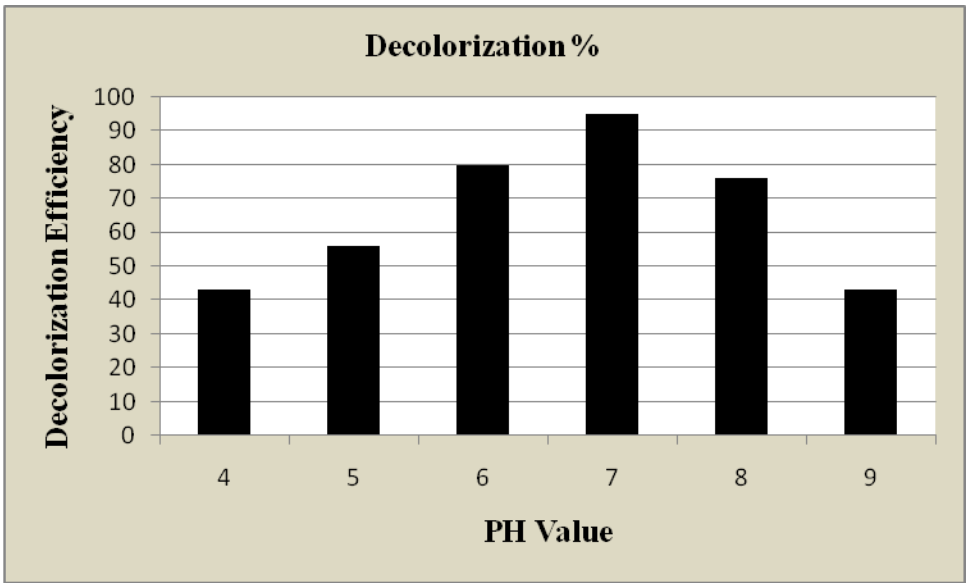


Figure.4 Influence of Dye concentration on dye decolorization efficiency

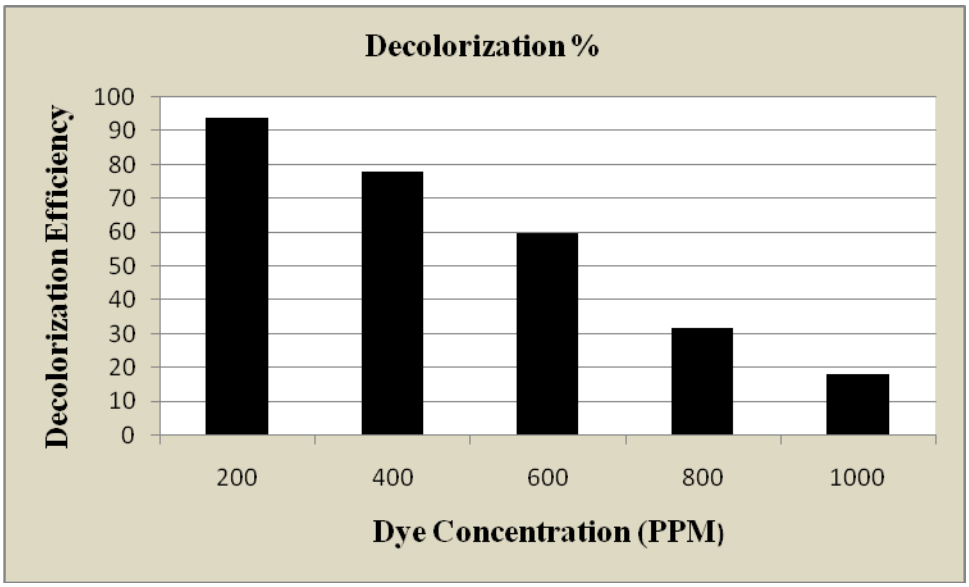
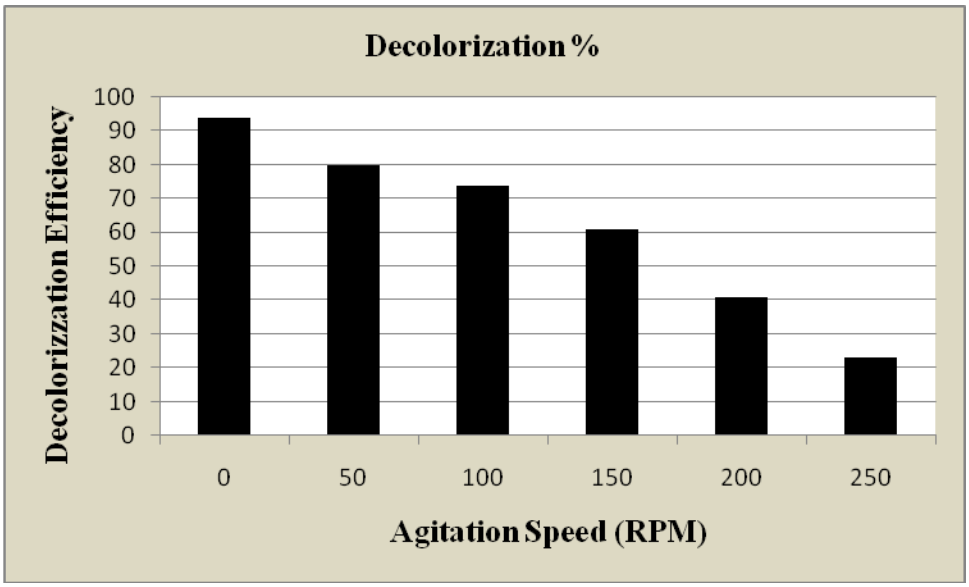


Figure.5 Influence of Agitation speed on dye decolorization efficiency



Influence of Agitation

Dye decolorization ability of the bacterial strain JHP-1 was found to be significantly decreased with upsurges in agitation speeds. At 200 rpm, the decolorization ability of JHP-1 was greatly inhibited (Fig. 5). Stationary conditions proved to be effective in optimizing decolorization efficiency of JHP-1. Bacteria mediated dye decolorization is often initiated by azo reductase enzyme. According to Chang and Lin (2000), 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.

Influence of Carbon sources

Various carbon sources were used to replace the original carbon source in the growth medium. Decolorization of Metanil Orange was not constitutive; dissimilar levels of

decolorization were found with different carbon sources. Among the several carbon sources investigated, Glucose was found to be maximizing the decolorization efficiency of JHP-1 (Table 2). According to Barathi *et al.,* (2020), the breakdown of glucose results in the fabrication of reduced metabolites including NADH and FADH that leads to the improved decolorization capability.

Influence of Nitrogen sources

Various organic nitrogen sources (peptone, yeast extract, tryptone and beef extract) were used to replace the original N_2 source in the growth medium. Among them, yeast extract was found to be the superior source in maximizing decolorizing ability of the consortium (Table 3).

The metabolism of yeast extract is considered to be essential for the regeneration of NADH that acts as electron donor for the reduction of azo bonds. Similar results were reported by Deng *et al.,* (2008); Vijayanand *et al.,* (2017) and Barathi *et al.,* (2020_b).

Author Contributions

Hena S. Das: Investigation, formal analysis, writing—original draft. Reshma Girirajan: Validation, methodology, writing—reviewing. Rajalakshmi Balaji:—Formal analysis, writing—review and editing. S. Vijayanand: Investigation, writing—reviewing. J. Hemapriya: Resources, investigation 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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