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Decolorization, Degradation and Kinetic Study of Textile Dye Navy Blue HE2R using Immobilized Bacterial Consortium PMB11

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ABSTRACT

Bacterial Consortium PMB11 showed 91% decolorization of textile dye Navy blue HE2R (50 ppm) in liquid broth within 6 h. The higher decolorization rate (75.8) was observed with bacteria consortium than the individual strains. The immobilized consortium PMB11 were showed complete decolorization of dye solution in flask within 15 h. The column was run for 7 days continuously passed dye solution (50 ppm) through column. The beads were viable for seven days in column that showed the efficiently decolorizing activity. Immobilized consortium PMB11 degraded Navy blue HE2R into different metabolites was confirmed by various analytical techniques like UV-vis spectroscopy, FTIR and HPLC.

1. Introduction

Now days, in order to produce quality product, a number of dyes are used, producing strongly colored effluents which has now become of critical environmental concern. Majority of the colored effluents consist of dyes, released to the environment from textile dyestuff and dyeing industries. Color pollution has always being an increasing problem, particularly associated with the reactive azo dyes, because of their strong color which leads to aesthetic problems and obstructs light penetration and oxygen transfer into water bodies [1], bio-recalcitrance and potential toxicity due to its mutagenic/carcinogenic properties [2].

Several physico-chemical techniques have been used for treating wastewater containing dyes but the methodologies appear to face several technical and economic limitations and lead to the generation of toxic by-products and further create secondary pollution problems [3,4]. Alternatively, a number of biotechnological approaches have been suggested in recent literature as of potential interest in combating this pollution source in an eco-efficient manner [5].

Biotechnology relies up on the pollutant degrading capacities of naturally occurring microbial consortium in which bacteria play central role. The advantages of using microbes for remediation include; their features like their natural occurrence ease of manipulation, high adaptability, cheap production, fast growth rate, easy availability to treat large volumes of wastewater due to rapid kinetics and high selectivity [6] provide desirable qualities of bacterial community for the bioremediation. Microbial consortia are usually used without analyzing the constituent microbial populations for environmental remediation and complexity of the microbial consortium enables them to act on a variety of pollutants. The utilization of microbial consortia has considerable advantages over the use of pure cultures in the degradation of synthetic dyes. The individual strains may attack the dye molecule at different positions or may use decomposition products produced by another strain for further decomposition [7]. As the catabolic activities of microorganisms in mixed consortium complement each other, obviously synergistic role or syntrophic interactions present in the mixed communities can lead to complete mineralization of azo dyes [8,9]. The biodegradation of dyes is mainly enzymatically catalyzed reaction involving various oxidative enzymes (lignin peroxidase, laccase and other oxidases) [10], reductive enzymes (azo reductase and nonspecific reductases) [7] and to some extent by *N*-demethylase to mineralize synthetic dyes [11,12]. The degradation of azo dyes is often initiated by an enzymatic step that involves cleavage of azo linkages with the aid of an azoreductase and an electron donor [13]. Generally, it is assumed that the first step in the biodegradation of azo compounds is the reduction to the corresponding amines, a reaction catalyzed by azoreductase. Then the resulting aromatic amines are further degraded by multiple-step bioconversion, aerobically or anaerobically [14]. The literature information about the kinetics of decolorization and the environmental factors affecting the decolorization rates is relatively scarce. Monoazo dye decolorization has been reported to follow first-order kinetics with respect to dye concentration by several authors [15] whereas other reports mention zero-order [16] or even half-order kinetics [17].

The objective of this work is to investigate the potential of immobilized consortium PMB11, decolorization and degradation of textile dye. It is widely known that immobilized cells offer many advantages: reusability of the same biocatalyst, control of reactions and the non-contamination of products [18]. Immobilization of living microorganisms has been described as useful in biological wastewater treatment [19]. A whole cell culture, the enzymes could be continually replenished. Immobilized cultures tend to have a higher level of activity and are more flexible to environmental troubles such as pH, or exposure to toxic chemical concentrations than suspension cultures [20, 21]. Immobilization results in a high local microbial population density even in a bioreactor.

2. Experimental Methods

2.1 Chemicals

Bacteriological peptone, yeast extract, beef extract, agar-agar powder, sodium alginate, calcium chloride anhydrate, monobasic sodium phosphate, dibasic sodium phosphate were obtained from Hi-media Laboratories Pvt. Ltd., Mumbai, India. Ethyl acetate HPLC grade methanol and NaCl were obtained from SD-Fine, Mumbai, India. Textile Navy blue HE2R was obtained from local textile industry Ichalkaranji, India.

2.2 Bacterial Strains

Dye decolorizing bacterial strains *Bacillus odyssey*, *Proteus* sp. and *Morganella morganii* were previously isolated from soil contaminated with textile processing and dye manufacturing unit Ichalkaranji (India). Patil [7] reported the consortium of these strains effectively and more rapidly decolorized the textile dyes.

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2.3 Decolorization of Navy blue HE2R by Immobilized Cells

2.3.1 Cell Immobilization Methods

Each bacterial strain was separately grown in the nutrient medium for 24 h. After incubation, bacterial cells were harvested by centrifugation (6000 rpm for 20 min). The 2 g cells of each bacterial strains were mixed into the 4 mL sodium alginate solution (4%), stirred it without clump formation. The mixture was extruded through a needle in the 200 mL calcium chloride (2% in phosphate buffer) by stirring. The formed beads were removed from calcium chloride solution and rinsed with phosphate buffer (50 mM, pH 7.4). The obtained beads were ~3 mm in diameter and used for the decolorization. All the procedures were performed at room temperature (27±3 °C).

2.3.2 Kinetic Study of Navy Blue HE2R Decolorization

Kinetic study of Reactive blue HE2R decolorization was performed in Erlenmeyer flask and glass column by using immobilized consortium beads. Beads of mixed bacterial culture were mixed and added in 250 mL Erlenmeyer flask containing 100 mL Navy blue HE2R solution (50 ppm). Decolorization experiment was performed at room temperature (27±3 °C) at static condition

Additionally, mixed bacterial culture (consortium PMB11) beads were packed in glass column having 30 cm height and 2 cm diameter and Navy blue HE2R decolorization was studied. After decolorization of dye, aliquot (3 mL) withdrawn was centrifuged (6,000 rpm, 10 min) and residual dye content (%) in the supernatant was measured at 620 nm. Decolorization was expressed in terms of percentage and was calculated as:

% Decolorization = [(Initial O.D. - Final O.D.) / Final O.D.] X 100

The average decolorization rate was calculated by using following formula.

Average decolorization rate = (Conc. of Dye x % decolorization x 100) / (100 x Time)

2.4 Degradation Analysis

2.4.1 Metabolite Extraction

Take decolorized solution and add equal amount of ethyl acetate in it. Shake well and keep this for 20 min. Collect the ethyl acetate layer and separate it using separating funnel. Then evaporate ethyl acetate overnight and add few drops of HPLC grade methanol in evaporated plate containing crystals of metabolite. Then collect the metabolite. The metabolite is further taken for analytical studies.

2.4.2 UV-Spectrophotometer

UV-visible spectral analysis was carried out using UV-vis spectrophotometer (Hitachi U 2800, Japan) and changes in its absorption spectra (400-800 nm) were recorded. The culture supernatant obtained after various hour of decolorization was used for UV-vis analysis.

2.4.3 High Performance Liquid Chromatography (HPLC)

HPLC analysis was performed in an isocratic waters 2690 system equipped with dual absorbance detector, using C18 column (symmetry, 4.6 x 250 mm) and HPLC grade methanol used as a mobile phase with a flow rate of 1 ml min^{-1} for 10 min and UV detector at λ_{max} of respective dyes and there metabolites.

2.4.4 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis was carried out in the mid IR region of 400-4000 cm^{-1} with 16 scans speed using Perkin Elmer 783 spectrophotometer and compared with control dye. Following figure shows the FTIR analysis of control dye and metabolite.

3. Results and Discussion

3.1 Structure of Navy Blue HE2R Dye

The reactive Navy Blue HE2R dye structure having the sulphonic group, amino group and azo bonds (-N=N-). The dye is used in textile and paper industries. Dye chemical structure is shown in Fig. 1.

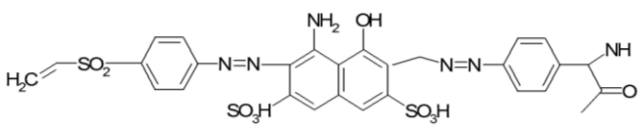

Fig. 1 Structural formula of Navy Blue HE2R

3.2 Decolorization

3.2.1 Decolorization and Average Rate of Decolorization in Liquid Broth

Dye decolorizing bacterial strains *Bacillus odyssey*, *Proteus* sp. and *Morganella morganii* were previously isolated from the soil sample from Ichalkaranji, India [7]. Also reported the consortium of these strains effectively and more rapidly decolorized textile dyes includes reactive Navy blue HE2R. The pure cultures of these isolates were tested individually for their decolorization ability in liquid medium, these cultures showed complete decolorization of Reactive Blue HE2R (50 ppm). The individual strains *Bacillus odyssey* SUK3, *Proteus* sp. SUK7 and *Morganella morganii* SUK5 showed the ability to decolorize Reactive Blue HE2R (50 ppm) 86, 83 and 86% within 60, 50 and 45 h respectively, while the bacterial consortium PMB11 decolorized 91% within 6 h. When all bacterial cultures were mixed and inoculated together in liquid medium, complete decolorization of Reactive Blue HE2R was observed in short time as compared to individual bacterial strains. Also the average decolorization rate of dye by bacterial consortium PMB11 was very high than individual bacterial strains (Fig. 2). That the suggesting a synergistic role of the bacterial species to each other in decolorization. The individual strains may attack the dye molecule at different positions or may use degradation products produced by another strain for further degradation.

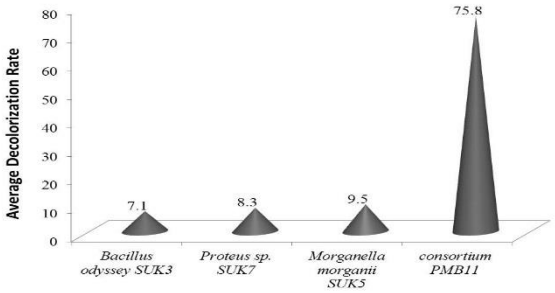

Fig. 2 Average decolorization rate of Navy blue HE2R

3.2.2 Decolorization by Immobilized Consortium

Sodium alginate immobilized consortium PMB11 beads (5 g) were showed complete decolorize dye solution in plain water (50 ppm) within 15 h in the flask. The decolorization time was increase when used immobilized consortium than the free consortium cells. It indicates that bacterial strains may require growth nutrient for their growth and metabolic activity as well as free cell environment for their synergistic role during decolorization process. Also indicated the entrapped cells may slower the production of enzymes which are responsible for dye decolorization. But an advantage with the immobilized cells is used repeatedly for the decolorization of dye.

3.2.3 Decolorization in Glass Column

Decolorization study with immobilized beads of consortium PMB11 was carried out using glass column. The beads were packed in glass column and Reactive Blue HE2R dye solution (50 ppm) was passed through column. The complete (99%) decolorization was observed when dye solution passed through beads packed column. In an hour 10.40±1 mL decolorized solution were collected. The decolorization efficiency of the immobilized cells was keep surprisingly up to seven days. Decolorization of dye can faster by using glass column which were packed with immobilized beads of consortium PMB11 than the same prepared immobilized beads used in flask study.

Entrapment is the most widely used technique for immobilization of whole cells, and alginate is a suitable matrix material because it is non-toxic and the method used for its gelatin is mild towards the microorganisms [22-24]. Furthermore, Columns of calcium alginate gel pellets have excellent physical properties when used as a cell immobilization support [25]. Calcium alginate is widely used as entrapment carriers for immobilization of the enzymes and whole cells because of its biocompatibility, cheapness and simplicity [26, 27].

3.3 Enzymatic Analysis

The data shown in Table 1 represents the enzyme present in the control cells of all bacterial strains and consortium. Lignin peroxidase, laccase, tyrosinase, NADH-DCIP reductase and aminopyrine N-demethylase were found to be present in the control cells. Presence of these biotransformation enzymes gives an idea about the ability of bacteria for biotransformation of certain xenobiotic compounds.

Table 1 Enzyme activities in bacterial cell

Enzyme	*B. odysseyi* SUK3	*M. morganii* SUK5	*Proteus* sp. SUK7	Consortium PMB11
Lignin peroxidase [a]	0.135±0.005	0.22±0.04	0.380 ± 0.076	0.20±0.032
Laccase [a]	0.035±0.004	0.002±0.001	0.005 ± 0.001	0.047±0.014
Tyrosinase [a]	0.015 ±0.001	0.001±0.001	0.003 ± 0.001	0.014±0.005
DCIP reductase [b]	0.917±0.008	0.557±0.01	8.183 ± 0.097	3.23±0.02
Aminopyrine *N*-demethylase [c]	13.25±0.001	25.12±0.03	3.140 ± 0.044	39.25±0.012

[a] *enzyme unit min^{-1}mg protein^{-1}*, [b] *µg DCIP reduced min^{-1}mg protein^{-1}*
[c] *n moles of formaldehyde librated mg protein^{-1}*

The several reports have showed that the lignin modified enzymes are efficiently involved in dye decolorization [28]. Similarly, various reductases such as, NADH-DCIP reductase [29], azoreductase [30] have been reported earlier for the biotransfor- mation of dyes. Potential applications of oxidoreductases have also been illustrated for the dye decolorization [31]. Demethylation reactions for the biodegradation of Methyl violet [32] and Crystal violet [11] were reported previously. Presence of various enzymes concludes the communal action of these enzymes in the dye decolorization process by consortium.

3.4 Analysis of Degraded Metabolites

3.4.1 UV-Vis Spectral Analysis

Spectrophotomerically the dye showed maximum absorbance at 620 nm, which was decreased after decolorization (Fig. 3). This analysis has been used to confirm that decolorization process was due to biodegradation and is not merely the visual decolorization. If dye removal is attributed to biodegradation, either major visible light absorbance, the peak would completely disappear or a new peak will appear.

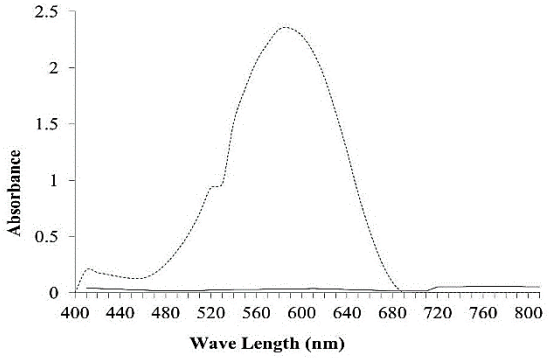

Fig. 3 UV-vis analysis of Navy Blue HE2R before and after decolorization

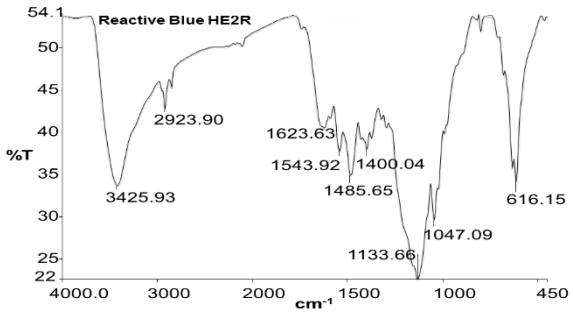

Fig. 4 FTIR analysis of Reactive blue HE2R

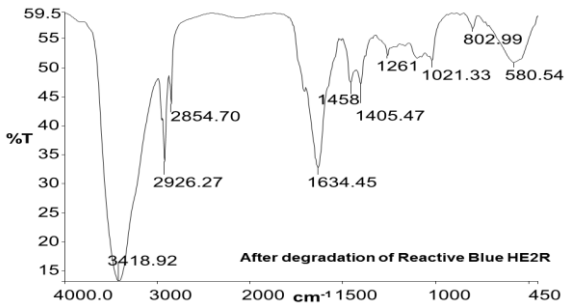

Fig. 5 FTIR analysis of degradation metabolites of Reactive blue HE2R by immobilized consortium

3.4.2 Fourier Transform Infrared Spectroscopy (FTIR)

The Figs. 4 and 5 shows the FTIR analysis of control dye and after degradation of dye respectively. Reactive Navy blue HE2R showed the peaks at 3425.9, 3 2923 .9, 1623.6 and 1543.9 cm^{-1} were for NH structure of secondary amides, CH structure of alkanes, N=N structure of azo compound and N=O structure of aromatic nitro compound respectively. The peak at 1485, 1400.04, 1299.06, 1133.66 and 1046.09 cm^{-1} indicated C=C structure of aromatic homocyclic compound, CH definition of alkenes, CH definition of alkenes, CH definition substituted benzenes and S=O structure of sulfonic acid respectively.

After degradation of dye metabolite were showed peaks at 3418. 9, 2926.2, 2854.7, 1707.3, 1634.45, 1458.66, 1405.47, 1261.32, 1021.33 and 802.9 cm^{-1} for NH structure of secondary amides, CH structure of alkanes, CH structure of aldehydes, C=O structures of ketones, CH structure of alkanes, CH definition of alkanes, OH definition of alcohols, ONO_2 vibration of nitrates, COH structure of primary alcohols and CH definition of tri-substituted alkanes. The FTIR spectrum indicated that the peaks of metabolites formed after degradation, were significantly different than those present in the spectrum of the dye during the biotransformation process. Also in dye spectra showed a peak at 1623.6 cm^{-1} completely disappear in metabolite spectra. This FTIR result indicated azo bond from completely break in to different metabolite down by immobilized consortium PMB11.

3.4.3 High Performance Liquid Chromatography (HPLC) Analysis

HPLC elution profile of Navy blue HE2R and metabolites extracted after its degradation showed different retention times (Figs. 6 and 7). Navy blue HE2R showed major peak at retention time 2.77, whereas degradation product showed major peaks at retention time 0.454, 0.747, 0.851 and 2.171. HPLC analysis was confirmed the biodegradation of Navy blue HE2R in to different metabolites.

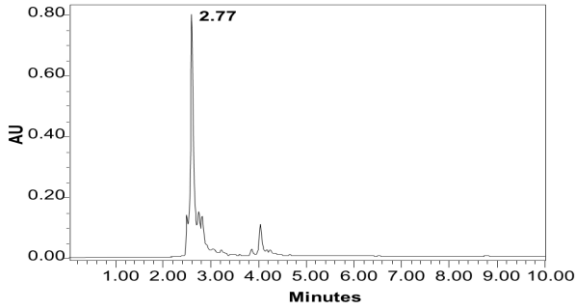

Fig. 6 HPLC elution profile of Reactive blue HE2R

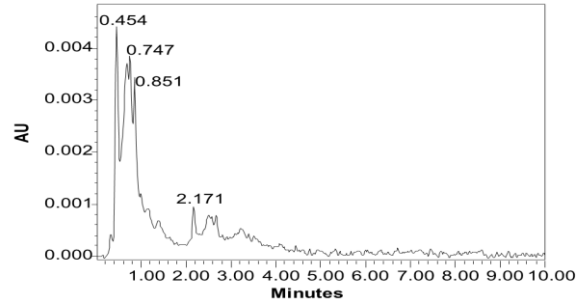

Fig. 7 HPLC elution profile of Reactive blue HE2R degradation by immobilized consortium

4. Conclusion

The immobilized bacterial consortium bears tremendous potential for the removal of Reactive blue HE2R. Studies on biotransformation enzymes may play their major role in dye degradation. To knowledge this work may be major report regarding the Reactive blue HE2R degradation using immobilized consortium, showing efficient decolorization and degradation. However, the model studies are needed to apply on large scale.

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