

Multiple approaches towards decolorization and reuse of a textile dye (VB-B) by a marine bacterium *Shewanella decolorationis*

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Abstract

Textile dye Victoria Blue-B (VB-B) was approached in two different ways, one to get rid of the color for its easy disposal to the environment and the other is to re use the decolorized water for coloring the same dye. *Shewanella decolorationis* (MBTD16) isolated from Dona Paula bay, identified by 16s rRNA gene and its action over decolorization was monitored by FTIR, UV-Vis spectrum and a color scanner. Dye removal index (DRI) increased L*, a*, b* 91.585, -2.856 and -0.132 against 62.29,-4.93 and -20.75 within 42 h as a first report. A maximum extent of decolorization (94.83 %) could be achieved with minimum dye concentration of 50 mg L⁻¹. The colored water treated by free and immobilized bacterial cells tested to reuse (VB-B dye) could give 35-50 % more color than the original. Process parameters optimized to achieve maximum decolorization, indicated pH 7, Temperature 32±2°C, inoculum size 8 % with co-substrates of glucose and yeast extract 5g L⁻¹ for its supremacy. Synthesis of lignin peroxidase, and tyrosinase augmented in strain *S. decolorationis* only after being exposed in to the dye signifies the enzymes in decolorization and it was confirmed through one way ANOVA. Results obtain by this work could suggest that *S. decolorationis* can be used very well to decolorize the textile dye and the same water could be recycled to get back its original color by adding around half the quantity of dye. Thus by the use of water, dye and pollution levels could be minimized.

Keywords Decolorization. Victoria blue-B. *Shewanella* sp. Lignin peroxidase. Tyrosinase

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1 Introduction

The textile industry utilizes about 10,000 different classes of dyes with an annual production of 7×10^5 metric tons worldwide, of which about 5–10 % are lost in industrial effluents (Ciullini et al. 2008). Once these dyes are discharged into aqueous ecosystems it reduces the penetration of sunlight into deeper layers by which it inhibits the photosynthetic activity and deteriorates the water quality (Saratale et al. 2009). These dyes also pose a major environmental problem because of their cytotoxicity and genotoxicity (Sandhya et al. 2008). Hence the decolorization of textile dye has been a major environmental concern in day – today – life (Asad et al. 2007).

VB-B dye ($C_{33}H_{32}ClN_3$), a basic blue 26 with the C.I. 44045, falls under the class of tyriarylmethane dye and extensively used for wool, silk, and cotton (Ayed et al. 2010). These dyes could cause strong coloration and toxicity in the waste water which has an irritating effect on eyes and respiratory system (Schulte et al. 1988) and to higher extent it may even promote tumor growth in some species of fish (Cho et al. 2003).

The merits of microbial remediation to improve degradation of various textile dyes have been worked out by many researchers (Telke et al. 2008; Shedbalkar and Jadhav 2011). Decolorization of these dyes is mainly caused by enzymatic actions (Saratale et al. 2011) and in principle the azoreductase, lignin peroxidase and laccase are involved in biodegradation of these dyes (Chen et al. 2003; Hatvani and Mecs 2001). A search of recent literature on bioremediation of VB-B dye did not provide many reference, only a very few studies were indicated by Gomaa et al. (2008) and Chen et al. (2011).

The textile industry uses high volumes of water throughout its operations, from the washing of fibres to bleaching, dyeing and washing of finished products. On an average, approximately 200 L of water are required to produce 1 kg of textile materials. The large volumes of wastewater generated also contain a wide variety of chemicals, used throughout processing. These can cause damage if not properly treated before being discharged into the environment (<http://www.indiantextilejournal.com>). There are many companies working to reduce water use within the industry through conservation efforts, water recycling, dye sublimation, and other new technologies.

All the technologies that have been investigated, bioremediation has emerged as the most desirable approach for cleaning up many environmental pollutants. Apparently there is a need to develop novel

biological decolorization processes leading to more effective clean up of textile dyes with minimum water use. Keeping the above background in mind a marine bacterium *Shewanella decolorationis* was isolated and tested for decolorizing VB-B dye and characterized to reuse the decolorized water back in to the textile industry through immobilization process. The merits of the color index, reduction in water use and the dye cost were discussed.

2 Materials and Methods

2.1 Dye and Chemicals

VB- B dye ($\lambda_{\text{max}} = 615\text{nm}$) used in this study was purchased from Hi-Media India, Pvt. Ltd, Mumbai. The color index and the structure of the dye are shown in Table 1. The 1000 mg L^{-1} of dye was prepared in double distilled water and used as a stock. Solutions of desired concentration were obtained by successive dilution. Chemicals related to enzymatic analysis i.e., Tartaric acid, n-Propanol and Catechol were purchased from Merck-Germany. NADH, mono sodium phosphate, di sodium phosphate and sodium alginate were purchased from Sigma- Aldrich U.S.A. Hydrogen peroxide and calcium chloride was purchased from S.D fine chem ltd., Mumbai, India.

2.2 Isolation, screening and maintenance of dye decolorizing bacteria

Water and sediment samples were collected from the bay area of Dona Paula, Goa. Samples were serially diluted from 10^{-2} – 10^{-5} and then plated on Nutrient Agar (M001– Hi-Media). Isolated colonies were screened for dye removal capability. The isolated pure colonies were exposed to 25 – 150 mg L^{-1} of VB-B added with glucose and Yeast extract 1.0 g L^{-1} (Decolorized media (DM)) and incubated for 3–4 days in static condition. Color intensity of the dye was measured at 12 h intervals. The bacterial isolates showing positive indications of growth and decolorization were gradually raised to high concentration of the dye (50 – 500 mg L^{-1}). Potential cultures were sub - cultured frequently and maintained in nutrient agar slants and stored in sealed tubes at $4 \text{ }^{\circ}\text{C}$. The same bacterial isolate were also stored in glycerol vials at $-80 \text{ }^{\circ}\text{C}$.

2.3 Molecular identification of dye degrading strains

The genomic DNA was extracted from the isolate by using DNeasy kit (Qiagen). 16S rRNA genes of the bacterial isolates were amplified using universal forward and reverse 16S rDNA primers 27F [5'-AGA GTT TGA TCC TGG CTC AG-3'] and 1492R [5'-GGT TAC CTT GTT ACG ACT T-3']. Nucleotide sequencing for these samples was performed using a 3130xl genetic analyzer (Applied Biosystems-Foster City, USA) at National Institute of Oceanography, Goa, India. The nucleotide sequences of the isolates obtained were compared with the sequences available in public database using BLAST software (www.ncbi.nlm.nih.gov). Neighbor-joining method was employed to construct the phylogenetic tree using MEGA4 software (Tamura et al. 2007) and the maximum likelihood method was adopted for calculating the evolutionary distance.

2.4 Decolorization assay

A loopful of microbial culture was inoculated in 100 mL of nutrient broth (NB) (M088-Hi-Media) and incubated on rotary shaker for 24 h at 125 rpm at 33±2°C. After 24 h the culture was centrifuged at 10,000 rpm for 10 min at 4 °C. Supernatant was discarded. The cell pellet was washed and resuspended in 0.85 % of NaCl and used for further studies.

One mL of the bacterial inoculums prepared as mentioned above (approximately 10⁵ cells mL⁻¹) was inoculated in to a 250 mL Erlenmeyer flask containing DM with 100 mg L⁻¹ of the VB-B dye. The same composition was used for all decolorization studies except optimization studies for dye concentration. The flasks were incubated in the dark at 33±2°C for 42 h under static condition. Every 6 h an aliquot of 4 mL was removed from the flask centrifuged at 10,000 rpm for 10 min at 20 °C and the absorbance values of clear supernatants were measured using UV-Vis Spectrophotometer, (Jasco-V-530, Japan) at 615 nm. All the experiments were performed in triplicate and the decolorization activity was measured.

Decolorization percentage was calculated using the formula:

$$\text{Decolorization (\%)} = \frac{\text{Initial absorbance}-\text{Final absorbance}}{\text{Initial absorbance}} \times 100$$

2.5 Optimization of different parameters

The decolorization of VB-B by MBTD16 was studied with respect to various incubation times (0–42 h) with an interval of 6 h, temperature from 10 °C–50 °C with every 10°C interval, pH 4–10, inoculum size 1–10 %, and dye concentrations of 50, 100, 150, 200, 250, 300, 350, 400, 450, 500 mg L⁻¹. All the experiments were carried out in static conditions. Co-substrates like glucose, sucrose, dextrose, maltose, lactose and xylose were added at the rate of 5g L⁻¹ of carbon source and beef extract, yeast extract, peptone, ammonium sulfate, casein and tryptone as nitrogen sources.

2.6 Immobilization studies

2.6.1 Decolorization of the dye by immobilized Bacterial cells

MBTD16 cells were grown in the NB overnight and centrifuged at 10,000 rpm for 10 min at 4 °C and kept at 0.85 % of NaCl. Approximately 10⁵ cells mL⁻¹ were immobilized into 3 % calcium alginate (1:3). Immobilized cell beads were put into 250 mL flasks and treated with the dye prepared in the DM and kept in static for 42 h and the decolorization rates were measured as usual. At the end of 42 h the immobilized cells were transferred aseptically into media containing dye for next cycles and it continued till third cycle.

2.6.2 Reuse of VB-B dyed water

The dye containing water treated with free cells of *S. decolorationis* for 42 h was centrifuged and the cell free supernatant was used for recoloring the same dye. In case of immobilized cell beads they were separated by filtration and the water was used for re-dyeing. 100 mg L⁻¹ of VB-B dye was freshly added to the both the waters and tested for its color intensity by measuring the L*a*b* values under premier color scanner (SS5100A, India). Freshly prepared dyes were also measured in a similar way and then compared.

2.6.3 Dye removal index

The first step is the calculation of the geometric distance from the treated dye CIE L*a*b* location to the ideal bleach point where a* = b* = 0, and L* = 100. The distance for the bacterially treated dye is subtracted from the distance of the control. The difference is then divided by the starting distance (no

bacterial control) to produce an index value (Dye Removal Index, DRI) that signifies the percentage of original color removed by the bacteria. The calculations were performed following Sharpe (1996), using the equations below.

$$\text{DRI} = -100[\Delta R^2/R_i^2]$$

R^2 = Distance from Ideal Bleach Point

$$= a^2 + b^2 + (100 - L)^2$$

$$\Delta R^2 = R_2^2 - R_1^2 = R^2 \text{ (for treated dye water)}$$
$$= -R^2 \text{ for control test water}$$

2.7 Enzyme activities

2.7.1 Preparation of cell free extracts

The bacterial isolate were grown in NB at 33 ± 2 °C for 24 h and centrifuged at 7,000 rpm for 15 min at 4 °C. These cells were suspended in 50 mM potassium phosphate buffer (pH 7.4) and sonicated (Ultrasonicator: Model No. SKL 250D) by keeping the sonicator as the output of 70A, and giving 16 strokes each of 9s, with 6s interval for 4 min at 4 °C. This homogenate was centrifuged and supernatant was used as an extracellular enzyme source and the cell pellets were used for intracellular enzyme source (Kalme et al. 2007).

2.7.2 Enzyme assays

Lignin peroxidase activity was determined by monitoring the formation of propanaldehyde at 300 nm in a reaction mixture (pH 3.5) containing 2.5 mL 100 mM n-propanol, 250 mM tartaric acid, 10 mM H₂O₂ (Kalyani et al. 2008). Tyrosinase activity was determined in a reaction mixture of 2 mL, containing 0.01 % catechol in 0.1 M phosphate buffer (pH 7.4) at 495 nm (Kalme et al. 2007). Azoreductase activities were measured according to the method of Ozdemir et al. (2008). One unit of enzyme activity was defined as the amount of enzyme that catalyses the oxidation of 1 μmol of NADH/min. All the enzymatic reaction was carried out at 31 ± 2 °C where reference blanks contained all components except the enzyme. All enzyme assays were run in triplicate and the average values taken to calculate unit of enzyme activity.

2.8 Decolorization analysis of VB-B.

Decolorization was monitored by UV-Vis spectroscopy (shimadzu/Japan) and the biodegradation was analyzed by Thin Layer Chromatography (TLC) and Fourier Transform Infrared Spectroscopy (FTIR). Biodegraded metabolites of VB-B were extracted from the culture supernatant with an equal volume of ethyl acetate. The extract was dehydrated over anhydrous Na₂SO₄ and evaporated to dryness in rotary vacuum evaporator (Equitron Reteva- 8763). The crystals obtained were dissolved in small volume of HPLC grade methanol and the same sample was used for UV-vis spectroscopic analysis. The samples were scanned between 300–800 nm and compared with control. The biodegraded VB-B was characterized by FTIR and the spectrum of fraction was recorded on a Nicolet 5700 (Thermo Electron Corporation, Madison, WI, USA). The extracted sample was mixed with KBr pellet and scanned in the range of 4000–400 cm⁻¹. The KBr pellet was used as blank. TLC was carried out on an alumina plate coated with silica gel 60 F254 (Merck, Germany) using mobile phase solvent system methanol and water in the ratio of 19:1 and the spots were visualized under UV chamber.

2.9 Statistical analysis

Data were analyzed by one-way ANOVA with turkey scheffe alpha multiple comparison test. Readings were considered significant when P was <0.05 by using Predicted Analytical Software 18.0 (PASW).

3 Results and Discussion

3.1 Molecular identity of the potential strain

The isolate (MBTD16) used in this study was identified on the basis of 16S rRNA gene sequencing. The closest neighbor in GenBank database was found to be *Shewanella decolorationis* with the homology of 99.49 %. The sequence was submitted to GenBank with an accession number of JQ965805. The phylogenetic relationship of the isolate is shown in Fig. 1 (other sequences used in the tree were retrieved from GenBank database).

3.2 Optimization of various environmental parameters

3.2.1 Static and Shake flask cultures

The decolorization efficiency of the bacterium on VB-B dye studied by static and shake flask cultures indicate its maximum decolorization rate of 85.87 % by MBTD16 within 42 h under static condition. Decolorization rates were found to decrease when the organisms were kept on shake flasks and resulted in 54.36 % (Table 2). In this case the organism preferred to grow at low oxygen levels and that could be maintained during static condition. This was supported by many researchers who say that the decolorization reactions were mainly through bacterial cells not by an abiotic factor (Phugare et al. 2011; Chen et al. 2003; Kolekar et al. 2008). Our studies also highlighted the significance of static condition for better decolorization.

3.2.2 Effects of pH, Temperature and Inoculum size on decolorization

It might be inferred that the decolorization rates increased when the pH was increased up to pH 7 later on it started to decrease when the pH were increased from 8–10. Maximum decolorization 93.32 % was observed at pH 7. Temperature 32 ± 2 °C showed maximum decolorization of about 92.42 %. Temperature rise above 32 ± 2 °C to 50 °C shows a slight reduction in decolorization rates. Different inoculum concentration indicates that maximum decolorization rate of 87.97 % can be obtained with the inoculum concentration of 8 % (v/v). Further increase from 8 % leads to lower decolorization.

The rate of decolorization with many dyes was higher at neutral pH and slightly decreased towards acidic or alkaline pH. This was supported by many researchers (Saratale et al. 2009; Mahmood et al. 2011). In general most of the bacteria preferred the pH between 6.7–7.5 and the temperature 30–37°C unless they were of acidophilic, alkalophilic, psychrophilic or thermophilic nature. Since this organism is a normal heterotrophic one it has a better adaptation towards room temperature and neutral pH. The effects of pH might be interrelated to the transfer of dye molecules across the cell membrane which is considered as the limiting factor for decolorization (Saratale et al. 2011). Higher temperature could affect the thermal inactivation of microbial cells that leads to poor decolorization (Angelova et al. 2008).

Various researchers working on different inoculum sizes said that the rate of increase in decolorization was not very significant beyond 8 % (Kumar et al. 2009; Mathew and Madamwar 2004; Shahvali et al. 2000). Increased concentration of inoculum size may raise the decolorization rates in the beginning but they were found to be inhibitory in later stages (Venil and Lakshmanaperumalsamy 2010). Our studies also showed that beyond 8 % of inoculum concentration the decolorization was not very significant. This could be caused by the bacteria due to over population and competitiveness.

3.2.3 Effects of incubation time for decolorization

MBTD16 strain showed a better decolorization from 15.61 to 86.73 % when we increased the incubation time from 6 to 42 h. Time required for bacterial action in the reactive media may depend upon the growth rate and activity of the microorganism. Further the catabolism or the breakdown of the organic material depends on the toxicity of the reactive media. The strain which is used in this study is known for its faster growth rate and reaction. This helps to decolorize the VB-B in a shorter residence time of 42 h. Gomaa et al. (2008) observed the decolorization rate of VB was 41.09 % at 4 days of incubation condition by *Phanerochaete chrysosporium*. Here we could bring the merit of our organism by decolorizing 85 – 90% of the VB-B dye within a short span of 42 h.

3.2.4 Effect of co-substrates on decolorization

Generally microorganisms require complex organic sources, such as yeast extract, peptone, or a combination of both for growth and activity (Khehra et al. 2005). The present study shown that the DM prepared with the combination of glucose and yeast extract gave high decolorization rates while comparing with individual performance against nitrogen or carbon source. An additional carbon source added to the DM such as glucose, sucrose, dextrose, maltose, lactose and xylose showed its maximum decolorization between of 26.60 – 67.26 % respectively. Similarly the nitrogen sources added to the DM also results that very low decolorization rates in comparison with nitrogen and carbon together in the DM.

A moderate decolorization activity of Reactive Red 195 by *Enterococcus faecalis* was observed in the presence of glucose (56.76 %) (Mate and Pathade 2012). The decolorization rate of Navy blue HER (100 %) by *Trichosporon beigelii* was higher when yeast extract was used as nitrogen source (Saratale et al. 2009a). The nitrogen sources can regenerate NADH, which acts as an electron donor for the

reduction of textile dyes by microorganisms, and therefore effective decolorization was observed (Chang et al. 2000).

Hence, we interpret that glucose and the yeast extract in the DM which can act as a main constituent of cellular material and helps to produce coenzymes in the MBTD16 where the organism were able to decolorize the dye maximum. Whereas the co-substrates glucose and yeast extract did not perform well when they were added independently to the medium.

3.2.5 Dye concentration

High decolorization (94.83 %) was obtained with 50 mg L⁻¹ of VB-B (Table 2). Higher concentration of 500 mg L⁻¹ yielded lower decolorization rates 6.59 %. Saratale et al. (2009) found that decolorization rate of Reactive brilliant red by *Trichosporon beigelii* was reduced by 50 % when they raised the dye concentration from 150 to 200 mg L⁻¹. Higher concentration of the dye may inhibit the bacterial activities and create inadequate biomass concentration to uptake the dye (Jadhav et al. 2007). Similar results were also observed in earlier studies in decolorizing triphenylmethane by *Kurthia sp* (Sani and Banerjee 1999).

3.3 Immobilization studies

3.3.1 Decolorization of VB-B dye by immobilized bacterial cells

The immobilized bacterial cells tried for the decolorization of VB-B dye showed that they could decolorize the dye in quantities equivalent to the free cells within 42 h. The rate of decolorization measured by the L* a* b* values and the DRI given in the table 3a for the free cells showed that they could remove the color by 96 %. Immobilized cells in the first cycle were able to decolorize 69.25 % and the second cycle 52.61 % and 30.25 % in third cycle (Fig. 2). Free cells are expected to give more than the immobilized because of their direct contact with the materials but the advantage over the entrapped cells here is the cell free supernatant for its reuse. Bioremediation of textile effluent using immobilized bacterial cells is an effective method for treating textile effluent and can be a good substitute for conventional remediation processes. This will be useful for the treatment of not only textile effluent but also industrial effluents of any kind (Ajao et al. 2011).

3.3.2 Reuse of decolorized water

Reuse of the decolorized water showed that the water treated with free cells could get back the color intensity higher than 38 % of its original. The water treated with immobilized cells could give more than 49.37 % and the heated recycled water could give 34.18 % more color (Fig. 3). This result explains the quantum of dye reduction nears about 35–50 % when we go for coloring the treated water. The color intensity tested in the recycled water by color differences in the cylindrical color bases black to white (L*) red-green (a*) and yellow to blue (b*) gave huge variations in its reflectance. This shows the strong color intensity against each combination while using the recycling technique. The feasibility of dye liquor reuse depends on the dye used and the shade required on the fabric or yarn as well as the type of process involved. It has already been applied whilst disperse dyeing polyester, reactive dyeing cotton, acid dyeing nylon and basic dyeing acrylic, on a wide variety of machines. However, commission dyeing where the shades required are much more varied and unpredictable would make the reuse of dye liquor difficult. But, given the right conditions dye liquor could be reused up to 10 times before the level of impurities limits further use (<http://www.sswm.info/sites>). The bacterium MBTD16 used in this study clearly expresses its application to reusability by giving a high reflectance (Table 3b). High reflectance indicates its darkness or high coloration.

Two things need to be discussed in this work. One is decolorization and the other is recycling of the treated water by adding the color. The first point is very clearly to minimize the color intensity of the effluent before we dispose of it to safe-guard the environment. The second aspect may raise a question like why can't we use the same water without treatment for re-dyeing. Even though we do not know the exact mechanism of the dyeing process in the industry the treatment of decolorization may help to remove the unwanted salts and other impurities which may cause turbidity and low attachment to the fabrics (Asamudo et al. 2005). Further, the treatments may enhance the binding properties.

Use of immobilization techniques like entrapment restricts direct involvement of bacteria in the dye while coloring. Results of the above study insist that the experimental bacteria have a huge potential in both the applications.

3.4 Enzyme studies

The bacterial strain MBTD16 did not support much of their enzyme production externally. When this organism are introduced into VB-B dye their extracellular enzyme production was restricted, while on the other hand, the internal secretion of these enzymes were uplifted and increased to manifold (Table 4). This shows that when the bacteria are exposed to the dye they may be under severe stress; to overcome this, they secrete enzymes like lignin peroxidase and azo reductase. Majority of the cellular enzymes are found to be very active when the bacteria are in contact with the dye. For example the internal activity of lignin peroxidase increased from 0.06 ± 0.00 to 0.65 ± 0.02 during decolorization. Similar studies on lignolytic enzymes which are responsible for decolorization have been reported previously by Jadhav et al. (2008).

Various studies conducted on the enzyme responsible for decolorizing the textile dyes indicated that enzymes oxigenases/oxidases play an important role in decolorization (Gomaa et al. 2008). The mechanism behind the current study in enzyme action may be because that the high polarities of the cell walls of MBTD16 that the dye was internalized into the bacteria for degradation.

3.5 Decolorization analysis

UV-Vis spectral analysis (200–800 nm) carried for the same treated by MBTD16 at various time intervals is shown in Fig. 4. The peaks observed between 600–615 nm at 0 h were found to reduce their peak area after 12 h. Maximum decolorization by complete reduction in the peak area could be observed at the end of 42 h. Peaks decreased without any shift in λ max confirms its degradation at a particular portion of the compound till the experiment ends. Further it indicates the bacterial action and its metabolic process over the materials.

TLC analysis showed the appearance of two different spots in the sample containing the extracted metabolites after 42 h with the retardation factor (Rf) values of 0.59 and 0.55 in MBTD16. While the Rf value of control 0.78 reduced to the above values in the bacteria treated samples indicates it biodegradation of VB-B dye. Appearance of additional compounds may be the degradative products of the dye.

The FTIR spectrum of the VB-B (Fig. 5 (a)) showed a weak N-H band at 3442 cm^{-1} . The peak appeared at higher wave number at 3524 cm^{-1} indicating the symmetrical nature of the stretching bond (usually secondary and tertiary amines will not show peaks in this region). The C–N stretching frequency appears at 1289 cm^{-1} due to the aromatic nature of the VB-B dye. While the C–C stretching band of the aromatic ring was observed at 1584 cm^{-1} . The IR spectrum of the samples obtained after the treatment with bacteria VB-B (Fig. 5 (b)) did not show peak at 3442 cm^{-1} corresponding to N-H bond, shown in the spectrum. The FTIR spectrum of the treated samples on H-C-H asymmetric and symmetric stretch and alkane C–H bonds in the specific regions 2924 and 2854 cm^{-1} in comparison with control indicates the growth of bacteria enhanced biodegradation of VB-B to a certain extent.

4 Conclusions

Shewanella decolorationis MBTD16 used in this study showed its potential role in decolorizing the textile dye VB-B in a significant proportion within a short residence time of 42 h and the decolorized water could be very well used for recycling. Thus by equivalent quantity of water used in the first phase of coloring may be saved. This will help in a larger way to minimize the amount of water required for textile dye processing. Minor improvements of this technique to industrial applications may guarantee a better remediation solution to the textile industry. The above bacteria were able to synthesize the enzymes lignin peroxidase and azoreductase internally to overcome the stress created by the dye. During the decolorization process MBTD16 ultimately prove to withstand high concentrations of VB-B dye (500 mg L^{-1}) and could yield a moderate decolorization rate of 19.64% with the concentration of 400 mg L^{-1} . Results suggested from the current study not only indicate the cost reduction investment of the dyes by 35-50% subsequently it help to reduce the level of pollution in equivalent to the dye use.

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Table 1

Characteristics of the dye used in this study

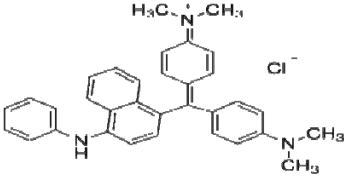
Dye	C.I. name	Chemical structure	C.I. number	Class	λ_{\max}
Victoria blue B	Basic blue 26		44045	Triarylmethane	615

Table 2

Optimization of the process parameters tested for maximizing the decolorization ability by MBTD16

Parameters	Values ranges tested	% of decolorization	
		Maximum	Minimum
Agitation	Static and Shaking	Static: 88.32±1.57	Static: 57.12±0.58
		Shake: 54.36±0.6	Shake: 38.19±0.57
pH	4,5,6,7,8,9,10	93.32±0.77- pH 7	16.76±0.62-pH 10
Temperature	10,20,30,40,50°C	92.41±1.74- 30°C	30.10±0.79- 50°C
Inoculums size	1,2,3,4,5,6,7,8,9,10 mL	87.97±1.90- 8 mL	9.86±1.45-1 mL
Incubation time	0-6-12-18-24-30-36- 42 h	86.73±1.07-42 h	15.61± 0.14(0 h)
Carbon sources* (5 g l ⁻¹)	glucose, sucrose, dextrose, maltose, lactose and xylose	67.26±0.46	26.60±1.58
		Glucose	Xylose
Nitrogen sources* (5 g l ⁻¹)	beef extract, yeast extract, peptone, ammonium sulfate, casein and trypton	66.15±2.81	43.20±3.31
		Yeast extract	Ammonium sulphate
Concentration of the dye	50-100-150-200-250-300-350-400-450- 500.	94.83±1.60 (50 mg L ⁻¹)	6.59±0.73 (500 mg L ⁻¹)

Mean ± Standard Deviation of triplicates.

*(Incubated under static, pH 7, Temperature 32±2°C, Inoculum size 8%).

Table 3a

'L'A'B' Values and the dye removal index of VB-B by bacteria treated sample

	L*	a*	b*	DRI (%)
Control	62.29	-4.93	-20.75	-
MBTD16	91.585	-2.856	-0.132	96

Table 3b

Increased level of dye reflectance measured in decolorized water added with additional dye concentration of 100 mg L⁻¹

	L*	a*	b*
Control	62.29	-4.93	-20.75
MBTD16	41.671	0.744	-61.414

Table 4

Various enzymes tested for their activities during decolorization

Enzyme	Enzyme production in NB		Enzyme production in the DM	
	MBTD16		MBTD 16	
	Ext	Int	Ext	Int
Lignin peroxide ^a	0.29±0.03	0.06±0.00	0.24±0.01 ^{***}	0.65±0.02 ^{***}
Tyrosinase ^a	0.01±0.00	0.01±0.00	0.00±0.00 [*]	0.08±0.01 ^{***}
Azo reductase ^b	0.60±0.02	0.00±0.02	0.09±0.00 ^{***}	0.26±0.03 [*]

Ext-Extracellular, Int-Intracellular.

Mean ± Standard Deviation of triplicates

^a - Enzyme unit's mg⁻¹ min⁻¹^b - Enzyme catalyses the oxidation of 1 μmol of NADH/min^{***} Significant at the level of P < 0.05,^{*}Not significant by One-way analysis of variance (ANOVA) with turkey scheffe alpha comparison test.

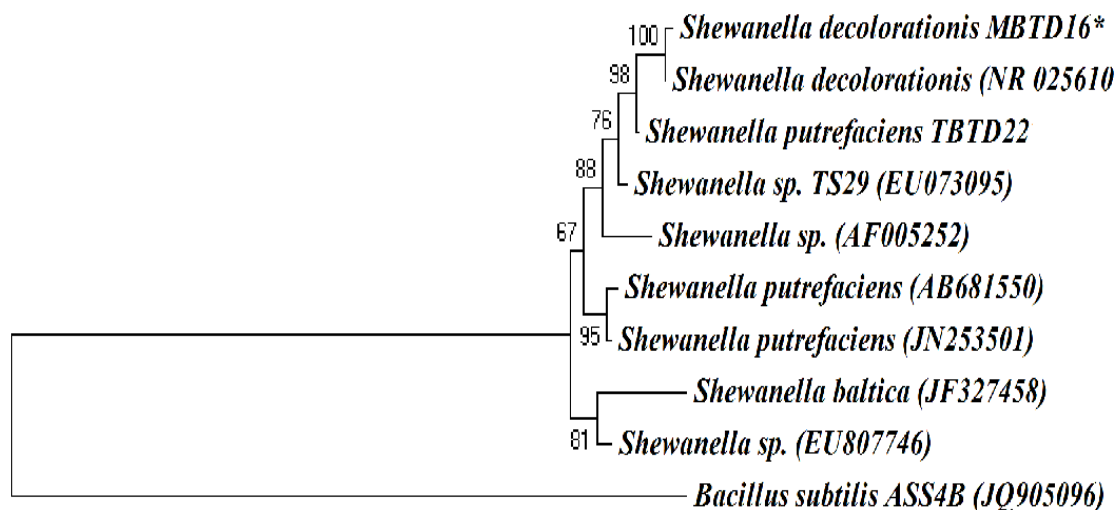


Fig. 1 The Neighbor-Joining tree based on evolutionary distances was computed using the Maximum Composite Likelihood method. Bootstrap values (expressed as percentages of 1000 replications) greater than 50% are shown at the branch points. *Bacillus subtilis* is used to assign an out group. Bacteria with Accession numbers were retrieved from GenBank database to know the relationship of our bacteria (marked as *)

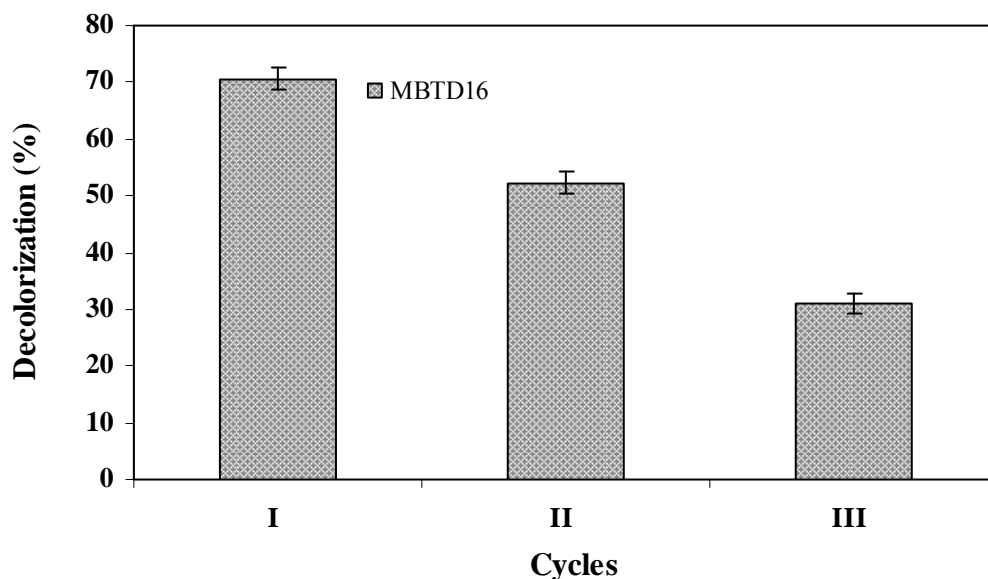


Fig. 2 Decolorization of textile dye VB-B by immobilized bacterial cells in different cycles and its cumulative rates

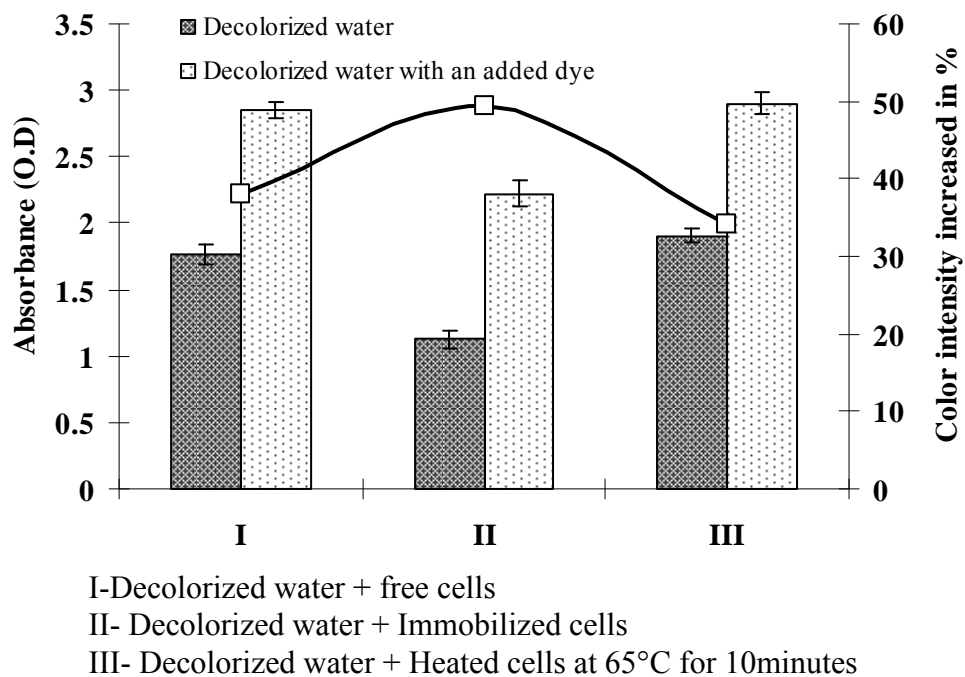


Fig. 3 Percentile increase over color intensity against the decolorized waters added with additional dye of 100 mg L⁻¹

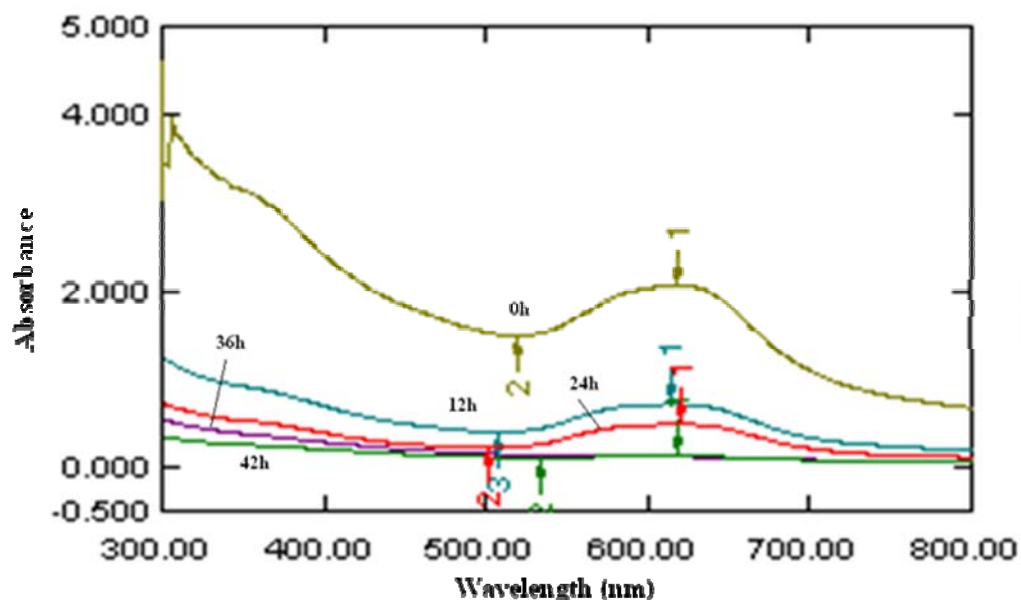
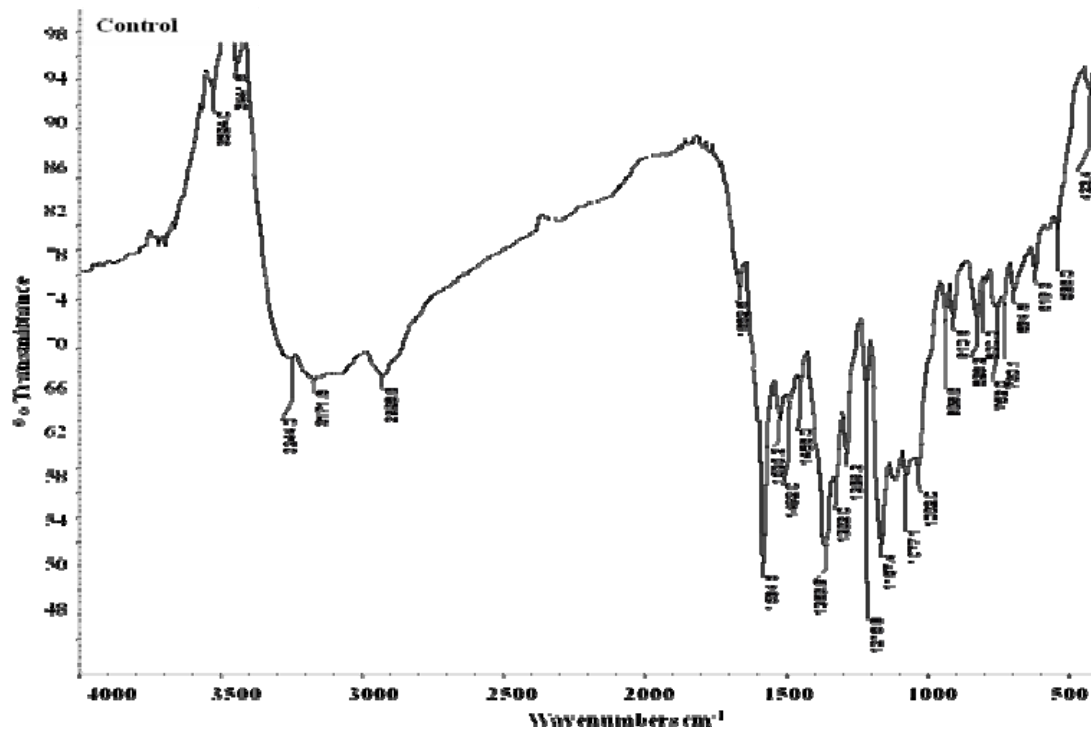


Fig. 4 Spectroscopic (UV-Visible) analysis of VB-B dye treated with the bacteria MBTD16

(a)



(b)

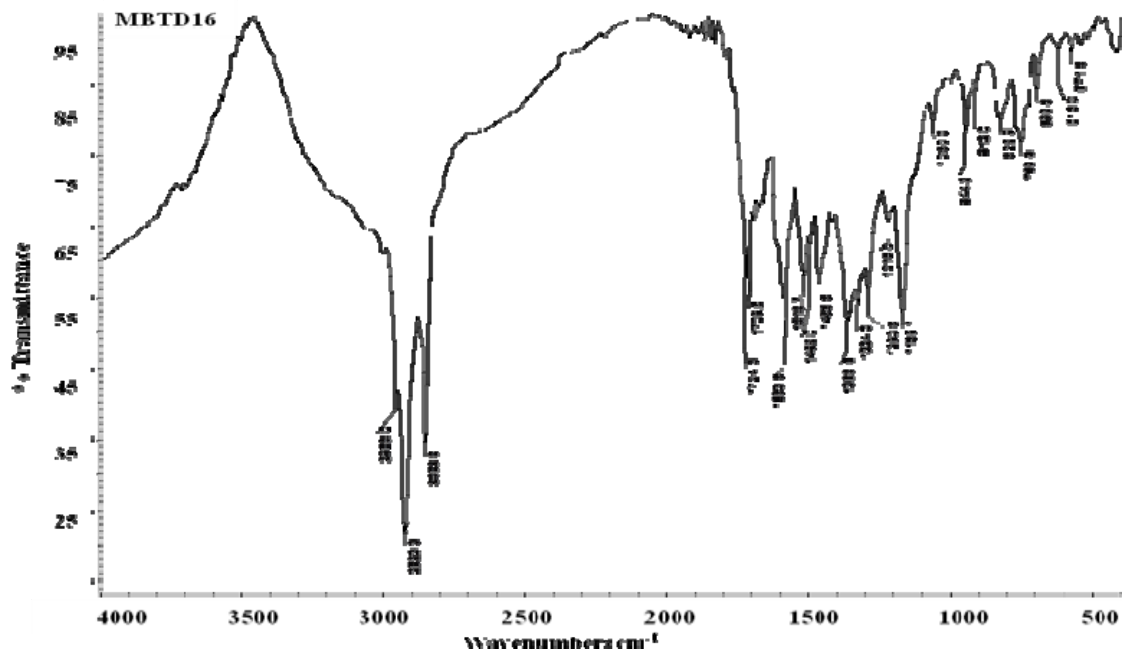


Fig.5 (a) and (b) FTIR analysis on the textile dye VB-B (Control and MBTD16)