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Enhanced biodecolorization of textile dye remazol navy blue using an isolated bacterial strain *Bacillus pumilus* HKG212 under improved culture conditions

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Abstract

Biodegradation of synthetic dyes has been proven as the most efficient, eco-friendly and cost-effective alternative for the treatment of textile dyes contaminated waste water. Remazole Navy Blue (RNB), an azo dye used extensively in the commercial textile industry constitutes hazardous recalcitrant, when released in the aqueous environment. In this research, an isolated bacterial strain identified as *Bacillus pumilus* HKG212 was studied for its potential to decolorize RNB dye. The isolated bacterial strain showed significant decolorizing capacity of more than 95% up to an initial dye concentration of 500 mg/L, within 48 hours and was efficiently tolerant up to a higher concentration of 1500 mg/L, under static condition. We investigated various physicochemical parameters to achieve maximum dye degradation by Bacillus pumilus HKG212. The optimal conditions for the decolorizing activity of *Bacillus pumilus* HKG212 were anaerobic culture environment with 10% innoculm volume and beef extract as a nitrogen source, at pH 8.0, and 30℃. Biodegradation of RNB dye was confirmed through UV-VIS Spectrophotometer, HPLC and FTIR analysis. Our findings confer the potential application of strain *Bacillus pumilus* HKG212 for decolorization of RNB dye and hence treatment of industrial wastewaters containing dye pollutants.

Keywords: Biodecolorization; *Bacillus Pumilus* HKG212; Dye; Remazole navy blue

Introduction

In the present scenario water pollution has become a major problem worldwide. Due to rapid industrialization the level of waste is increasing day by day and the toxic and hazardous chemicals released from various industries are causing threat to the biotic and abiotic components of various eco-systems. Among various industries, the textile dyeing and finishing industry has created a huge pollution problem. As more than 100,000 commercially available dyes (Fu and Viraraghavan 2001) are used in various processes of textile manufacture including dyeing and printing most of which are toxic, carcinogenic and recalcitrant in nature (Babu et al 2007; Chequer et al 2013; Kant 2012).

Around 10 to 20% of about 7×10^5 tons of dyestuffs (Zollinger 2002) being manufactured and used in dyeing processes may be found in the wastewater each year (Robinson et al 2001). Apart from that, textile industries use large amount of water in every step of processing of fabric materials and the used water released after processing are saturated with various toxic chemicals, having increased pH and temperature (Babu et al 2007). During the processing nearly 10-15% of dyes are lost along with the effluent (Kant 2012; Khaled et al 2009). The colored waste water released from such industries pollutes the soil and nearby water bodies. The colored and colloidal matter increases the turbidity of the river and sea water, which affects the light and oxygen penetration inside the water bodies which ultimately affects the aquatic ecosystem. When these colored effluents are discharged without proper treatment, it pollutes soil and ground water which causes various health hazards to human and animals. Hence, it becomes very necessary to degrade these toxic chemicals in to non-toxic products, before its release in to different water sources.

Currently, several strategies are available to remove dyes from the industrial effluent, before discharging them into main water body. These include several physical methods such as membrane technologies, chemical methods such as ozonation, physicochemical methods (like adsorption, chemical precipitation, flocculation, photolysis, and ion pair extraction) and biological processes such as biodegradation and bio-adsorption (Engade and Gupta 2010). Application of these physical and chemical methods of treatment has several shortcomings as they are costly, produce large amount of sludge which again produces an obvious disposal problem, lack of effective color reduction etc. (Banat et al 1996; Gogate and Pandit 2004; Vijayaraghavan et al 2013). Moreover, because of their high stability against light, temperature and oxidizing agents, most of the dyes form textile effluents escape from such conventional treatment processes and persist in the

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environment for a long time. Bioremediation technology provides an effective, cheap and eco-friendly method to remove the color from dye containing waste water (dos Santos et al 2007; Sudha et al 2014). A wide variety of microorganisms was found to be capable of degrading various textile dyes (Huang et al 2014; Jafari et al 2014; Shah et al 2013). Among them, bacterial decolorization was found to be nonspecific and comparatively more effective as compared to other forms of microbes. Many researchers have investigated a wide range of bacterial strains capable of decolorizing various textile dyes under certain environmental conditions (Chang et al 2001; Kaushik and Malik 2009; Togo et al 2008).

Current study deals with the isolation of potent bacterial strain from textile industry effluent waste water for their decolorization efficiency of the textile dye. Among the various categories of dyes, azo dyes are used frequently in textile industries because of its wide range of color spectrum, ease of application, bright colors and shades (Aksu et al 2007; Lee and Pavlostathis 2004). They are characterized by the presence of one or more azo groups -N=N- and the bond between two aromatic ring (Zollinger 2002). Remazol Navy blue, a widely used azo dye was chosen as the model dye for this study. Effects of different process parameters like pH, temperature, initial dye concentration, inoculum volume, aeration condition, carbon source and nitrogen source on dye decolorization capacity of the isolated strain were studied. Biodegradation of the dye was confirmed through various analytical techniques like UV-VIS spectrophotometer, FTIR and HPLC analysis.

Materials and Methods

Chemicals and Textile dye stuff used

All the chemicals and reagents used were of the highest purity and of analytical grade and were obtained from Merck Ltd., India. All the culture media, nutrient broth and nutrient agar used were obtained from Hi-Media Lab. Pvt. Ltd., India. Remazol Navy Blue used as the model dye for this study was obtained from a textile dye wholesaler H. Nagindas & Bros, Kolkata, India. A Stock solution of 10,000 ppm was prepared for RNB dye and all the experiments were done with these stock solutions.

Sample Collection

Textile waste water sample was collected from Viwandi, Mumbai, India. Collected water sample was brought to the laboratory in sterile plastic bottles and stored in the refrigerator at 4ºC for further study.

Isolation and screening and identification of dye decolorizing strain

Textile waste water sample was used as a source for isolation of dye degrading microorganisms. The waste water sample was subjected to acclimatization with increasing concentrations of textile dye RNB in a nutrient medium. Repeated transfers were carried until complete decolorization of the broth to isolate stable dye decolorizing strains. A small amount of the decolorized medium was spread on nutrient agar plates containing RNB dye. After 48 hrs of incubation, colonies showing zones of decolorization were isolated using streak plate technique. A number of strains were isolated and were further screened based on their dye decolorizing ability by performing a dye decolorization assay with RNB dye using UV-VIS spectrophotometer (UV-VIS spectrophotometer-3200, Labindia Instruments Pvt Ltd, India). The strain having the highest decolorizing activity was designated as M2C and selected for further studies. The strain was sent to Xcelris labs Ltd, Ahmedabad, India for 16S rDNA identification.

Dye Decolorization Assay

The dye decolorization capacity of the isolated strain was studied with respect to time and also with increasing concentrations of dye. All the decolorization experiments were done in triplicate and abiotic culture was taken as control for all the studies. Wave length for maximum absorbance (λ_{max}) of RNB dye was determined through spectrophotometric analysis and was found to be at 597 nm.

Dye Decolorization at different dye concentration

To study the effect of initial dye concentration on the rate of decolorization, 1% v/v of 24 hrs old microbial culture was inoculated to nutrient medium containing different concentrations of RNB dye (100-1500 mg/L). After 24 hrs of incubation at 30ºC, aliquot of decolorized medium was centrifuged at 10,000 r/min for 15 min to separate the bacterial cell mass. The clear supernatant was used to measure the decolorization at the absorbance maxima of the RNB dye at 597 nm.

Dye Decolorization with respect to time

Nutrient broth containing 300 mg/L of RNB was prepared and transferred to 30 ml culture bottles. The bottles were filled completely and sealed with screw caps to achieve anoxic condition. 1% v/v of 24 hrs old microbial culture was then inoculated to every bottle and incubated under static condition at 30ºC. The number of bottles incubated corresponded to the number of samples to avoid incorporation of air during repeated sampling. Un-inoculated control was also incubated under same condition to check the abiotic decolorization of the dye. Aliquot of culture media from respective vials were withdrawn at different time intervals and centrifuged at 10,000r/min for 15 min. Extent of decolorization was monitored by measuring the absorbance of the clear supernatant. The percent decolorization and average decolorization rate was measured.

$$\%Decolorization = \frac{Initial\ abs. - Observed\ abs.}{Initial\ abs.} \times 100 \quad (0.1)$$

$$Average\ Decolorization\ rate = \frac{I \times \%D \times 1000}{100 \times t} \quad (0.2)$$

Where I = Initial concentration of dye, % D = Percentage of dye decolorization after time t.

Effect of physicochemical conditions on dye decolorization

Effect of Aeration condition on dye decolorization

To study the effect of aeration condition on decolorization, inoculated dye containing medium was inoculated under three culture conditions, namely, static (no shaking), shaking (aerobic) and anaerobic. To maintain aerobic condition, culture tubes were kept in rotary shaker running at 120r/min. In order to maintain anaerobic condition, culture tubes were filled up to the neck and sealed with screw cap to avoid penetration of air and in the static condition, culture tubes were placed directly in the incubator.

Effect of pH on dye decolorization

To study the effect of pH on decolorization capacity of the isolated strain, pH of the sterile nutrient medium was adjusted to 4, 5, 6, 7, 8, 9 and 10 before dye addition. After pH adjustment, 300 mg/L of syringe filter sterilized dye was added and the medium were inoculated with 1% v/v of inoculum. After 24 hrs of incubation at 30°C under static condition, extent of decolorization was measured as mentioned earlier.

Effect of temperature on dye decolorization

Effect of incubation temperature on decolorization capacity of the isolated bacteria was studied by inoculating dye (300 mg/L) containing nutrient medium with 1% v/v of inoculum and incubating them under different temperature (20°C, 25°C, 30°C, 35°C, 40°C) in static condition. The extent of decolorization was measured as earlier.

Effect of Inoculum volume on dye decolorization

Sterile dye (300 mg/L) containing nutrient medium were inoculated with varying volume of inoculum (1%, 5%, 10%, 15%, 20%) to study the effect of inoculum volume on decolorization capacity of the bacteria.

Effect of Carbon and Nitrogen source on dye decolorization

Study of effect of carbon and nitrogen source on decolorization capacity of the isolated strain was done with semi-synthetic medium containing 100 mg/L of RNB dye. Different Carbon sources like glucose, sucrose, and mannose were added individually to the culture medium at a concentration of 1g/L. Similarly different Nitrogen sources (Ammonium Sulfate, Ammonium Chloride, Peptone, Yeast Extract and Beef Extract) were added to the medium individually and in combination with carbon source at a concentration of 0.5g/L to study the effect on decolorization process.

Analytical methods to study the dye degradation

Spectrophotometric analysis

Biodegradation study was done with UV- Visible spectral analysis. During the process of decolorization, samples were withdrawn at different time intervals and changes in absorbance peaks of decolorized supernatant of the medium was observed and compared with the control runs.

Analysis of degradation products by HPLC

HPLC unit equipped with (MD-2015 plus, Jasco, Japan) UV-VIS detector was used to study the degradation of RNB. Dye decolorized medium was centrifuged at 10,000 r/min for 10 min, the supernatant so obtained was filter sterilized with 0.22µm syringe filter and used directly for HPLC analysis. Untreated dye containing media acted as the abiotic control. Samples were eluted in C18 column (5µm, 4.6mm×250mm). The mobile phase used composed of 60% methanol, 38% water and 2% acetic acid. Degradation products were analyzed by comparing the retention time of elutes.

Analysis of degradation products by FTIR

For FTIR analysis, the decolorized medium was centrifuged at 10,000r/min for 10 min. The supernatant was extracted overnight with equal volume of ethyl acetate. The organic phase was then collected and concentrated to about 2 ml in a rotary evaporator. The concentrated solution was further dried and the dried residue was dissolved in methanol and used for analysis. FTIR analysis was carried out with (Nicolet^{TM} iS^{TM}10, Thermofisher Scientific, USA) spectrophotometer in the mid-infrared region of 400-4000cm^{-1} with 16-scan speed.

Results and discussion

Isolation, Screening and 16S rDNA-based identification of the isolate

Isolation of the bacterial strains was performed by serial dilution and spread plate method based on their ability to perform clear zone on nutrient agar plate containing RNB dye. Isolated strains were further screened based on their dye decolorizing ability in broth culture. On repeated transfer one consortium exhibited consistent and highest decolorization potential was designated as strain M2C and selected for further studies. 16s rDNA based molecular identification result of the isolated strain suggested that the strain M2C has highest similarity to *Bacillus pumilus* strain HKG212 (GenBank Accession Number: KJ741252.1) based on nucleotide homology and phylogenetic analysis (Fig. 1).

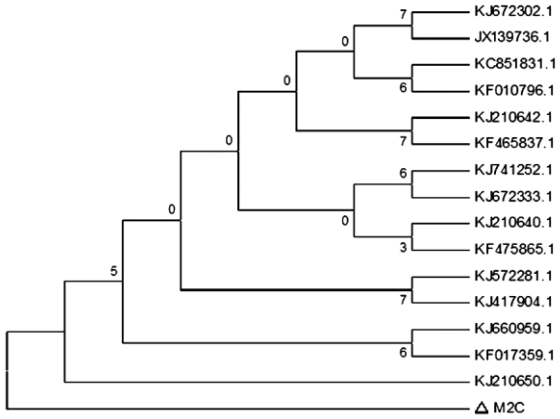

Figure 1: Phylogenetic tree of isolated bacterial strain M2C
KJ672302.1 - *Bacillus safensis* strain; JX139736.1 - *Bacillus safensis* strain
KC851831.1 - *Bacillus safensis* strain; KF010796.1 - *Bacillus safensis* strain
KJ210642.1 - *Bacillus safensis* strain; KF465837.*1* - *Bacillus pumilus* strain
KJ741252.1 - *Bacillus pumilus* strain; KJ672333.1 - *Bacillus safensis* strain
KJ210640.1 - *Bacillus safensis* strain; KF475865.1 - *Bacillus pumilus* strain
KJ572281.1 - *Bacillus pumilus* strain; KJ417904.1 - *Bacillus pumilus* strain
KJ660959.1 - *Bacillus pumilus* strain; KF017359.1 - *Bacillus safensis* strain
KJ210650.1 - *Bacillus safensis* strain; M2C- *Bacillus pumilus* strain

Decolorization assay of Remazol Navy Blue

Effect of dye concentration

Study of the effect of initial dye concentration on the efficiency of color removal of the isolated strain revealed that,

the bacterial strain is capable of decolorizing an acceptable high concentration of color. Decolorization with different initial RNB concentration ranging from 100 mg/L to 1500 mg/L was studied under anaerobic condition. At an initial dye concentration of 100 mg/L, 96% color removal was observed after 24 hrs of incubation while, in 48 hrs almost 99% decolorization was achieved. As the dye concentration was increased to 500 mg/L, 91% decolorization was achieved after 24 hrs of incubation which increased to 98% after 48 hrs of incubation. At a subsequently increased dye concentration of 1000 mg/L and 1500 mg/L, 85% and 80% decolorization was observed after 48 hrs, respectively (Fig. 2). This study clearly shows that, as the dye concentration is increasing, the bacterial strain is taking longer time for decolorization. Similar studies have been reported (Chang et al 2001; Shah et al 2013) in literatures where a decrease in the efficiency of decolorization was observed with increase in initial dye concentration. With subsequent increase in dye concentration toxic effect of dye and its metabolites became dominant, leading to inhibition in decolorization. The decrease in the efficiency of color removal with increase in concentration of dye can be due to toxic effect of dye and inadequate amount of biomass to uptake this higher concentration of dye and the ability of the enzyme to recognize the substrate efficiently at the very low concentrations (Jadhav et al 2007; Pearce et al 2003).

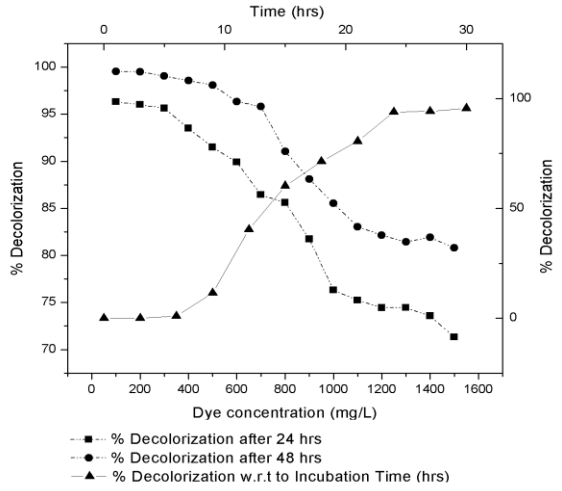

Figure 2: Effect of Initial dye concentration and Incubation time on % of decolorization

Time course study of decolorization of RNB

The decolorization assay with respect to time showed maximum decolorization of 95.5% for 300 mg/L dye RNB in 30 hrs at 30ºC in anaerobic condition (Fig. 2). Initially up to 6 hrs of incubation, no noticeable decolorization was observed probably indicating the lag period for acclimatization to culture environment and thereafter an increasing trend was noticed until 94.2% decolorization was achieved in 27 hrs. As evident, beyond 27 hrs very little change in decolorization percentage could be noticed. This may be due to the culture approaching the death phase that reduced the enzyme activity. Since, maximum decolorization of the dye was noticed after 24 hrs, this optimum time period was used for the subsequent experiments using strain M2C. Similar studies have been reported in various literatures (Ponraj et al 2011; Praveen Kumar and Bhat Sumangala 2012) where with increase in time the rate of decolorization increased and after a certain point of time it showed no further decolorization.

Effect of physicochemical parameters on the decolorization performance

Effect of aeration condition

Aeration condition has a tremendous impact on dye decolorization capacity of the bacterial strain. After incubating the dye in the culture medium for 24 hrs, M2C showed 85% decolorization at static condition which, further improved to 94.5% under anaerobic environment, while it reduced to 49.5% under shaking (aerobic) environment (Fig. 3). Similar to most of the bacterial decolorization process, maximum decolorization was achieved under anaerobic condition (Aksu et al 2007; Chen et al 2003; Sethi et al 2012). This result suggests that the isolated bacterial strain were capable of growing in deficiency of oxygen and can efficiently decolorize RNB dye in anaerobic conditions. In anaerobic condition azo group of the dye acts as the electron acceptor from the reduced electron carrier i.e. NADH, quinones etc. and gets reduced (Wuhrmann et al 1980). However, if the extra-cellular environment is aerobic, this reduction mechanism gets inhibited by oxygen, as the oxidation of the reduced redox mediator is mediated by oxygen rather than by the azo dye (Pearce et al 2003). Hence, the probable reason for decreased decolorization at shaking condition could be competition of oxygen and the azo compounds for the reduced electron carriers under aerobic condition (Chang et al 2001; Van der Zee et al 2001).

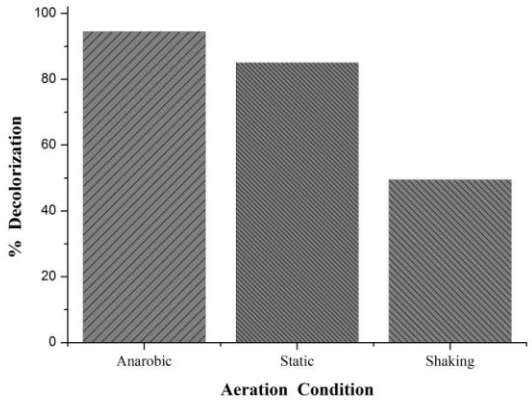

Figure 3: Effect of aeration condition on % decolorization of RNB dye

Effect of pH

Effect of pH is significant on treatment of industrial effluent containing dye. Textile waste water shows varying pH and mostly alkaline pH is maintained during the processing as it favors the addition and substitution mechanism between the cotton fibers and azo dyes (Walters et al 2005). The isolated strain M2C showed decolorization efficiency over a broad range of pH from 5-9. However, maximum decolorization of RNB (97.6%) was achieved at pH 8 within 24 hrs of incubation. At pH 6 and 7, the percentage decolorization was 93% and 95% respectively and the % of decolorization was found to decrease sequentially from pH 6 to 4 and was negligible at pH 4 (Fig. 4). The optimum range of pH for textile dye decolorization generally lies between 6.0 and 10.0 (Chen et al 2003; Guo et al 2007; Kılıç et al 2007). A similar study was depicted (Shah et al 2013) for the removal of azo dye Reactive red. They found that *Bacillus* species ETL-1982

was capable of decolorizing azo dye within a broad range of ph 6-11 and optimum pH was obtained at pH 9.0.

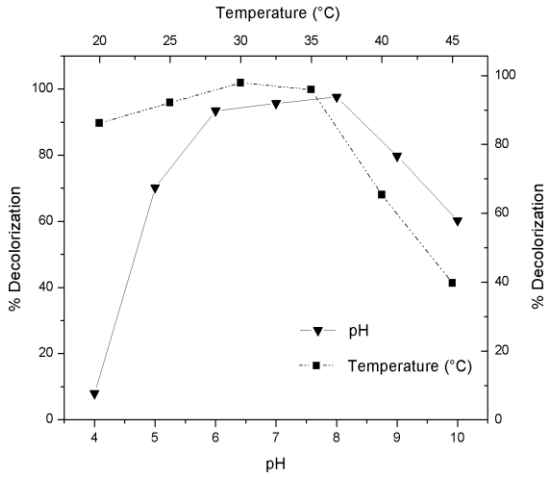

Figure 4: Effect of pH and Temperature on % Decolorization of RNB dye

Effect of temperature

Effect of temperature on dye decolorization efficiency of the isolated bacteria was studied for wide range of temperature from 20°C to 40°C. Maximum decolorization of 98% was achieved at 30°C. As the temperature decreased to 20°C, percentage of decolorization reduced to 86%. With increase in temperature above the optimum value, gradual decrease in decolorization potential of the bacteria was observed and at 45°C only 39% decolorization was achieved (Fig. 4). This suggests that the organism used is a mesophile and able to give a good decolorization in ambient temperature. In a similar kind of study (Sugumar 2012) for the decolorization of Reactive Orange 16 using *Nocardiopsis alba* strain optimum temperature was obtained at 30°C where maximum decolorization (92%) was achieved in 24hrs.

Effect of inoculum volume

Figure 5 depicts the change in the extent of dye decolorization in response to varying inoculum size. After 24 hrs of incubation at 30°C in static condition, 84% decolorization of RNB dye was achieved in the flasks containing 1% v/v of bacterial inoculum. With an increase in inoculum size, the extent of decolorization also increased and 90% decolorization was achieved in the flasks containing 10% v/v of inoculum. A further increase in volume of inoculum resulted in decreased decolorization efficiency of the bacteria. As it is a batch process, early depletion of nutrient occurs as the inoculum volume increased and hence biological process of decolorization involving microorganisms require an optimum amount of microbial cells. Similar kind of result were obtained in literature for effect of varying inoculum volume on dye decolorization (Kumar et al 2009; Mohan et al 2013). Kumar and associates used mixed microbial culture for decolorization of reactive azo dye and observed strength of 10% v/v as optimum inoculum volume. Mohan and co-workers used 10% concentration of *Bacillus* sp. and reported a maximum of 48.15% decolorization for Coractive Blue P-3R.

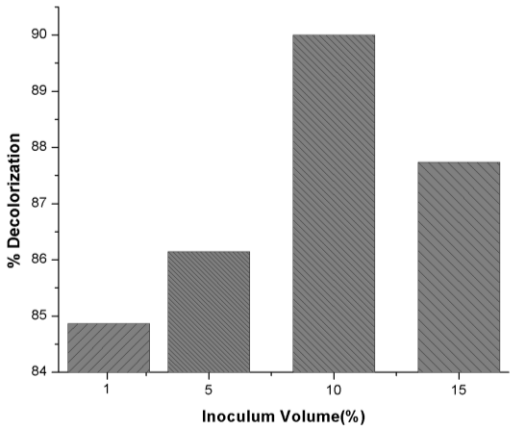

Figure 5: Effect of Inoculum volume on % decolorization of RNB dye

Effect of Carbon and Nitrogen source

The efficacy of M2C to decolorize Remazol Blue in presence of additional carbon (1%) and nitrogen sources (0.5%) was tested in order to obtain efficient and faster decolorization. The maximum percentage of decolorization was observed with beef extract (92.19%), while peptone and yeast extract showed a moderate decolorization value of 86.82% and 69.70% respectively, within 24 hrs (Fig. 6). Negligible decolorization was observed for NH_4Cl and $(NH_4)_2SO_4$ as supplements of nitrogen source.

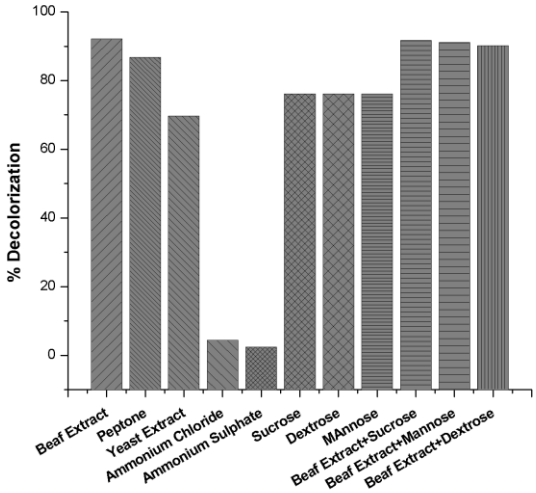

Figure 6: Effect of (a) Nitrogen source and (b) Carbon source on % decolorization of RNB dye

Effect of carbon source was evaluated with and without beef extract as a nitrogen source. Presence of sucrose, dextrose and mannose as carbon source without beef extract showed moderate decolorization around 76.19% in each case. On addition of beef extract along with sucrose, dextrose and mannose, the decolorization percentage was increased to 91.70%, 90.24% and 91.13% respectively (Fig. 6). This indicates that presence of Nitrogen source in media has a significant effect on the rate of decolorization, where as the presence of different carbon sources seems to be ineffective to promote the decolorization of NBR dye by the bacterial strain

M2C. Similar results were obtained in literature (Saratale et al 2009a, b), where using *Micrococcus glutamicus* and *Trichosporon beigelii* NCIM-3326 bacterial strain researchers observed the effect of nitrogen sources to supersede the effect of carbon sources under static conditions. Dye being a complex carbon ring is less preferred than a supplementary carbon source by the organisms. Hence the dye degradation potential of the organism decreases in presence of supplementary carbon source. In addition to that organic nitrogen sources acts as the source of electron donor to reduce the azo dyes by microorganisms (Hu 1994).

Analytical methods to study Biodegradation

Spectrophotometric analysis

If the dye removal is attributed to biodegradation, either the major visible light absorbance peak will completely disappear, or a new peak will appear (Yu and Wen 2005). Spectrophotometric analysis reported a gradual change is absorbance peak of RNB dye and its degradation product. The highest peak for RNB dye was observed in the visible region at 597nm in the control run which decreased gradually as the process of degradation proceeded. With increase in time the peak at 597nm gradually disappeared till it was completely invisible after 30 hrs suggesting the complete degradation of RNB by M2C within 30 hrs (Fig. 7). After 9 hrs of incubation, a new peak was observed at 263 nm which increased gradually and could be attributed to metabolic product formation after dye degradation.

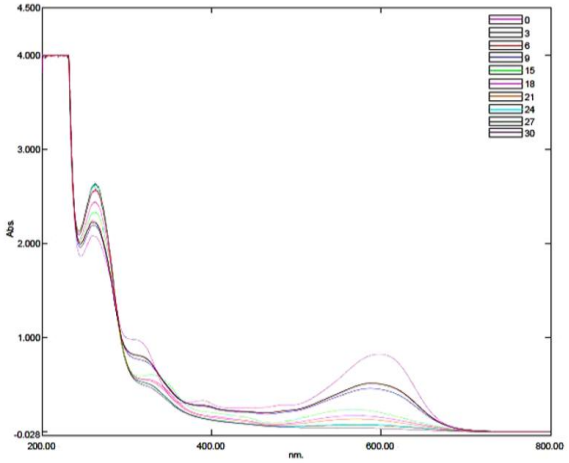

Figure 7: UV-Vis spectrum of RNB dye degradation for 30 hrs

HPLC Analysis

HPLC chromatogram of control dye RNB showed single peak at retention time of 3.7 min. (Fig. 8). After decolorization, the peak at 3.7 min. disappeared whereas two major peaks at 4.1 min. and 3.5 min. and two minor peaks at 5.25 min. and 8.88 min. with some other new peaks were observed (Fig. 9). The disappearance of the single peak may be attributed to the degradation of the parent dye and the appearance of new peaks may be attributed to the metabolites formed after biodegradation of the dye. It is thus reasonable to suggest that new peaks observed represent the degradation metabolites confirming the formation of additional aromatic metabolites. Similar findings for HPLC were reported for biodegradation of azo dye Rubine GFL and Red HE3B (Lade et al 2012; Phugare et al 2011).

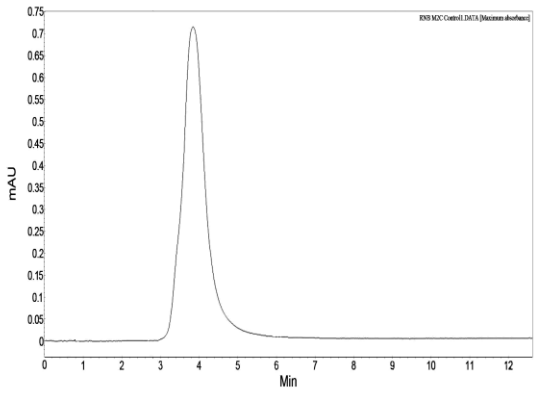

Figure 8: HPLC chromatogram of control for RNB dye

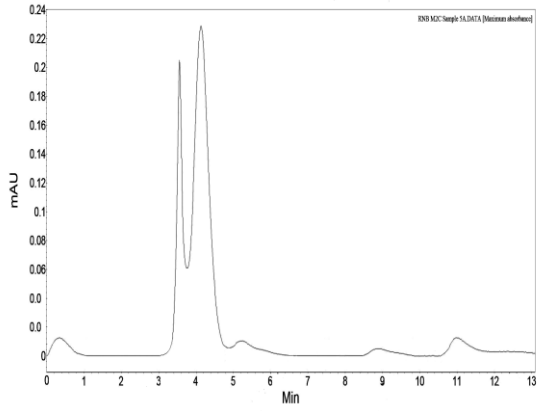

Figure 9: HPLC chromatogram of RNB dye after decolorization

FTIR Analysis

FTIR spectrum of control for RNB dye (Fig. 12a) showed peaks at 3359cm^{-1} for –C-H stretching of alcoholic or phenolic compound, 2974cm^{-1} for alkanes, 1775 for reactive carbonyl group, 1630cm^{-1} for –N=N- stretching of azo bonds, 1404cm^{-1} for –OH deformation of alcohols, 1017cm^{-1} for aliphatic phosphate group (P-O-C stretch). The FTIR spectrum of RNB metabolites after degradation (Fig. 12b) showed major peaks at 1645cm^{-1} for C=O stretch of amides, 1384cm^{-1} for alkanes CH_3 deformation, 1081cm^{-1} for cyanate (-OCN and C-OCN stretch), 599cm^{-1} for either alkyl halides or disulfides (C-S stretch). The disappearance of peak at 1630cm^{-1} gives the evidence of azo bond cleavage and appearance of different new peaks proves the formation of metabolites from cleavage of azo bond (Lade et al 2012; Phugare et al 2011).

Conclusion

From this study it can be concluded that, the isolated strain M2C which has been identified as *Bacillus pumilus* HKG212 is capable of effectively decolorizing textile azo dye, Remazole navy blue. Various physico-chemical parameters affecting the efficiency of decolorization of the bacterial strain were analysed and the optimum values for each parameters were determined. The optimal conditions for the decolorizing activity of *Bacillus pumilus* HKG212 were anaerobic culture environment with beef extract as nitrogen source supplementation, at pH 8.0, and 30 °C. The maximum decolorization efficiency against RNB dye achieved in this

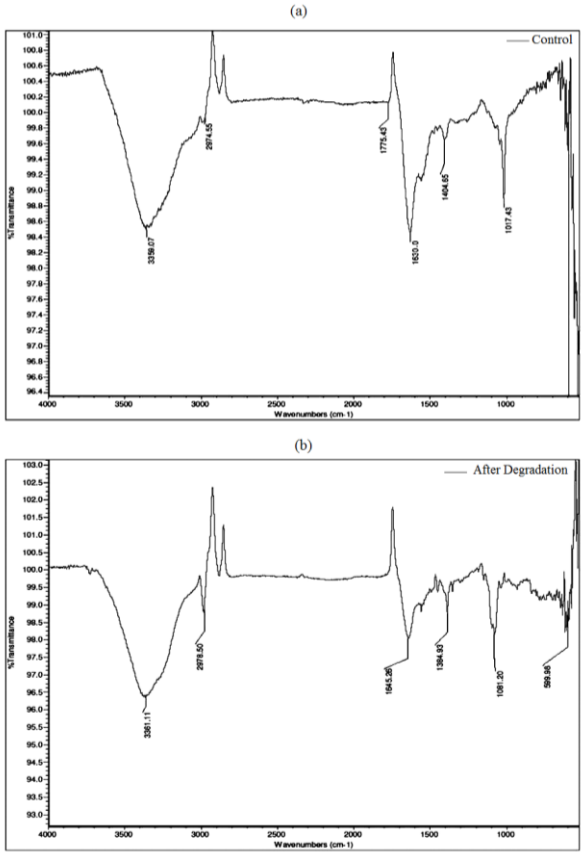

Figure 10: FTIR spectrum of (a) control RNB dye and (b) metabolites obtained after decolorization of RNB dye using strain M2C

study was more than 95% for an initial concentration up to 500 mg/L, within 30 hrs. *Bacillus pumilus* HKG212 showed decolorizing activity through a degradation mechanism rather than adsorption and it could tolerate high concentrations (up to 1500 mg L^{-1}) of RNB dye. Different analytical techniques like Spectrophotometric, HPLC and FTIR analysis confirmed the degradation of azo bond of the dye RNB by the isolated bacterial strain. Because of high degradative and decolorizing activity against a commonly used textile dye RNB, it is proposed that *Bacillus pumilus* HKG212 has a practical application in the biotransformation of common textile dye. Further pilot scale studies are necessary with this strain for real industrial applications and detailed study is desirable to explore the mechanism involved.

Acknowledgments

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