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Decolorization of Different Textile Azo Dyes using an Isolated Bacterium *Enterococcus durans* GM13

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ABSTRACT

Keywords

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Textile and other dye-stuff industries discharge various synthetic dyes into their effluent. The release of these synthetic dyes into the environment is a matter of concern due to the toxicity, mutagenicity, carcinogenicity and xenobiotic nature and causes serious pollution of soil, water & environment. The present study demonstrates the decolorization of three structurally different textile azo dyes (i.e. Reactive green-19 (RG-19), Remazol navy blue (RNB) and Reactive red-198 (RR-198)) using the isolated strain *Enterococcus durans* GM13 from textile industry waste water. The acclimatized strain was capable of decolorizing all the dyes in a wide concentration range and maximum decolorization efficiency has been achieved with 100 mg/L dye concentration. At 100 mg/L dye concentration, the organism was capable of decolorizing 87% RG-19, 91.3% RNB and 92% RR-198 dye within 24hr of incubation under static condition. Effect of different physico-chemical parameters on the decolorization efficiency of the isolated strain has been studied to achieve maximum decolorization efficiency. Biodegradation of the three model dyes were confirmed through UV-Vis spectral analysis.

Introduction

The discovery of man-made synthetic dyes in late 19th century replaced the large-scale market for natural dyes. Azo dyes contribute to the largest proportion of total synthetic dyes consumed annually in various industries such as textile, paper printing, cosmetics, leather etc. due to its versatile color range, better color fastness and stable chemical structure (He, Hu, & Li, 2004) (Asad, Amoozegar, Pourbabaee, Sarbolouki, & Dastgheib, 2007). Presence of electron withdrawing group in azo dyes makes them recalcitrant towards various

degradation processes (Singh, Singh, & Singh, 2014). However, reductive degradation of azo dyes under certain conditions produces a class of potentially dangerous chemical substances referred to as aromatic amines. Hence azo dyes and their degradation products are known for their toxic effect towards aquatic life, can be responsible for allergenic effects and are known to have carcinogenic and mutagenic effects to living organisms (Babu, Parande, Raghu, & Kumar, 2007) (Dias, Bezerra, Lemos, & Pereira, 2003) (Nigam, Banat,

Singh, and Marchant, 1996). Hence it becomes necessary to establish an efficient treatment method for the removal of toxic azo dyes from dye contaminated waste water before being released to the environment. Various physico-chemical methods for the treatment of these colored effluents practised till date have several drawbacks of being costly, inefficient color removal and production of secondary pollutant (Saratale, Saratale, Kalyani, Chang, & Govindwar, 2009). Biological treatment method of azo dye containing effluent using different microbial strains has been reported to be an effective and eco-friendly option by many researchers (K. C. Chen, Wu, Liou, & Hwang, 2003) (Jadhav, Dawkar, Ghodake, & Govindwar, 2008). However, bacterial decolorization of azo dyes has been reported to be faster as compared to other biological method of treatment (Chang, Chen, & Lin, 2004) (Kalyani, Telke, Dhanve, & Jadhav, 2009).

Organisms isolated from dye contaminated wastewater are believed to be more tolerant to high dye concentrations and can be used for the development of an effective bioprocess for the treatment of colored effluent. Current study deals with the decolorization of three different azo dyes using a bacterial strain isolated from textile industry wastewater.

Dye decolorization efficiency of the isolated strain has been evaluated in a wide concentration range for all the three model dyes. Effect of different physico-chemical parameters on the decolorization efficiency of the strain has been studied to achieve maximum decolorization potential. Degradation of the dye chromophore was confirmed through UV-Vis spectral analysis for the three model dyes before and after decolorization.

Materials and Methods

Dyes and Chemicals

Three different textile azo dyes Reactive green-19 (RG-19), Remazol navy blue (RNB), Reactive red-198 (RR-198) used in this study were purchased from a textile dye whole seller of Kolkata, India. Stock solutions of 10,000 mg/L concentration for the three model dyes were prepared and surface sterilised with 0.22 μm syringe filter. The desired concentrations of dye used in decolorization experiments were prepared by subsequent dilution of the stock solution. The structure and absorption maxima of the model dyes were presented in Table-1. All the media components used for bacterial, isolation, preservation and decolorization studies were purchased from Himedia (Mumbai, India).

Isolation, Screening and identification of dye decolorizing microorganism

Textile wastewater sample collected from Viwandi, Mumbai, India has been used as the source of dye degrading microorganism. Potent dye degrading bacterial strains were isolated by enrichment culture technique. 1 mL of textile waste water sample was inoculated to 100 mL of nutrient broth medium (Peptone 1g, Yeast extract 0.5g, NaCl 1g) containing mixture of three textile dye (RG-19, RNB & RR-198). After 48 hr of incubation at 30 °C under static condition, 1 mL of cell suspension was transferred to nutrient medium containing higher concentration of dye, and the process was continued till complete decolorization of the medium has been observed. A small portion of the decolorized medium was then spread on nutrient agar plate containing mixture of dye. After 48 hr of incubation at 30 °C, the organisms showing zone of decolorization were isolated and further screened based on

their highest decolorization potential. The strain showing highest decolorization potential was designated as M1A and chosen for further studies. The strain was sent to Xcelris labpvt. Ltd, Ahmedabad, India for 16s rRNA identification.

Decolorization Experiments

Batch decolorization experiments were conducted to study the dye decolorization efficiency of M1A for the decolorization of three model dyes RG-19, RNB and RR-198. All the decolorization experiments were performed in 250 mL Erlenmeyer flasks containing 100 mL of nutrient medium. Effect of different physico-chemical parameters affecting the decolorization efficiency of M1A has been studied by varying one factor at a time keeping the others constant with the basic conditions of temperature 32°C, pH 7.2, dye concentration 100 mg/L and 24hr of incubation period. Decolourization experiments were conducted at different incubation time, initial dye concentrations (100mg/L-1000 mg/L), inoculum volume, aeration condition, incubation temperature (20°C-45°C) and pH (5-10). Extent of dye decolorization was monitored using UV-Vis spectrophotometer at the absorption maxima of the dyes under investigation. The percentage decolorization was calculated using the following equation.

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

Results and Discussion

Isolation, screening and identification of dye decolorizing bacterial isolate

Dye decolorizing bacterial strains were isolated using serial dilution method based upon their ability to create clear zone in nutrient agar plate. A number of strains were

isolated and further screened to select isolates showing highest dye decolorizing ability. On repeated screening, one strain showing highest dye decolorization potential was selected and was designated as M1A. 16s rRNA based identification result of the isolate revealed that, the strain M1A has maximum similarity with *Enterococcus durans* GM13 (GenBank Accession Number: KC213474.1) based on nucleotide homology and phylogenetic analysis (Figure 1, Table-2).

Effect of incubation time

Biological decolorization of dyes is highly dependent on the incubation time as growth and metabolic rate of different bacteria varies with time. Decolourization of all the three model dyes by the isolated bacterial strain was recorded at different time intervals (Figure 2). The results showed that, M1A has achieved its maximum decolorization potential within 24 hr of incubation for all the three model dyes. Initially up to 10hr of incubation, a rapid decolorization efficiency has been observed which may contributes to the log phase when rapid metabolic rate and growth of microorganisms helped in fast reduction of dye chromophore.

Within 24hr of incubation M1A was able to achieve 86.56% decolorization of RG-19 dye, 91.08% decolorization of RNB and 92.23% decolorization of RR-198 at 32°C under static condition with 100 mg/L of dye concentrations and 5% inoculum volume.

The difference in percentage decolorization for three different dyes may be due to the difference in their structures. As maximum decolorization has been achieved within 24hr of incubation, rest of the studies were carried out taking this constant incubation time.

Effect of inoculum volume

In batch process where organisms grow in a fixed amount of nutrient, inoculum volume becomes an important factor affecting the overall efficiency of the process. Small inoculum volume slows down the overall rate of biological reaction which leads to decrease in rate of dye decolorization. However, inoculum volume larger than the optimum value results in early depletion of nutrient and microorganisms attain the death phase which leads to decrease in dye decolorization rate. Figure 3 depicts the change in the extent of dye decolorization of all the three model dyes in response to varying inoculums size of M1A. An increase in decolorization percentage has been observed with increase in inoculum volume from 1% (v/v) to 5% (v/v). However, with further increase in inoculum volume leads to decrease in decolorization efficiency of M1A strain for all the three model dyes. This may be due to early depletion of nutrient which leads to decreased metabolic rate, hence decolorization efficiency found to be reduced.

Effect of aeration condition

Aeration condition has a tremendous impact on azo dye decolorization capacity of the bacterial strains. Figure 4 depicts the color removal efficiency of the M1A under static, anaerobic and shaking condition. The strain M1A showed maximum decolorization both in static and anaerobic condition, however under shaking condition, a sharp decrease in decolorization efficiency has been observed. Most of the bacterial dye decolorization process of azo dyes prefers anaerobic environment as 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, Mechsner, & Kappeler, 1980)(Banat, Nigam, Singh, & Marchant,

1996) (Chen *et al.*, 2003). Similarly under static incubation condition, oxygen transfer is limited to the broth surface only and the cell cultures sediment in the bottom of the flasks and becomes oxygen depleted (Stolz, 2001)(Chen, 2002) which favours degradation of azo dyes. However, if the extra-cellular environment is aerobic (shaking condition), 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, Lloyd, & Guthrie, 2003). Similar findings were reported by many researchers where static and anaerobic conditions favour the reductive decolorization of azo dye molecule using different bacterial species (Ghodake, Jadhav, Tamboli, Kagalkar, & Govindwar, 2011) (Tripathi & Srivastava, 2011) (Singh *et al.*, 2014).

Effect of dye concentration

Concentration of dye is supposed to be an important parameter which can influence the decolorization efficiency of an organism. Higher dye concentrations can be toxic to microorganisms which may affect the microbial metabolism and growth, hence their decolorization efficiency gets affected. A wide range of concentration of the three model dyes were chosen to study the tolerance level and dye decolorization capacity of the isolated strain M1A. Our isolated strain M1A was found to be highly efficient in decolorizing the three model dyes (RG-19, RNB and RR-198) in all the tested concentrations of dyes (Figure 5). Highest decolorization has been achieved with 100 mg/L of dye concentration, at which 87.01%, 91.3% and 92% decolorization has been obtained for RG-19, RNB and RR-198 dye respectively. However, a decrease in dye decolorization efficiency was observed as the dye concentration increased from 100 mg/L to

1000 mg/L. A decrease in dye decolorization efficiency with increase in dye concentration may be due to the increasing toxic effect of dye and its degradation metabolites which affects the overall efficiency of the organism.

Effect of temperature

Incubation temperature plays an important role in microbial growth and activity. It is one of the vital parameter taken into consideration for the optimization of any kind of bioremediation process. Batch decolorization experiments were performed at different temperature levels ranging from 20°C to 45 °C for assessing optimal decolorization capacity of isolated strain M1A. A gradual increase in decolorization activity of M1A was observed with increase in temperature from 20 °C to 35 °C (Figure 6), showing maximum decolorization efficiency at 35 °C. At this optimum temperature the strain showed maximum decolorization of 87.78%, 94.23% and 95.5% for RG-19, RNB and RR-198 respectively.

However, a sharp decrease in color removal efficiency was observed as the temperature increased beyond 40 °C for all the three model dyes. The reduction in decolorization efficiency at higher temperature may be attributed to loss of cell viability or deactivation of enzyme activity (Saratale, Saratale, Chang, & Govindwar, 2011). Madhuri *et al.*, (2014) reported that *Enterococcus sp.* showed maximum decolorization of sulfonated diazo dye C.I. Direct Red 81 in the temperature range of 30-40°C which supports our finding.

Effect of pH

The hydrogen ion concentration showed profound effect on metabolic activity of microorganisms. 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, A., Santillo, D., & Johnston, 2005). Decolourization efficiency of the isolated strain has been studied in a wide range of pH from 5-10 (Figure 7).

The strain showed a good decolorization activity in a range pH 6-pH 9 and the maximum decolorization efficiency of M1A was achieved at pH 8 within 24hr of incubation for all the three model dyes. Decolourization efficiency found to be decreased substantially in higher acidic and alkaline conditions as extreme pH environment inhibits bacterial growth. Similar findings were reported by many researchers where the optimum range of pH for textile dye decolorization using different bacterial species lies between pH 6 to pH 10 (K. C. Chen *et al.*, 2003) (Guo *et al.*, 2007) (Kiliç, Nielsen, Yüce, & Dönmez, 2007). Sahasrabudhe *et al.*, (2014) reported that to obtain maximum decolorization of C.I. Direct Red 81 using *Enterococcus faecalis* strain YZ 66 the pH range of 5-8 has to be maintained.

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Table.1 Structure & Absorption maxima of model dyes used in this study

Dye	Molecular structure	Absorption maxima
Reactive Green-19 (anionic di-azo dye)		635nm
Remazol Navy Blue		597nm
Reactive Red-198		521nm

Fig.1 Phylogenetic tree of M1A

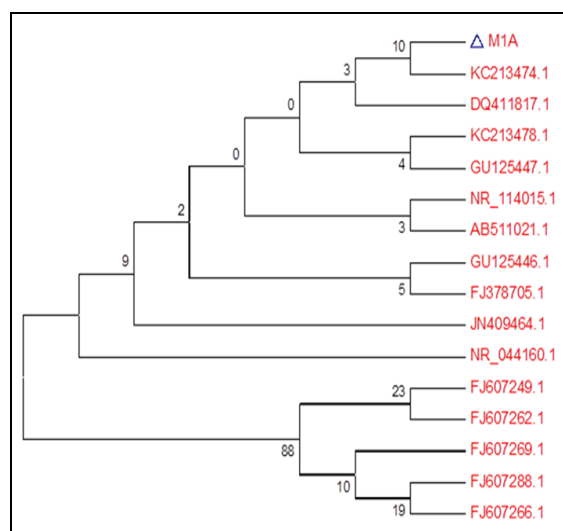


Table.2 Accession number of strains and their maximum identity with M1A

Accession	Description	Max ident
KC213478.1	<i>Enterococcus durans</i> strain GM18	99%
NR114015.1	<i>Enterococcus thailandicus</i> strain NBRC 101867	99%
JN409464.1	<i>Enterococcus thailandicus</i> strain SP15	99%
GU125447.1	<i>Enterococcus thailandicus</i> strain IMAU80025	99%
GU125446.1	<i>Enterococcus thailandicus</i> strain IMAU80024	99%
DQ411817.1	<i>Enterococcus sanguinicola</i> strain ss1743	99%
AB511021.1	<i>Enterococcus durans</i> , strain: C102901	99%
FJ378705.1	<i>Enterococcus sanguinicola</i> strain HN-S8	99%
NR_044160.1	<i>Enterococcus thailandicus</i> strain FP48-3	99%
KC213474.1	<i>Enterococcus durans</i> strain GM13	99%
FJ607288.1	<i>Enterococcus durans</i> strain KLDS6.0632	99%
FJ607266.1	<i>Enterococcus durans</i> strain KLDS6.0620	99%
FJ607269.1	<i>Enterococcus durans</i> strain KLDS6.0622	99%
FJ607249.1	<i>Enterococcus durans</i> strain KLDS6.0613	99%
FJ607262.1	<i>Enterococcus durans</i> strain KLDS6.0617	99%

Fig.2 Effect of incubation time on decolorization potential of M1A (at 100 mg/L dye concentration, pH 7.2, 32 °C)

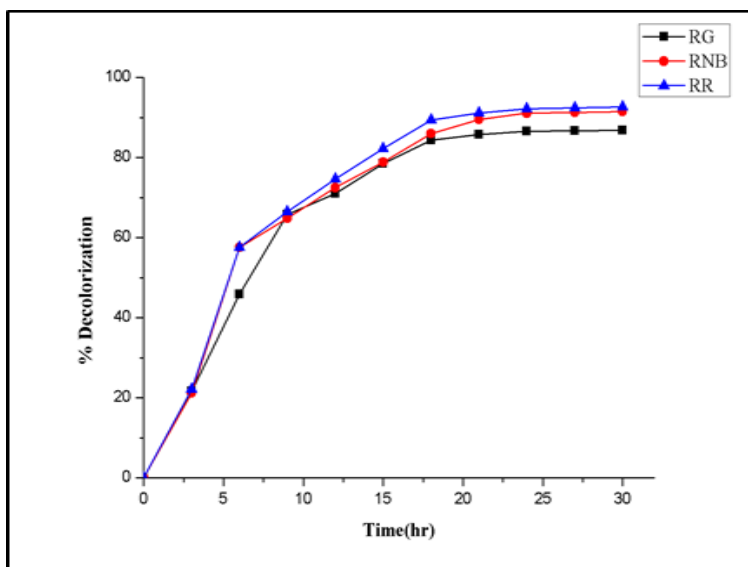


Fig.3 Effect of inoculum volume on decolorization efficiency of M1A (at 100 mg/L dye concentrations, pH 7.2, 32 °C)

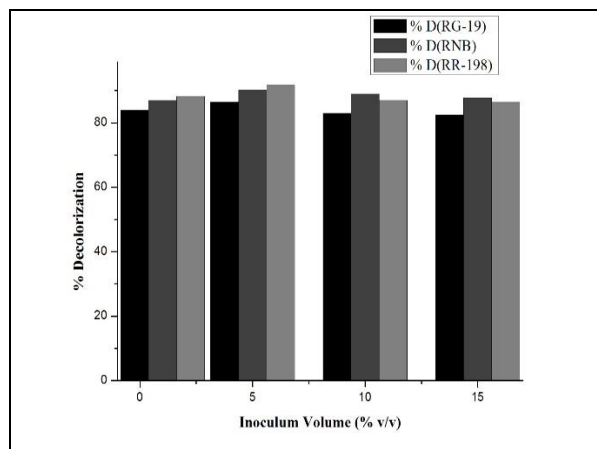


Fig.4 Effect of aeration condition on decolorization efficiency of M1A (at 100 mg/L dye concentration, pH 7.2, 32 °C)

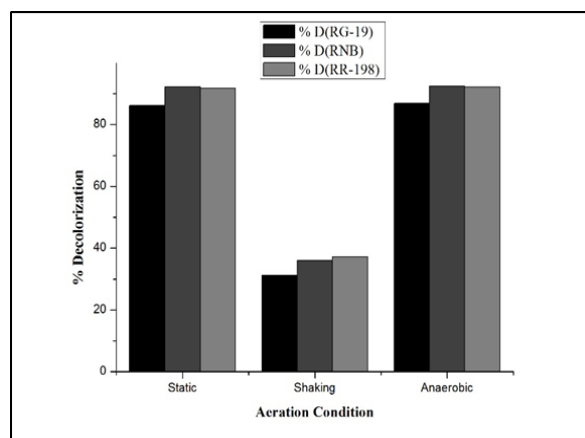


Fig.5 Effect of dye concentration on dye decolorization efficiency of M1A (at 100 mg/L dye concentration, pH 7.2, 32 °C)

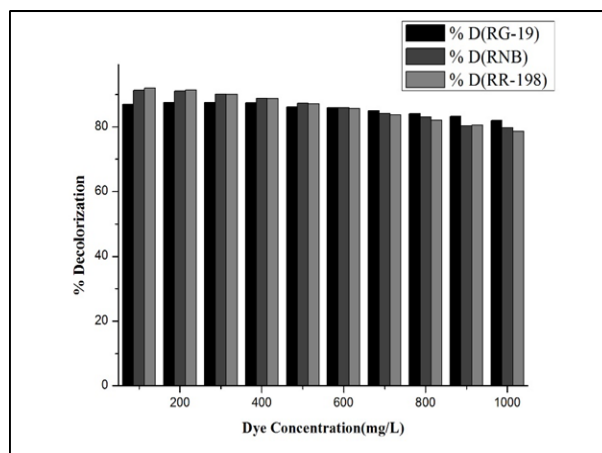


Fig.6 Effect of incubation temperature on dye decolorization efficiency of M1A (at 100 mg/L dye concentration, pH 7.2)

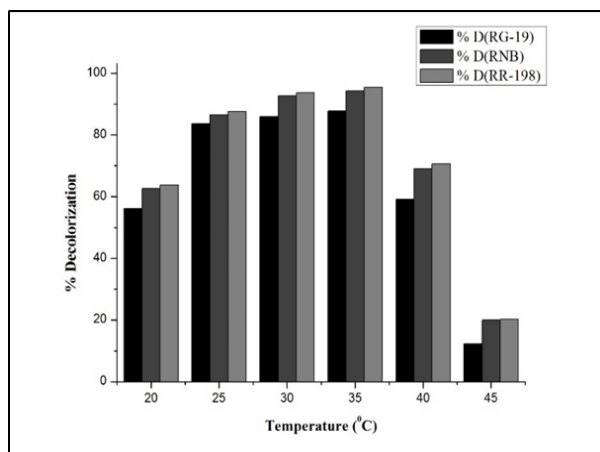


Fig.7 Effect of pH on dye decolorization efficiency of M1A (at 100 mg/L dye concentration, 32 °C)

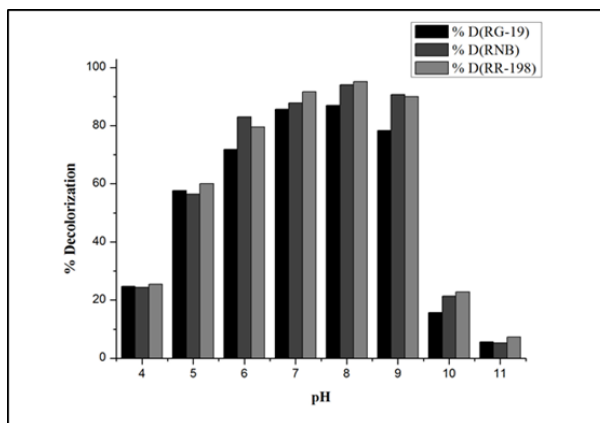
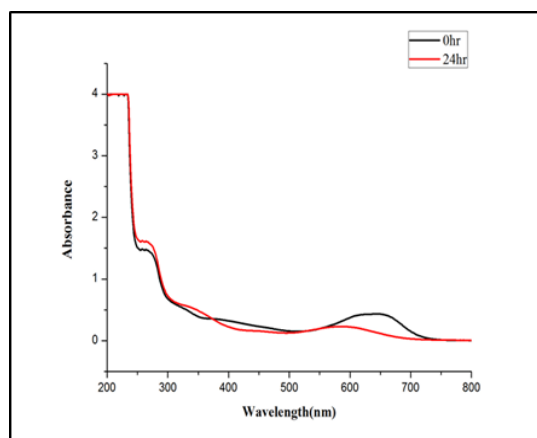
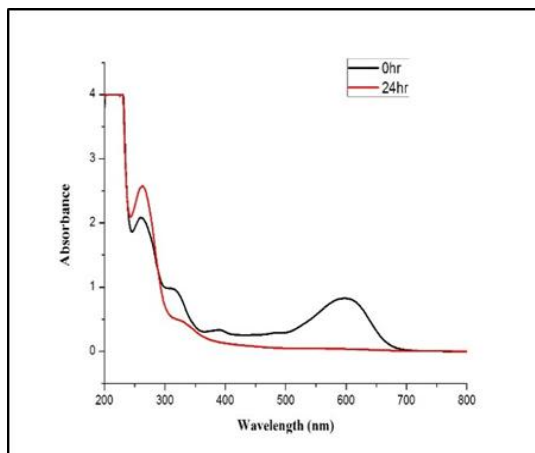


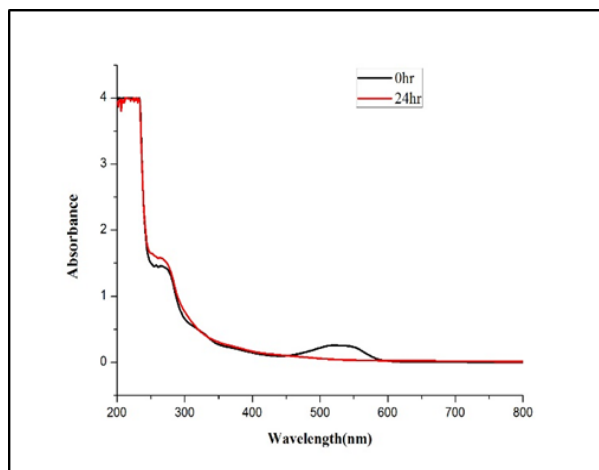
Fig.8 UV-Vis spectral scan of (a) RG-19, (b) RNB and (c) RR-198 before and after decolorization using M1A strain



(a) RG-19



(b) RNB



(c) RR-198

Sahasrabudhe *et al.*, (2014) reported that to obtain maximum decolorization of C.I. Direct Red 81 using *Enterococcus faecalis* strain YZ 66 the pH range of 5-8 has to be maintained.

The decrease in peak at the respective absorption maxima of all the three dyes suggests the cleavage of -N=N- bond by the anaerobic reductive degradation of the dye chromophore. However, we can observe a difference in UV-Vis spectral pattern between all the three dyes. This could be due to the difference in structure of dyes which follow different degradation pathways

and formation of different intermediate products.

In conclusion, it can be concluded from the above study that, our isolated strain M1A isolated from textile wastewater is highly efficient in decolorization of different textile dyes in a wide concentration range. The organism was able to achieve 82-87% decolorization of RG-19, 80-91% decolorization of RNB and 78-92% decolorization of RR-198 dye within 24hr of incubation at 35 °C under static condition in a wide concentration range of 100 mg/L to 1000 mg/L. Through 16s rDNA molecular

characterization techniques M1A was identified as *Enterococcus durans* GM13 (GenBank Accession Number: KC213474.1). Effect of different physico-chemical parameters on dye decolorization efficiency of M1A has been studied. The organism was found to be mesophilic in nature, showing its maximum decolorization activity at 35°C temperature. Neutral to slightly alkaline pH was found to be favourable for achieving maximum decolorization potential of M1A strain. 5% inoculum volume and static incubation condition was found to be effective for maximum decolorization of all the three tested dyes.

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