The Pharma Innovation Journal 2018; 7(2): 175-182

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2018; 7(2): 175-182

www.thepharmajournal.com
Received: 23-12-2017
Accepted: 24-01-2018

Anamika Ghatak
Department of Microbiology, Taradevi Harakhchand Kankaria Jain College 6, Ram Gopal Ghosh Road, Cossipore, Kolkata, West Bengal, India

Suchismita Das
Department of Microbiology, Taradevi Harakhchand Kankaria Jain College 6, Ram Gopal Ghosh Road, Cossipore, Kolkata, West Bengal, India

Correspondence
Suchismita Das
Department of Microbiology, Taradevi Harakhchand Kankaria Jain College 6, Ram Gopal Ghosh Road, Cossipore, Kolkata, West Bengal, India

Characterization of bacterial strains isolated from natural sources involved in decolourization of malachite green dye

Anamika Ghatak and Suchismita Das

Abstract
The wastes generated from the industrial dyes confer serious hazard to soil and water bodies. Various physicochemical methods have been employed for their elimination; however, owing to the less efficiency and their expensive nature, interest has been generated for searching microbial agents capable of decolorizing these dyes. In this study three bacterial strains were isolated from industrial effluent contaminated Ganges water and nearby locality having high effectiveness to decolorize Malachite green (MG), a triphenylmethane dye widely used in industries. The bacterial isolates were partially characterized as gram negative, Indole and MR negative, VP and citrate positive. The optimal MG decolourization conditions were when 50 ppm (mg/lt) dye was added in medium of pH 8.0 at 37 ºC. The strains decolorized MG in the range of 74 - 82% within just 4 hours and almost decolorized the dye (91-96%) within 30 hours of addition. The decolorization of other dyes such as congo red, nigrosine, crystal violet and safranine was further exploited in these strains. The results provide evidence that the strains are potential candidates that can be exploited for industrial waste-water treatment.

Keywords: Malachite green, Congo red, Dye decolourization, Ganges water

Introduction
In modern life, growth in industrialization and urbanization has resulted in discharge of large amount of toxic wastes into the environment. The wastes generated from the textile, leather, paper, cosmetics, food and plastic industries often contain synthetic dyes which are complex combinations of chlorinated compounds, pigments, other dyestuffs and inorganic compounds. The wastes when enter the water and soil bodies, contribute to environmental toxicity and carcinogenicity [1], thereby creating detrimental effects for both land and aquatic ecosystems [2]. They alter the pH, increases BOD (biological oxygen demand), block the penetration of light in water bodies and affect the photosynthesis of aquatic plants [3]. Major class of synthetic dyestuffs include triphenylmethane, azo, phthalocyanine and anthroquinone dyes. Dyes are aromatic, water-soluble, dispersible organic colorants and are resistant towards degradation [4, 5]. Around 100,000 industrially used dyes are known and on an average one million tons of dyes are produced, throughout the year, where 10-15% of total used dyes are released in the environment as waste [6-8].

Malachite green, a triphenylmethane dye ($C_{23}H_{25}ClN_2$) is an important group of basic dyes, also known as Basic green 4. MG is extensively used in textile industry, as food additive, fungicide and fish farming industry due to its relatively low cost, ready availability and high efficacy against fish microbial pathogens [9]. Malachite green and its reduced form leucomalachite green may persist in edible fish tissues for extended periods of time [10]. MG is known to cause irritation in the gastrointestinal tract of humans and when contacted with skin, it creates redness and pain. Accordingly MG is highly toxic to both aquatic and terrestrial ecosystems, causing severe environmental and health hazards. It has been banned by the US Food and Drug Administration in food related use and is listed as a carcinogenic compound [11].

Removal of dyes from industrial effluents has been studied profoundly by researchers. The synthetic dyes have shown resistance towards several conventional techniques such as ozonation, photooxidation, adsorption, activated carbon, froth floatation, reverse osmosis etc. [12]. These methods are less efficient, costly and produce secondary pollutants which are very difficult to dispose [13]. Industrial effluents produced are markedly variable in chemical composition having low BOD/COD ratios [14] which affects the efficacy in removal.

It has been reported that microorganisms can play a very significant role in decomposition and ultimate mineralization of these dyes [15, 16]. Environmental biotechnology is based on the ability of microorganism (both bacteria and fungi) to decompose larger chemical compounds which are xenobiotics [16-18]. Moreover, such decolorization and degradation ability of microbes serves as an environmental friendly and cost-effective alternative over chemical decomposition process [19, 20]. Substantial efforts are hence required for bioengineering the intrinsic capabilities of microorganisms to clean up the environment with more effectiveness by replacing the conventional remediation methods [21, 22].

The present study describes the microbial decolorization of industrially used MG and other dyes like congo red, nigrosine, crystal violet, safranine etc. by three bacterial strains isolated from Ganges water and the nearby contaminated soil in an industrial locale, Cossipore, Kolkata. The objective of the study was to analyse different physicochemical parameters for optimal decolorization of the reactive dyes.

2. Materials and Methods

2.1 Dyes, chemicals and microbiological media

All the dyes (malachite green, congo red, crystal violet, nigrosine and safranine) and chemicals used were of highest purity and analytical grade. Reagents and media components were procured from E-Merck and Himedia respectively.

2.2 Sample collection and isolation of bacterial strains

Samples of Ganges water and soil from nearby locality were collected from an industrial neighbourhood, Cossipore, Kolkata. The sample collection was done three independent times and experiment for isolation of potential strains was performed in triplicates. The samples were serially diluted and were plated on nutrient agar medium containing 25 parts per million (ppm or mg/lt) MG. Of the several isolates, three potential bacterial strains capable of decolorizing MG were isolated; one from Ganges water (named G5) and two from surrounding soil (named R1 and R5). The isolated bacterial strains were maintained on nutrient agar slants and stored in refrigerator at 4^0C.

2.3 Morphological and biochemical characterization of isolated strains

The three isolated bacterial strains were characterized by routine bacteriological methods i.e. colony morphology, growth on selective media (EMB and McConkey agar), gram character, cell arrangement, growth at different pH, temperature, and salt percentages. They were further characterized by performing biochemical tests like IMViC, fermentation of different carbohydrates, urea hydrolysis, starch hydrolysis etc.

2.4 MG Decolourization assay

Malachite green decolourization was monitored by the decrease in absorbance at 619 nm, the λ_{max} of MG. Inoculums of R1, R5 and G5 were given independently in 50 ml nutrient broth at pH 7.0 and were grown overnight at 37^0C. The following day, the MG dye was added in each culture at concentration of 50 ppm and were further incubated at 37^0C under static condition. At different time intervals, 2 ml cultures were collected and cells were removed by centrifugation and the clear supernatant was taken for absorbance measurement at 619 nm using UV-Vis spectrophotometer. For all the assays, a control set was maintained in which 50 ppm dye was added in nutrient broth but not inoculated with bacteria (uninoculated control). The percentage of decolourization of effluent was determined using the following formula:

$$\text{Percentage of decolourization} = \frac{[(\text{Initial absorbance} - \text{Final absorbance})]}{\text{Initial absorbance}} \times 100$$

Initial absorbance is the OD value just after addition of dye and final absorbance is the OD value at the respective time-points. All the assays were repeated at least three independent times and the data in the graph is represented as Mean ± SE.

2.5 Standardization of optimum parameters of MG decolorization

a. Determination of appropriate concentration of MG

To optimize the appropriate concentration of MG decolourization by the three bacterial strains, the dye was added at different concentrations of 12.5 ppm, 25 ppm, 50 ppm, 100 ppm and 200 ppm in 50 ml overnight bacterial cultures of R1, R5 and G5 respectively. The control sets were addition of different concentrations of dye (as above) in uninoculated nutrient broth. The culture flasks were kept under static condition at 37^0C and 2 ml cultures were collected for absorbance measurement at different time-points after addition of dye.

b. Assay of MG at different time points

The MG decolourization assay was performed for maximum of 30 hrs under static condition at 37^0C after addition of 50 ppm dye in the 50 ml overnight bacterial cultures of R1, R5 and G5. After addition of dye, samples for absorbance measurement were collected at seven different time-points of 2 hours (hrs), 4 hrs, 8 hrs, 14 hrs, 18 hrs, 24 hrs and 30 hrs. The control set was prepared in the same manner as mentioned earlier.

c. Determination of optimum temperature

To determine the optimum temperature of MG decolourization, the three bacterial strains R1, R5 and G5 were grown overnight at various temperatures of 14 ºC, 20 ºC, 28 ºC, 37 ºC and 45 ºC respectively. Growth at 14 ºC and 45 ºC were poor and best growth was found to be at 37 ºC. After addition of 50 ppm MG dye in the overnight grown cultures, they were further incubated at the same temperatures as above under static condition. For absorbance measurement, 2 ml cultures were taken at different time-points after addition of dye. The control sets were same as discussed before and was grown at similar temperatures as the experimental sets.

d. Determination of optimum pH

To determine the optimum pH of MG decolourization by the three bacterial strains, 50 ppm MG dye was added to the overnight grown bacterial cultures grown in nutrient broth at 37 ºC at different pH of 6, 7, 8, 9 and 10 respectively. Growth of the bacterial strains was less at pH 6 and 10 and best growth was at pH 7 and 8 respectively. After addition of dye, the flasks were kept under static condition and 2 ml cultures were collected at different time-points for absorbance measurement. The control sets were as referred earlier and was grown at different pH of 6-10.

e. Assay at different N sources

The three bacterial strains were grown for overnight in nutrient broths containing different nitrogen sources of peptone, tryptone, urea and gelatin respectively. The following day, 50 ppm MG dye was added in the bacterial cultures and the flasks were kept under static condition at 37 ^{o}C. Samples for absorbance measurement were collected at different time-points after addition of MG dye. The control sets were prepared as discussed earlier in different N sources.

2.6 Decolourization assay using different dyes

The three bacterial strains R1, R5 and G5 were further assessed for their effectiveness in degrading other reactive dyes like congo red (λ_{max} 495 nm), crystal violet (λ_{max} 586 nm), nigrosine (λ_{max} 570 nm) and safranine (λ_{max} 515 nm). The assays performed, were exactly similar to MG assays and 50 ppm of the different dyes were added to the overnight grown bacterial cultures of pH 7 at 37 ^{o}C. After addition of the dyes, the flasks were further incubated under static condition and samples for absorbance measurement were collected at different time-points. The control sets were as discussed before using different dyes.

3. Results and discussion

3.1 Sample collection and isolation of bacterial strains

A total of thirty two different bacterial isolates were obtained from Ganges water and the surrounding soil. Among them, three best strains were selected based on their potential to decolourize 25 ppm MG on nutrient agar plates and were named as R1 and R5 (from soil) and G5 (from Ganges water). The MG decolorizing ability of the three strains was further assayed in liquid broth containing different concentrations of dye in the range 12.5-200 ppm.

3.2 Morphological and biochemical characterization of isolated strains

The three isolated microorganisms (R1, R5 and G5) were partially identified by conventional microbiological and biochemical techniques (Table 1). Colony and growth characteristics of the isolates were observed on nutrient agar media, EMB and McConkey agar. The three strains were found to be gram negative short rods and their gram character was further established by their growth on McConkey agar medium. All of them have shown growth between 14 ^{o}C to 45 ^{o}C with optimum growth at around 37 ^{o}C and poor growth at lower and higher temperatures respectively. The strains have shown growth between pH 6-10 with best growth at around pH 7- 8. Different biochemical tests such as Indole production, citrate utilization, carbohydrate utilization (lactose, dextrose, sucrose, fructose, maltose, mannitol, and starch), urease activity, etc. were performed with the strains. The three strains may be non-faecal as they all gave negative Indole and MR tests and positive VP and citrate tests. Among the three strains, only R1 did not show lactose fermentation and it is only G5 who gave positive urease test and is being capable of hydrolyzing urea.

3.3 Standardization of optimum parameters of MG Decolourization

The ability to decolorize MG was assayed at variable concentrations of dye from 12.5 to 200 ppm (Figure 1A). After addition of dye, samples were collected at 2 hrs, 4 hrs and at 24 hrs. The percentages of decolorization at these three time-points are shown in Table 2A, and Figure 1A is the graphical representation of the same of only at 4 hrs. At 4 hrs after addition of dye, the three strains have shown decolourization in the range of 64-69% at 12.5 ppm, 67-72% at 25 ppm, 74-82% at 50 ppm, 68-74% at 100 ppm and 64-69% at 200 ppm respectively (Figure 1A). The strongest decolorization ability was shown by R5 strain and the lowest was by G5 at every different concentrations of MG. The maximum MG decolorization was shown at 50 ppm and interestingly the dye was almost decolorized (> 90%) within 24 hrs of addition (94% for R1, 95% for R5, 89% for G5; Table 2A). At higher concentrations of 100 ppm and 200 ppm (Figure 1A) significant amount of decolorization indicates the high tolerance level of the bacterial strains. However the reduction in the efficiency of decolourization might be due to the toxic effect of the dye on the metabolic activities of the cells as has been shown in earlier studies [23].

A time-course study on decolourization of MG was done for the three strains and the result is shown in Figure 1B. The dye amount added was 50 ppm as all the three strains have shown optimum dye decolourization at this concentration (Table 2A, Figure 1A). Even after 2 hrs of addition of the dye, the % of decolourization was as high as 70% for R1, 72% for R5 and 67% for G5. At 4 hrs after addition of dye, 75% decolorization was shown by R1, 82% by R5 and 74% by G5. After 24 hrs of addition, the increase in decolourization was 19% for R1, 14% for R5 and 15% for G5, as compared to 4 hrs of addition. As the MG dye was almost decolorized by the three bacterial strains within 30 hrs of addition of dye (91% to 96%), the assay was not continued further. It is interesting to note, that the maximum decolourization of MG was made at very early hours (within 2 - 4 hrs) and attained saturation by 18 hrs, with not much further increase in the later hours.

To determine the optimum temperature and pH of MG decolorization, the assay was performed at different temperatures and pH. The dye amount added was 50 ppm and samples were collected at different time-points after addition of the dye. The values of percentage of decolorization at all time-points are shown in Table 2B (for temperature) and Table 2C (for pH), and the graphical presentation shown in Figures 1C (of temperature) and 1D (of pH) are of values at 4 hrs time-point. The optimum temperature was found to be 37 ^{o}C for all the three strains, although their decolourization ability was moderate even at 28 ^{o}C (~ 41 - 49%). The reduced decolourization ability at very lower or higher temperatures might be due to deactivation or denaturation of the enzymes which are involved in decolourization as discussed in earlier studies [24]. The optimum pH for decolourization was found to be at pH 8; however the decolorization percentages at pH 7 and pH 9 were extremely close to values at pH 8. It is to be noted, that all earlier assays on dye decolourization with different amount of MG and at different time-points were done at 37 ^{o}C in nutrient broth at pH 7.

The MG decolourization capabilities of the three strains were further explored in nutrient broth containing various N sources like urea, tryptone and gelatin [25, 26] other than the standard peptone containing nutrient broth. The amount of MG added was 50 ppm and measurements for absorbance were done at different time-points after addition of the dye. The percentages of decolorization for all time-point are presented in Table 3A and the values at 4 hrs have been presented graphically for comparison (Figure 2). The maximum MG decolourization for R1 and R5 strains was when urea was used as the N source (83% and 90% respectively) and for G5 when tryptone was used as the N source (79%). Decolourization by the bacterial strains in urea

or tryptone medium was quite significant and comparable to that of standard peptone containing nutrient broth. However in gelatin containing medium, the % of decolourization was in the range of 27-36%, which was much less as compared to other N sources. The enhancement of MG decolorization in presence of urea by R1 and R5 is similar to earlier observations [26], where it was shown that decolorization of several textile dyes were significantly increased in presence of urea. An interesting fact is that both R1 and R5 lacking urease activity (Table 1) shows higher MG decolorization capability (83 and 90%) than G5 (66%), a urease positive strain (Table 1). G5 is capable of hydrolysing urea and shows reduced efficiency in decolorizing MG. This explains that urea might be playing some role in facilitating the decolorization of the MG dye.

Table 1: Morphological and Biochemical Characterization of R1, R5 and G5 strains

Characteristics/Test	R1	R5	G5
Colony Characteristics	Small round, entire edge, convex upper surface	Entire edge, convex upper surface, off- white	Entire edge, small, convex upper surface, off-white
Gram character	Gram negative	Gram negative	Gram negative
Cell shape and arrangement	Very small rods, isolated and some in clusters	Very small rods, isolated and some in clusters	Very small rods, isolated and some in clusters
Growth at different temperature (14 - 45^{0}C)	Optimum growth at 37 ^{o}C	Optimum growth at 37 ^{o}C	Optimum growth at 37^{0}C
Growth at different pH (pH 6.0 – 10.0)	Optimum growth at around pH 7-8	Optimum growth at around pH 7-8	Optimum growth at around pH 7-8
Growth at different salt concentration (3-7%)	+	+	+
Growth on EMB agar	+, no metallic sheen	+, no metallic sheen	+, no metallic sheen
Growth on McConkey agar	+	+	+
Lactose fermentation	-	+	+
Dextrose fermentation	+	+	+
Sucrose fermentation	+/-	+	+
Fructose fermentation	+	+	+
Maltose fermentation	+	+	+
Mannitol fermentation	+	+	+
Indole production	-	-	-
Methyl red	-	-	-
Voges -Proskauer	+	+	+
Citrate Utilization	+	+	+
Hydrolysis of Starch	-	-	-
Hydrolysis of Urea	-	-	+

Table 2

Table 2A: Determination of appropriate concentration of MG

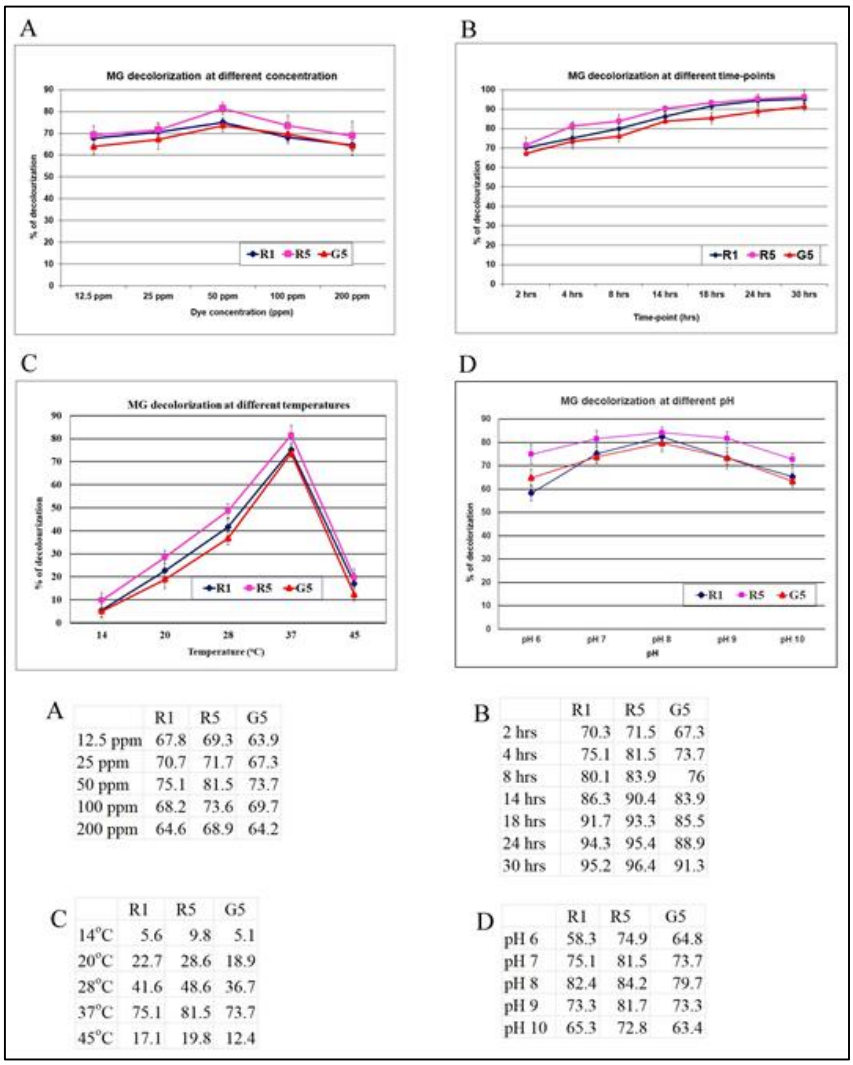
R1 strain				R5 strain				G5 strain			
Dye conc.	**2 hrs**	**4 hrs**	**24 hrs**	**Dye conc.**	**2 hrs**	**4 hrs**	**24 hrs**	**Dye conc.**	**2 hrs**	**4 hrs**	**24 hrs**
12.5 ppm	63.8	67.82	91.4	12.5 ppm	63.11	69.29	87.6	12.5 ppm	59.4	63.9	77.7
25 ppm	65.3	70.7	92.4	25 ppm	67.6	71.7	89.8	25 ppm	63.4	67.3	83.9
50 ppm	70.3	75.1	94.3	50 ppm	71.5	81.5	95.3	50 ppm	67.3	73.7	88.9
100 ppm	63.2	68.2	79.6	100 ppm	64.5	73.6	85.9	100 ppm	64.8	69.7	79.3
200 ppm	57.9	64.6	68.2	200 ppm	60.4	68.9	81.1	200 ppm	55.6	64.2	72.7

Table 2B: Determination of optimum temperature

R1 strain				R5 strain				G5 strain			
Temp (^{o}C)	**2 hrs**	**4 hrs**	**24 hrs**	**Temp (^{o}C)**	**2 hrs**	**4 hrs**	**24 hrs**	**Temp (^{o}C)**	**2 hrs**	**4 hrs**	**24 hrs**
14	5.2	5.6	11.6	14	7.8	9.8	16.7	14	4.6	5.1	10.11
20	15.6	22.7	32.6	20	21.6	28.6	38.7	20	12.7	18.9	26.7
28	25.8	41.6	49.2	28	36.7	48.6	58.7	28	24.7	36.7	43.7
37	70.3	75.1	94.3	37	71.5	81.5	95.3	37	67.3	73.7	88.9
45	13.6	17.1	28.9	45	14.6	19.8	33.7	45	8.8	12.4	22.8

Table 2C: Determination of optimum pH

R1 strain				R5 strain				G5 strain			
pH	**2 hrs**	**4 hrs**	**24 hrs**	**pH**	**2 hrs**	**4 hrs**	**24 hrs**	**pH**	**2 hrs**	**4 hrs**	**24 hrs**
pH 6	49.4	58.3	79.9	pH 6	57.2	74.9	83.5	pH 6	54.4	64.8	74.7
pH 7	70.3	75.1	94.3	pH 7	71.5	81.5	95.3	pH 7	67.3	73.7	88.9
pH 8	74.7	82.4	96.7	pH 8	77.6	84.2	97.9	pH 8	71.7	79.7	89.9
pH 9	70.8	73.3	83.8	pH 9	72.7	81.7	91.5	pH 9	74.9	73.3	74.8
pH 10	64.3	65.3	73.1	pH 10	63.9	72.8	81.8	pH 10	68.8	63.4	69.7



A

	R1	R5	G5
12.5 ppm	67.8	69.3	63.9
25 ppm	70.7	71.7	67.3
50 ppm	75.1	81.5	73.7
100 ppm	68.2	73.6	69.7
200 ppm	64.6	68.9	64.2

B

	R1	R5	G5
2 hrs	70.3	71.5	67.3
4 hrs	75.1	81.5	73.7
8 hrs	80.1	83.9	76
14 hrs	86.3	90.4	83.9
18 hrs	91.7	93.3	85.5
24 hrs	94.3	95.4	88.9
30 hrs	95.2	96.4	91.3

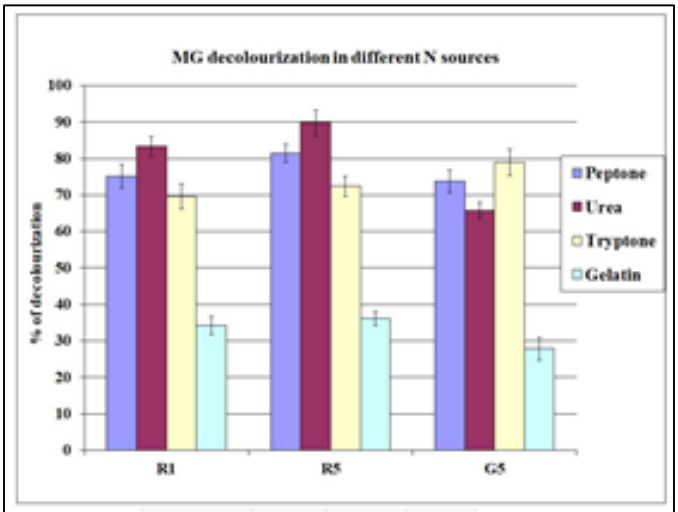
C

	R1	R5	G5
14°C	5.6	9.8	5.1
20°C	22.7	28.6	18.9
28°C	41.6	48.6	36.7
37°C	75.1	81.5	73.7
45°C	17.1	19.8	12.4

D

	R1	R5	G5
pH 6	58.3	74.9	64.8
pH 7	75.1	81.5	73.7
pH 8	82.4	84.2	79.7
pH 9	73.3	81.7	73.3
pH 10	65.3	72.8	63.4

Fig 1: Decolorization of Malachite green by R1, R5 and G5 strains. (A) at different concentrations of MG dye from 12.5 to 200 ppm, (B) at different time points from 2 hrs to 30 hrs, (C) at different temperatures from 14 ºC to 45 ºC and (D) at different pH from 6 to 10. The data is representation of three independent experiments and expressed as Mean ±SE. The graphs and their corresponding percentages of decolorization values of A, C and D, are at 4 hrs time-point after addition of MG dye.



	R1	R5	G5
Peptone	75.1	81.5	73.7
Urea	83.4	89.8	65.7
Tryptone	69.6	72.4	78.9
Gelatin	34.2	36.1	27.8

Fig 2: Decolorization of Malachite green by R1, R5 and G5 strains in different N sources of peptone, urea, tryptone and gelatin (at 4 hrs time-point after addition of MG dye). The data is representation of three independent experiments and expressed as Mean ±SE.

3.4 Decolourization of different dyes

The three bacterial strains were further assessed for their abilities to decolourize other reactive dyes, which are commonly used in industries [27-29]. Most of these dyes are recalcitrant and act as potent carcinogen and mutagen. Four such dyes assayed were congo red, crystal violet, nigrosine and safranine and the concentration of various dyes added was 50 ppm each; which was decided based on the optimum amount of MG decolorization by the three strains (Figure 1A). Table 3B shows the percentages of decolourization at all time-points and Figure 3 represents graphically the values at 4 hrs. Among different dyes, the MG has been decolorized the maximum and R5 strain being the most efficient. The safranine dye was decolorized in the range of 54-67% by the different strains with maximum being by R5 strain. The crystal violet was decolorized within the range of 58-67% and maximum by the R1 strain. The % of decolourization of Congo red was 42% by R1, 44% by R5 and 31% by G5, which was lower than other dyes. Nigrosine was found to be minimally decolourized by the three strains (26% by R1, 40% by R5 and 16% by G5 strain).

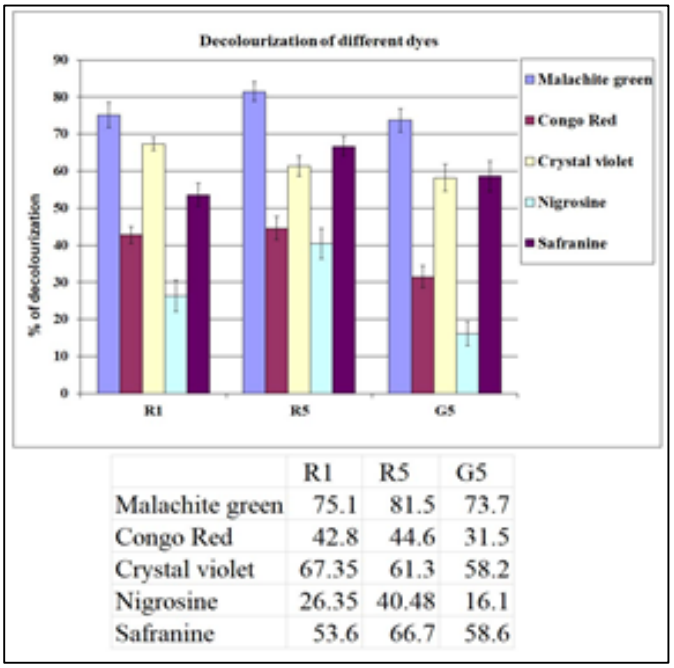
Table 3

Table 3A: MG decolorization assay in different N sources

R1 strain				R5 strain				G5 strain			
Nitrogen sources	**2 hrs**	**4 hrs**	**24 hrs**	**Nitrogen sources**	**2 hrs**	**4 hrs**	**24 hrs**	**Nitrogen sources**	**2 hrs**	**4 hrs**	**24 hrs**
Peptone	70.3	75.1	94.3	Peptone	71.5	81.5	95.3	Peptone	67.3	73.7	88.9
Urea	72.3	83.4	93.1	Urea	75.6	89.8	93.5	Urea	56.7	65.7	44.2
Tryptone	60.2	69.6	88.2	Tryptone	63.6	72.4	90.3	Tryptone	73.2	78.9	94.5
Gelatin	25.6	34.2	43.1	Gelatin	28.2	36.1	47.7	Gelatin	22.4	27.8	36.6

Table 3B: Decolorization assay of different dyes

R1 strain				R5 strain				G5 strain			
Dyes	**2 hrs**	**4 hrs**	**24 hrs**	**Dyes**	**2 hrs**	**4 hrs**	**24 hrs**	**Dyes**	**2 hrs**	**4 hrs**	**24 hrs**
Malachite green	70.3	75.1	94.3	Malachite green	71.5	81.5	95.3	Malachite green	67.3	73.7	88.9
Congo Red	33.33	42.8	47.8	Congo Red	46.73	44.6	47.9	Congo Red	23.62	31.5	36.2
Crytal violet	64.17	67.35	73.6	Crytal violet	56.8	61.3	66.8	Crytal violet	43.18	58.2	66.7
Nigrosine	24.8	26.35	31.3	Nigrosine	36.45	40.48	43.8	Nigrosine	14.57	16.1	20.7
Safranine	34.9	53.6	67.8	Safranine	45.6	66.7	77.9	Safranine	45.2	58.6	67.9



	R1	R5	G5
Malachite green	75.1	81.5	73.7
Congo Red	42.8	44.6	31.5
Crystal violet	67.35	61.3	58.2
Nigrosine	26.35	40.48	16.1
Safranine	53.6	66.7	58.6

Fig 3: Decolorization by R1, R5 and G5 strains of different dyes like malachite green, congo red, crystal violet, nigrosine and safranine (at 4 hrs time-point after addition of the dyes). The data is representation of three independent experiments and expressed as Mean ±SE.

Although MG is not approved by the U.S. Food and Drug Administration, its worldwide use will probably continue due to its relatively low cost, ready availability and efficacy. During decolorization, the dye MG is enzymatically converted to leucomalachite green [30] and different enzymes like laccase, azoreductase, hydroxylases, lignin peroxidase, triphenyl methane reductase etc. [31] are involved in such conversions. Among these, the enzyme laccase is a multicopper phenol oxidase that decolorizes such dyes through a non-specific free radical mechanism which forms phenolic compounds [32]. Guaiacol is used as an *in vitro* substrate for enzymatic assays of laccase [33]. Surprisingly we have found that there was only ~35-41% MG decolorization; when the same experiments of dye decolorization were done in nutrient broth containing 2 mM guaiacol compared to 70-80% decolourization in absence of guaiacol (data not shown). This suggests that one of the possible enzymes involved in decolourization might be laccase as when both MG and guaiacol are present, there might be some competition and less of MG dye gets degraded.

Future scope of the study would be identification of the isolated strains at molecular level and to investigate the pathway and the involvement of various enzymes in decolorization.

4. Conclusion

Biotreatment offers simple, economical and efficient alternatives for efficient removal of dye. The bacterial strains isolated from natural sources, can be used as a good source for waste water treatment. The three isolated strains have shown maximum MG decolourization capability at 50 ppm dye and 73-81% removal of colour was completed within 4 hrs of addition of dye under static condition. The optimum temperature and pH for decolourization of MG was found to be 37 ^{o}C and at pH 8 respectively. Medium containing different N sources like tryptone and urea has favoured MG decolorization whereas presence of gelatin has shown lower decolourizing ability. The strains decolorized other reactive dyes like crystal violet (58-67%) and safranine to a significant extent (53-67%) and nigrosine and congo red dyes to a lesser extent. From this work it can be concluded that the three

isolated strains are capable in decolorizing MG and other reactive dyes and further characterization of the pathway involved in decolorization will be supportive for them to be used for bioremediation of industrial effluents.

5. Acknowledgments
We are thankful to Shree S.S. Jain Sabha for providing us financial support and to Sri L. Kankaria (Secretary), Prof. O.P. Singh (Rector) and Dr. M. S. Sengupta (Principal) for their full cooperation. We are also grateful to Shri T.N. Seth and Sri A. Mahato for their technical assistance.

6. References

1. Kaur A, Vats S, Rekhi S, Bhardwaj A, Goel J, Tanwar S *et al*. Physico-chemical analysis of industrial effluents and their impact on the soil microflora. Procedia Environmental Sciences. 2010; 2:595-599.
2. Ahmed TF, Sushil M, Krishna M. Impact of dye industrial effluents on physicochemical characteristics of Kshipra river, Ujjain City, India. International Research Journal of Environmental Sciences. 2012; 1:41-45.
3. Puvanesswari N, Muthukrishnan J, Gunasekaran P. Toxicity assessment and microbial degradation of azo dyes. Indian J Exp Biol. 2006; 44:618-626.
4. Chawla A, Saharan BJ. Novel *Castellaniella denitrificans* SA13P as a Potent Malachite Green Decolorizing Strain. Applied and Environmental Soil Science. 2014; 2014:1-7.
5. Aksu Z, Tezer S, Biosorption of reactive dyes on the green alga *Chlorella vulgaris*. Process Biochemistry. 2005; 40:1347-1361.
6. Maguire RJ. Occurrence and persistence of dyes in a Canadian river. Water Science Technology. 1992; 5:265-270.
7. Kim SJ, Soda M. Batch decolorization of molasses by suspended and immobilized fungus of *Geotrichum candidum*. Journal of Bioscience and Bioengeneering. 1999; 88:586-589.
8. Mohan SV, Rao CN, Prasad KK, Karthikeyan J. Treatment of simulated Reactive Yellow 22 (azo) dye effluents using *Spirigyra* species. Waste Management. 2002; 22:575-582.
9. Culp SJ, Beland FA. Malachite green: a toxicological review, Journal of the American College of Toxicology. 1996; 15:219-238.
10. Henderson AL, Schmitt TC, Heinze TM, Cerniglia CE. Reduction of malachite green to leucomalachite green by intestinal bacteria. Applied and Environmental Microbiology. 1997; 63:4099-4101.
11. Srivastava S, Sinha R, Roy D. Toxicological effects of malachite green. Aquatic Toxicology. 2004; 66:319-329.
12. Jadhav JP, Parshetti GK, Kalme SD, Govindwar SP. Decolourization of azo dye methyl red by *Saccharomyces cerevisiae* MTCC 463. Chemosphere. 2007; 68:394-400.
13. Forgacs E, Cserhati T, Oros G. Removal of synthetic dyes from wastewaters; A review. Environmental International. 2004; 30:953-971.
14. Bisschops IAE, Spanjers H. Literature review on textile wastewater characterization. Environmental technology. 2003; 24:1399-1411.
15. Daneshvar N, Khataee AR, Rasoulifard H, Pourhassan M. Biodegradation of dye solution containing Malachite green: optimization of effective parameters using Taguchi method. Journal of Hazardous materials. 2007; 143:769-776.
16. Chen CH, Chang CF, Liu SM. Partial degradation mechanisms of malachite green and methyl violet B by *Shewanellade colorationis* NTOU1 under anaerobic conditions. Journal of Hazardous Materials. 2010; 177:281-289.
17. Fu Y, Viraraghavan T. Fungal decolorization of dye wastewaters: a review. Bioresource Technology. 2001; 79:251-262.
18. Junghanns C, Krauss G, Sclosser D, Potential of aquatic fungi derived from diverse freshwater environments to decolourize synthetic azo and anthraquinone dyes. Bioresource Technology. 2008; 99:1225-1235.
19. Jain RK, Kapur M, Labana S, Sarma PM, Lal B, Bhattacharya D *et al*. Microbial diversity: application of microorganisms for the biodegradation of xenobiotics. Current Science. 2005; 89:101-112.
20. Mali PL, Mahajan MM, Patil DP, Kulkarni MV. Biodecolourization of members of triphenylmethane and azo group of dyes. Journal of Scientific and Industrial Research. 2000; 59:221-224.
21. Jang MS, Lee YM, Kim CH, Lee JH, Kang DW, Kim SJ *et al*. Triphenyl methane reductase from *Citrobacter sp*. Strain KCTC18061P: purification, characterization, gene cloning and overexpression of a functional protein in *Escherichia coli*. Applied and Environmental Microbiology. 2005; 17:7955-7960.
22. Li-LT, Hong Q, Yan X, Fang GH, Ali SW, Li SP. Isolation of a malachite green degrading *Pseudomonas* sp. MDB-1 strain and cloning of the tmr2 gene. Biodegradation. 2009; 20:769-776.
23. Murugesan K, Arulmani M, Nam IH, Kim YM, Chang YS, Kalaichelvan PT. Purification and characterization of laccase produced by a white rot fungus *Pleurotos sajor-caju* under submerged culture condition and its potential in decolorization of azo dyes. Appl Microbiol Biotechnol. 2006; 72:939-946.
24. Wanyonyi WC, Onyari JM, Shiundu PM, Mulaa FJ. Enzymatic decolorization of Malachite green dye by a newly isolated *Bacillus cereus* strain wwcp1. IOSR Journal of Environmental Science, Toxicology and Food technology. 2014; 8:58-64.
25. Santhi R, Nalina Sundari MS. Optimization of Malachite green decolourization by *Pseudomonas aeruginosa* MTCC 424. The Pharma Innovation. 2015; 3:11-15.
26. Mahmood R, Sharif F, Ali S, Umar Hayyat M. Enhancing the decolorizing and degradation ability of bacterial consortium isolated from textile effluent affected area and its application on seed germination. The Scientific World Journal. 2014; 2015:1-9.
27. Fulekar MH, Wadgaonkar SL, Singh A. Decolurization of dye compounds by selected bacterial strains isolated from dyestuff industrial area. International Journal of Advancements in Research & Technology. 2013; 2:182-192.
28. Durve AA, Gupta AR, Naphade SR. Decolourization of textile dyes and biological strains isolated from industrial effluents. Advances in Applied Science Research. 2012; 3:2660-2671.
29. Khan S, Mathur N. Biodegradation of different dye by bacterial strains isolated from textile effluents of Western Rajasthan, India. International Journal of Current Microbiology and Applied Sciences. 2015; 4:994-1001.

30. Rao KV. Inhibition of DNA synthesis in primary rat hepatocyte cultures by malachite green: A new liver tumor promoter. Toxicol Letter. 1995; 81:107-113.
31. Sudha M, Saranya Y, Selvakumar G, Sivakumar N. Microbial degradation of azo dyes: A review. International Journal of Current Microbiology and Applied Sciences. 2014; 3:670-690.
32. Couto SR, Toca Herrera HR. Industrial and biotechnological applications of laccases: A review. Biotechnology Advances. 2006; 24:500-513.
33. Sheikhi F, Ardakani MR, Enayatizamir N, Rodriguez-Couto S. The determination of assay for laccase of *Bacillus subtilis* WPI with two classes of chemical compounds as substrates. Indian J Microbiol. 2012; 52:701-707.