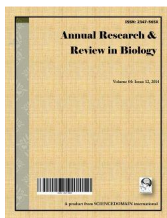

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Isolation and Identification of Orange M2R and Green GS Dye Decolourizing Bacteria from Textile Sludge (Soil) Samples and Determination of Their Optimum Decolourization Conditions

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Authors' contributions

This work was carried out in collaboration between both authors. Author RS designed the study, Author FAA managed the sample collection and laboratory analyses. Authors RS and FAA managed the literature searches, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Objectives: Azo dye accounts for majorly produced synthetic dye substances in industries, posing a threat to all possible life forms. This study was focused to isolate azo dye "Orange M2R" and "Green GS" degrading bacterial strain from textile effluent soil samples and optimization of their optimum physio-chemical growth conditions.

Methodology: To achieve above-mentioned objective, sludge samples were collected from textile industrial area and were applied to 1%, 3% and 5% dye containing SM broth to observe the dye degrading capability of those samples that contain acclimatized bacteria. ABIS microbiology software (Advanced Bacterial Identification Software) was used to justify and determine the identity of these bacteria with the aid of results obtained from the biochemical tests

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that were undertaken.
Results: Bacterial strains identified in this study were *Enterococcus termitis*, *Enterococcus camelliae, Bacillus farraginis, Bacillus muralis, Paenibacillus macerans, Bacillus decolorationis, and Macrococcus brunensis.* Out of these isolates *Enterococcus termitis, Bacillus farraginis, Paenibacillus macerans, Bacillus decolorationis* emerged out to be most potent decolourizer, being selected for further studies. *Bacillus farraginis* was identified as the best decolourizer of OM2R (Orange M2R) dye that decolourized 98% of the dye and *Paenibacillus macerans* showed maximum decolourization on GGS(Green GS) dye that decolourized 97% of the dye. The effect of pH, NaCl, temperature and initial concentration of dye was studied with an aim to determine the optimal conditions required for maximum decolourization. The research showed different decolourization rate with varying parameters. The optimum pH for decolourization of OM2R and GGS dye was 7.0, the optimum NaCl concentration for decolourization was 2%, initial dye concentration was 1% and the temperature was 37°C for optimum decolourization by the selected isolates.
Conclusion: The findings are well acclimatized and have potentials for bioremediation in textile waste effluent treatment plants.

Keywords: Azo dye; bioremediation; optimum growth; decolourization; OM2R.

1. INTRODUCTION

The textile industry in Bangladesh accounts for 45% of all industrial employment and contributes 5% to the total national income [1]. This industry consumes a large quantity of water for various processes and discharges equally large volumes of wastewaters containing variety of pollutants and colouring matters like azo dyes. Azo dyes are widely known dyestuff used in industries and hence commonly released in the environment [2]. It is estimated that over 280,000 tons of textile dyes are discharged in industrial effluent every year, worldwide. Therefore, pollution from these discharge contaminated with dyestuff is becoming alarming [3]. Textile wastewater is highly coloured which mainly block the penetration of sunlight thereby retarding the growth of aquatic animals and plants; it also contains the dissolved toxic substance and carcinogens [4,5]. Pollution caused by dye effluent is mainly due to durability of the dyes in wastewater [6].

Various physiochemical methods can be used for the removal of azo dyes from the wastewater. Some of these methods are effective but are quite expensive because they generate significant amounts of chemical sludge waste whose disposal in a secure landfill increases process cost. Also, there is disposal problem of such waste material to a proper place that also limits the use of these methods [7,8,9].

Biological treatment is the most economic and eco-friendly process due to least running cost, no hazardous chemicals are required and very low non-toxic sludge are produced. Biological methods involve the use of bacteria, fungi and algae.

The bacterial reduction of the azo dye is usually nonspecific and bacterial decolourization is normally faster. A wide range of aerobic and anaerobic bacteria have been extensively reported as degraders of azo dyes. Some strains of aerobic bacteria use azo dyes as sole source of carbon and nitrogen [10]; others only reduce the azo group by special oxygen-tolerant azo reductases.

The present study was aimed to isolate and identify dye degrading bacterial strain capable of decolorizing Orange M2R and Green GS dye commonly used in textile industries of Bangladesh. In addition, the effects of various physical and nutritional conditions on decolorization of these dyes were also studied. This will eventually lead to the treatment of waste effluents which will be beneficial to Bangladeshi textile industry.

2. MATERIALS AND METHODS

2.1 Sample Collection

Soil samples were aseptically collected from textile effluent disposal area, in Savar (23°51′30″N 90°16′00″E). Sterile plastic containers were used to carry the soil samples. The soil samples named A, B, C and D were stored in sterile plastic bags at 4°C for later use. Two different azo dyes Orange M2R and Green GS were procured from Orient Chemicals, Bangladesh and were of industrial grade. All the other chemicals used were of analytical grade.

2.2 Preparation of Soil Suspension

1 g of soil sample was taken from each sample A, B, C and D and then homogenously suspended individually with 100 ml of 0.85% NaCl solution by vortexing. Isolation of bacteria from soil samples was done through serial dilution method after culturing the isolates in SM broth with Azo dye at 37°c for 24 hours incubation at static condition.

2.3 Media Preparation for Inoculation

The composition of SM broth for 1000 ml is as follows- Glucose 10 g/L, Di potassium Hydrogen Phosphate 0.6 g/L, Peptone 10 g/L, Potassium di Hydrogen Phosphate 1.9 g/L, Magnesium Sulphate 1 g/L, Yeast Extract 1 g/L and then adjust the pH to 6.0 to 6.4. The media contained glucose only at the enrichment stage to isolate single cell colony. After the isolation of single colony, the final media contained all important nutrients and buffers except any carbon source.

2.4 Isolation, Screening of Dye Decolourizing Bacteria

The suspension of each soil (effluent) sample was individually applied to 1% dye containing SM broth media to observe the dye degrading capability of those samples that contain acclimatized bacteria. For each soil sample (A, B, C and D) 2 conical flasks were prepared that contained 200 ml SM broth with glucose and 1% of 1 ml dye. One was kept as a control and another conical flask was inoculated with prepared soil sample suspension. All the conical flasks (control and inoculated) were incubated at 37°C at static condition.

After 5 days each conical flask was compared with the control and decolourization was observed. As each of the sample (A, B, C and D) contained mixture of different types of microorganism in high concentration so dilution technique up to 10^{-4} and 10^{-5} were performed and then plated on nutrient agar plates through spread plate technique and incubated for 24 hours at 37°C to obtain single colony of desired isolates. Twenty different bacterial colonies based on their morphological characteristics were selected. The stock cultures of screened bacterial isolates were maintained routinely on the nutrient agar medium and stored at 4°C.

Screening was done to find out the efficient bacterial strains capable of decolorizing the dye OM2R and GGS. Total 20 primarily screened isolates were enriched in nutrient broth media individually for 24 hours at 37°c then inoculated (always added ratio was 200 µl of bacterial inoculums in 50 ml of dye containing media) in 1% dye (both OM2R and GGS) containing SM broth media without glucose to evaluate individual Azo dye decolourization ability by analyzing the OD (absorbance at 590 nm for OM2R and at 510 nm for GGS) of each sample. Then from the initial four OM2R dye decolourizing bacteria and four GGS dye degrading bacteria best two for each (AO & CO for OM2R dye and BG and CG for GGS dye) were chosen for further analysis.

2.5 Biochemical Characteristics of the Isolates

Biochemical tests were done to detect and confirm the presence of microorganisms after the observation of the single isolated colonies. Several biochemical tests were done among them were Gram staining, Starch hydrolysis, TSI test, Simmon's citrate test, Oxidase test, Catalase test, Nitrate reduction, Nitrate Broth media, MRVP, Indole test, Urease, Acid production from carbohydrate and growth at 6.5% NaCl solution. Each of these experiments were repeated twice.

2.5.1 Catalase test

The hydrogen peroxide used here is broken down to water and oxygen. The test was done in a slide one drop of the 3% hydrogen peroxide was given over the surface of a glass slide. Test organism was taken from the nutrient agar plates and places it on the reagent drop. The presence or the absence of the bubbles or foam was observed. This determines whether the organism was capable of catalase activity [11].

2.5.2 Oxidase test

This test was done for the morphological identification of *Pseudomonas aeruginosa* or *Neisseria species*. It is a filter paper spot test. Two drops of oxidase reagent p-aminodimethylaniline oxalate were added to the surface of growth of test organisms onto the filter paper. The test organisms were picked from nutrient agar plates and smeared in the filter paper. Two drops of oxidase reagent p-aminodimethylaniline oxalate were added to the surface of growth of test organisms onto the filter paper. The colour change from pink to maroon and to purple were observed. Positive test colour

change took place in 10-30 second, negative test no colour change or light pink colour [12].

2.5.3 Nitrate reduction test

Nitrate broth consists of beef extract (3 g/l), peptone (5 g/l), potassium nitrate (5 g/l). Nitrate broth was prepared in which the inoculums from the culture was transferred and incubation done for 24 hrs at 37°C .Secondly five drops of nitrate reagent A was added followed by five drops of nitrate reagent B after the incubation period. Observation of red colour indicated a positive result. The cultures in which no appearance of red colour minute quantity of zinc added. Then the convertion of the red colour was observed. Based on this the organisms capable of nitrate reduction was determined [13,14].

2.5.4 TSI test

The triple sugar iodine agar was prepared and the filled in the test tubes 7 ml per test tube. Test organisms was picked up from the nutrient agar plates by needle and stabbed into the TSI consisting of dextrose, lactose and sucrose butt. The tubes are kept for incubation for about 24 hrs at 35°C. If the organism was capable of fermenting all three sugars then it would produce yellow (acidic) colour in the butt, whereas if the slant and the butt appeared red (alkaline) colour then the organism was a non-fermenter. A Black precipitation in the butt indicated the formation of hydrogen sulphide. If CO_2 was produced there would be crack and bubbles in the medium. The absence of yellow colour in the butt and slant indicated negative result [14].

2.5.5 Gram staining

A smear was prepared by mixing loopful of culture with a drop of saline. It was then left to air dry for some time with some heat fixation. Then the crystal violet was added on the smear and washed with tap water after 60 seconds. Gram's Iodine was also added on the fixed culture and after 60 seconds the solution was poured off and the slide washed with tap water. After that few drops of ethanol was added for decolourization. After 5 seconds it was rinsed off. Safranin was used as a counter stain for 40 to 60 seconds and washed off. The whole slide was air dried and was observed under microscope [14].

2.5.6 MRVP test

The test organism from fresh culture was inoculated into the test tube containing 10 ml MR-VP broth. The broth consist of peptone, dextrose and potassium phosphate. It was kept for inoculation at 37°C for 24 hrs. 5 ml of the inoculum was transferred into another test tube for VP test.

In 5 ml of MR-VP broth containing tube 5 drops of Methyl Red was added. Positive result shows a red ring in the test tube. Negative result gives a yellow colour. The other test tube containing 5 ml of MR-VP broth was used here. The test tube contains 5 ml of the desired bacterial culture. Thirdly 10 drops of Baritts reagent A was added and the culture shaken. Next the Baritts reagent B was added and the culture shaken again. They were kept for 15 minutes to allow them to react with each other. The red colour showed a positive result and yellow or no colour changed was indicated as negative result [14].

2.5.7 Simmon's citrate test

This test indicated the organism capacity to utilize citrate as a carbon source. Simmon citrate agar consists of sodium citrate as the sole source of carbon, ammonium dihydrogen phosphate as the sole source of nitrogen, other nutrients, and the pH indicator bromthymol blue. Firstly the test organism was inoculated into the Simmon's agar slants by the means of streak inoculation. The cultures were incubated for 24 hrs at 37°C. The citrate positive cultures showed blue coloration and the citrate negative cultures showed no growth and medium remain green [14].

2.5.8 Carbohydrate fermentation

The ingredients were tryptiase- 10g/l, NaCl-5 g/l, Phenol red 0.018/l, sugar (Glucose, Lactose and Sucrose) 5 g/l. The experimental organism was inoculated into the phenol red lactose, dextrose and sucrose broth by loop inoculation. In this step shaking of the fermentation tube might force a bubble of air into the inverted gas vial displacing the medium. They were incubated at 37°C for 24 hrs. Based on the colour change of the carbohydrate broth cultures and the presence or absence of gas bubbles the organism capable of fermenting carbohydrate substrate with the production of acid and gas was determined [14,15].

2.5.9 MIU test

In this method the test organism was taken from the fresh subculture by a needle. The needle was

stabbed into the MIU media. This test was done for motility, indole and urease test. After incubation of 24hr if the stab line of the media turned hazy then it was motility positive, if the media turned pink it is urease positive and after adding 10 drops of Kovacs reagent a red ring appeared in the media then it was considered as indole positive [13,16].

2.5.10 Starch hydrolysis test

Starch agar was prepared by adding 3 g/l of beef extract, 10 g/l of soluble starch and 15 g/l of agar then it was autoclaved at 37°C for 24 hours. An inoculum from a pure culture was streaked on sterile plates of starch agar. Then the plates were incubated for 24 hr. Finally Iodine reagent was added to flood the growth. The presence of clear zone was positive that means they could digest starch thus indicates presence of alpha amylase [14,17].

2.6 Determination of Optimum De-colourization Condition

The bacterial isolates were optimized at pH values 5, 6, 7 and 8 and at temperatures 30, 37, 45 and 55°C in 50 ml SM broth media without glucose with corresponding dye (OM2R and GGS) for 5 days and then OD was measured at 590 nm for OM2R and at 510 nm for GGS with the help of a spectrophotometer (UVmini-1240V) to find the optimum growth condition. The dye concentration was set at 1%, 3% and 5% and sodium chloride concentrations of 2%, 4%, 6% and 8% were adjusted. All the experiments were aseptically performed twice and the results were the average of collected data.

2.7 Decolourization

Decolorization ability of bacterial isolates were analyzed using spectrophotometer (UVmini-1240V) every 24 h at optimum wavelength 590 nm for OM2R and at 510 nm for GGS. Based on the reduction in absorbance, the percentage of de-colorization was estimated. All the experiments were repeated twice the results were the average of collected data.

The dye decolourization efficiency of bacterial isolates was calculated by the formula of Cheriaa et al. [18].

$$\text{De-colorization (\%)} = [I - F] \times 100 / I$$

Where, I = initial absorbance; F = final absorbance of decolorized medium.

3. RESULTS AND DISCUSSION

During isolation process a total of 36 of bacterial colonies were observed. From those colonies 20 bacterial strains were isolated as most of the colonies were morphologically similar. The isolates AO, BO, CO, DO, AG, BG, CG, and DG were screened on the basis of the ability to degrade the dye efficiently more than 60%. All the isolates were Gram negative except AG, BG and DG (Table 1). BO, DO and DG were cocci shaped whereas rest were rod shaped bacterium (Table 1). All negative results recorded for MIU test except non motile AG and motile CG isolate (Table 1). No positive result was observed for Simmon's Citrate test. All the samples were responsive to nitrate reduction test and all of them showed the ability to grow at 6.5% NaCl solution (Table 1).

Finally, the test results were analyzed in ABIS online software [19], which is a laboratory tool for bacterial identification. Based on the morphological characteristics, Biochemical test and ABIS software the isolates were identified as *Enterococcus termitis* (Isolate AO), *Enterococcus camelliae* (Isolate BO), *Bacillus farraginis* (Isolate CO), *Unknown taxon* (Isolate DO), *Bacillus muralis* (Isolate AG), *Paenibacillus macerans* (Isolate BG), *Bacillus decolorationis* (Isolate CG) *and Macrococcus brunensis* (Isolate DG).

3.1 Effect of Different Concentration of Dye on Decolourization

Percentage decolourization of OM2R dye by AO (*Enterococcus termitis)* and CO *(Bacillus farraginis)* was found to vary with different concentrations (1-5 g/l) when studied for 120 hours at 37°C. Maximum decolourization rate was 98% of 1% OM2R dye by CO *(Bacillus farraginis*) however as concentration of dye increased up to 3% and 5% the decolourization rate was decreased respectively to 94% and 90% (Table 2). It was because of the toxic nature of azo dyes. The Percentage decolourization was found to be decreasing with the increase of dye concentration [20]. Similar pattern of result was observed for isolates AO (*Enterococcus termitis)*. At 1% OM2R dye concentration the decolourization percentage was 93% of dye and at 3% and 5% dye concentration decolourization was dramatically same that was 87% of dye.

Table 1. Morphological and biochemical characteristics of bacterial isolates obtained from the textile sludge

Isolate no	Sample Isolate Name	Gram stain		MIU			Catalase	Oxidase	Simmon's Citrate	MR	VP	Casein hydrolysis	Nitrate reduction	Starch hydrolysis	Carbohydrate									Growth at 6.5%NaClsoln.	Growth at 7% NaClsoln.	Growth at 10% NaClsoln.	Growth at 15% NaClsoln.	Growth at 45^0 C	Aerobic growth	Presumptive organism
		+/-	Shape	Motility	Indole	Urease									Sucrose	Lactose	Glucose	Fructose	Maltose	Trehalose	Galactose	Mannitol	Arabinose							
1.	AO	-	rod	-	--	-	+	+	-	+	-	_	+	-	-	+	-	+	-	-	+	-	+	+	+	_	-	+	+	*Enterococcus termitis*
2.	BO	-	cocci	-	-	-	+	+	-	-	-	-	+	-	+	-	+	-	+	+	-	-	+	+	-	_	-	+	+	*Enterococcus camelliae*
3.	CO	-	rod	-	-	-	+	+	-	+	-	-	+	-	-	-	-	-	-	-	+	-	-	+	+	_	-	+	+	*Bacillus farraginis*
4.	DO	-	cocci	-	-	-	+	+	-	+	-	-	+	-	-	+	-	+	-	-	+	-	+	+	+	_	-	+	+	*Unknown taxon*
5.	AG	+	rod	-	+	-	+	+	-	-	-	-	+	-	-	+	+	-	-	-	+	-	+	+	+	_	-	+	+	*Bacillus muralis*
6.	BG	+	rod	-	-	-	+	+	-	+	-	-	+	-	+	+	+	+	+	+	-	+	+	+	+	_	-	+	+	*Paenibacillus macerans*
7.	CG	-	rod	+	-	-	+	+	-	+	-	+	+	-	+	-	+	+	+	+	+	+	-	+	+	_	-	+	+	*Bacillus decolorationis*
8.	DG	+	cocci	-	-	-	+	+	-	-	-	-	+	-	-	-	+	+	+	+	+	+	-	+	+	_	-	+	+	*Macrococcus brunensis*

+ =*Positive reaction; - = Negative reaction*

In the case of GGS dye decolourization, a maximum decolourization rate was observed 97% of dye by BG *(Paenibacillus macerans)* and then 93% of dye by CG *(Bacillus decolorationis)* at 1% dye concentration. Both of the isolates were similarly effective at 3% dye concentration, decolourization rate was respectively 94% and 93% of dye. However at 5% dye concentration the degradation rate was found to be a slump for both of the isolates that was respectively 81% and 77% of dye (Table 3). Normally the dye concentration in the effluent varies within a range of 0.1-0.2 g/l [21]. In comparison with this range the dye concentration used in this study was much higher (10-50 g/l) .This result indicate that the isolated bacterial strains showed efficiency in dye decolourization under in-vitro laboratory conditions.

3.2 Effect of Different pH on Dye Decolourization

The pH and temperature are important factor for the optimal physiological performance of microbial cultures and decolorization of dyes [20]. The effect of pH was studied at pH values 5.0, 6.0, 7.0 and 8.0.The temperature was fixed at 37°C. At pH 7.0 both AO (*Enterococcus termitis)* and CO (*Bacillus farraginis)* gave maximum decolourization respectively 90% and 89% of dye [Fig 1]. Dye decolourization rate was almost similar over the pH range of 5.0 and 6.0 that was around 75% by both of AO (*Enterococcus termitis*) and CO *(Bacillus farraginis)*. A swift reduction in the decolourization was observed at pH 8 by AO (*Enterococcus termitis*) and CO *(Bacillus farraginis)* respectively 59% and 52% of dye. Those results suggest that acidic pH values may influence the stability of the enzyme causing denaturation. Hang et al. [2] found that azoreductase performance was affected by pH, with 2.5 times better dye reduction at pH 7-9 than below pH 7. These findings corresponded well to the best decolourization found between pH 7-9.5 [22].

Maximum decolourization rate was attained at pH7.0 by BG (*Paenibacillus macerans)* 92% of dye and CG *(Bacillus decolorationis)* 88% of dye [Fig 2]. Majority of the azo dye reducing bacterial species reported so far were able to reduce the

Table 2. Percentage of decolourization Azo dye (OM2R) by AO and CO at different concentration in SM media after 120h

Concentration of OM2R	% of decolourization by AO (*Enterococcus termitis)*	% of decolourization by CO (*Bacillus farraginis)*
1%	93%	98%
3%	87%	94%
5%	87%	90%

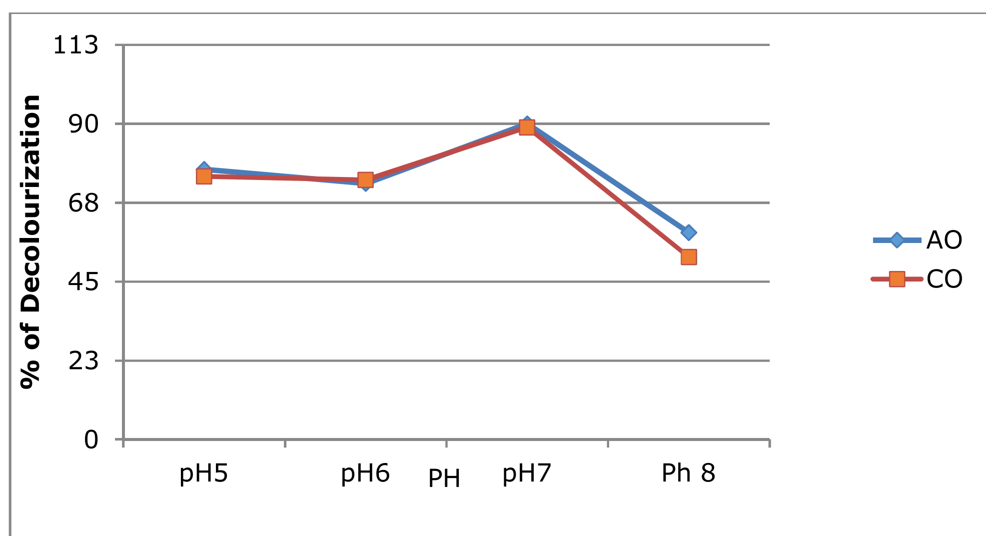

Fig. 1. Effect of different pH on decolourization of OM2R dye by the isolates AO and CO

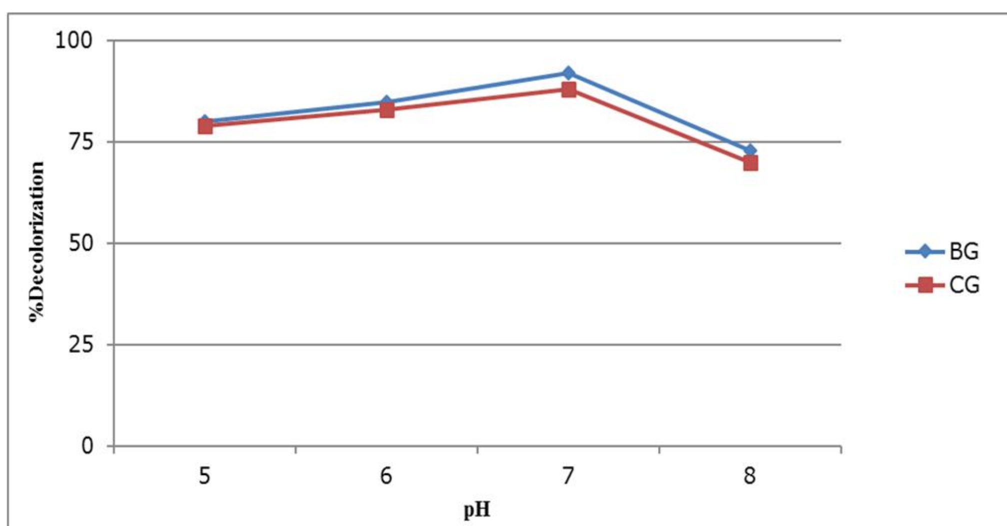

Fig. 2. Decolourization of GGS dye at varying pH by the isolates BG and CG

Table 3. Percentage of decolourization Azo dye (GGS) by BG and CG at different concentration in SM media after 120h

Concentration of OM2R	% of Decolourization by BG (*Paenibacillus macerans*)	% of Decolourization by CG (*Bacillus decolorationis*)
1%	97%	93%
3%	94%	93%
5%	81%	77%

dye at pH near 7 [2,23,24]. The requirement of near neutral pH for optimum growth had been reported in several studies [25,26]. The results indicate that a pH increase from 5.0 to 7.0 enhanced the decolourization of GGS dyes. At pH5 the decolourization rate was 80% and 79% of dye respectively by BG *(Paenibacillus macerans)* and CG (*Bacillus* decolorationis). A little improvement was observed at pH 6 that was 85% of dye by BG *(Paenibacillus macerans)* and 83% of dye by CG *(Bacillus decolorationis)*. At pH 8.0 the decolourization rate decreased dramatically that was 73% and 70% respectively by BG*(Paenibacillus macerans)*and CG*(Bacillus decolorationis)*. It was observed that better decolourization rate was around pH 6 and pH 7 for both of OM2R and GGS dye by the selected isolates.

3.3 Effect of Different Concentration of NaCl on Azo dye Degradation

Percentage decolourization of OM2R by selected isolates were found to vary with different concentration (2-8 g/l) of NaCl when studied for 120 hours at 37°C. Maximum decolourization of OM2R by AO (*Enterococcus termitis)* and CO *(Bacillus farraginis)* was observed 81% and 71% respectively at 2% NaCl but the percentage decolourization was found to be decreasing with the increase of NaCl concentration. The decolourization attained by AO (*Enterococcus termitis)* at 37°C for 4%, 6% and 8% NaCl was 60%, 43%, and 37% and for CO (*Bacillus farraginis)* it was 58%, 40% and 34% respectively [Fig 3]. Kargi et el. [27] mentioned that high salt concentrations (>1% salt) are known to cause plasmolysis and/or loss of cell activity.

Similarly, at 2% NaCl concentration the degradation percentage of GGS dye were 89% and 81% respectively by BG (*Paenibacillus macerans)* and CG (*Bacillus decolorationis*). The decolourization attained by BG (*Paenibacillus macerans*) at 37°C for 4%, 6% and 8% NaCl was 72%, 62%, and 40% and for CG (*Bacillus decolorationis*) it was 65%, 54% and 36% respectively as evident from (Fig. 4).

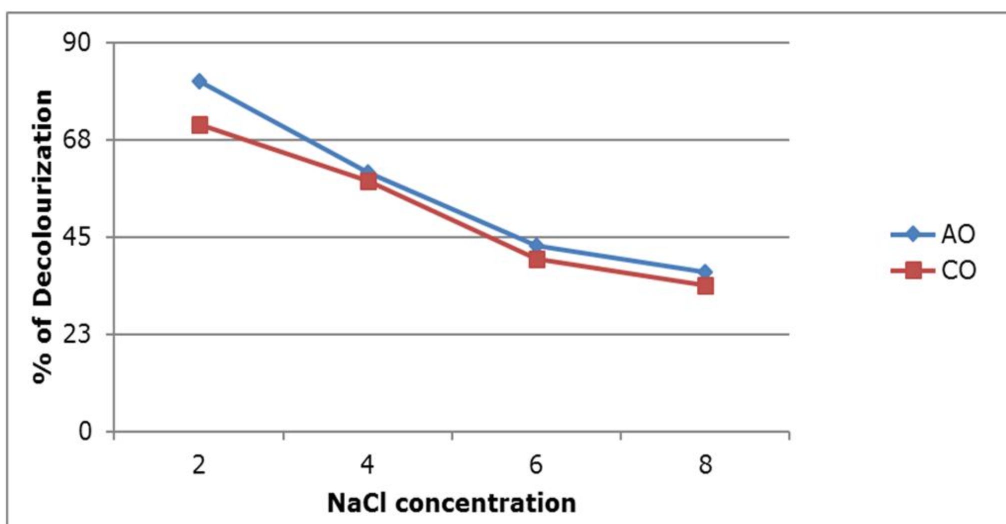

Fig. 3. Decolourizationof OM2R at varying NaCl concentration by the isolates AO and CO

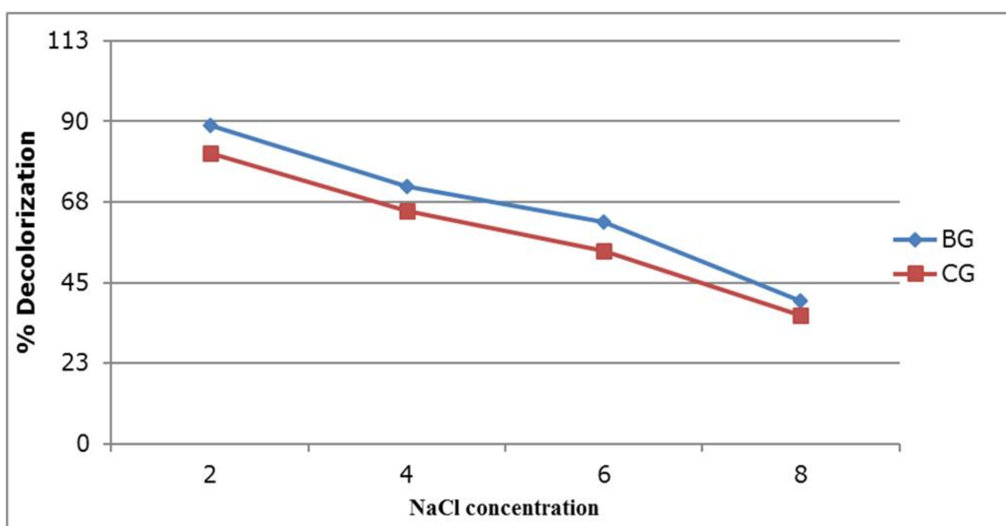

Fig. 4. Decolourization of GGS dye at varying NaCl concentration by the isolates BG and CG

3.4 Effect of Different Temperature on Dye Degradation

To determine the optimum temperature for dye decolourization a temperature range of 30°C – 55°C was examined. The optimum temperature for OM2R dye decolourization was 37°C for both of the AO *(Enterococcus termitis)* and CO *(Bacillus farraginis)* attained a maximum decolourization of 93% and 92% of dye respectively. Angelova et al. [28] found that the azo bond reduction rate rose with an increased temperature, a maximum rate around 40°C, 3-5 times faster than at 20°C. At 30°C and 45°C the degradation rate for OM2R by AO (*Enterococcus termitis)* was 82% and 72% of dye and for CO *(Bacillus farraginis)* it was 84% and 82% of dye respectively. Then a low decolourization of 59% and 57% of dye was detected at 55°C by AO (*Enterococcus termitis)* and CO *(Bacillus farraginis)* isolates respectively. Temperatures above 55°C were not studied since results shown that the increase from 37°C to 45°C promoted a marginal decrease in dye decolourization as evident from (Fig. 5).

The optimum temperature for GGS dye decolourization was 37°C for both of the BG (*Paenibacillus macerans)* and CG *(Bacillus decolorationis)* attained a maximum

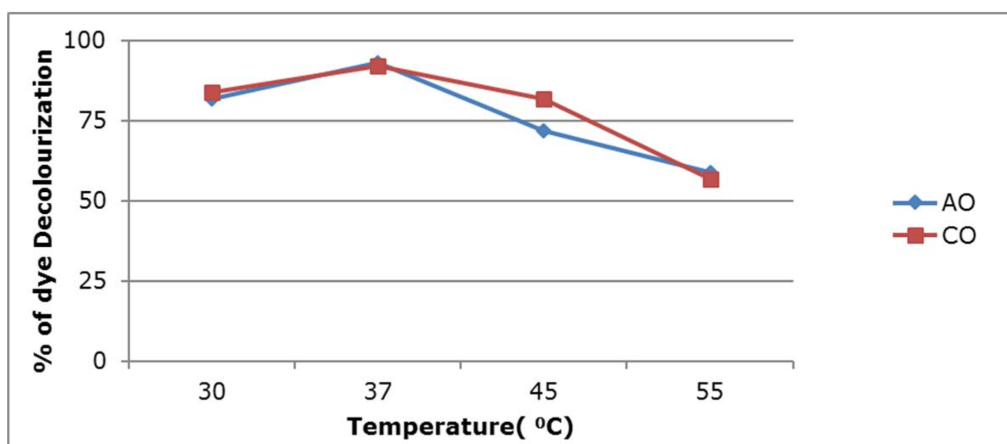

Fig. 5. Decolourization of OM2R at varying temperature by the isolates AO and CO

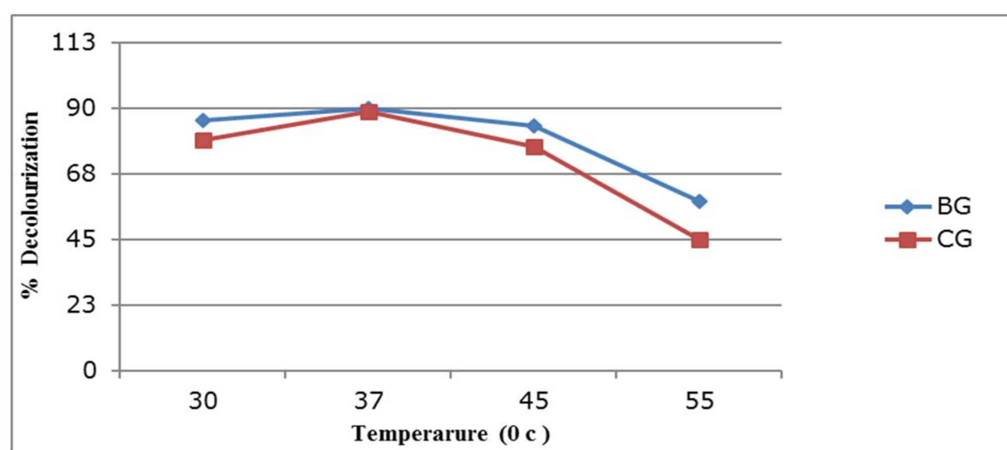

Fig. 6. Decolourization of GGS dye at varying temperature by the isolates BG and CG

decolourization of 90% and 89% of dye respectively. In case of BG at 30°C and 45°C the decolourization percentage was 86% and 84% of dye respectively whereas by CG it was 79% and 77% of dye respectively. No improvement in dye decolourization was observed at temperatures above 45°C. Where a low decolourization of 58% and 45% of dye was detected at 55°C by BG (*Paenibacillus macerans)* and CG *(Bacillus decolorationis)* isolates respectively as evident from (Fig. 6). BG (*Paenibacillus macerans)* has a broad range of compatibility from 30°C to 45°C. Within the optimal values of temperature, the lowest temperature was selected as the optimum temperature since this leads to lower energy costs.

4. CONCLUSION

Colour removal of textile and dye stuff plant with a continuous discharge of great quantity of remaining dyes to the environment has been a major concern in waste water treatment. Biotreatment offers easy, cheaper and effective alternative for color removal of textile dyes. Thus, according to this present study we can strongly conclude that the bacterial isolates like *Enterococcus termitis, Bacillus farraginis, Bacillus decolorationis,* and *Paenibacillus macerans* species were a good microbial source for textile effluent treatment, in biological degradation of textile dye. However, potential of culture need to be demonstrated for its application in treatment of real dye bearing waste waters using appropriate bioreactors. In future the obtained bacterial isolates can be used as microbial consortium to remove textile dyes from complex textile effluent and hence a greener solution to environmental pollution. Furthermore, if we can isolate the genes that are responsible for dye degradation and can develop genetically

modified bacteria will be more robust and can be commercialized.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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