BIOREMEDIAL POTENTIAL OF MARINE BACTERIUM

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ABSTRACT

Reactive Red 81 was selected as model textile dye. The bacterial strain *Marinobacter santoriniensis DR-11* isolated from natural marine environment was screened for decolourization of Reactive Red 81. Optimization was carried out with respect to incubation conditions, carbon source, nitrogen source, pH and temperature. It was found that the optimum pH and temperature were 7.0% and 37°C respectively. Glucose and yeast extract at 1.0 % concentration was found to give maximum decolourization. Further, it was found that the culture has brought about almost 98.00% decolourization of a recalcitrant dye, Reactive Red 81(3000µg/ml) at shaking condition within 24 hours. The present isolate also showed the degradation as evidenced from the reduction in the term of COD mg/L to the extent of 88.00 % within 24 hours. The biodegradation was monitored by GC-MS analysis. This study revealed the enormous biodegradation abilities of indigenous marine bacterial flora. The toxicity of the degraded products was checked by the microbial toxicity and fish toxicity and was revealed the degradation of Reactive Red 81 into non-toxic product by *Marinobacter santoriniensis DR-11*.

KEYWORDS: Reactive Red 81, Bioremediation, GC-MS, Microbial Toxicity, Fish Toxicity.

INTRODUCTION

Textile industries utilizes large quantities of water and chemicals (Chakraborty *et al.*, 2003[1], Mondol *et al.*, 2015). The discharge of highly colored synthetic dye effluents into inland
and coastal waters is an environmental problem of growing concern. Approximately, 10,000
different dyes and pigments are used industrially and over 0.7 million tons of synthetic dyes
are produced worldwide. A broad variety of particulars chemicals such as softeners, wetting
agents and stain releasing agents are used and several of these compounds become part of the
final product, whereas the rest are removed from the fabric and laden into the effluent stream
Two per cent of dyes that are produced are discharged directly in aqueous effluent and 10%
are subsequently lost during the textile coloration process. These colorants are environmental
pollutants. With increasing industrialization, the magnitude of the problem is constantly on
rise and needs a believable microbial solution. Thus, screening of microflora with effective
decolorizing ability could evolve new indigenous strains to be used as bioremediation tools
for removal of these dyes. Most of the textile industries are located on the coastal areas
because of low cost transportation and available source of water, but the dye containing
effluents are directly discharged in natural marine water bodies which in turn affect the
marine flora and fauna very adversely. The disposal of these textile dyes into the aquatic
environment causes serious damage, since they may affect significantly the photosynthetic
activity of hydrophyles by reducing light penetration, decreases the gas solubility which
ultimately affect water quality and also they may be toxic to some aquatic organisms due to
their breakdown products. Therefore, industrial effluents containing dyes must be treated
before their safe discharge into the environment. Although several physico-chemical methods
have been used to eliminate the color from wastewater, but these are generally expensive and
produce large amount of sludge. More often these conventional modes of treatment lead to
the formation of some harmful side products leading to the secondary pollution problem and
requires additional cost for regeneration of treatment system. Therefore, the search for
efficient, eco friendly and cost effective remedies for wastewater treatment has been initiated
as a best alternative. The capability of bacteria to metabolise azo dyes has been reported by
different authors (Singh and Singh, 2015[7], Karthikeyan, 2015[8]; Jasińska *et al.*, 2015[9]). The
use of marine bacteria able to degrade the dyes in presence of salt could help to prevent
costly dilution to lower the salinity or the removal of salt by physico-chemical methods
before biological treatment. Additionally, it is known that the traditional pollutant
biodegradation is less efficient or does not function when salinity increases above that of the
sea. One remedy for the removal of these xenobiotic compounds in the saline environment is
the use of marine bacteria that have natural ability to tolerate such saline conditions.
Therefore use of such halotolerant bacteria can be useful for the degradation of textile dyes under saline conditions.

**MATERIALS AND METHODS**

**Dyes and Chemicals**

The textile dye Reactive Red 81 (Table 1) was procured from Sigma-Aldrich (USA). Dye solution was prepared by dissolving it in distilled water before each experiment. All the chemicals used were of highest purity and analytical grade.

**Table 1: The structural information of textile diazo dye used in the present study**

<table>
<thead>
<tr>
<th>C.I. name and Common name</th>
<th>Wavelength $\lambda_{\text{max}}$ (nm)</th>
<th>Chemical structure, Molecular formula, Molecular weight and composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive Red 81</td>
<td>510nm</td>
<td>![Chemical structure image] Molecular Formula = C$<em>{29}$H$</em>{21}$N$_5$O$_8$S$_2$ Formular Weight = 631 Composition = C(55.14%) H(3.35%) N(11.09%) O(20.26%) S(10.15%)</td>
</tr>
<tr>
<td>Direct Red 5B</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Acclimatization and isolation of microorganisms**

The acclimatization of the micro flora from soil + homogenized material to reactive red 81 dye and NaCl concentrations were carried out in different containers. For this, the dye solutions were added to the containers in increasing concentration for the period of 2 months. Same procedure was repeated for different NaCl concentrations.

**Bacterial Identification by 16s rRNA Sequences and Phylogentic Relationship**

The phylogentic identity of the present bacterium was determined by BLAST result. The sequences were aligned by using alignment software Clustal X2. Phylogeny calculations and dendrograms was then constructed by using MEGA 4.0 Software package with Neighbor joining (NJ) methods (Saitou and Nei, 1987$^{[10]}$). Bootstrap analysis (Felsenstein, 1985$^{[11]}$) was then conducted using 1000 replicates data samplings.

**Optimization of Decolorization Conditions**

**Effect of pH and Temperature**

In the experiments with different pH, all the optimal conditions were kept constant except pH and the variation in percentage decolorization with respect to change in pH was studied. The
physical parameters like pH and temperature were optimized for the maximum decolorization of the dye. Nutrient medium was inoculated with 3000µg/ml (final dye concentrations) of the dye, 7.0% final NaCl concentration in separate tube, a 24 hours old culture of the promising strain was inoculated in all these tube of different pH and temperature and kept for incubation for 24 hours. Temperature is the most important factor for decolorization of dye. Growth of microorganism is a cumulative activity of a large number of reactions mediated by enzymes. Therefore, rate of microbial growth and these enzymatic reactions is directly proportional to the each other. Thus these enzymatic reactions are directly influenced by temperature. However in most of cases with increase in temperature growth also increases but it decreases suddenly at extreme upper and lower limits of temperature. In order to determine the effect of temperature on decolorization of the selected dye under study by using the promising halophilic microbial strain, a series of experiments were performed. All these tubes of nutrient medium having the different NaCl were incubated at respective temperatures Viz. (25°C, 30°C, 37°C, 40°C and 50°C) for 24 hours. The decolorization of the dye was monitored spectrophotometrically.

**Effect of Shaking and Non-Shaking Condition**

The tube containing nutrient medium added with dye with concentration of 3000µg/ml and NaCl having final concentration 7.0% were incubated at optimum temperature and optimum pH for 24 hours. While all the experimental conditions were kept constant as described above. Then the second set of tube was incubated at constant shaking conditions. The tube was inoculated separately with the dye specific promising isolate. After optimum incubation time, samples were withdrawn from the tubes and the rate of decolorization was measured spectrophotometrically at its specific absorbance maxima. A comparison was done between shaking vs. static condition for decolorization potential of dye by the selected strain.

**Effect of Carbon and Nitrogen sources on percent decolorization**

The promising isolate was inoculated in nutrient broth containing 3000µg/ml of final dye concentration, 7.0% NaCl concentration and different 1% carbon and nitrogen sources Viz. Glucose, Lactose, Sucrose, Maltose, Fructose, Ammonium chloride, urea, peptone, beef extract and yeast extract. These tubes were then incubated at optimum temperature for 24 hours.
Measurement of Decolorization Extent

The decolorized broth samples after decolorization of the dye were centrifuged at 10,000rpm for 10min in Cooling Centrifuge (BIOLAB-BL-165R) for to separate the cell mass. Dyes concentrations of the decolorized broth were determined by comparing the absorbance of the decolorized broth with that of the known concentrations of the dyes. This was used for calculating the dye removal rate (mg/L/h) and was expressed in percent decolorization of the dyes (Chu and Chen, 2002[12]). The percentage of decolorization of the dye was find out by using spectrophotometer (Systronics-106 model) at its respective absorbance maxima of dyes and the following formula,

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

Percent Decolorization in Various Media

Percent Decolorization in Nutrient Broth and Half (½) Strength Nutrient Broth

The promising isolate was studied for the decolorization of the dye in the nutrient medium (Peptone – 1.0g, NaCl – 0.5g, Beef Extract – 0.3g, Distilled Water – 100.0ml, pH – 7.2). The 100µl of 24 hours old culture of the isolate was inoculated in 25ml of nutrient medium containing 3000µg/ml of dye and 7.0% NaCl. This was then incubated at ambient temperature for 24 hours for decolorization of the dye. Additionally, half strength nutrient broth (peptone – 0.5g, NaCl – 0.25g, Beef Extract – 0.15g, Distilled Water – 100.0ml) was also used to test for the ability of isolate DR-11 to decolorize the dye Reactive Red 81 with same dye concentration and salinity.

Percent Decolorization by Cell Free Extract

The cell free extract was prepared to study the effect of enzymes (intracellular and extracellular) on decolorization/degradation of dye. The cells grown in nutrient broth having 7.0% NaCl concentrations were separated by centrifugation using cooling centrifuge (BIO-LABS 165-R) at 7000rpm for 20min at 4°C. The supernatant was used as a crude extracellular enzyme source. After centrifugation, the cell pellet was resuspended in 50mM phosphate buffer pH – 7.4. The cell suspension in the buffer was properly cooled and lysed by using Ultrasonicator (Sonic-Vibra Cell System – 130) the output was kept 50amp with 6 strokes of 25s each, time interval kept was 2min at 4°C. This homogenate was centrifuged at 10000 rpm for 10 min to separate the cell debris. The intracellular enzymes present in the buffer were then used for further decolorization and degradation studies. Both the crude
extracellular and intracellular enzymes contents were then added with 10,000µg/ml concentration of dye solution and 20% NaCl concentration observed for dye decolorization. The percent decolorization studies were monitored by using spectrophotometer (Systronics – 106 model).

**Determination of Chemical Oxygen Demand (COD)**
Percent COD reduction value of the dye decolorized by potential isolate DR-11 was calculated by COD analysis using K$_2$Cr$_2$O$_7$ as a strong oxidizing agent under reflux conditions.

**Extraction and analysis of biotransformed products**
The biodegradation of Reactive Red 81 was monitored by GC-MS analysis. After decolorization, 100ml sample was withdrawn. Centrifugation was carried out at 10,000 rpm and the metabolites were extracted from supernatant using equal volume of Dichloromethane. The treated Reactive Red 81 dye was characterized by Gas Chromatography and Mass Spectroscopy.

**Confirmation of Dye Degradation by GC-MS Technique**
To study the products formed after degradation of azo dye Reactive Red 81, decolorized samples were analyzed by GCMS. The promising isolate was inoculated in 100mL of sterile nutrient broth containing 3000µg/ml of dye Reactive Red 81 and 7.0% NaCl. The broth was then incubated at ambient temperature for 24 hrs in separate flasks. The decolorized broth was then centrifuged at 10,000 rpm for 15 minutes in cooling centrifuge. Centrifugate was mixed with equal amount of dichloromethane in separating funnel. Samples were shaken vigorously for 15 minutes and kept for 10-15 minutes to separate solvent and liquid phases. After separation, liquid phase was discarded and solvent phase allowed for partial evaporation. Partially evaporated samples were analyzed by GCMS technique.

**Detection of Degraded Products**
The degraded products were detected by GC-MS technique.

**Prediction of Dye Degradative Metabolic Pathway**
The metabolic pathway of dye degradation was then proposed with the help of GC-MS analysis report.
Microbial Toxicity Testing of Dye Degraded Products

Agar well bioassay is the most common technique used for the evaluation of the microbial toxicity. This can be achieved by microbial toxicity tests with the original dye and its biodegradation products. The microbial toxicity was tested on three test microorganisms Viz. *Azotobacter sp.*, *Pseudomonas sp.* *Rhizobium sp.*. The 24 hours old culture of the test organisms were used for the toxicity testing. After the confirmation of dye degradation, the degraded solution (decolorized broth) obtained after treatment with the present organism was poured in the wells prepared in nutrient agar previously spreaded with the test organisms. These plates were incubated at ambient temperatures for 24 hours. The zone of inhibition around the wells proved the toxicity of the dye and their degraded products.

Phytotoxicity Study

Toxicity of original dye Reactive Red 81 and treated dye was observed by seed germination experiments. Phytotoxicity was studied on *Triticum spp.* The pots containing earth (soil) were labeled for the respective isolates, the dye (positive control), nutrient broth (negative control) and water. Seeds of *Triticum spp.* (20 nos.) were sowed in all pots. The dye, nutrient broth and the water were added in the respective pots. The degraded dye solutions were centrifuged at 10,000 rpm before adding to the soil in pot to remove the cell mass. All the solutions were added for consecutive 9 days. The results (root and shoot lengths of each plant) were noted after 9 days of growth. The control of this test was added with plain normal water. The negative control was sterile nutrient broth in which the decolorization reaction took place.

Fish Toxicity Study

The mature healthy marine fish like *Indian Salmon* having uniform size (Width = 2.2 ± 1.0mm Length = 2.5 ± 0.2cm, Fresh weight = 157 ± 10mg) were acclimatized in an aquarium (20L) for 10 days after obtaining from a fish farm. The aquarium had fine planktonic population to serve the marine fish and for to oxygenate marine water a submerged hydrophyte Viz. *Ceratophyllum demersum* was also supplied in an aquarium tank. Before starting the bioassay, the fish were starved in dechlorinated water for 24 hours and then were exposed to five test concentrations of textile dye like 800µg/ml, 1000µg/ml, 1500µg/ml, 2000µg/ml and 3000µg/ml. Further going in another aquarium (20L) which was taken as a control filled with the tap water. The experiments were carried out in triplicates for a test concentration of dye or control. Each triplicates having 10 healthy fish. The water from test aquarium or test concentration of dye in another aquarium was replaced after every 24
hours of inoculation (APHA, 1989\textsuperscript{[13]}). After 96 hours of exposure, results were recorded in the form of mortality. Further going, the graphical estimation of LC\textsubscript{50} values were calculated for 96 hours (Claude E. Boyd., 2005\textsuperscript{[14]}).

**RESULTS**

**Isolation and phylogenetic Identification of marine bacterium**

The promising bacterium was isolated from the acclimatized soil on nutrient agar containing 7.0\% NaCl and was identified by using biochemical observations and 16s rRNA analysis technique. From the analysis the isolate was identified as *Marinobacter santoriniensis DR-11*. The phylogenetic tree was developed by using Neighbor joining method by Kimura-2-parameter with 1000 replicates in MEGA 4.0 (Fig. 1).

**Gene Bank Accession Number**

Phylogenetic position was done by searching the National Center of Biotechnology Information (NCBI) BLAST. The 16s rRNA sequence of *Marinobacter santoriniensis DR-11* is availed under the GenBank accession number HF563062.

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![Phylogenic placement of *Marinobacter santoriniensis DR-11* (EMBL Accession No. HF563062).](image)

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Gene sequences showing relationships among strain DR-11 and the closest type strain species of *Marinobacter santoriniensis* DR-11. Numbers at nodes indicate percentage of bootstrap support based on a Neighbor-joining analysis of 1,000 resampled datasets. Bar 0.005 substitutions per nucleotide position.

**Optimization of Conditions**

**Effect of Static and Shaking Condition**

Bacterial isolate was further selected for its ability to decolourise the dye Reactive Red 81 in nutrient medium containing 7.0% NaCl concentration and 3000µg/ml dye concentration under static and shaking condition (120rpm) at for 24 hours. Reactive Red 81 was found to be completely decolourised by DR-11 culture within 24hrs. incubation under shaking condition at faster rate.

![Fig. 2 Percent Decolorization Reactive Red 81 in static and shaking condition in 24 hrs at \( \lambda_{\text{max}} - 510\text{nm} \)](image)

**Effect of arious physico-chemical conditions on decolourisation**

Effect of different physicochemical factors as pH, temperature, carbon sources and nitrogen sources on the decolourisation of Reactive Red 81 dye by DR-11 was studied at shaking condition (120rpm) in order to increase the decolourisation of the dye.

**Effect of pH and Temperature**

The decolourisation of Reactive Red 81 was carried out in a medium containing 7.0% NaCl concentrations and dye (3000µg/ml) at different pH (5.0, 6.0, 7.0, 9.0 and 11.0) by selected
bacterial isolate DR-11 under shaking condition and decolourization were measured at 510nm. It was found that decolourisation was highest at pH 7.0. Least decolourisation observed at pH 11.0. Increase in pH from 7.0 to 11.0 proved to have an inverse effect on decolourisation Reactive Red 81 by DR-11 (Fig. 3). It was observed that the decolourization of Reactive Red 81 increased at neutral pH but has been drastically affected after pH 7.0. Further, different temperatures viz. 25ºC, 30ºC, 37ºC, 40ºC and 50ºC were used for decolourisation of Reactive Red 81 by selected potential isolate DR-11. The decolourisation of the dye was carried out at its previously optimized initial pH value in an incubator shaker (120 rpm) for 24 hours. The results showed that the decolourisation of Reactive Red 81 by DR-11 was found maximum at 37ºC (Fig. 3). The decolourisation of Reactive Red 81 was less at 25ºC but it was increased at 37ºC. It was found to be slightly decreased at 40ºC. Overall, an increase in temperature from 25ºC to 35ºC found to have significant positive correlation with percent decolourisation.

![Graph showing percent decolorization of Reactive Red 81 at different pH and temperatures in 24 hrs at λmax-510nm](image)

**Fig.3** Percent Decolorization of Reactive Red 81 at different pH and temperatures in 24 hrs at λmax-510nm

**Effect of 1% Carbon Source on percent decolorization of dye**

The selected bacterial isolate was further used for the decolorization assay in nutrient medium containing (0.5%-7.0%) NaCl concentrations and Glucose, Lactose, Sucrose, Maltose and Fructose as carbon sources and 3000μg/ml concentration of dye. The results of percent decolorization of dye Reactive Red 81 in presence of different carbon sources is given in Fig. 4.
Fig. 4 Percent Decolorization in presence of different (1%) Carbon sources in 24 hrs at \( \lambda_{\text{max}} \) 510nm

Effect of 1% Nitrogen Source on percent decolorization of dye

The different inorganic and organic nitrogen sources were used to study their effects on dye decolourisation in the medium (0.5%-7.0%) NaCl concentrations with optimized carbon source as glucose with pH 7.0. These media were inoculated with DR-11 culture and incubated at 37°C and 120 rpm for 24 hours. The addition of nitrogen sources proved to have a boosted effect on the dye decolourisation. Out of five nitrogen sources screened, yeast extract showed complete decolourization after 24 hours. Mycological peptone and Beef extract was also found to be an effective nitrogen sources which showed maximum decolourisation with 24 hrs. of incubation. The other nitrogen sources was not found be so operative in decolourisation (Fig. 5).

Fig. 5 Percent Decolorization in presence of different (1%) Nitrogen sources in 24 hrs at \( \lambda_{\text{max}} \) 510nm
Decolorization Assay

Percent Decolorization in Nutrient Broth
The promising bacterial isolate was used for to examine the decolorization in nutrient broth having 7.0% NaCl concentrations and 3000µg/ml concentration of dye. The results of percent decolorization of dye Reactive Red 81 by the selected isolate is given in Fig. 6.

Percent Decolorization in Half (½) Strength Nutrient Broth
The promising bacterial isolate was also examined for the decolorization in half strength nutrient broth a having 7.0% NaCl concentrations and 3000µg/ml concentration of dye. The results of percent decolorization of dye Reactive Red 81 in half strength nutrient broth is given in Fig. 6.

Percent Decolorization by Cell-Free Extract
Further going, the promising isolate was examined for the decolorization in Cell-Free extract. The results of percent decolorization of dye Reactive Red 81 in cell-free extract is given in Fig. 6.

Percent COD reduction
To evaluate the biodegradation of Reactive Red 81 by Marinobacter santoriniensis DR-11, the determination of percentage of mineralization (represented by COD removal) was carried out by measuring the initial and final organic content. The percent COD reduction was obtained to the extent of 88.00% by DR-11 culture with optimized cultural conditions upon 24 hours of treatment of dye.

![Fig. 6 Percent Decolorization of Reactive Red 81 in Nutrient Broth, half Strength Nutrient Broth and by Cell-Free extract in 24 hrs at λmax-510nm](image-url)
**Legend:**  
CFE = Cell Free Extract, HSNM = Half Strength Nutrient Medium, NM = Nutrient Medium

**Confirmation of Dye Degradation by GC-MS Technique**

The biodegradation of the dye Reactive Red 81 was monitored by GC-MS analysis. The GCMS analysis report showed that the dye Reactive Red 81 was degraded into smaller fragments having molecular weight and are given in Fig. 7 and Table 2 by *Marinobacter santoriniensis DR-11*. The result indicated the complete decolourisation and deformation of the structural confirmation that was responsible for the color.

![GCMS analysis report of degraded products of Reactive Red 81 dye by *Marinobacter santoriniensis DR-11*](image)

**Detection of the Dye Degraded Products**

The degraded products were detected by GC-MS technique and are given in Table 2.

**Table 2- Detection of the Dye Degraded Products**

<table>
<thead>
<tr>
<th>Name of the Degraded Products Formed</th>
<th>Molecular Weights of the Degraded Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-[(4-aminophenyl)diazenyl]benzenesulfonic acid</td>
<td>277.29904</td>
</tr>
<tr>
<td>prop-1-ene-2-sulfonic acid - 1-[(2Z)-but-2-en-1-yl]-2-methyl diazene (1:1)</td>
<td>220.28924</td>
</tr>
<tr>
<td>(1E)-buta-1,3-dien-1-amine - prop-1-ene (1:1)</td>
<td>111.18482</td>
</tr>
<tr>
<td>(3Z)-penta-1,3-diene</td>
<td>68.11702</td>
</tr>
<tr>
<td>ethanesulfonic acid - dimethyl diazene (1:1)</td>
<td>168.21468</td>
</tr>
<tr>
<td>(1E)-buta-1,3-dien-1-amine - ethene (1:1)</td>
<td>97.15824</td>
</tr>
<tr>
<td>(2Z)-but-2-enal</td>
<td>70.08984</td>
</tr>
<tr>
<td>oxomethanesulfonic acid</td>
<td>110.08918</td>
</tr>
<tr>
<td>(3E)-4-aminobuta-1,3-dien-1-one</td>
<td>83.0886</td>
</tr>
</tbody>
</table>
Proposed Dye Degradative Metabolic Pathway

We proposed the biodegradation pathway of dye Reactive Red 81 on the basis of GC–MS data (Fig. 8). The degradation products of Reactive Red 81 were analyzed by GC-MS and the results indicated that the reductive cleavage (Fig. 8) may be responsible for azo dye degradation by *Marinobacter santoriniensis* DR-11.

![Proposed Dye Degradative Metabolic Pathway](image)

**Fig. 9:** Probable Degradation Pathway of Reactive Red 81 by *Marinobacter santoriniensis* DR-11

**Microbial Toxicity Testing of Dye Degraded Products**

Microbial toxicity of the textile azo dye Reactive Red 81 was studied on microorganisms *Viz.* *Azotobacter sp.*, *Pseudomonas sp.* and *Rhizobium sp.* The toxicity of the dye and its
degradation products was studied by the agar well assay. The results showed that the wells which were poured with decolorized broth had no zone of inhibition and wells with original dye solution had a zone of inhibition. This confirmed that the original dye solution 3000µg/ml was toxic to the bacteria but its degradation products were non toxic to the bacteria.

Phytotoxicity Testing

The bioassay for dye toxicity was carried out for measuring the effect of dye Reactive Red 81 (3000µg/ml) on plant parameters Viz. shoot and root length and percent seed germination of Triticum aestivum. The toxicity of Reactive Red 81 was indicated by a decrease in root and shoot growth as well as seed germination rate. The results further showed the reduced toxicity in the treated dye sample, with increased germination rate as well as significant growth in shoot and root for Triticum aestivum, as compared to the dye (Table 3). This way, phytotoxicity studies revealed the biodegradation of the dye Reactive Red 81 by DR-11 culture resulted in its detoxification.

Table 3: Phytotoxicity analysis of untreated and treated dye Reactive Red 81

<table>
<thead>
<tr>
<th>Plant under study: Triticum aestivum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nature of the sample</td>
</tr>
<tr>
<td>-----------------------</td>
</tr>
<tr>
<td>Control (Distilled water)</td>
</tr>
<tr>
<td>Reactive Red 81 (3000µg/ml)</td>
</tr>
<tr>
<td>Treated dye sample</td>
</tr>
</tbody>
</table>

Fish Toxicity Testing

The evaluation of fish toxicity in control as well as in tests revealed no mortality even after 96 hours of test period. Throughout the test period of 6 hours the fishes were found to be healthy with no side effects whereas in test aquarium with the original dye solution all the fishes died within a period of 8 hours proving original textile dye concentrations were highly toxic to the marine water fish like Indian Salmon (Table 4 and Fig. 10). It is observed from the Table 4 and Fig. 10 that at 0µg/ml, 800µg/ml, 1000µg/ml, 1500µg/ml, 2000µg/ml and 3000µg/ml concentrations, the percentage survival was 100%, 60%, 50%, 40%, 30% and 20% respectively after 96 hours of test period. According to Claude E. Boyd (2005), the LC50 value was calculated by plotting the survival percentage against concentrations of original dye Reactive Red 81 solution. It is evident from the Table 4 and Fig. 10 that the percentage survival was decreased with increasing concentrations of original textile dye Reactive Red
81. The concentrations of Reactive Red 81 that is lethal to 50% of the organisms exposed to it in a toxicity test i.e. the \( LC_{50} \) value was found to be 1400µg/ml.

Table 4- The percentage survival against concentrations of original Reactive Red 81 dye solutions.

<table>
<thead>
<tr>
<th>Concentrations (µg/ml)</th>
<th>Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>800µg/ml</td>
<td>60</td>
</tr>
<tr>
<td>1000µg/ml</td>
<td>50</td>
</tr>
<tr>
<td>1500µg/ml</td>
<td>40</td>
</tr>
<tr>
<td>2000µg/ml</td>
<td>30</td>
</tr>
<tr>
<td>3000µg/ml</td>
<td>20</td>
</tr>
</tbody>
</table>

\( LC_{50} \) value is 1400µg/ml

DISCUSSION

Rani et al.,(2016)\(^{(15)}\) isolated halotolerant bacterial strain *Bacillus flexus* capable of effectively decolorize Acis Blue 113 at different dye concentrations. In the present study a halophilic bacterial strain was isolated capable of decolorizing the diazo dye Reactive Red 81 at 7.0% NaCl concentration within 24 hours. Few researches successfully got the microbes which decolorizes the dye but with increasing concentration of dye the bacterial growth decreased. Bacteria capable of utilizing dyes as their carbon source. Azo dyes are deficient of carbon source therefore color removal is feasible only with co metabolite conditions (Shellina Khan and Nishi Mathur, 2015\(^{(16)}\)). Additionally, nitrogen sources acts as the source of electron donor to reduce the azo dyes by microorganisms (Adya Das et al., 2015\(^{(17)}\)).
results were obtained in the present study in which complete removal of dye within 24 hours obtained when 1% carbon and nitrogen sources were used. In the present study the isolated marine bacterium could completely decolorize the diazo dye Reactive Red 81 at shaking condition in 24 hours. This was in contrast to the results obtained by Suganya, K. and Revathi, K. (2016)\cite{18} in which significance of static conditions for better decolorization has been highlighted. In accordance with other reports, the present study revealed the excellent decolorization (100%) capacity of *Marinobacter santoriniensis DR-11* in presence of 1% yeast extract but this decolorization was best when compared with the nutrient medium (Adya Das *et al.*, 2015). It was found from the phytotoxicity study that the seed germination rate was high in case of treated dye sample when compared to original dye sample. Further, root and shoot length were also observed better in case of treated dye sample. This results were in accordance with the study conducted by Baljeet Singh Saharan *et al.*, (2015)\cite{19}. Initially with the increase in pH value from 3.0 to 7.0 decolorization increased which was maximum at 7.0 pH. Similarly, further increase in pH from 7.0 to 9.0 had negative effect on decolorization capacity of bacteria. The isolates showed gradual increase in decolorization from 25°C to 35°C with maximum decolorization at 35°C. As the temperature increased further from 35°C to 45°C, there was sharp decline in decolorization capacity in all the isolates (Walaa Salah El-Din Mohamed, 2016).\cite{20} However, in the present study, the isolated bacterium showed maximum decolorization at pH 7.0 and temperature 37°C in 24 hours. Geetha *et al.*, (2016)\cite{21} studied the decolorization of Alizarin red S by *Pseudomonas sp.* and *Escherichia coli*. They optimized the conditions for maximum dye decolorization and was found to be 1% peptone 1% glucose, pH 7.0, 37°C, 500mg/L dye concentration, combination of 1% peptone and 1% glucose and 50 immobilized bacterial cells per 100ml of Mineral Salt Medium. The highest decolorization rate was found to be 78.04 % and 69.17% for *Escherichia coli* and *Pseudomonas sp.* respectively. In the present study, the optimized conditions for maximum dye decolorization is pH 7.0, temperature 37°C, 1% glucose, 1% yeast extract and 1% beef extract was found to be the best carbon and nitrogen sources for complete removal of dye Reactive Red 81 in 24 hours by *Marinobacter santoriniensis DR-11*. Jagwani *et al.*, (2013)\cite{22} reported maximum COD reduction of 67% and 83 % of ROM2R dye in 24 hours and 48 hours of inoculation respectively by Consortium VSS. The mineralization of dye is indicated by this high rate of COD reduction (Hassan *et al.*, 2002).\cite{23} The present marine bacterium could reduce the COD up to 88.00% in 24 hours at 7.0% salinity. The study which was carried out by Krishnaswamy V. and Namasivayam V. (2010)\cite{24} isolated a moderately halophilic bacterial consortium from marine environment
which showed maximum degradation of dye in the presence of yeast extract at 50g/L of NaCl and pH 7.0. Our results were in strong accordance of the research conducted by Guo et al., (2008a)[25], in which the Halomonas strains grew well and completely decolorized K-2BP where either peptone or yeast extract was present in the medium; however, sucrose, glucose, glycerol, starch and lactose resulted in lower rates of growth and decolorization of these dyes.

CONCLUSIONS

From the present study it can be concluded that, the isolated strain DR-11 has been identified as Marinobacter santoriniensis is capable of effectively decolorizing textile diazo dye, Reactive Red 81. Different physico-chemical factors affecting the efficiency of decolorization of the bacterial strain were also analysed and the optimum values for each factors were determined. The optimal conditions for the decolorizing activity of Marinobacter santoriniensis DR-11 were aerobic culture environment with yeast and beef extract as nitrogen sources supplementation, at pH 7.0, and 37°C. Marinobacter santoriniensis DR-11 showed potential decolorizing activity through a degradation mechanism rather than adsorption and it could tolerate high concentrations (up to 3000 µg/ml) of Reactive Red 81. The analytical technique GC-MS analysis confirmed the degradation of azo bond of the diazo dye Reactive Red 81 by the present isolated bacterial strain.

In conventionality with similar literatures, the present decolorizing bacterial isolate was found to successful in decolorization, degradation and mineralization of dye Reactive Red 81. The biological treatment not only decolorized the recalcitrant dye solution but also reduced the cost of effluent treatment process. In this study bacterial isolate Marinobacter santoriniensis DR-11 was isolated for the decolorization study based on its higher potentials to decolorize Reactive Red 81. Further research on this strain could be needed to explore new techniques and tools to evolve viable microbial solutions for the treatment of dyeing industrial effluent. The present study clearly demonstrates the native bacterial community capable of decolorizing and degrading different types of dyes originating from textile industries and therefore can be exploited for bioremediation of textile dyes containing wastes, due to its potentiality to degrade toxic reactive dyes into non-toxic simpler forms. This bioremedial approach would help in developing promising green technology for the treatment of such textile dyes to achieve sustainable cleaner production.
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