RESEARCH ARTICLE

RETRACTED: Optimization of conditions for the biological treatment of textile dyes using isolated soil bacteria [version 1; peer review: retracted]

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Retraction


https://doi.org/10.12688/f1000research.13757.1) by Shafkat Shamim Rahman and colleagues, has been retracted by F1000Research on grounds of misconduct by the first author. Following publication of the article, the editorial team at F1000Research were notified by Romana Siddique, from BRAC University, that the data presented in this paper significantly overlaps with the data in her recently published article: Siddique and Alif; ARRB, 22(5): 1-12, 2018; Article no.ARRB.38637; https://doi.org/10.9734/ARRB/2018/38637.

In response to our queries to the authors, the second and last author listed on this article, Fahim Ahmed Alif and M. Mahboob Hossain, have stated that they were not aware of the submission of this article to F1000Research, and did not agree to be authors. We have evidence which confirms their statement. After further investigation by the F1000Research team, and a separate investigation by BRAC University, it has become clear that Shafkat Shamim Rahman was not involved with the research presented in this paper, and that the decision to submit and publish the article was taken independently by him, and not his listed co-authors. BRAC University has confirmed that Shafkat Shamim Rahman is not currently based at their institution.

Abstract

Background: In the 21st century, environmental pollution has been acknowledged as one of the major problems. The textile and dyeing industries contribute a major portion by discharging intensely complex effluent consisting of highly noxious azoic dyes.

Methods: In this study, biological treatment using acclimatized microorganisms were employed in search of a cheap and eco-friendly substitute for color removal from textile waste. The microbial inocula were isolated from effluent soil samples and then applied to flasks containing azo dyes as the only source of carbon for decolorization.
**Results:** Biochemical tests postulated predominance of *Enterococcus* and *Bacillus* bacterial strains. CO isolate or *Bacillus farrarinis* emerged as the best decolorizer of Orange M2R dye, decolorizing 98% of the dye. BG isolate or *Paenibacillus macerans* showed maximum decolorization on Green GS dye that decolorized 97% of the dye. The optimum physiochemical condition for decolorization of OM2R and GGS dye was pH 7.0, 2% NaCl conc., 1% initial dye conc. and 37°C temperature by the selected isolates.

**Conclusions:** The findings were validated and have the potential for bioremediation in textile waste effluent treatment plants.

**Keywords**
Azo dye; decolorization; Enterococcus; Bacillus; optimization

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**Author roles:** Rahman SS: Conceptualization, Formal Analysis, Validation, Writing – Original Draft Preparation, Writing – Review & Editing; Alif FA: Data Curation, Formal Analysis, Investigation, Methodology, Resources; Hossain MM: Conceptualization, Project Administration, Supervision

**Competing interests:** No competing interests were disclosed.

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Retracted: 12 Jun 2018
Editorial note:

23rd May 2018: Significant concerns have been raised about the overlap in the data presented in this paper, and that of another recently published article [Siddique and Alif; ARRB, 22(5): 1-12, 2018; Article no.ARRB.38637; https://doi.org/10.9734/ARRB/2018/38637]. In addition, the second and last author listed on this article, Fahim Ahmed Alif and M. Mahboob Hossain have stated that they were not aware of the submission of this article to F1000Research, and did not agree to be authors. We are currently investigating this and have suspended all peer review activity in the meantime.
**Abbreviations**

OM2R - Orange M2R; GGS - Green GS; NaCl - sodium chloride; NaOCl - sodium hypochlorite; WAO - wet air oxidation; SM - salt media; NA - nutrient agar; O.D. - optical density; MR - methyl red; VP - Voges Proskauer; MIU - Motility Indole Urease Test; ml - milliliter; g/L - gram per liter.

**Introduction**

Industrialization has expanded in every corner of the globe in the 21st century and the textile industry has emerged as a leading sector. It uses thousands of tons of synthetic dyes (azo) annually. A large portion of those goes into water bodies untreated. Carcinogenic and recalcitrant molecules present in the dye penetrate into the ecosystem and harm every member of the system. Humans use the polluted water directly for daily necessities which may result in diseases such as cancer, new genetic mutations or changes in the DNA etc. becoming an epidemic.

The textile industry in Bangladesh accounts for 45% of all industrial employment and contributes 5% to the total national income. The industry employs nearly 4 million people, mostly women. Despite the significant economic contribution, it has brought with it a range of environmental problems, mostly pollution of water resources. The textile industry consumes large quantities of water for various processes and discards equally large volumes of wastewaters containing a variety of pollutants and coloring agents such as the azo dye.

It is estimated that over 2,80,000 tons of synthetic dyes are discharged in industrial effluent every year worldwide. Therefore, pollution from these discharges contaminated water bodies is becoming alarming. This sector is placing a major burden of water pollution in Bangladesh.

Textile wastewater is highly colored, resulting in the blocking of the majority of sunlight, thereby reducing the growth of aquatic animals and plants; it also contains the dissolved toxic substance and carcinogens. The serious damage of pollution is caused mainly due to the durability of the dyes in the environment. The dye wastewater is extremely toxic to both aquatic fauna and flora, crop plants, and human beings.

At present, there are several techniques that can be employed in dye removal from effluents. However, these methods are varied in efficiency due to the variety of existing dyes and to the effluents complexity, and the combination of various methods may be considered since each method showed its limitations. There are three categories of existing methods: physical, chemical and biological. Physical methods like Coagulation/Flocculation, Adsorption, Membrane filtration, and Ion exchange are expensive. Chemical methods like Fenton’s reagent, Ozone, Photochemical, Sodium hypochlorite (NaOCl), Electrolysis and wet air oxidation are not cost effective and produce toxic byproduct.

Biological treatment, in the form of bacterial degradation, has been mainly applied in the removal of azo dyes, which generally is resistant to aerobic degradation. However, its degradation was observed in anaerobic conditions, but aromatic amines are formed as a final product, which can be toxic, mutagenic and carcinogenic. Under these anaerobic conditions, it is not possible to degrade the aromatic amines formed, which in turn are only degraded in an aerobic environment. Thus, to achieve a complete degradation of azo dyes, a method that combines aerobic treatment of the dyes with the mineralization of aromatic amines under aerobic conditions should be applied.

This research aimed to identify effective dye degrading bacteria from effluent soil samples and optimize the physiochemical condition for their optimum growth.

**Methods**

**Soil sample and azo dye collection**

Four soil samples (A: 23°54'52.6"N, 90°15'32.6"E; B: 23°47'34.1"N, 90°20'05.3"E; C: 23°54'54.0"N, 90°20'05.3"E; D: 23°56'52.8"N, 90°00'12.1"E) were aseptically collected from a textile effluent disposal area, in Savar, Hemayetpur and Atia Bazaar in June 2015. Sterile plastic containers were used to carry the soil samples. The samples were stored at Microbiology and Biotechnology Research Laboratory under Dpt. of MNS, BRAC University in sterile plastic bags at 4°C (to keep the microorganism viable for later use). Commercially available azo dyes (Meera Dyestuff Industries, India) were collected from Mitford Textile Ltd in Dhaka.

**Inoculation in dye-containing media, isolation and screening**

One gram of each of the soil (effluent) samples (A, B, C, D) were taken to prepare a homogenous suspension. The suspension of each sample was individually applied to sixteen 1% dye solution of each sample was individually applied to sixteen 1% dye concentration media. 1% solution of OM2R (Meera Dyestuff Industries, India) were collected from Mitford Textile Ltd in Dhaka.

Biological treatment, in the form of bacterial degradation, has been mainly applied in the removal of azo dyes, which generally is resistant to aerobic degradation. However, its degradation was observed in anaerobic conditions, but aromatic amines are formed as a final product, which can be toxic, mutagenic and carcinogenic. Under these anaerobic conditions, it is not possible to degrade the aromatic amines formed, which in turn are only degraded in an aerobic environment. Thus, to achieve a complete degradation of azo dyes, a method that combines aerobic treatment of the dyes with the mineralization of aromatic amines under aerobic conditions should be applied.

This research aimed to identify effective dye degrading bacteria from effluent soil samples and optimize the physiochemical condition for their optimum growth.
best four selected isolates were cultured in 50 ml SM broth with three different dye concentrations of 1%, 3% and 5%; for pH (5 to 8; adjusted by adding drops of basic NaOH or acidic diluted HCl in the solution) (Model: E-201-C, China), for NaCl tolerance (added 2%, 4%, 6% and 8% concentrated Sigma brand solution to the broth and incubated), and for incubation (30°C, 37°C, 45°C and 55°C) and incubated (Model: SAARC) at 37°C (except temperature parameter) for a period of 5 days with corresponding dye OM2R and GGS. Then, O.D. was measured by adjusting the wavelength at 590nm for OM2R and 510nm for GGS (Model: UV-VIS spectrophotometer UVmini-1240; Shimadzu, Kyoto, Japan). Each experiment was repeated to validate the results. The representative data is the average of all results.

Decolorization was measured by: Decolorization (%) = \( \frac{(\text{Initial O.D.} - \text{Final O.D.})}{\text{Initial O.D.}} \times 100 \)

**Biochemical characterization**

Gram staining and biochemical tests were performed on the bacterial isolates according to the Microbiology Laboratory Manual. Standard protocols were followed by gram staining and then the dried slides were observed under a microscope. Motility tests, enzyme tests (indole utilization, urease test, citrate utilization, oxidase test, catalase test, starch hydrolysis, nitrate reduction), fermentation tests (carbohydrate fermentation, methyl red test, Voges-Proskauer test, arabinose test, fructose test, galactose test, glucose test, lactose test, maltose test, mannitol test, sucrose test, trehalose test) and salt tolerant tests were conducted according to individual standard protocols.

**Results**

Four isolates AO, BO, CO and DO were collected from effluent soil samples preliminarily inoculated for 5 days. After 5 days of reaction, 99% and 93% decolorization were achieved with the BG isolate in 1%, 3% and 5% dye concentration media respectively. CG isolates demonstrated a lesser rate in all three parameters (Table 2; Dataset 1). pH optimization showed, highest rate (90%) in pH 7 by AO isolate in OM2R dye and 92% by BG isolate in the same parameter. AO isolate also showed a slightly higher response in 2% NaCl media compared to CO. Both isolates resulted in gradually reduced rates of decolorization in 6%, 8% NaCl containing media. For GGS dye, BG isolate was superior to all other isolates achieving 81% decolorization in 2% NaCl. The optimum temperature was 37°C in all four isolates. AO achieved 93% decolorization and BG 90% in two different dye-containing media. This decolorization rate gradually decreased with temperature elevation (Table 3; Dataset 2). In summary, 1% dye concentration, pH 7, 2% NaCl and 37°C appeared to be the optimum physiochemical condition for dye decolorization.

**Biochemical tests results**

AO, BO, CO and DO isolates were gram -ve rod and AG and BG were gram -ve coccoid bacteria. BO and DO isolates were gram -ve cocci and AG and DG were +ve cocci and +ve coccoid. Other biochemical results (Table 4) also showed all the isolates were catalase, oxidase, nitrate and aerobic growth positive. All were capable of tolerating 6.5% NaCl in media and withstand 45°C. All recorded negative results for urease, citrate, VP, starch hydrolysis and 10%, 15% NaCl tolerance. Motility, Indole, MR, Casein hydrolysis, 7% NaCl soln. tolerance, carbohydrate tests produced mixed results (A mixture of positive and negative results, which indicated the disparity of a wide range of characteristics in these primarily unidentified organisms) (Table 4). Finally, software analysis using ABIS (Version: July 29, 2015) based on morphology characteristics and biochemical tests postulated AO as Enterococcus termitis, BO as Enterococcus camelliae, CO as Bacillus farraginis, AG as Bacillus muralis, BG as Paenibacillus macerans, CG as Bacillus decolorationis and DG as Macroccus brunensis. DO isolate remain unidentified.

### Table 1. Results of O.D. at fifth day after de-colorization of 1% concentration dye in SM broth at room temperature

<table>
<thead>
<tr>
<th></th>
<th>1st O.D.</th>
<th>2nd O.D.</th>
<th>Avg. O.D.</th>
<th>Decolorization (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AO</td>
<td>0.009</td>
<td>0.007</td>
<td>0.008</td>
<td>93</td>
</tr>
<tr>
<td>BO</td>
<td>0.025</td>
<td>0.037</td>
<td>0.031</td>
<td>74</td>
</tr>
<tr>
<td>CO</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>99</td>
</tr>
<tr>
<td>DO</td>
<td>0.025</td>
<td>0.021</td>
<td>0.023</td>
<td>81</td>
</tr>
<tr>
<td>AG</td>
<td>0.021</td>
<td>0.013</td>
<td>0.017</td>
<td>89</td>
</tr>
<tr>
<td>BG</td>
<td>0.009</td>
<td>0.013</td>
<td>0.011</td>
<td>93</td>
</tr>
<tr>
<td>CG</td>
<td>0.009</td>
<td>0.009</td>
<td>0.009</td>
<td>94</td>
</tr>
<tr>
<td>DG</td>
<td>0.043</td>
<td>0.033</td>
<td>0.038</td>
<td>76</td>
</tr>
</tbody>
</table>
### Table 2. Results of O.D. & de-colorization (%) with different concentrations of dye.

<table>
<thead>
<tr>
<th></th>
<th>O.D. &amp; De-colorization (%) in 1% conc.</th>
<th>O.D. &amp; De-colorization (%) in 3% conc.</th>
<th>O.D. &amp; De-colorization (%) in 5% conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1       Day 2       Day 3       Day 4       Day 5</td>
<td>Day 1       Day 2       Day 3       Day 4       Day 5</td>
<td>Day 1       Day 2       Day 3       Day 4       Day 5</td>
</tr>
<tr>
<td>Control</td>
<td>0           0           0           0           0</td>
<td>0           0           0           0           0</td>
<td>0           0           0           0           0</td>
</tr>
<tr>
<td>AO</td>
<td>0.031       0.025       0.019       0.013       0.009</td>
<td>0.093       0.041       0.036       0.024       0.020</td>
<td>0.018       0.013       0.008       0.007       0.004</td>
</tr>
<tr>
<td>CO</td>
<td>0.020       0.011       0.009       0.007       0.005</td>
<td>0.039       0.038       0.036       0.034       0.032</td>
<td>0.057       0.056       0.054       0.053       0.051</td>
</tr>
<tr>
<td>BG</td>
<td>0.025       0.018       0.013       0.009       0.005</td>
<td>0.078       0.077       0.075       0.074       0.073</td>
<td>0.098       0.097       0.096       0.095       0.094</td>
</tr>
<tr>
<td>CG</td>
<td>0.033       0.026       0.019       0.015       0.011</td>
<td>0.042       0.040       0.038       0.036       0.034</td>
<td>0.091       0.090       0.089       0.088       0.087</td>
</tr>
</tbody>
</table>

### Table 3. Results of O.D. & de-colorization (%) with different parameters at day 5.

<table>
<thead>
<tr>
<th></th>
<th>pH 5</th>
<th>pH 6</th>
<th>pH 7</th>
<th>pH 8</th>
<th>2% NaCl</th>
<th>4% NaCl</th>
<th>6% NaCl</th>
<th>8% NaCl</th>
<th>30°C</th>
<th>37°C</th>
<th>45°C</th>
<th>55°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AO</td>
<td>0.031</td>
<td>0.040</td>
<td>0.047</td>
<td>0.054</td>
<td>0.075</td>
<td>0.073</td>
<td>0.071</td>
<td>0.069</td>
<td>0.062</td>
<td>0.059</td>
<td>0.058</td>
<td>0.055</td>
</tr>
<tr>
<td>CO</td>
<td>0.034</td>
<td>0.038</td>
<td>0.042</td>
<td>0.049</td>
<td>0.070</td>
<td>0.067</td>
<td>0.065</td>
<td>0.063</td>
<td>0.056</td>
<td>0.054</td>
<td>0.052</td>
<td>0.048</td>
</tr>
<tr>
<td>BG</td>
<td>0.042</td>
<td>0.055</td>
<td>0.051</td>
<td>0.069</td>
<td>0.098</td>
<td>0.096</td>
<td>0.094</td>
<td>0.092</td>
<td>0.086</td>
<td>0.087</td>
<td>0.085</td>
<td>0.083</td>
</tr>
<tr>
<td>CG</td>
<td>0.044</td>
<td>0.061</td>
<td>0.080</td>
<td>0.098</td>
<td>0.106</td>
<td>0.104</td>
<td>0.102</td>
<td>0.100</td>
<td>0.096</td>
<td>0.096</td>
<td>0.095</td>
<td>0.093</td>
</tr>
</tbody>
</table>

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Table 4: Results of biochemical and sugar tests of the isolates collected from Nutrient agar.

| Isolate no | Sample Isolate Name | Gram stain | MIU | Carbohydrate | Catalase | Oxidase | Simmons Citrate | MR | VP | Casein hydrolysis | Nitrate Reduction | Starch Hydrolysis | 45°C | 6.5% NaCl soln. | 7% NaCl soln. | 10% NaCl soln. | 15% NaCl soln. | Aerobic Growth | Presumptive Organism               |
|------------|---------------------|------------|-----|--------------|----------|---------|-----------------|----|----|-----------------|-----------------|-----------------|-----|----------------|-------------|----------------|----------------|----------------|----------------|----------------|-----------------------------------|
| 1. AO      | - rod               | -/±        | +   | -            | +        | -       | -               | +  | -  | -               | +               | -               | -   | +              | -            | -              | -              | -              | +              | Enterococcus termitis              |
| 2. BO      | - cocci             | - -        | +   | -            | +        | -       | +               | -  | -  | +               | +               | -               | -   | -              | -            | -              | -              | +              | -              | Enterococcus camelliae              |
| 3. CO      | - rod               | - -        | -   | -            | -        | -       | -               | +  | -  | +               | -               | -               | -   | +              | -            | -              | -              | -              | +              | Bacillus farraginis                |
| 4. DO      | - cocci             | - -        | +   | -            | +        | -       | -               | +  | -  | +               | -               | -               | -   | +              | -            | -              | -              | +              | -              | Unknown texon                     |
| 5. AG      | + rod               | - -        | +   | +            | +        | -       | +               | -  | -  | -               | +               | -               | -   | +              | -            | -              | -              | +              | -              | Bacillus muralis                  |
| 6. BG      | + rod               | - -        | +   | +            | +        | -       | +               | +  | -  | +               | +               | -               | +   | -              | -            | -              | -              | -              | +              | Paenibacillus macerans              |
| 7. CG      | - rod               | + -        | +   | +            | +        | -       | +               | -  | -  | +               | -               | -               | -   | +              | -            | -              | -              | +              | -              | Bacillus decolorationis            |
| 8. DG      | + cocci             | - -        | +   | +            | -        | +       | +               | +  | -  | +               | +               | -               | -   | +              | -            | -              | -              | +              | +              | Macroccoccus brunensis              |

+/± = Positive reaction; - = Negative reaction
Discussion

Most of the isolates from the different soil samples were identified as *Bacillus* species. Dye decolorizing ability of isolates was investigated independently. CO (*B. farraginis*) showed the highest dye decolorization capacity (98%) in SM broth media containing 1% OM2R dye for 5 days at 37°C. However, as the concentration of dye increased up to 3% and 5% the decolorization rate decreased to 94% and 90% respectively, because of the intensity of azo dyes. On GGS dye degradation BG (*P. macerans*) showed 97% decolorization. This was similarly effective at 3% dye concentration (94%), however, the rate was found to slump at 5% dye concentration (81%).

pH is one of the important abiotic factors that affect the growth and metabolic homeostasis. The effect was studied at different pH values (5 – 8). At pH 7.0 AO (*E. termitis*) gave maximum decolorization (90%). A similar trend was observed at pH range of 5.0 and 6.0, with a swift reduction (59%) observed at pH 8 by AO (*E. termitis*). These results suggest that acidic pH values may influence the activity of the enzyme causing denaturation. Chang et al. (2001)11 found that azo reductase performance was affected by pH, with 5 times better dye reduction at pH 7.0, than below pH 4. These findings correspond well with the high decolorization found between pH 7 - 9.39.

In the case of GGS, the maximum decolorization rate was attained at pH 7.0 by BG (*P. macerans*) at 92%. The majority of the azo dye reducing bacterial species reported so far were able to reduce azo dye at pH near 7.0-3. The requirement of near neutral pH for optimum growth had been reported in several studies.13-15 Results indicate that a pH increase from 5.0 to 7.0 enhanced the decolorization of GGS dyes. At pH 5 the decolorization rate was 80% of dye by BG (*P. macerans*). A small increase was observed at pH 6 (85%) with an abrupt decrease at pH 8.0 (73%). It was observed that better decolorization rates were around pH 6 - 7 bands for both of OM2R and GGS dye by the selected isolates.

Decolorization percentage of OM2R by selected isolates was found to vary with different concentration (2 - 8 g/L) of NaCl when studied for 120 hours at 37°C. Maximum decolorization of OM2R by AO (*E. termitis*) was observed as 81% at 2% NaCl, but the percentage decolorization of dye started to decrease with increases of NaCl concentration (Table 3; Dataset 228). The decolorization attained by AO (*E. termitis*) at 35°C for 4%, 6% and 8% NaCl was 60%, 43% and 37%. Kargi and Dincer (1996)36 mention that high salt concentrations (2-10% salt) are known to cause plasmolysis and/or loss of cell activity.

Similarly, at 2% NaCl concentration the degradation percentage of GGS dye was 85% by BG (*P. macerans*). The decolorization attained by BG (*P. macerans*) at 37°C for 4%, 6% and 8% NaCl was 72%, 67% and 40% (Table 3; Dataset 228).

To determine the optimum temperature for dye decolorization a temperature range of 30°C –55°C was examined. As seen in Table 3 the optimum temperature for OM2R dye decolorization was 37°C. The AO (*E. termitis*) attained a maximum decolorization of 88%. Angelova et al. (2008)37 found that the azo bond reduction rate rose with an increased temperature, maximum rate of around 40°C, 3–5 times faster than at 20°C. At 30°C and 45°C the degradation rate for OM2R by AO (*E. termitis*) was 82% and 72% of dye. A low decolorization of 59% of the dye was detected at 55°C by AO (*E. termitis*) isolate. Temperatures above 55°C were not studied since results showed that the increase from 37°C to 45°C promoted a marginal decrease in dye decolorization (Table 3; Dataset 228).

The optimum temperature for GGS dye decolorization was 37°C for the BG isolate (*P. macerans*), attaining a maximum decolorization of 90% of dye. In the case of BG, at 30°C and 45°C the decolorization percentage was 86% and 84% of dye respectively. No improvement in dye decolorization was observed at temperatures above 45°C, with low decolorization of 58% and 45% of dye detected at 55°C by BG (*P. macerans*) isolate as evident from Table 3. BG (*P. 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.

Conclusion

Traditional wastewater treatment is inefficient and remains a threat to environment18. Biotreatment offers an easy, cheap and effective alternative for color removal of textile dyes19. Hence, economic and eco-friendly techniques using bacteria can be an alternative method. The present study strongly concluded that
the bacterial isolates *E. termitis*, *E. camelliae*, *B. decolorationis*, *P. macerans* species were a good microbial source for textile effluent treatment, in biological degradation of textile dye. However, decolorization potential of the isolates needs to be validated by demonstration in appropriate bioreactors before its application.

**Data availability**

Dataset 1: Results of optical density (O.D) & de-colorization (%) with different concentrations of dye. Average O.D. calculated from 1st and 2nd O.D. 10.5256/f1000research.13757.d19816427

Dataset 2: Results of optical density (O.D) & de-colorization (%) with different parameters at day 5. Average O.D. calculated from 1st and 2nd O.D. 10.5256/f1000research.13757.d19816528

### Competing interests

No competing interests were disclosed.

### Grant information

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### References


for the biological treatment of textile dyes using isolated soil bacteria.  

Data Source


Data Source

Publisher Full Text

Publisher Full Text

Publisher Full Text

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