RESEARCH ARTICLE

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

Shafkat Shamim Rahman 1,2, Fahim Ahmed Alif 1, M. Mahboob Hossain 1

1Department of Mathematics and Natural Sciences, BRAC University, Dhaka, 1212, Bangladesh
2United Surgical (BD) Ltd, Kadda, Gazipur, 1702, Bangladesh

Retraction

The article titled “Optimization of conditions for the biological treatment of textile dyes using isolated soil bacteria” ([version 1; referees: peer review discontinued], F1000Research 2018, 7:351 https://doi.org/10.12688/f1000research.13757.1) by Shafkat Shamim Rahman and colleagues, has been retracted by F1000Research on grounds of misconduct by the first author.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 farraginis 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
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.
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 go 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 discharges 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 wastewaters is becoming alarming. This sector in place is the major source 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 wastewater. Azo dyes are widely known coloring agents used in industries and hence commonly released in the environment. The dye wastewaters are 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 dye, a method that combines anaerobic 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°50′52.6″N, 90°15′32.6″E; B: 23°47′44.1″N, 90°18′34.1″E; C: 23°46′54.0″N, 90°20′05.3″E; D: 23°56′52.8″N, 90°01′01.2″E) were aseptically collected from an effluent disposal area, in Savar, Hemayetpur and Arinbazar 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 Jet 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 containing (eight Orange M2R - OM2R and eight Green GS containing (eight Orange M2R - OM2R and eight Green GS) SM broth media (glucose - 10 g/L; #G8270, dipotassium phosphate - 0.6 g/L; #1551128 USP, peptone - 10 g/L; #1551139 USP, magnesium sulfate - 1 g/L; #M2643, yeast extract - 1 g/L; #P7750, monopotassium phosphate - 1.9 g/L; #1551118 USP, sodium chloride - 1 g/L; #Y1625, pH: 6.0 - 6.4; Sigma Aldrich, St Louis, MO, USA) to detect the dye degrading capability. 1% solution of OM2R and GGS was made by adding 0.5g of dye into the 50ml dye mixture of different types of microorganism, up to 10-4 and 10-5 dilutions were performed, and spread plate technique applied on NA plates followed by incubation for 24 hours at 37°C to obtain eight soil isolates (AO, BO, CO, DO, AG, BG, CG, DG). Selected isolates, based on morphology, were enriched in NA media and incubated for 24 hours at 37°C. The plates were sealed, refrigerated at 4°C and were frequently subcultured.

Media optimization

Optimum growth conditions for the isolates were identified applying different physiochemical state (dye concentration, pH, NaCl concentration, temperature) to the time of growth. The

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.
Biochemical characterization

Gram staining and biochemical tests were performed on the bacterial isolates according to the Microbiology Laboratory Manual\(^1\). 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 tolerance tests\(^2\) 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. The soil samples were cultured in SM broth containing 1% 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:

\[
\text{Decolorization (\%)} = \left( \frac{\text{Initial O.D.} - \text{Final O.D.}}{\text{Initial O.D.}} \right) \times 100
\]

Dye concentration optimization

After five days of reaction, CO isolate achieved 98% decolorization in SM broth containing 1% OM2R dye. The same rates were also observed in 3% dye concentration. AO lagged in all three (1%, 3% and 5%) reactions. Degradation rate gradually decreased at higher concentration dye-containing media. Intriguingly, CO isolates resulted in 93% decolorization for both 1% and 3% dye concentration.

In GGS dye-containing media, 97%, 94% and 81% decolorization were achieved with the BG isolate in 1%, 3% and 5% dye concentration media respectively. CO isolates demonstrated a lesser rate in all three parameters (Table 1; Dataset 1\(^3\)).

In summary, 1% dye concentration, pH 7, 2% NaCl and 37°C appeared to be the optimum physicochemical condition for dye decolorization.
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>Day 1</td>
<td>Control 0 0 0 0 0</td>
<td>Day 1 0 0 0 0</td>
<td>Day 1 0 0 0 0</td>
</tr>
<tr>
<td></td>
<td>AO 0.031 74 0.025 79 0.013 84 0.009 93</td>
<td>AO 0.041 72 0.036 74 0.024 82 0.020 86</td>
<td>AO 0.047 72 0.036 78 0.030 82 0.024 85</td>
</tr>
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<td></td>
<td>CO 0.020 83 0.011 90 0.009 93 0.007 98</td>
<td>CO 0.029 78 0.022 84 0.015 89 0.011 92</td>
<td>CO 0.038 77 0.030 82 0.021 87 0.020 88</td>
</tr>
<tr>
<td></td>
<td>BG 0.025 85 0.018 89 0.013 92 0.009 95</td>
<td>BG 0.031 83 0.021 90 0.019 89 0.016 91</td>
<td>BG 0.079 63 0.066 69 0.051 76 0.044 80</td>
</tr>
<tr>
<td></td>
<td>CG 0.033 80 0.026 85 0.019 89 0.015 91</td>
<td>CG 0.042 76 0.032 86 0.022 88 0.017 90</td>
<td>CG 0.098 54 0.078 63 0.062 71 0.054 75</td>
</tr>
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</table>

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

<table>
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<tr>
<th></th>
<th>pH 5 0 0</th>
<th>pH 6 0 0</th>
<th>pH 7 0 0</th>
<th>pH 8 0 0</th>
<th>2% NaCl 50 0</th>
<th>4% NaCl 50 0</th>
<th>6% NaCl 50 0</th>
<th>8% NaCl 50 0</th>
<th>30°C 0 0 0</th>
<th>37°C 0 0 0</th>
<th>45°C 0 0 0</th>
<th>55°C 0 0 0</th>
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<td>Control</td>
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<td>Control 0 0 0 0 0 0 0 0 0 0 0 0 0</td>
<td>Control 0 0 0 0 0 0 0 0 0 0 0 0 0</td>
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<tr>
<td></td>
<td>AO 0.031 77 0.040 73 0.018 90 0.051 59 0.031 81 0.054 60 0.032 87 0.075 37 0.023 82 0.008 93 0.040 72 0.053 59</td>
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<tr>
<td></td>
<td>CO 0.034 75 0.038 74 0.020 89 0.059 52 0.049 71 0.057 58 0.076 40 0.079 34 0.022 86 0.010 92 0.025 82 0.055 57</td>
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<td>BG 0.042 80 0.055 85 0.051 92 0.089 73 0.019 89 0.045 72 0.070 62 0.098 43 0.031 86 0.025 90 0.035 84 0.075 58</td>
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<td></td>
<td>CG 0.044 79 0.061 83 0.080 88 0.098 70 0.035 81 0.058 65 0.086 54 0.100 36 0.045 70 0.028 99 0.049 77 0.093 45</td>
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</table>
Table 4: Results of biochemical and sugar tests of the isolates collected from Nutrient agar.

<table>
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<th>Isolate no</th>
<th>Sample Isolate Name</th>
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<th>Shape</th>
<th>Motility</th>
<th>Indole</th>
<th>Urease</th>
<th>Glucose</th>
<th>Fructose</th>
<th>Sorbitol</th>
<th>Mannitol</th>
<th>Malitol</th>
<th>Arabinose</th>
<th>Galactose</th>
<th>Trehalose</th>
<th>Inositol</th>
<th>Citrate</th>
<th>MR</th>
<th>VP</th>
<th>45° C</th>
<th>6.5% NaCl soln.</th>
<th>7% NaCl soln.</th>
<th>10% NaCl soln.</th>
<th>15% NaCl soln.</th>
<th>Aerobic Growth</th>
<th>Presumptive Organism</th>
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<tbody>
<tr>
<td>1. AO</td>
<td>- rod</td>
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<td>-</td>
<td>+</td>
<td>Enterococcus termitis</td>
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<td>2. BO</td>
<td>- cocci</td>
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<td>+</td>
<td>Enterococcus camelliae</td>
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<td>3. CO</td>
<td>- rod</td>
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<td>+</td>
<td>Bacillus farraginis</td>
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<td>4. DO</td>
<td>- cocci</td>
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<td>5. AG</td>
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<td>+</td>
<td>Bacillus muralis</td>
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<td>6. BG</td>
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<td>+</td>
<td>Paenibacillus macerans</td>
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<td>7. CG</td>
<td>- rod</td>
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<td>+</td>
<td>Bacillus decolorationis</td>
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<td>8. DG</td>
<td>+ cocci</td>
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<td>-</td>
<td>+</td>
<td>Macrococcus brunensis</td>
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</table>

+/= 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*) showed maximum decolorization (90%). A similar rate 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)14 found that azo reductase performance was affected by pH, with 15% better dye reduction at pH 7.0 than below pH 5. These findings correspond well to the high decolorization found between pH 7 - 9.35.

In the case of GGS, the maximum decolorization rate was attained at pH 5 by BG (*P. macerans*) at 92%. The majority of the azo dye reducing bacterial species reported so far were able to reduce dyes at pH near 7.0-7.2. The requirement of near neutral pH for optimum growth had been reported in several studies.15-16. Results indicate that a pH increase from 5.0 to 7.0 enhances 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 was found to decrease with increases of NaCl concentration (Table 3; Dataset 2). The decolorization attained by AO (*E. termitis*) at 37°C for 4%, 6% and 8% NaCl was 60%, 43%, and 37%. Kargi and Dincer (1996)36 mention that high salt concentrations (≥1% salt) are known to cause plasmolysis and/or loss of cell viability.

Similarly, at 2% NaCl concentration the degradation percentage of GGS dye was 99% by BG (*P. macerans*). The decolorization attained by BG (*P. macerans*) at 37°C for 4%, 6% and 8% NaCl was 72%, 65%, and 40% (Table 3; Dataset 2). The optimum temperature for dye decolorization at 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. AO (*E. termitis*) attained a maximum decolorization of 33%. 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 shown that the increase from 37°C to 45°C promoted a marginal decrease in dye decolorization (Table 3; Dataset 2).

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. The optimal 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 environment.18. Biotreatment offers an easy, cheap and effective alternative for color removal of textile dyes.19. 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.d198164

**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.d198165

### Competing interests

No competing interests were disclosed.

### Grant information

The author(s) declared that no grants were involved in supporting this work.

### Acknowledgements

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### References


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