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ELUCIDATION OF MICROBIAL DECOLOURIZATION AND DEGRADATION EFFICIENCY OF RECALCITRANT-CARCINOGENIC AZO DYE CONGO RED BY NOVEL BACTERIAL STRAINS

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ABSTRACT
Novel bacterial strains capable of decolorizing textile dyes were isolated from dye contaminated soil obtained from the vicinity of African Textiles Industry Challawa, Kano - Nigeria. The Carcinogenic azo dye Congo red used widely in various industrial processes poses terrific environmental concern, causing major health problems to human beings. In the present study, decolourization of Congo red by newly isolated antibiotics sensitive novel bacterial strains from dyes contaminated soil of a treatment plant capable of decolorizing organic dyes were investigated and identified as Rhodococcus rhodochrous and Pseudomonas aeruginosa strains by 16S rDNA gene sequence analysis. Decolorization of Congo red dye using these bacterial strains was assayed by screening and optimizing various parameters to determine the optimal conditions required for maximum decolourization. Rhodococcus rhodochrous and Pseudomonas aeruginosa showed maximum decolourization upon incubation for 24 hrs at 37 °C and pH 7. Enhanced decolourization was achieved by amendment with glucose and peptone as well as carbon and nitrogen sources in the culture medium. The bacterial strains showed great potential in the decolourization of Congo red dye up to 96.1 % for Rhodococcus rhodocchrous and 91.1 % for Pseudomonas aeruginosa in the aqueous environment at optimal conditions. High performance liquid chromatography (HPLC) analysis confirmed that dye decolourization occurred due to the breakdown of dye molecules into colourless end products. The less toxic nature of the dye degraded products was observed by microbial toxicity assay of agricultural significant bacteria and this can be exploited for the bioremediation of different classes of textile dyes and their derivatives containing wastes.

Keywords*: Antibiotics sensitive, bacterial strains, decolourization, microbial toxicity, textile dyes*

1. Introduction

Textile industry generated wastewater as a complex mixture of many pollutants such as heavy metals, chlorinated compounds, pigments and dyes (Celia and Suruthi, 2016; Hamisu *et al.,* 2016). It is estimated that approximately 15 % of the dyestuffs are lost in the industrial effluents during manufacturing and processing operations (Khaled *et al.,* 2009; Soni *et al.,* 2016). The presence of textile dye even in low concentration in effluent is highly visible and undesirable (Rajabi *et al.,* 2016). There are more than 100,000 commercially available dyes with over 700,000 tons of dyestuff produced annually (Pirbazari *et al.,* 2015; Buthiyappan *et al.,* 2016). Synthetic dyes are chemically diverse and divided into azo, triphenylmethane or heterocyclic/polymeric structures (Cheubarn *et al.,* 2008). These dyes are designed to be stable and long lasting colorants and are usually recalcitrant in natural environment. After release into water bodies, these dyes have negative impact on photosynthesis of aquatic plants and the azo group (N = N) in dyes are converted to aromatic amines which are possible human carcinogens (Banat *et al.,* 1996). Some dyes and their breakdown products also have strong toxic and mutagenic effect on living organisms (Mahmood *et al.,* 2016). Discharge of textile dyes without appropriate treatment may lead to bioaccumulation that may incorporate into food chain and upset human health (Mahbub *et al.,* 2012; Celia and Suruthi, 2016). Considering this effluent load, physicochemical methods viz, membrane filtration, reverse osmosis, ozonation, electrochemical destruction, photocatalysis, ion exchange, coagulation, use of activated carbon, Fenton's reagent, and chemical flocculation are mostly the widely used treatment methods adopted by industries. These methods are expensive and produce high amount of chemical-containing secondary pollution (Banat *et al.,* 1996; Jadhav *et al.,* 2008).

On the other hand, biological treatment based on microbial transformation of textile dyes hold promises in providing a lower treatment cost and a more efficient means of effluent treatment (Dos Santos *et al.,* 2007). A number of microorganisms namely *Pseudomonas*, *kurthia*, *Aeromonas*, *Proteus mirabilis*, *Rhodococcus globerulus*, *Bacillus* spp., *Micrococcus luteus*, *Staphylococcus aureus* and white rot fungus-*Phanerochaete,* have previously been reported to possess the capability of decolorizing textile dye (Zimmermann *et al.,* 1984; Swamy & Ramsay, 1999; Chen *et al.,* 2008; Joshi *et al.,* 2008; Mahbub *et al.,* 2011). This study was undertaken to investigate the ability of two selected indigenous novel bacterial strains for their ability to degrade synthetic commercial dye 'Congo red' with the logical application of optimum

kinematics to determine an effective and efficient decolourization and degradation under varying environmental conditions.

2. Materials and Methods

2.1 Collection of Samples / Sampling Area

The soil samples were collected from dyes contaminated soils polluted by effluents emanating from the Common Effluent Treatment Plant of African Textiles Industry, Kano-Nigeria, in sterile plastic containers. The samples were brought to the laboratory and processed within 48 hrs. These samples were used in the isolation of indigenous adapted microbes.

2.2 Chemicals and dyestuffs

Microbiological media and ingredients were purchased from Oxoid and Aldrich (USA) and all other chemicals used in the study were of analytical grade and obtained from Oxoid and Aldrich. The textile dye subjected to investigation was courteously provided by the African Textiles Industry Kano, Nigeria.

2.3 Isolation of bacterial strains from textile effluents

The present studies were carried out using soil samples collected from sites polluted by effluents from the textile industry. Bacterial strains having potentials for the degradation of textile industry dye Congo red were isolated from the soil sample through enrichment technique using Congo red (50 mgL^{-1}) supplemented nutrient broth and MSM media. The composition of MSM used was (gL^{-1}): Na_2HPO_4 3.6, KH_2PO_4 1.0, $MgSO_4$ 1.0, $(NH_4)_2SO_4$ 1.0, $CaCl_2.2H_2O$ 0.1, and Fe (NH_4) citrate 0.01. To this media, trace element solution (10 ml) of the following composition was added: ZnSO4.7H2O 10.0, $MnCl_2.4H_2O$ 3.0., $CoCl_2.6H_2O$ 1.0, $NiCl_2.6H_2O$ 2.0, $Na_2MoO_3.2H_2O$ 3.0, H_3BO_3 30.0 and $CuCl_2.2H_2O$ 1.0. Final pH of the medium were adjusted by pH meter (Santorious Professional Meter PP15) to 7.0 using 1N HCl and 1N NaOH solution (Khehra *et al.,* 2008).

2.4 Screening for decolorizing of dye by bacterial strains

In order to select the potential and efficient Congo red dye degrading bacteria, the isolates was subjected to screening in liquid medium (MSM). Therefore, an inoculums of 10 ml of absorbance (A_{600}) at 600 nm was used to inoculate 90 ml of MSM supplemented with 50 mgL^{-1} of Congo red dye, and media was dispensed in 250 ml of Erlenmeyer flask, the flask were separately incubated under shaking (150 rpm) as well as static condition at 37 °C for seven (7) days. The aliquot (3 ml) of the culture media was aseptically withdrawn after every 24 hrs and centrifuged at 10,000 rpm for 15 min. The supernatant obtained after centrifuging was read at 612 nm i.e. λ_{max} of reactive Congo red dye using a scanning UV-visible spectrophotometer (Agilent 8453). Biotic and abiotic controls were run side by side. The decolourization activity (%) of each isolates was calculated using the following equation (Adedayo *et al.,* 2004).

$$Decolourization\ (\%) = \frac{\text{Initial dye concentration} - \text{Residual dye concentration}}{\text{Initial dye concentration}} \times 100 \quad (i)$$

2.5 Identification of dyes decolorizing bacterial strains

Bacterial cells from cultures were collected by centrifugation at 10,000 rpm for 10 min. The identification of strains was performed by using standard morphological, gram staining, biochemical and molecular characterization methods as described by Franciscon *et al.* (2012). The sequential digestion was done by lysozyme (2.5 mg/ml, 37 °C for 1 h) and proteinase K (200 mg/ml in 1 % SDS, 55 °C for 1 h), followed by incubation in 1 % CTAB and 0.7 M NaCl at 65 °C for 15 min. Ice cold ethanol was used for the precipitation of DNA after extraction with phenol/chloroform, and then dissolved in ddH_2O. The universal 16S rDNA primers 8f (5′-GAG TTT GAT CAT GGC TCA G-30) and 1495r (5′-CTA CGG CTA CCT TGT TAC G-30) were used and PCR amplification was performed in total reaction volume of 20 µl by using Master Mix RED (150 mM Tris-HCl, pH 8.5, 40 mM $(NH4)_2SO_4$, 4.0 mM $MgCl_2$, 0.2 % Tween 20; 0.4 mM dNTPs; 0.05 U/µl Ampliqon *Taq* DNA polymerase; inert red dye and a stabilizer).

The PCR programming used were 94 °C for 5 min, 30 cycles at 94 °C for 1 min, 54 °C for 1 min and 72 °C for 3 min and final extension was at 72 °C for 10 min (Su *et al.,* 2007). The amplified PCR product was detected on 0.8 % agarose gel. Nucleotide sequences of purified PCR products were determined by sequencing, using two universal primers 8f and 1495r (Staley *et al.,* 2001). The length of 16S r DNA gene sequence was found to be 387 base pairs and this sequence was subjected to BLAST search tool in gene database of NCBI (http://www.ncbi.nlm.nih.gov/blast/blast.cgi) and corresponding sequences were downloaded. ClustalW multiple sequence alignment tools were used for the alignment of sequences (Ooi *et al*., 2007).

2.6 Culture maintenance

Bacterial strains were routinely maintained on BHBY medium containing 100 mg/L Congo red dye and stored at 4 °C. Decolourization experiment was set up from acclimatized culture (grown for 24 h) with 6 x 10^8 cells/mL culture density (1.0 O.D at 600 nm) as inoculums.

2.7 Establishment of antibiotics susceptibility assay

The indigenous bacterial isolates were subjected to antibiotics susceptibility assay using the bauer-kirby method that has been standardised by NCCLS (Adzitey *et al.,* 2013) and evaluated by the methods of National Committee for Clinical Laboratory Standards. Isolates grown overnight on Nutrient agar were suspended in sterile normal saline (0.9 % w/v NaCl) with turbidity was equivalent to 0.5 Macfarlan's standard. Antibiotic multi disc; Dodeca universal I (DE001) and V (DE013), Dedeca G1 minus (DE003) and G2 minus (DE010), Dodeca Pseudo 1 (DE020) were gently and aseptically placed on the agar plates to ensure complete contact

using a sterile forceps, and all plates incubated (Gallenkamp England model IH -150) at 37 °C for 18 hrs (Mills-Robertson *et al.,* 2003). The diameters of zones of inhibition measured after incubation to the nearest millimetre were interpreted using the chart adopted from National Committee for Clinical Laboratory Standards (Jones *et al.,* 2002).

2.8 Effect of different environmental parameters on dye decolourization

As the biological decolourization of Congo red depends on various cultural and environmental parameters such as pH (5 - 9); temperature (30 – 45 °C); inoculums size i.e. 2 – 10 % (v/v); carbon sources (Glucose, sucrose, maltose, lactose, fructose and mannitol); nitrogen sources including organic and inorganic (yeast extract, peptone, potassium nitrate, ammonium sulphate and sodium nitrate); concentration of glucose i.e. 0 – 4 gL^{-1}; yeast extract i.e. 0 - 2 gL^{-1}; different initial Malachite green dye concentration i.e. 50 - 300 mgL^{-1}; NaCl i.e. 0 - 100 gL^{-1}; and metal compounds (10 mgL^{-1}) including $CuCl_2$, $CoCl_2$, $CdCl_2$, $AgNO_3$ and $HgCl_2$. The classical method of process optimization was adopted by altering one parameter at a time in each experiment, while keeping the previously optimized parameters at constant. Decolourization efficiency at the respective optimized conditions for each of the selected isolates was determined. In addition, the relationship between the growth and the decolourization process was also monitored. For this, optical density (OD) at 300 – 600 nm was measured using UV-vis spectrophotometer (Agilent 8453) (Khan, 2015).

2.9 Estimation of biological oxygen demand (BOD) and chemical oxygen demand (COD)

1ml of initial medium containing dye solution, decolourized medium, distilled water was added to airtight BOD bottles sample 1, sample 2, and blank respectively. Place desired volume of water in a suitable bottle and add 1ml of each of Phosphate buffer, $MgSO_4$, 4FeCl and seeding/L of water. Before use 3 bring dilution water was brought to 20 °C. Dilution water was aerated with organic free filtered air. All the bottles are kept in BOD incubator at 20 °C for 5 days. After incubation 1ml of $MnSO_4$, Alkali iodide solution and sulphuric acid was added to form brown color solution. After colour formation they were titrated against their $NaSO_4$ for their BOD values and the readings were noted.

$$BOD_{mg/l} = \frac{(B - T) \times 250}{S} \qquad (ii)$$

where; B (v) = volume of $NaSO_4$ used for blank, T (v) = volume of $NaSO_4$ used for sample 2, S (v) = volume of sample.

While for COD, the chemical oxygen demand was measured by the standard Potassium dichromate method. 1 ml of initial medium containing dye solution, decolorized medium, distilled water was added to COD tube sample 1, sample 2, and blank respectively (Mahbub *et al.,* 2012). Then 1.5 ml of distilled water and reducing agent potassium dichromate and 3.5 ml COD acid were added to each tube. Duplicates were put up for all the tubes. All the tubes were kept in the COD incubator at 148 °C for 2 hrs. After incubation the entire content were transferred to a conical flask. A drop of ferroin indicator was added to it and was titrated against FAS in the burette and readings were noted.

$$COD_{mg/l} = \frac{(A - B)\ N \times \text{Equivalent weight of } O_2}{\text{Volume of Sample}} \qquad (iii)$$

where; A = Volume of FAS used for blank, B = Volume of FAS used for sample, N = Normality of FAS – 0.1, Equivalent weight of oxygen

COD values were compared between the initial medium containing dye solution and decolorized medium.

2.10 High performance liquid chromatography (HPLC) analysis

UV-vis absorption of the control and treated Congo red dye samples were observed in the range 300 – 800 nm using a scanning UV-visible spectrophotometer (Agilent, 8453). Congo red dye concentration was monitored at 615 nm i.e. λ_{max} of Congo red using standard dye solution. The degradation products of rb 35 was evaluated by HPLC (Shimadzu, SCL-10AVP) equipped with a reverse phase C -18 column (Princeton, 4.6 nm x 150 mm) at 25 °C and a dual wavelength by isocratic method. The mobile phase was methanol with flow rate of 1 $mLmin^{-1}$ for 10 min. (Surwase *et al.*, 2013). The 10 µl of sample was extracted manually into injector port.

2.11 Toxicity assessment

The toxicity of dyes (1000 mg L^{-1}) and its biodegraded products was assayed for their toxicity effect by performing microbial toxicity on the agricultural important bacterial flora by using well diffusion assay according to the method reported earlier (Sheth and Dave, 2009; Gottlieb *et al.,* 2003; Mali *et al.,* 2000). Two well of 5.0 mm diameter were made on the respective media containing plates and filled with untreated dye (1000 mgL^{-1}) and with decolorized centrifuged broth. The plates were incubated at 30 ± 1 °C for 48 hrs. Zone of inhibition surrounding the well represent the index of toxicity and were measured after 48 hrs.

3. Results and Discussion

A total of eighteen (18) morphologically distinct colonies were isolated, purified and screened for the decolourization and degradation of azo dyes from sludge sample incubated under shaking and static conditions. Of all the isolates, six (6) bacterial isolates were further screened for dyes degradation and finally two (2) bacterial isolates was selected on the basis of dyes tolerance i.e. resistance in minimal medium containing 2 % Congo red. The isolates under static condition showed remarkable decolourization compared to shaking. The two bacterial strains with the potentials of 90 % decolourization (96.1 and 91.1 % respectively) were selected and subjected to identification, antibiotic

susceptibility assay and optimization. On the basis of their cultural, biochemical properties, gram's staining reaction and finally through genes sequence analysis. The isolates were identified and designated as *Rhodococcus rhodochrous* and *Pseudomonas aeruginosa* ARSKS20. Microorganisms used for biodegradation purpose should not be pathogenic or not to be resistant to antibiotics, therefore antibiotics susceptibility of the culture towards wide range of antibiotics was evaluated and observed that it showed susceptibility to major antibiotics (Tab. 1).

The pH of the culture medium is another significant parameter and plays a vital role for the optimal physiological performance of microbial cells and has a noticeable effect on cell growth. For decolourization process an optimal pH was selected from the range 5 – 9. At pH 7 best decolourization results (78.5 %) were observed by *R. rhodochrous,* while at pH 8 the decolourization percentage was reduced to 63.4 %, meaning decolourization was achieved from pH 7 and 8 respectively. At pH 5, 6 and 9, further reduction in percentage decolourization of both isolates was observed (Fig. 2a). Incubation temperature is a critical process parameter and it varies among different microorganisms, and slight changes in temperature may affect growth and ultimately rate of decolourization. In the case of *R. rhodochrous* and *P. aeruginosa* highest colour removal was achieved at 35 ^{o}C (96.2 %) and 37 ^{o}C (86.2 %) respectively. For both the isolates a decline in the decolourization percentage was observed at higher temperature ranges as shown in Fig. 2b.

Generally decolourization rate increases with an increase in inoculums size. The effect of inoculum size on decolourization was tested and it was found that at an inoculums size of 2 % only 20 – 24 % decolourization was achieved with both strains. Upon further increase in inoculums size to 4, 6 and 8 %, progressive increase in decolourization ability (32 – 34 %, 39 – 45 % and 65 – 69 %) were observed and maximum decolourization (88 – 92 %) occurred at an inoculums size of 10 % (Fig. 2c). Decolourization process solely depends on the presence of nutritional supplements (co-substrate) that acts as electron donor for dye reduction. In the presence of glucose as a co-substrate, *R. rhodochrous* gave 69.6 % decolourization, while *P. aeruginosa* showed up to 62.8 % colour removal among various carbon sources (0.1 gL^{-1}) tested. With all other carbon sources the rate of decolourization was not significant (Fig. 2d). Results of effective glucose concentration evaluation depicted that in the control (0 mgL^{-1} glucose). The extent of colour removal was greatly inhibited and insignificant decolourization percentage i.e. 48.4 and 30.7 % was observed in the case of *Rhodococcus* and *Pseudomonas* sp respectively. With an increase in glucose concentration a progressive in colour removal was observed. At 1 gL^{-1} glucose concentration maximum decolourization of 95.2 and 96.1 % was attained in the case of *Rhodococcus* and *Pseudomonas* respectively. Nitrogen sources are also considered essential supplements for the decolourization of dyes by microbial formulation, so different organic and inorganic nitrogen sources were evaluated for the decolourization process. *Rhodococcus* and *Pseudomonas* species showed remarkable decolourization in the presence of yeast extract with 95.8 and 92.5 % decolourization observed. Other nitrogen sources could not yield exciting results as decolourization process was less pronounced as shown in Fig. 2e. With a progressive increase in concentration of yeast extract from 0 – 1 gL^{-1}, a linear increase in decolourization rate was observed and 1 gl^{-1} was found to be the optimum concentration, as 92.3 – 95.3 % decolourization by the selected isolates was detected at this concentration (Fig. 2g).

The toxicity nature of dyes compounds greatly have an inhibitory effect on the decolourization process at higher concentration, so the influence of different dye concentration was evaluated by taking 100, 200, 300, 400, 500 mgL^{-1} of Congo red. At an initial concentration of 50 mgL^{-1}in which 92.8 and 95.6 % decolourization was demonstrated by both *Rhodococcus* and *Pseudomonas* sp. At 100 mgL^{-1} dye concentration more than 85 % decolourization was attained. A progressively decreasing trend in decolourization rate was observed on further increase in dye concentration (Fig. 2h). Textile effluents generally contains chloride salts of sodium and potassium, which are frequently employed for salting out of dyes concentration and are therefore discharged into the effluent. Hence decolourizing efficiency of both isolate over a range of NaCl concentration (0 – 100 mgL^{-1}) was assessed under static condition at 37 ^{o}C. Results indicated that in the control medium (0 mgl^{-1} NaCl) 94.3 and 92.4 % decolourization was observed in the case of *Rhodococcus* and *Pseudomonas* sp respectively. By increasing concentration from 0 – 20 mgl^{-1} no severe inhibitory effect was noticed as decolourization was still more than 80 %. However, both organisms displayed good decolourization between 0 – 20 mgl^{-1}, even at 40 gL^{-1} NaCl concentration (Fig. 2i). Textile effluents frequently contain different metal compounds along (coupled) with different salts, which can interfere with microbial mediated decolourization process. To check their effect the medium was supplemented with metal compounds. Results showed that *Rhodococcus* specie displayed variable respond as it exhibit decolourization percentage with $CuCl_2$ (92.6 %), $CdCl_2$ (55.2 %), $CoCl_2$ (88.8 %), $AgCl_2$ (27.4 %) and $HgCl_2$ (32.3 %). While Pseudomonas specie was found to be affected by all the metal compounds with a decolourization percentage of $CoCl_2$ (54.5 %), $CuCl_2$ (85.6 %), $CdCl_2$ (38.2 %), $AgCl_2$ (24.7 %) and $HgCl_2$ (25.1 %) (Fig. 2j).

At optimized cultural condition a decolourization percentage of 96.1 % was achieved with *Rhodococcus* sp. A gradual increase in rate of decolourization resulted with maximum activity after 96 hrs. Later on no significant increase was observed. Comparison of growth pattern with that of decolourization showed a relative trend over a period of 64 hrs. After this the growth levelled off but decolourization rate continued to increase for 96 hrs (Fig. 1). The highest

decolourization percentage obtained with *Pseudomonas* sp. at its optimal condition was 91.1 %. The rate of decolourization progressively increased and reached its climax after 112 hrs of incubation. On extended incubation no further increase in the decolourization percentage was observed (Fig. 1). The BOD determination was used to determine the relative oxygen requirements of dye solution. BOD of all the samples decreases from 7.4 – 1 mg/l (Fig. 3a). The test measures the oxygen utilized during a specified incubation period for the biochemical degradation of organic matter (carbonaceous demand) and the oxygen used to consume an organic material such as sulfides and ferrous iron. It also may measure the oxygen used to oxidize reduce forms of nitrogen (nitrogenous demand). The chemical oxygen demand was measured by calculating the amount of oxidizing agent i.e., $K_2Cr_2O_4$ consumed during oxidation of organic matter (biodegradable and non-biodegradable) under acidic conditions. Chemical oxygen demand of degraded dye solution gets considerably reduced after degradation. COD of the solutions after degradation showed significant decrease from 16 – 2.3 mg/l (Fig. 3b).

The HPLC analysis of Congo red show single peak at retention time 1.187 min. After decolourization three new peaks were observed at retention time 1.447, 2.069, and 2.369 min. Dye decolourization and degradation was strongly supported in published research reports on HPLC analysis (Kalyani *et al.,* 2009; Surwase *et al.,* 2013; Agrawal *et al.,* 2014). The toxicity of Congo red (1000 mgL^{-1}) and its metabolites obtained after degradation was evaluated by well diffusion method against agricultural important bacteria such as *Enterobacter cloaca* and *Azotobacter vinelandii* that are known to be responsible for starch hydrolysis and nitrogen fixation in soil, respectively. Toxicity of Congo red was reduced by 78.3 and 67.8 % respectively, in *Enterobacter cloacae* and *Azotobacter vinelandii* after treatment with *R. rhodochrous* and *P. aeruginosa* (Tab. 2). Results are in close agreement with earlier report (Gomare *et al.,* 2009).

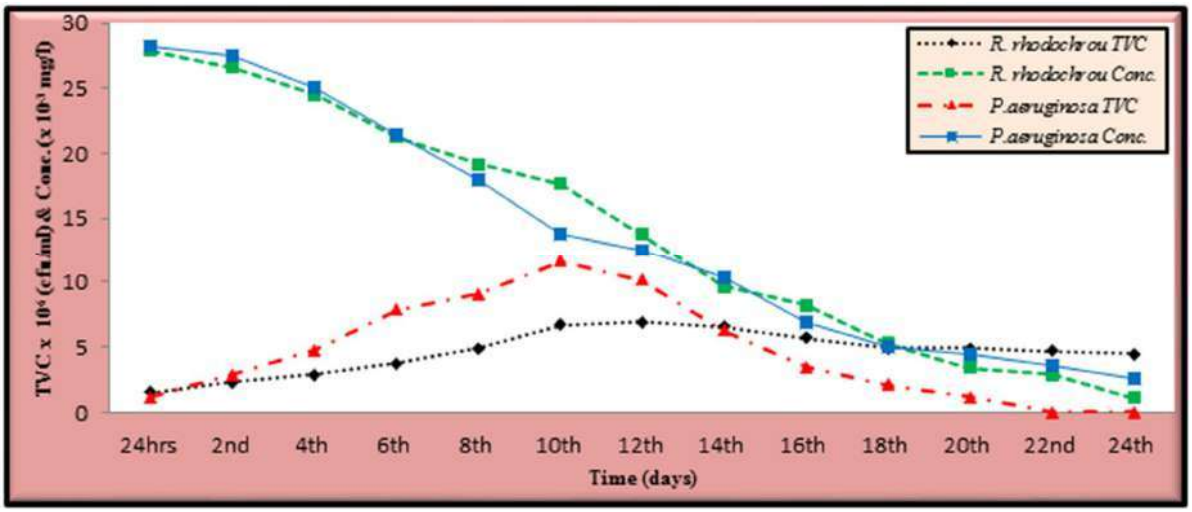


Figure 1. Growth profile of mixed culture of *Rhodococcu*s sp and *Pseudomonas* sp in minimal salt medium containing Congo red dye as a sole carbon source and energy, pH 7, (37 °C)

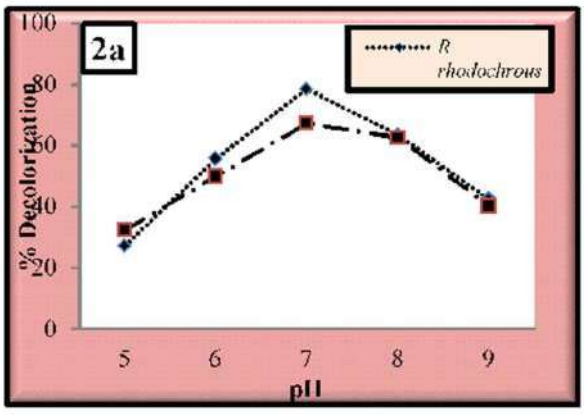


Figure 2a. At pH 7 best decolourization results (78.5

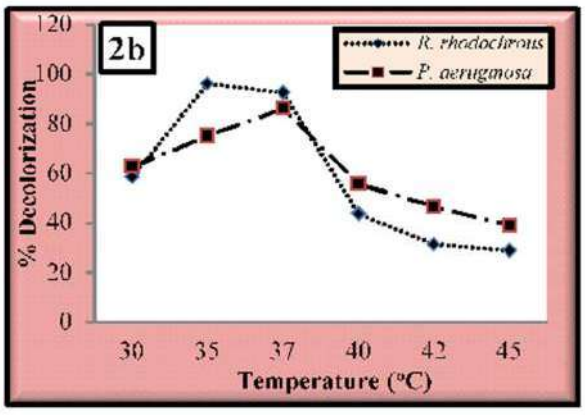


Figure 2b. *R. rhodochrous* and *P. aeruginosa* highest

%) were observed by *R. rhodochrous*. At pH 8 decolourization percentage was reduced to 63.4 %

colour removal was achieved at 35 ^{o}C (96.2 %) and 37 ^{o}C (86.2 %). A decline in the decolourization percentage was observed at higher temperatures

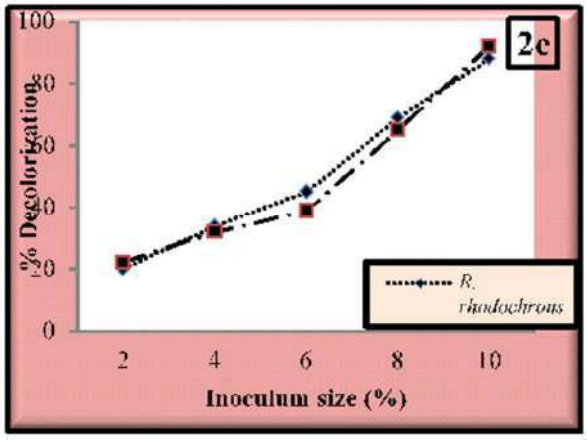


Figure 2c. Decolourization rate increases with an increase in inoculums size. Maximum decolourization (88 – 92 %) occurred at an inoculums size of 10 %

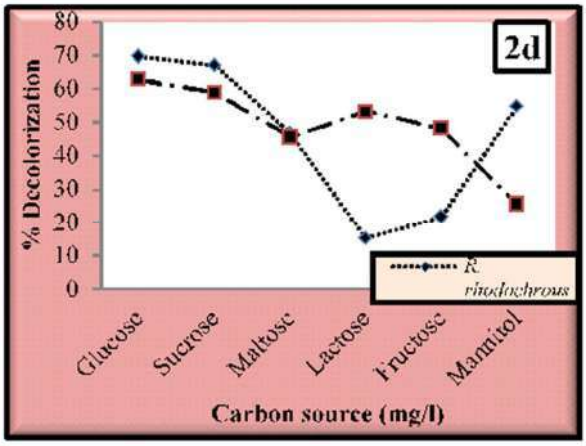


Figure 2d. Using glucose as a co-substrate, *R. rhodochrous* gave 69.6 % decolourization, while *P. aeruginosa* showed up to 62.8 %

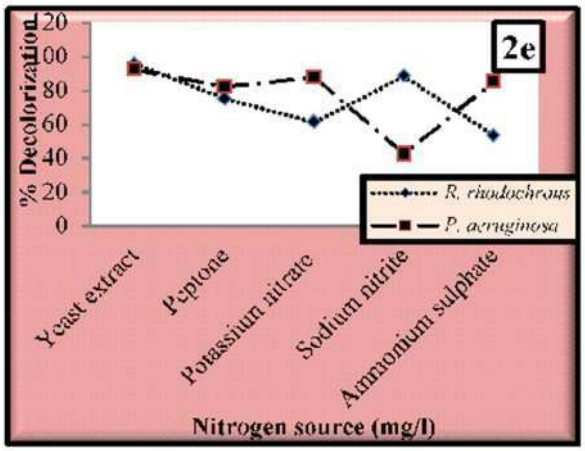


Figure 2e. Different organic and inorganic nitrogen sources were evaluated. *Rhodococcus* and *Pseudomonas* sp showed remarkable decolourization in the presence of yeast extract with 95.8 and 92.5 % decolourization observed

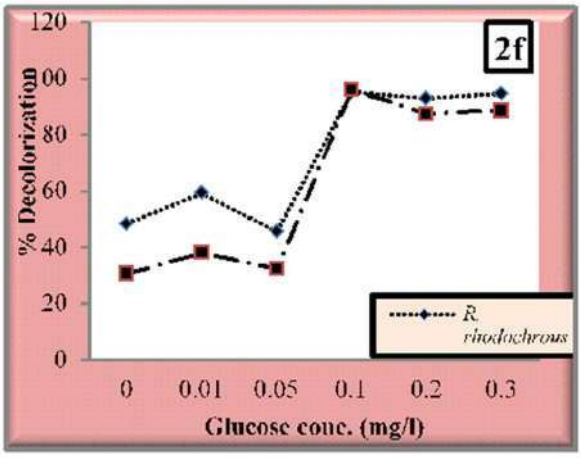


Figure 2f. With an increase in glucose concentration, a progressive in colour removal was observed. At 1 gL^{-1} glucose concentration maximum decolourization of 95.2 and 96.1 % was attained in the case of *Rhodococcus* and *Pseudomonas* species

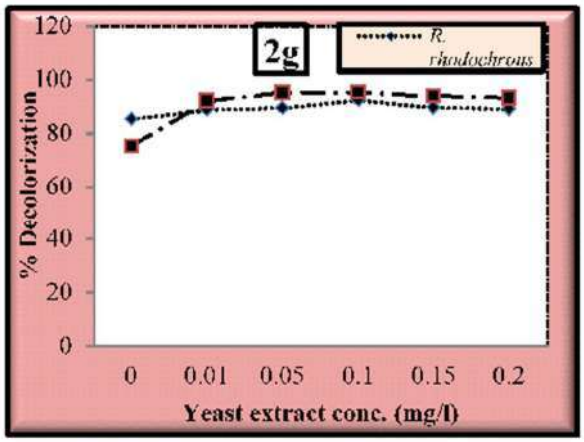


Figure 2g. Increase in concentration of yeast extract from 0 – 1 gL^{-1}, an increase in decolourization rate was observed and 1 gl^{-1} was found to be the optimum concentration, as 92.3 – 95.3 % decolourization by was detected

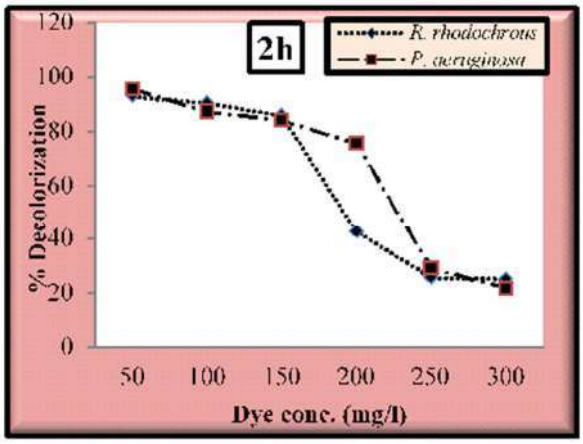


Figure 2h. At concentration of 50 mgL^{-1}. 92.8 and 95.6 % decolourization was demonstrated by both *Rhodococcus* and *Pseudomonas* sp. At 100 mgL^{-1} dye concentration, 85 % decolourization was attained

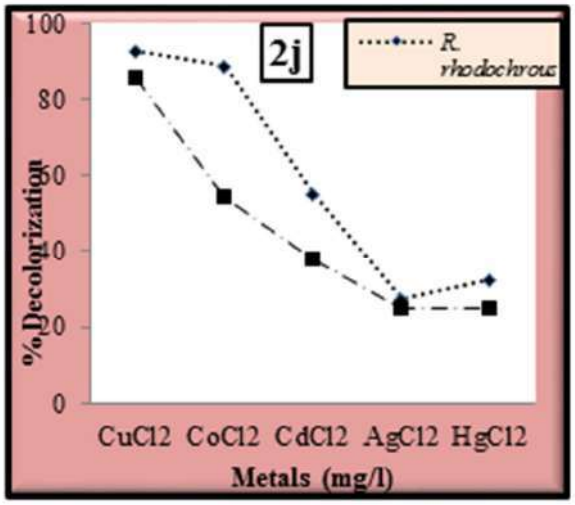


Figure 2i. Both organisms displayed good decolourization between 0 – 20 mgl^{-1}, and even at 40 gL^{-1} NaCl concentration

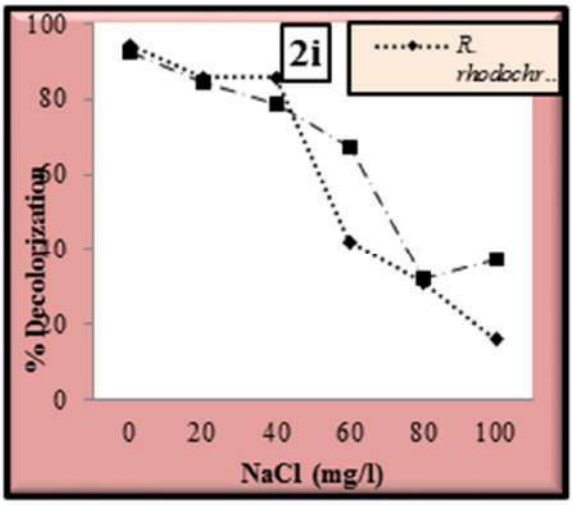
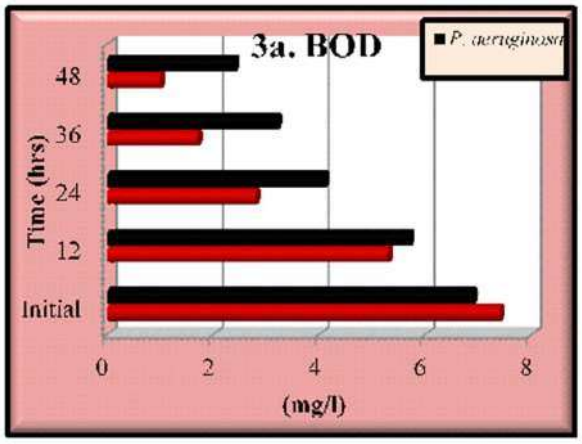


Figure 2j. *Rhodococcus* sp showed variable response to decolourization in metallic compounds, and *Pseudomonas* sp was affected by all the metal compounds



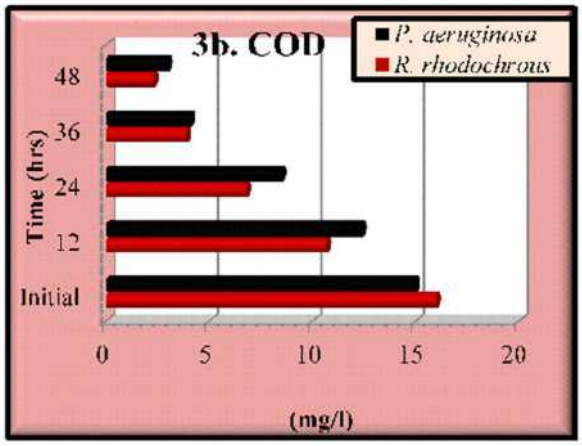


Figure 3a. The BOD determination was used to determine the relative oxygen requirements of dye solution. BOD of all samples decreases from 7.4 – 1 mg/l.

Figure 3b. Chemical oxygen demand (COD) of the degraded dye solutions after degradation showed significant decrease from 16 – 2.3 mg/l.

4. Conclusion

Bioremediation has proved to be a very effective method in encountering the textile dye pollution in an eco-friendly manner. This approach creates a promising hope for remediation of the environment which is polluted by hazardous dyes. The present study clearly demonstrates that the bacterial community in textile effluents of African Textile Company, Challawa Kano has the ability to degrade and decolorize various types of dyes used in such industries. The potential of these bacteria can be exploited to remove residual dye in textile wastes. Further detailed study is needed to transfer the laboratory findings to the field, the exposition of fertilizer action, the discovery of new, more efficient fertilizers or any locally available nutrient supplement and their ways of application, including the coupling of major biochemical cycles, such as nitrogen and phosphorus.

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TABLE 1. ANTIBIOTICS SUSCEPTIBILITY OF *RHODOCOCCUS RHODOCHROUS AND PSEUDOMONAS AERUGINOSA*

No.	*R. rhodochrous*				*P. aeruginosa*			
	Antibiotics	**Code**	**Conc.**	**Zone of inhibition (mm)**	**Antibiotics**	**Code**	**Conc.**	**Zone of inhibition (mm)**
1	Amikacin	AK	30 mcg	33	Amikacin	AK	30 mcg	28
2	Amoxycillin	AMX	10 mcg	28	Amoxycillin	AMX	10 mcg	31
3	Ampicillin	AMP	10 mcg	13	Ampicillin	AMP	10 mcg	16
4	Ampicillin/Sulbactam	A/S	10/10	32	Ampicillin/Sulbactam	A/S	10/10	29
5	Augmentin	AMC	30 mcg	22	Augmentin	AMC	30 mcg	24
6	Azithromycin	AZM	30 mcg	18	Azithromycin	AZM	30 mcg	20
7	Cefadroxil	CFR	30 mcg	19	Cefadroxil	CFR	30 mcg	18
8	Cefepime	CPM	30 mcg	42	Cefepime	CPM	30 mcg	40
9	Cefixime	CFM	5 mcg	0	Cefixime	CFM	5 mcg	0
10	Cefoperazone	CPZ	75 mcg	36	Cefoperazone	CPZ	75 mcg	38
11	Cefotaxime	CTX	30 mcg	34	Cefotaxime	CTX	30 mcg	33
12	Cefpodoxime	CPD	10 mcg	0	Cefpodoxime	CPD	10 mcg	0
13	Ceftazidime	CAZ	30 mcg	38	Ceftazidime	CAZ	30 mcg	38
14	Ceftriaxone	CTR	30 mcg	26	Ceftriaxone	CTR	30 mcg	25
15	Cefuroxime	CXM	30 mcg	19	Cefuroxime	CXM	30 mcg	18
16	Chloramphenicol	C	30 mcg	20	Chloramphenicol	C	30 mcg	20
17	Ciprofloxacin	CIP	5 mcg	49	Ciprofloxacin	CIP	5 mcg	50
18	Clindamycin	CD	2 mcg	0	Clindamycin	CD	2 mcg	0
19	Cloxacillin	COX	1 mcg	20	Cloxacillin	COX	1 mcg	18
20	Erythromycin	E	15 mcg	38	Erythromycin	E	15 mcg	36
21	Gentamicin	GEN	10 mcg	29	Gentamicin	GEN	10 mcg	29
22	Levofloxacin	LE	5 mcg	45	Levofloxacin	LE	5 mcg	44
23	Lomefloxacin	LOM	10 mcg	37	Lomefloxacin	LOM	10 mcg	38
24	Lomefloxacin	LOM	30 mcg	48	Lomefloxacin	LOM	30 mcg	46
25	Meropenem	MRP	10 mcg	54	Meropenem	MRP	10 mcg	52
26	Netillin	NET	30 mcg	34	Netillin	NET	30 mcg	33
27	Ofloxacin	OF	5 mcg	43	Ofloxacin	OF	5 mcg	42
28	Pefloxacin	PF	5 mcg	39	Pefloxacin	PF	5 mcg	38
29	Penicillin	PI	10 unit	19	Penicillin	PI	10 unit	18
30	Piperacillin	PI	100	39	Piperacillin	PI	100	38
31	Rifampicin	RIF	5 mcg	12	Rifampicin	RIF	5 mcg	12
32	Roxithromycin	RO	30 mcg	30	Roxithromycin	RO	30 mcg	30
33	Sparfloxacin	SPX	5 mcg	45	Sparfloxacin	SPX	5 mcg	45

34	Streptomycin	S	10 mcg	30	Streptomycin	S	10 mcg	30
35	Tetracycline	TE	30 mcg	11	Tetracycline	TE	30 mcg	10
36	Ticarcillin	TI	75 mcg	33	Ticarcillin	TI	75 mcg	32
37	Tobramycin	TOB	10 mcg	37	Tobramycin	TOB	10 mcg	37
38	Vancomycin	VA	30 mcg	0	Vancomycin	VA	30 mcg	0

TABLE 2. TOXICITY OF CONGO RED AND ITS METABOLITES BY BACTERIAL STAINS

Bacterial strains	**Inhibitory zone diameter (cm)**	
	Congo red	**Degradation product**
Enterobacter cloacae	1.06 ± 0.08	0.23 ± 0.07*
Azotobacter vinelandii (nifHDK)	1.74 ± 0.04	0.57 ± 0.06*

Values are mean of three experiments (±) SEM. Significantly different from Congo red dye at *$P < 0.001$ by ANOVA with Turkey - Kramer comparison test

6. References

Adedayo, O., Javadpour, S., Taylor, C., Anderson, W. A., and Moo-Young, M. (2004). Decolourization and detoxification of methyl red by aerobic bacteria from a wastewater treatment plant. *World Journal of Microbiology and Biotechnology*, *20*(6), 545-550.

Adzitey, F., Ali, G. R. R., Huda, N., Cogan, T., and Corry, J. (2013). Prevalence, antibiotic resistance and genetic diversity of Listeria monocytogenes isolated from ducks, their rearing and processing environments in Penang, Malaysia. *Food Control*, *32*(2), 607-614.

Agrawal, S., Tipre, D., Patel, B., and Dave, S. (2014). Optimization of triazo Acid Black 210 dye degradation by Providencia sp. SRS82 and elucidation of degradation pathway. *Process Biochemistry*, *49*(1), 110-119.

Banat, I. M., Nigam, P., Singh, D., and Marchant, R. (1996). Microbial decolorization of textile-dyecontaining effluents: a review. *Bioresource Technology*, *58*(3), 217-227.

Buthiyappan, A., Aziz, A., Raman, A., Daud, W., and Ashri, W. M. (2016). Recent advances and prospects of catalytic advanced oxidation process in treating textile effluents. *Reviews in Chemical Engineering*, *32*(1), 1-47.

Celia, M. P., and Suruthi, S. (2016). Textile dye degradation using bacterial strains isolated from textile mill effluent. *IJAR*, *2*(3), 337-341.

Chen, B. Y., Chen, W. M., Wu, F. L., Chen, P. K., and Yen, C. Y. (2008). Revealing azo-dye decolorization of indigenous *Aeromonas hydrophila* from fountain spring in Northeast Taiwan. *Journal of the Chinese Institute of Chemical Engineers*, *39*(5), 495-501.

Cheunbarn, T., Cheunbarn, S., and Khumjai, T. (2008). Prospects of bacterial granule for treatment of real textile industrial wastewater. *International Journal of Agriculture and Biology*, *10*(6), 689-692.

Dos Santos, A. B., Cervantes, F. J., and van Lier, J. B. (2007). Review paper on current technologies for decolourisation of textile wastewaters: perspectives for anaerobic biotechnology. *Bioresource technology*, *98*(12), 2369-2385.

Farouk, H. U., Raman, A. A. A., and Daud, W. M. A. W. (2016). TiO_2 catalyst deactivation in textile wastewater treatment: Current challenges and future advances. *Journal of Industrial and Engineering Chemistry*, *33*, 11-21.

Franciscon, E., Grossman, M. J., Paschoal, J. A. R., Reyes, F. G. R., and Durrant, L. R. (2012). Decolorization and biodegradation of reactive sulfonated azo dyes by a newly isolated *Brevibacterium* sp. strain VN-15. *Springer Plus*, *1*(1), 37.

Gomare, S. S., Kalme, S. D., and Govindwar, S. P. (2009). Biodegradation of navy blue-3G by *Brevibacillus laterosporus* MTCC 2298. *Acta Chim Slov*, *56*, 789-796.

Gottlieb, A., Shaw, C., Smith, A., Wheatley, A., and Forsythe, S. (2003). The toxicity of textile reactive azo dyes after hydrolysis and decolourisation. *Journal of Biotechnology*, *101*(1), 49-56.

Jadhav, U. U., Dawkar, V. V., Ghodake, G. S., and Govindwar, S. P. (2008). Biodegradation of Direct Red 5B, a textile dye by newly isolated *Comamonas* sp. UVS. *Journal of Hazardous Materials*, *158*(2), 507-516.

Jones, R. N., Della-Latta, P., Lee, L. V., and Biedenbach, D. J. (2002). Linezolid-resistant *Enterococcus faecium* isolated from a patient without prior exposure to an oxazolidinone: report from the SENTRY Antimicrobial Surveillance Program. *Diagnostic microbiology and infectious disease*, *42*(2), 137-139.

Joshi, T., Iyengar, L., Singh, K., and Garg, S. (2008). Isolation, identification and application of novel bacterial consortium TJ-1 for the decolourization of structurally different azo

dyes. *Bioresource Technology*, *99*(15), 7115-7121.

Kalyani, D. C., Telke, A. A., Dhanve, R. S., and Jadhav, J. P. (2009). Ecofriendly biodegradation and detoxification of Reactive Red 2 textile dye by newly isolated *Pseudomonas* sp. SUK1. *Journal of Hazardous Materials*, *163*(2), 735-742.

Khaled, A., El Nemr, A., El-Sikaily, A., and Abdelwahab, O. (2009). Removal of Direct N Blue-106 from artificial textile dye effluent using activated carbon from orange peel: Adsorption isotherm and kinetic studies. *Journal of Hazardous Materials*, *165*(1), 100-110.

Khan, M. (2015). *Bacterial decolorization of textile industry effluent* (Doctoral dissertation, CCSHAU).

Khehra, M. S., Saini, H. S., Sharma, D. K., Chadha, B. S., and Chimni, S. S. (2005). Decolorization of various azo dyes by bacterial consortium. *Dyes and Pigments*, *67*(1), 55-61.

Mahbub, K. R., Ferdouse, J., and Anwar, M. N. (2011). Demonstration of decolorization of various dyes by some bacterial isolates recovered from textile effluents. *Bangladesh Journal of Scientific and Industrial Research*, *46*(3), 323-328.

Mahbub, K. R., Mohammad, A., Ahmed, M. M., and Begum, S. (2012). Decolorization of synthetic dyes using bacteria isolated from textile industry effluent. *Asian journal of biotechnology*, *4*(3), 129-136.

Mahmood, S., Khalid, A., Arshad, M., Mahmood, T., and Crowley, D. E. (2016). Detoxification of azo dyes by bacterial oxidoreductase enzymes. *Critical reviews in biotechnology*, *36*(4), 639-651.

Mali, P. L., Mahajan, M. M., Patil, D. P., and Kulkarni, M. V. (2000). Biodecolourisation of members of triphenylmethane and azo group of dyes. *Journal of Scientific and Industrial Research*, *59*(3), 221-224.

Mills□Robertson, F., Crupper, S. S., Addy, M. E., and Mensah, P. (2003). Antibiotic resistance and genotyping of clinical group *B Salmonella* isolated in Accra, Ghana. *Journal of applied microbiology*, *94*(2), 289-294.

Ooi, T., Shibata, T., Sato, R., Ohno, H., Kinoshita, S., Thuoc, T. L., and Taguchi, S. (2007). An azoreductase, aerobic NADH-dependent flavoprotein discovered from *Bacillus* sp.: functional expression and enzymatic characterization. *Applied microbiology and biotechnology*, *75*(2), 377-386.

Pirbazari, A. E., Pargami, N. R., Ashja, N., and Emami, M. S. (2015). Surfactant-coated Tea Waste: Preparation, Characterization and its Application for Methylene Blue Adsorption from Aqueous Solution. *Journal of Environmental & Analytical Toxicology 2015*, 5(5) 1-11.

Rajabi, A. A., Yamini, Y., Faraji, M., and Nourmohammadian, F. (2016). Modified magnetite nanoparticles with cetyltrimethylammonium bromide as superior adsorbent for rapid removal of the disperse dyes from wastewater of textile companies. *Nanochemistry Research*, *1*(1), 49-56.

Sheth, N. T., and Dave, S. R. (2009). Optimisation for enhanced decolourization and degradation of Reactive Red BS CI 111 by Pseudomonas aeruginosa NGKCTS. *Biodegradation*, *20*(6), 827-836.

Soni, H., Kumar, J. N., Patel, K., and Kumar, R. N. (2016). Photocatalytic decoloration of three commercial dyes in aqueous phase and industrial effluents using TiO2 nanoparticles. *Desalination and Water Treatment*, *57*(14), 6355-6364.

Surwase, S. V., Deshpande, K. K., Phugare, S. S., and Jadhav, J. P. (2013). Biotransformation studies of textile dye Remazol Orange 3R. *3 Biotech*, *3*(4), 267-275.

Swamy, J., and Ramsay, J. A. (1999). The evaluation of white rot fungi in the decoloration of textile dyes. *Enzyme and Microbial Technology*, *24*(3), 130-137.

Zimmermann, T., Gasser, F., Kulla, H. G., and Leisinger, T. (1984). Comparison of two bacterial azoreductases acquired during adaptation to growth on azo dyes. *Archives of Microbiology*, 138(1), 37-43.