



Decolourization of Vat Dyes by Bacterial Isolates Recovered from Local Textile Mills in Southwest, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author SOA designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed literature searches. Authors SAB and AKA managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Waste water emanated from the use of synthetic dyes obtained from different textile and other dyestuff industries require treatment before they are discharge into the environment to prevent groundwater contamination. Considerable interest has been on decolourisation and degradation of dyes by microorganisms due to its efficiency and duration of treatment. In this study bacterial isolates were recovered from textile dye effluent and used in decolourization of textile dyes to non-toxic metabolites.

Study Design: Effluent samples were collected from two different local textile mills in Abeokuta, Ogun state, Nigeria.

Place and Duration of Study: Effluent sample were collected from local textile mills in Abeokuta, Ogun state during the dry season in the month of November and December, 2013

Methodology: Ability of bacterial strains isolated from textile wastewater were screened for vat dyes decolourization and high decolourization potential isolates were used for decolourization of different vat dyes.

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Results: Thirty-four bacterial strains were isolated from textile wastewater. Screening of the thirty-four bacterial isolates on Luria-Bertani Agar medium supplemented with 100 mg/L of dye revealed four bacterial isolates as potential isolate for dye-decolourisation. The four bacterial isolates are: *Bacillus firmus*, *Bacillus macerans*, *Staphylococcus aureus* and *Klebsiella oxytoca*. Decolourisation of vat black dye using all the potential four isolates showed that *Bacillus macerans* had highest decolourising potential of 75.04% while *Bacillus firmus* had the lowest decolourising ability of 41.42%. *Klebsiella oxytoca* had the highest decolourisation potential of 69.68% for vat brown dye while *Staphylococcus aureus* had the least decolourisation potential (33.33%). *Bacillus firmus* after 5 days showed the highest vat red dye decolourisation of 81.27% while *Staphylococcus aureus* had the least decolourisation activity of 34.67%.

Conclusion: Application of the bacterial strains under natural environmental conditions in the decolourisation is an indication of its ability and effectiveness in treatment of wastewater containing dye.

Keywords: Vat dyes; decolorisation; bacteria; wastewater.

1. INTRODUCTION

Poly-aromatic molecules like synthetic dyes give permanent color to textile fibres and some other materials. Generally, dyes are coloured compounds, which are capable of being fixed in fabric and are classified in accordance with their application. Synthetic dyes are extensively used in textile, paper, pharmaceutical, food and cosmetic industries [1,2,3]. Rapid industrialisation and tremendous increase of human population increase the conventional solid and liquid waste which create problems to environment and mankind. Textile industry is known for using large quantities of water and variety of chemicals [4]. One of the most important problems is the textile dye pollution to land and water [5].

Most of the commonly used dyes are synthetic and triphenylmethane dyes with about 30%-40% of their usage of the dye total consumption. They have several applications in paper, plastics, bacteriology and histopathological staining specimens, cosmetics, foods e.t.c and can also be applied on nylon, wool, silk and cotton extensively processes [6,7].

Colour in water bodies reduces light penetration, alter the pH, COD and BOD and thereby make aquatic life very difficult and dye house effluents are therefore of serious environmental concern [8].

Unused dyestuff (10-50%) enters the wastewater directly due to inefficiency in dyeing processes and also all dyes do not bind to the fabric [9]. The discharge of the wastewater into the environment causes severe environmental problems, obstruct

light penetration and produce toxic effects on fauna and flora. Highly coloured effluents containing dye decrease photosynthesis activity in aquatic life due to reduced light penetration and affect gas solubility in water bodies [10].

In some cases, compounds from synthetic azo dyes and their degradation intermediates are toxic, causes cancer and can cause mutation in humans and other animals [11]. Adsorption, membrane filtration, precipitation, oxidation, coagulation are some of the physical and chemical methods used for textile effluent treatment but these methods have some demerits like production of large amounts of sludge which require safe disposal and are very expensive [12]. Hence the need for effective treatment method becomes very imperative. Biological process as an alternative method involves the use of microorganisms are cost effective, eco-friendly nature and produce less sludge are now being used as an alternative method [13]. Meanwhile, low decolorisation efficiency limits the use of fungi for the treatment of textile effluent [14,15]. Higher degree of mineralization and degradation of dyes can be achieved by bacteria aerobically or anaerobically or combination of both under optimum conditions [14]. Bacterial degradation initial step is the reductive cleavage of bond by biotransformation using enzyme reaction under static or anaerobic conditions and formation of colorless aromatic amine [9] and are further degraded to simpler non-toxic forms by multiple step bioconversion anaerobically or aerobically [9]. Microorganisms uses their metabolic activities in bioremediation leading to the transformation of organic pollutants into carbon dioxide and water or harmless metabolites [16].

Whole cells were used in this study for degradation experiments despite its numerous demerits so as to obtain high dye degradative, adaptive and potential isolates. Some of the limitations in using whole cells are: effluent concentration or toxicity might affect the metabolic activities of the cell, Some of the effluent can lyse the cell wall of whole cells and cause cell lysis. Whole cells might not take up enough effluent compared to immobilized cell, enzyme, set-up much likely to have unwanted by-products since many other enzymes or materials in addition to the desired materials or enzymes may be present [17].

Itoku, located at Abeokuta city in Nigeria is an area commonly known for the use of dye especially vat dyes for dyeing of different textile. Unfortunately, the wastewater that emerges from the dye house is disposed into the environment without treatment. Discharge of dyes into the environment without treatment pollute soil, water and environments, hence the need for the removal of these dye pollutants is of great importance. Due to paucity of knowledge in decolorization or degradation of vat dye, this study aimed at the isolation and identification of effective bacterial strains with decolorisation of vat dyes commonly used in textile industries of Abeokuta, Nigeria.

2. MATERIALS AND METHODS

2.1 Dyes

Vat dyes (black, brown and red) used in this study was of industrial grade and purchased from local market of Itoku, Abeokuta, Nigeria.

2.2 Effluent Collection

Effluent samples (500 mL each) were collected from two local textile dyeing sites in Itoku, ITK 1 and ITK2 (N 7° 9' 23" Lat and E 3° 20' 33" Long) Ogun state. Effluents were collected into sterile bottles and transported to the laboratory under cold storage for further studies.

2.3 Physicochemical Characterization of Textile Effluent

Physical and chemical parameters (pH, temperature, biochemical oxygen demand (BOD), chemical oxygen demand (COD) and electrical conductivity) of effluent were determined using standard method for the examination of wastewater [18].

2.4 Isolation of Microorganisms

Microbial isolation from effluent was carried out according to [19]. Total heterotrophic bacterial count was carried out using spread plate method. Aliquot (0.1 ml) of the serially diluted solution of the effluent were plated out on sterilized Luria bertani (LB) plates in triplicates. Inoculated plates were incubated in an incubator (Gallenkamp, U.K.) at 28°C for 24 h. The population of the aerobic heterotrophic bacterial count was recorded.

2.5 Screening of Dye-decolourising Bacteria

Dye-decolourising bacteria were screened according to [20]. Bacterial isolates obtained from LB agar plates were streaked on Luria Bertani (LB) Agar supplemented with 100 mg/L of vat red dye and the plates were incubated at 27°C for 48 hours. Colonies that showed clear decolourisation zones around them were selected as efficient isolates for decolourisation of dye and are used for further dye decolourisation studies.

2.6 Decolourisation Experiments

Decolourisation experiments were carried out in Erlenmeyer flasks containing 100 mL of mineral salt medium containing (100 mg/L of each dye), 0.1 g of yeast extract and sucrose and adjusting pH to 7. Flasks were inoculated with 5 mls of inoculum. Set-up was incubated in a shaker incubator (Gallenkamp, UK) at 110 rpm for 5 days. After five days, decolorization activity was determined by monitoring absorbance on a spectrophotometer at 540 nm [21]. Uninoculated dye medium served as control. Decolorizing activity was expressed in terms of percentage decolourisation according to [22].

% decolorization

$$= \frac{\text{Initial Absorbance} - \text{Final Absorbance}}{\text{Initial Absorbance}} \times 100$$

3. RESULTS AND DISCUSSION

Table 1 showed the result of the physico-chemical parameters of effluent. It was observed that the pH of effluent ranged between 10.5 and 11.7 with effluent from ITK2 being the highest while ITK 1 had the lowest pH. Alkaline pH values of effluent could be due to the use of high amounts of salts during dyeing process.

Temperature of the effluents ranged from 48°C to 55°C. The highest temperature of 55°C was observed in ITK 2 effluent while lowest temperature of 48°C was observed in ITK 1 effluent. High temperature can be attributed to the use of hot water during dyeing process. Electrical conductivity (EC) ranged between 267.19 $\mu\text{S}/\text{cm}$ to 311.08 $\mu\text{S}/\text{cm}$ with ITK 2 effluent showing highest conductivity while ITK 1 was least conductive. High EC values could be as a result of high concentration of ions in water. The highest BOD of 50.83 was at ITK 2 while the lowest BOD of 49.99 was at ITK 1. The highest and lowest value of COD was 81.50 and 77.49 at ITK 1 and ITK 2 respectively.

BOD and COD values obtained from this study are within the WHO permissible limits while temperature, pH and electrical conductivity were not within WHO permissible limits (Table 1).

Result of Total Heterotrophic Bacterial Count (THBC) of textile effluents of local textile mills at Itoku showed that high total heterotrophic count value of 3.61×10^8 CFU/mL was obtained from Itoku 1 (local textile mills) while lowest heterotrophic count value of 3.36×10^8 CFU/mL was obtained from ITK 2 local textile mills (Table 2). High values of THBC are due to the adaptability of the isolates to the dye terrain and the isolates have develop different enzyme system for the dye utilization.

Out of 34 bacterial isolates from the different effluent samples, *Klebsiella oxytoca*, *Bacillus firmus*, *Staphylococcus aureus* and *Bacillus macerans* were selected based on their efficiency of dye degradation ability on LB plates

supplemented with dye and they were used for further decolourisation studies.

Decolourisation of dye by bacterial isolates in the dye solution could be as a result of transfer of electrons from the bacterial cytoplasm to the dye solution and also could be as a result of metabolism. Decolourisation studies showed that *Bacillus macerans* had highest decolourisation potential of 75.04% for vat black dye after 5 days of exposure while *Bacillus firmus* had the least decolourisation activity of 41.42% (Fig. 1).

Fig. 2 shows the decolourisation of vat brown after 5 days by the different dye-decolourising isolates. However, *Klebsiella oxytoca* had the highest decolourisation potential of 69.68% for vat brown dye while *Staphylococcus aureus* had the least decolourisation potential (33.33%).

Bacillus firmus after 5 days showed the highest vat red dye decolourisation of 81.27% while *Staphylococcus aureus* had the least decolourisation activity of 34.67% (Fig. 3).

Toxic materials and coloured spent dyes are found inside wastewater discharged from textile industry but this wastewater is recalcitrant. Physical and chemical procedures used in remediation of toxic waste water, however generate highly hazardous sludge which make the use of natural microbes to degrade this dye in water to become very imperative [16]. The chosen bacterial isolates were able to utilize the vat dyes used in this study very well and among the different bacterial strains used for the decolourisation experiments, *Bacillus* species had highest decolourization potential.

Table 1. Analysis of constituent of the effluent

Parameters	WHO permissible limits	ITK 1	ITK2
pH	6.5- 8.5	10.5	11.7
Temp	(< 35°C)	48 °C	55 °C
BOD	< 500	49.99	50.83
COD	< 1000	81.50	77.49
Electrical conductivity	2500	267.19	311.08

Key: ITK 1 – Itoku site 1, Abeokuta, ITK 2 – Itoku site 2, Abeokuta

Table 2. Total heterotrophic bacterial count at local textile mills

S/No	Sample code	Sample location	10^8 CFU/mL (Mean \pm SD)
1	ITK 1	Itoku 1	3.61 ± 1.27
2	ITK 2	Itoku 2	3.36 ± 1.05

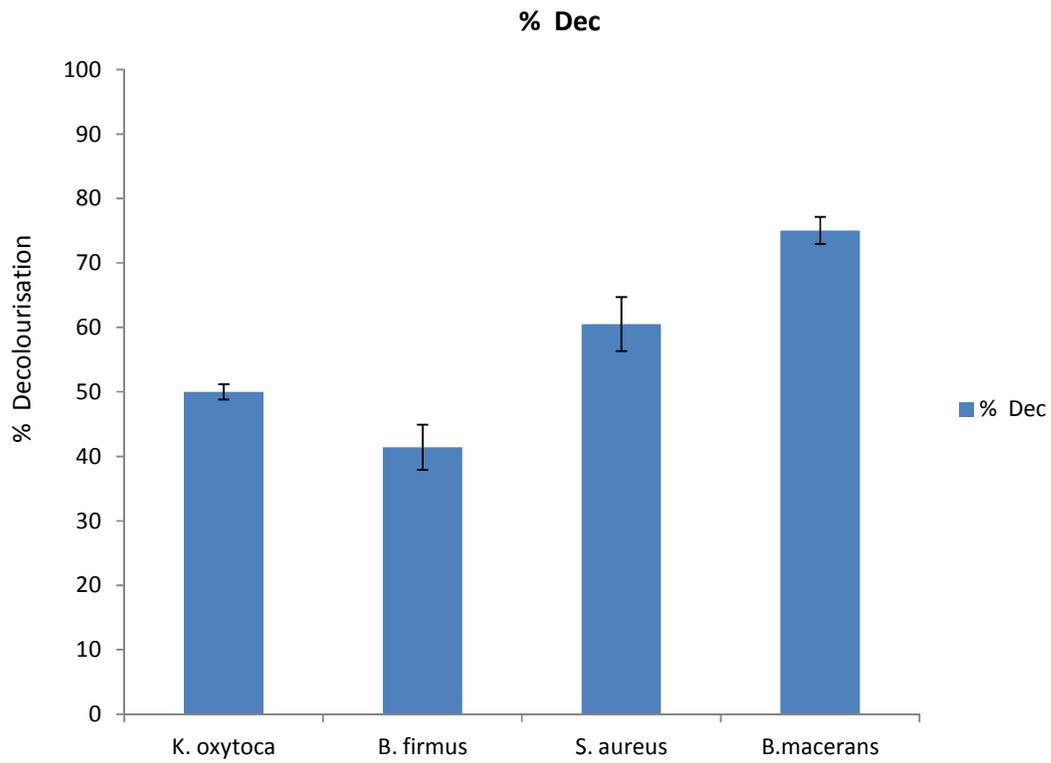


Fig. 1. Percentage decolourization of vat black dye

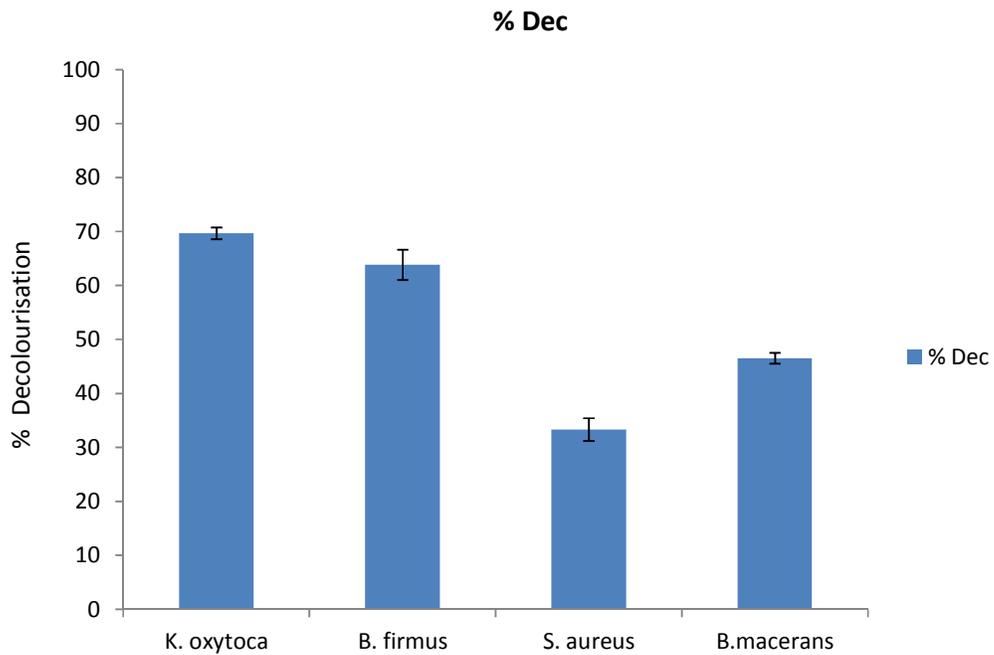


Fig. 2. Percentage decolourization of vat brown dye

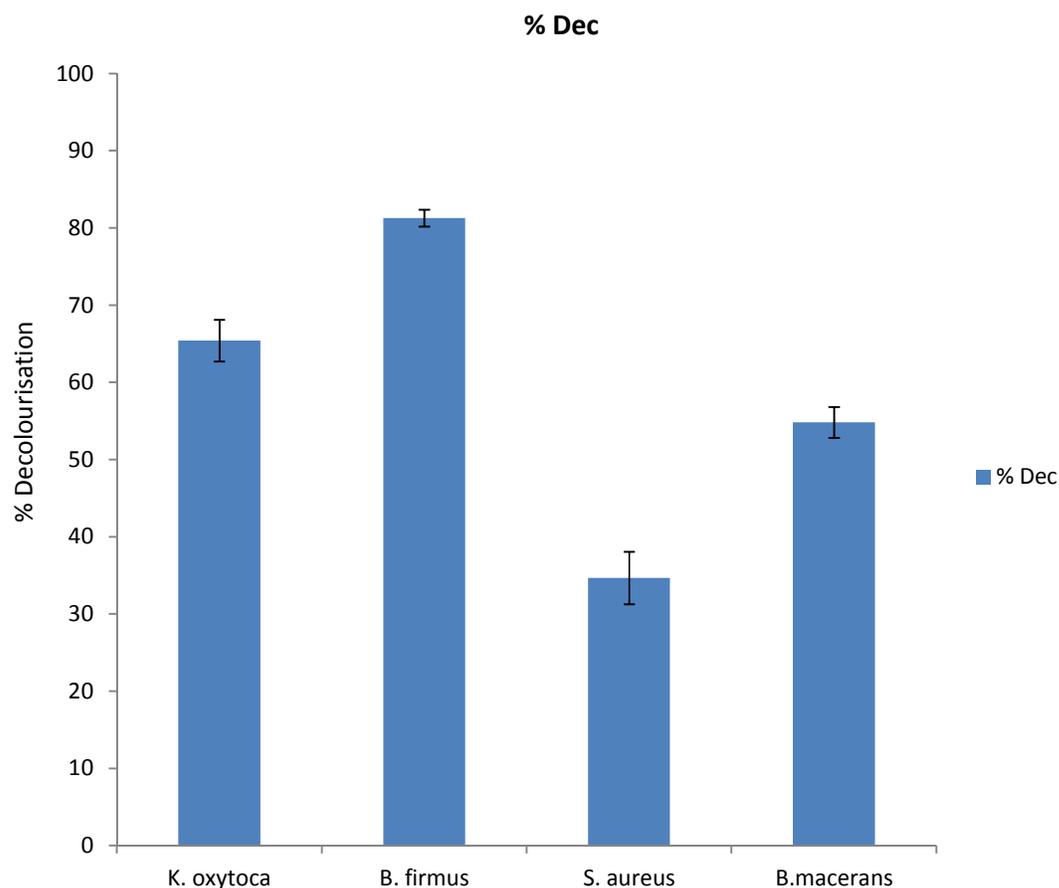


Fig. 3. Percentage decolourization of vat red dye

Highly adapted organisms to high dye concentration in wastewater have been isolated by several authors from areas near textile industries complex. Continuous exposure of the microbial population to dye enriched wastewater could result to high total heterotrophic count values. Bacterial isolates especially the viable ones can thrive, multiply and be found naturally in the bottom of soil or effluent in the presence of overlying water containing high dye concentration.

4. CONCLUSION

Bacterial isolates were able to decolourised the dye at different degrees or levels. The results imply that *Bacillus macerans* is a potential isolate for decolourisation of vat black, *Bacillus firmus* to be efficient isolate for degradation or decolourisation of vat brown and vat red dye.

Mineralisation and decolourisation of dyes by bacterial enzymes have been developed under certain environmental conditions [23,24,25]. Bacteria from dye or local dye environment are easily adapted to the environment because the bacteria are originated from the dye contaminated textile waste water of the local environment and have develop enzyme for the dye decolourisation. Therefore, treatment of wastewater contaminated with dye using *Bacillus macerans*, *Klebsiella oxytoca* and *Bacillus firmus* is an effective method.

However, the potential for bioremediation of dye-polluted wastewater using these isolates at large scale should be considered.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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