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Comparative Study of the Potentials of *Aspergillus terreus*, *Bacillus* species and *Chlorella vulgaris* on the Bio-Remediation of Reactive Red 198 (RR198) Dye

[*1]Sani, Z. M., [2]Dalhatu, A. S. and [1]Ibrahim, S.

[1]Department of Biological Sciences, Bayero University, Kano, Nigeria

[2]Department of Biological Sciences, Kaduna State University, Kaduna, Nigeria

[*]Corresponding Author: zmsani.bio@buk.edu.ng; +2348027988187

Abstract

Re-dyeing of fabric materials using synthetic dyes (such as reactive dyes) is fast spreading in metropolitan Kano which causes serious damage to the ecosystems. This study was carried out to compare the potentials of *Aspergillus terreus, Bacillus* species and *Chlorella vulgaris* in bioremediation and adsorption of reactive red 198 (RR198) dye used in fabric re-dyeing. This was achieved through inoculation of pure cultures of the organisms in the dye solution. The highest percentage adsorption for all the test organisms was recorded after 48 hours of inoculation, with *Chlorella vulgaris* displaying 86.4%, *Bacillus* species, 84.4% and *Aspergillus terreus*, 69.8% of dye adsorption. The results showed statistically significant difference in dye adsorption among the three species with Chlorella vulgaris having the highest adsorption potential compared to the *Bacillus* species and *Aspergillus terreus*. The adsorption process fitted with the Freundlich's isotherm, revealing a multilayer adsorption pattern. There is need for the introduction of better strategies that detoxify dyes before discharging into the environment to avoid further contamination.

Keywords: *Aspergillus terreus*, *Bacillus* species, *Chlorella vulgaris*, Reactive red 198 (RR198) dye.

INTRODUCTION

The technological advancement in the textile industry led to the emergence of new dyeing practices using synthetic dyes of different colour shades (Sani and Abdullahi, 2019; Renne, 2020). The waste generated from textiles re-dying is causing serious damage to the environment (Ahmad *et al*., 2012).

Microorganisms have served in nature for many years in the breakdown of complex human, animal, and plant wastes maintaining the continuity of life from generation to generation (Rosenberg and Zilber-Rosenberg, 2016). Such organisms are ideally suited to the tasks as they possess enzymes that allow them utilize the contaminants for survival (Chen *et al*., 2003).

The breakdown of harmful compounds contained in dyes into safe substances has been reported by several researchers (Sani and Banerjee, 1999; Palanivielan *et al*., 2013). The process is said to be an effective tool where a number of indigenous microorganisms are used for the treatment of industrial dye effluent (Dubey *et al*., 2003). Among these microorganisms, bacteria are most commonly used for various bioremediation processes (Chen *et al*., 2003). Isolation of bacterial The study was carried out to compare the dye remediation potential among the fungal, culture capable of degrading azo dyes started in 1970s with report of *Bacillus subtilis* (Gomez *et al*.,2000), and *Pseudomonas* species being the most active degrader isolated from aerobic dyeing house wastewater treatment facility (Young and Juan, 2001). *Phanerochaete chrysosporium* (a white rot fungus) has also been used extensively for decolourization of dyes in wastewaters due to its ability to synthesize lignin degrading exo-enzymes such as lignin and manganese peroxidases (LiP and MnP respectively) or Laccases (Kirk *et al*., 1992; Namdhari *et al*., 2012). Microalgae of different species (*Chlorella vulgaris*, *Chlorella pyrenoidosa*, *Chlorella sorokiniana*, *Scenedesmus* species, *Chara* species, *Dunalielia* species and *Oscillatoria* species) have been used as adsorbents/bioaccumulators of dye from industrial wastewater (Kotoula *et al*., 2020). This is due to their ability to grow fast and mitigate carbon dioxide in wastewater (Andrade and Andrade, 2018) as well as produce important molecules such as fatty acids, phenolic compounds, volatile compounds, sterols, proteins, amino acids, peptides, vitamins, polysaccharides and pigments (Katheresan *et al*., 2018). bacterial and algal species.The outcome of this research would provide valuable data that

could inform local strategy, review of existing environmental and health policies.

MATERIALS AND METHODS

Wastewater containing Reactive red 198 (RR198) dye was collected from Kofar Na'isa dyeing pit. Microorganisms used in this study were from the fungal, bacterial and algal species. *Aspergillus terreus* and *Bacillus* species were isolated from dye-contaminated soil of Kofar Na'isa dyeing pit, while *Chlorella vulgaris* was collected from Department of Plant Biology, Bayero University, Kano.

Isolation of *Aspergillus terreus* and *Bacillus* species

Aspergillus terreus was isolated using the dilution plating and direct isolation methods as described by Al-Mohanna (2016). Media (Potato dextrose agar (PDA) and Potato dextrose broth (PDB)) used, were prepared according to the method of Cheesbrough (2000). One gram of the dye-contaminated soil sample was placed in a sterilized test-tube, to which 9 ml of distilled water was added (stock solution). The solution was mixed thoroughly and allowed to sediment for 15 minutes. Five sterilized test-tubes labeled (10^{-1} - 10^{-5}) were arranged accordingly with each containing 9 ml of distilled water. Using a sterilized syringe, 1 ml of the stock solution was transferred into the test-tube labeled 10^{-1} which was mixed carefully. Using another sterilized syringe, 1 ml from the 10^{-1} test-tube was transferred to the second test-tube labeled 10^{-2}. The dilution subsequently continued to the fifth test-tube, giving dilutions of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} respectively. Individual sterilized syringes were also used to transfer aseptically 1 ml from each dilution into each of the corresponding labeled petri-dishes. The prepared PDA was aseptically poured into each petri-dish, swirled to have a thorough mixture and allowed to solidify, afterwards incubated at 37°C for 5 days. *A. terreus* was further sub-cultured for another 5 days on PDB for the assay (Benson, 1998).

The isolation *Bacillus* species was done using the pour plate and streak culture methods as described by Lee *et al*. (2013). Nutrient agar and nutrient broth were used for culturing the isolate as described by Cheesbrough (2000). One gram of the dye-contaminated soil sample was placed in a sterilized test-tube, to which 9 ml of distilled water was added (stock solution). The solution was mixed thoroughly and allowed to sediment for 15 minutes. Five sterilized test-tubes labeled (10^{-1} - 10^{-5}) were arranged accordingly with each containing 9 ml of distilled water. Using a sterilized syringe, 1 Where,

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Adsorption Assay

Aspergillus terreus was cultivated on potatoes dextrose broth for 5 days at room temperature (37°C) as described by Al-Mohanna (2016). The 5 day old mycelium (0.4 grams) was generated for the assay. *Bacillus* species biomass was generated according to the method of Ngui *et al*. 2013, which involved sub-culturing on Nutrient broth (NB) medium at 37°C for 24 hours in an incubator shaker (Innova 4000) at 150 rpm. After 24 hours, the solution was centrifuged (Centromix Selecta 540) at 10,000 rpm for 10 minutes, to separate the supernatant and pellet (bacterial cells) 4.79×10^{6} cells/ml of *Bacillus* species was used for the assay. Bold Basal medium (BBM) was used for cultivation of *C. vulgaris* at 30°C with proper aeration (with an air pump - Shining beach SB660) and 4.47×10^{7} cells/ml was generated.

The generated biomass for each of the three species wasseparately placed in a test-tube containing 1 ml of dye wastewater and 5 ml of normal saline. The solution was mixed with an auto-vortex mixer and incubated at 37°C. After 24 and 48 hours the solution was centrifuged and the absorbance of supernatant was recorded using a spectrophotometer (model 722) at wavelength 650 nm. The amount of dye adsorbed per gram of the biomass and percentage adsorption of the dye by the biomass of individual species were calculated using equations 1 and 2 respectively.

$$Qe = A - B \times \frac{V}{M} \quad - \quad - \quad - \quad - \quad - \quad - \quad 1$$

$$Adsorption\ (\%) = \frac{(A-B)}{A} \times 100 \quad - \quad - \quad - \quad - \quad 2$$

Qe = Concentration of dye at equilibrium
A = Initial concentration of dye in solution
B = Final concentration of dye in solution
V = volume of solution in millilitre, and
M = quantity of biomass (Ngui *et al.*, 2013; Verma *et al.*, 2015; Vikrant *et al.*, 2018)
All experiments were performed in triplicates and the numerical values were expressed as mean ± standard error and analyzed by one-way analysis of variance (ANOVA) using Microsoft Excel 2007. Readings were considered significant when P less than 0.05. Freundlich's isotherm was used to explain whether the adsorption process displayed a monolayer or multilayer pattern (Mahmoud *et al.*, 2016).

Freundlich's equation is given by;
$Qe = Kf \times B^n$
Where, Qe = Concentration of dye at equilibrium, Kf = Freundlich's constant and n = Slope of graph (log Qe versus log B) (Abel *et al.*, 2020).

RESULTS

The result for percentage adsorption by the three species (*Aspergillus sterreus*, *Bacillus* species and *Chlorella vulgaris*) is presented in Figure 1. The maximum percentage adsorption was at 48 hours and *Chlorella vulgaris* was observed to have the adsorption of about 86.4%.

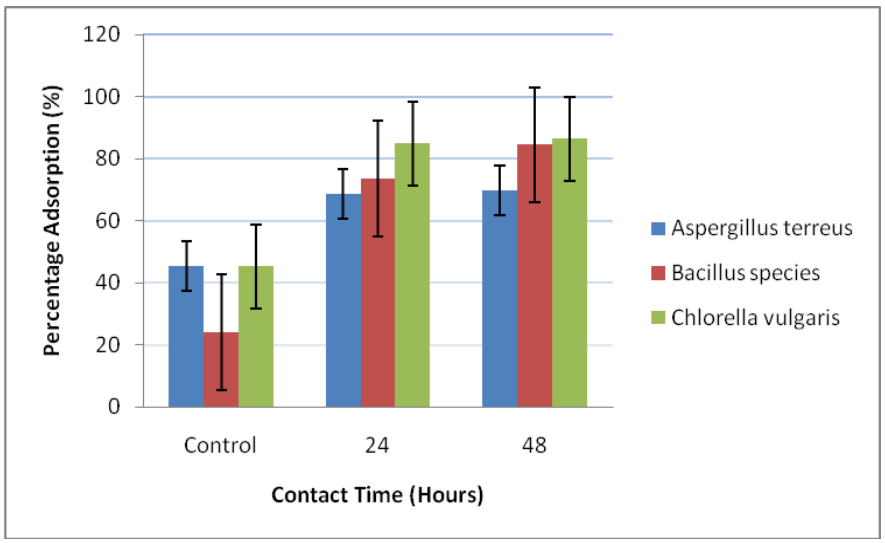

Figure 1: Percentage Adsorption of RR198 by the three Species

Table 1 shows the linear regression data for Freundlich's Isotherm for RR198 adsorption by the three species with *Bacillus* species having the highest Kf value.

Table 1: Linear regression data for Freundlich's Isotherm for RR198 Adsorption by the three Species

Microorganism	Kf (L/mg)	N	R^2
Aspergillus terreus	443.79	2.0	0.99
Bacillus species	26491	0.8	0.95
Chlorella vulgaris	14.63	0.1	0.88

Plate I below shows the decrease in dye colouration after 48 hours of inoculation of the three organisms in the dye solution. The assay with *Bacillus* species (c) displayed a clearer solution than that with *A. terreus* (d) and *C. vulgaris* (b), even though spectrophotometrically *C. vulgaris* had the highest percentage adsorption.

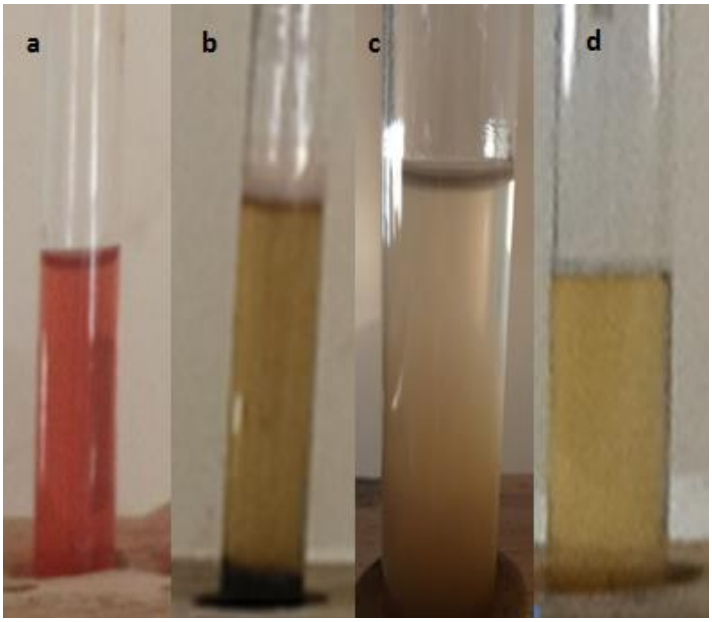

Plate I: shows the decrease in dye colouration after 48 hours of inoculation
(a) RR198 solution (control)
(b) Adsorption by *C. vulgaris* at 48 hours
(c) Adsorption by *Bacillus* species at 48 hours
(d) Adsorption by *A. terreus* at 48 hours

DISCUSSION

The maximum percentage adsorption was at 48 hours and when the incubation period was extended it was observed that spectrophotometric readings were declining which may be due to cellular activity. Previous researches have reported *C. vulgaris* to breakdown and remove certain compounds and nutrients in wastewater, as such, is capable of degrading azo compounds contained in reactive dyes to attain permissible limit for discharge into the environment (Subashini *et al.*, 2018). The dye adsorption is dependent on dye concentration, period of algal inoculation and quantity of algal biomass (Ezenweani and Kadiri, 2017). Chin *et al.* (2020) reported that *C. vulgaris* decolourized methylene blue by surface adsorption through electrostatic interaction to 84% within 3 days. Furthermore, the ability of *C. vulgaris* to decolourize a variety of azo dyes through algal azoreductase enzyme was also reported by El-Sheekh *et al.* (2009). The dye removal may be attributed to the accumulation of dye ions on the surface of algal biopolymers and diffusion of the dye molecules from aqueous phase onto solid phase of the biopolymer (Gupta *et al.*, 2006).

Bacillus species have been reported to have the ability to degrade different classes of dyes commonly used in the textile industry (Khalid *et al.*, 2012). *Bacillus* species are extensively used in degradation of dyes and other toxic effluents (Saratale *et al.*, 2011). For example, *Bacillus* sp. VUS decolourized Navy Blue 2GL at 94% within 18hours (Mullai *et al.*, 2017). *B. fusiformis* KMK5 also perfectly (100%) decolourized Disperse Blue 79 and Acid Orange 10 within 48hours as reported by Mullai *et al.* (2017). Saranraj *et al.* (2010) also isolated *Bacillus subtilis* from the textile dye effluent sample and tested it remediating capability against some reactive dyes.

This result agrees with the findings of Karim*et al.* (2018) also revealing in their study that two *Bacilli* species were able to moderately decolourize reactive dye (Bezema Red S2-B) at 37°C within 6 days when tested as individual monocultures. Maheswar and Sivagami (2016) also studied the remediation of reactive red M5B using *B. subtilis* and *B. cereus* and the results obtained were 71.5% and 72.5% respectively. Decolourization of azo red dye by two bacterial species *Bacillus megaterium* and *Bacillus cereus* under optimum conditions was revealed to be 95% and 98% respectively (Shah *et al.*, 2013). Ito *et al.* (2018) observed that during biosorption, decolourization of dyes starts with the adsorption of the dyes on bacterial cell surface, and then the colour on the stained cells disappears within a period of time depending on the rate of metabolic activity of the bacteria.

Aspergillus terreus has been found to be very effective in the decolourization and degradation of textile wastewaters because of the presence of various nonselective enzyme systems, which can act upon a wide range of substrates, enabling them to survive under

harsh conditions (Kaushik and Malik, 2009; Rohilla *et al*., 2012). The secretion of laccase, lignin peroxidases, and manganese peroxidase helps them in degrading the recalcitrant components of the wastewater (Christian *et al*., 2005). *Aspergillus* species are able to adsorb different dyes through production of biomass which act as adsorbent (Fu and Viraraghavan, 2001; Bhole *et al*., 2004). (Karthikeyan *et al*. (2010) and Wang *et al*. (2008) also revealed an *Aspergillus* species to have remediated Congo red dye and reactive brilliant red K-2BP to 94.7% within 120hours.Sen*et al*. (2016) stated that decolourization is usually accomplished by adsorption or enzymatic degradation.

Freundlich's isotherm constant, Kf revealed high values indicating an excellent adsorption capacity by all three species. A graph of log Qe against log B was plotted which revealed linear regression with R^2values less than one, thus, showing the occurrence of a multilayer adsorption pattern.

CONCLUSION

In conclusion, the results of the study revealed that all the three species had the capacity to adsorb the reactive red 198 dye as there was significant decrease in colour affinity visually and spectrophotometrically. The highest percentage adsorption was recorded after 48 hours of inoculation, with *Chlorella vulgaris* having the highest value, then, *Bacillus* species and *Aspergillus terreus*. The results showed that there is statistically significant difference of dye adsorption among the three species with *Chlorella vulgaris* having the highest adsorption potential compared to the *Bacillus* species and *Aspergillus terreus*. The adsorption process fitted with the Freundlich's isotherm, revealing a multilayer adsorption pattern.

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