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Bioremediation of Reactive Dyes by *Bacillus megaterium* and *Bacillus velezensis*

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ABSTRACT

Background and Objective: The use of synthetic dyes in fabric re-dyeing has become widespread and is one of the major sources of environmental pollution in urban Kano, Nigeria. This research was carried out to assess the potential of bacterial species isolated from one of the major dyeing sites in Kano: The Kofar Na'isa dyeing pit in the remediation of reactive dyes. **Materials and Methods:** The bacterial species (*Bacillus megaterium* QM B1551 and *Bacillus velezensis* EH9) were isolated and identified from the dye-contaminated soil using dilution, pour plate and streak culture techniques. The isolated organisms were used to assess bioremediation (biosorption and bio-decolourisation) potential on dye wastewater. **Results:** From the results, the highest dye removal efficiency by enzymatic action and biomass biosorption was recorded after 48 hrs, at pH 11.3 and a temperature of 37°C. The dye removal by biosorption and bio-decolourisation were within the ranges of 73.2-93.4 and 29.4-84.2% for *B. velezensis* and 49.3-92.5 and 26.5-92.3% for *B. megaterium*, respectively. Dye removal increased with an increase in contact time due to the growth of new bacterial cells. Freundlich's isotherm model was best fitted for the biosorption of the dyes with a strong linear correlation coefficient, R^2 ranging from 0.923-0.999 (*Bacillus velezensis*) and 0.909-1.000 (*Bacillus megaterium*). **Conclusion:** It was concluded that the bacterial species can be used in the effective remediation of reactive dyes, which in turn may greatly reduce environmental pollution.

KEYWORDS

Bacillus megaterium, *Bacillus velezensis*, bio-decolourisation, biosorption, pollution, reactive dyes, wastewater

INTRODUCTION

Wastewater management strategy for the future has to meet the benefits of humanity which include safety, respecting principles of ecology and compatibility with other habitability systems[1]. For these reasons, wastewater management technologies using microorganisms like bacteria are of great importance as most of them are natural pollutant decomposers, thus, may reduce environmental damage. The selection of bacterial species for biological treatment depends upon the chemical composition of the dye wastewater/effluent[2]. Bacterial isolates from soil and sludge samples belonging to *Bacillus*, *Alcaligenes* and *Aeromonas* species have been reported to have high dye removing potential[3].

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The use of bacterial culture for degradation of synthetic dyes started in the 1970s with a report of *Bacillus subtilis* and *Pseudomonas* species being the most active degraders isolated from aerobic dyeing house wastewater treatment facility[4,5]. An NADH-dependant azoreductase of the *Bacillus* species strain (SF) was found to be responsible for the decolourisation of azo dyes[6]. Enzymes play an important role in the remediation of synthetic dyes such as azo dyes. Enzymes involved in the degradation of azo dyes are mainly peroxidases[7]. The microbes utilise carbon, nitrogen and sulphate found in an effluent medium for nourishment. Decolourisation efficiency could be further increased and prolonged by supplementing the effluent medium with other cheaper effective carbon or energy source such as sucrose, starch and hydrolysed starch. Bacteria that can degrade dye effluent from the textile industry under aerobic conditions are *Pseudomonas* species, *Alcaligenes* species, *Sphingomonas* species, *Rhodococcus* species and *Mycobacterium* species, which have also been applied for bioremediation of pesticides[8]. Also, phosphate removal (which leads to eutrophication of lakes) is an important aerobic degradation carried out by certain heterotrophic bacteria[9]. Some bacteria such as *Bacillus* and *Pseudomonas* species are capable of storing energy in form of intracellular polyphosphate, thus removing phosphorus from the environment by biomass uptake[9].

Shi *et al.*[10] observed that certain bacterial species harbour strong enzymatic machinery necessary to remediate the recalcitrant azo bonds in dyes which can be used for building sound bioremediation systems to control environmental pollution before discharging into the terrestrial and/or aquatic environment.

Bacterial biosorption is mainly used for the removal of pollutants from effluents contaminated with pollutants that are not biodegradable, like metal ions and dyes. However, their isolation, screening and harvesting on a larger scale may be complicated but still, remain one of the efficient ways of remediating pollutants[11]. This research was aimed at assessing the potential of bacterial species isolated from the Kofar Na'isa dyeing pit in the remediation of reactive dyes.

MATERIALS AND METHODS

Study area: The study was carried out at the Research Laboratory, Department of Biological Sciences, Bayero University, Kano, Nigeria from December, 2019 to May, 2021.

Research protocol: Wastewater containing individual reactive dyes (Reactive Red 198 (RR198), reactive yellow 176 (RY176), reactive green 19 (RG19), reactive orange 122 (RO122), reactive red 195 (RR195) and reactive violet 1 (RV1) were collected in sterilized sampling bottles from a local fabric re-dyeing pit at Kofar Na'isa, Kano, Nigeria.

The bacterial species (*B. megaterium* QM B1551 and *B. velezensis* EH9) were isolated from dye-contaminated soil of Kofar Na'isa Dye Pit, Kano, Nigeria. Pure cultures of the species were sub-cultured on nutrient agar and broth at 37±2°C to generate biomass for assays[12,13]. The bacterial cells were harvested after 24 hrs by centrifuging (Centrifuge 80-2) at 10,000 rpm for 15 min. An approximate constant number of cells determined using a Neubauer chamber (Marienfeld)[14] were used for the biosorption assay, whereas, the supernatant obtained from centrifugation was utilized for the bio-decolourisation assay.

In the biosorption assay, 4.79×10^6 cells mL^{-1} of *B. megaterium* and *B. velezensis* were placed separately in individual test tubes containing 1.0 mL of wastewater (separate for each dye-RR198, RY176, RG19, RO122, RR195 and RV1) and 5.0 mL of distilled water. The initial absorbance of the solution was taken after mixing with an auto-vortex mixer and incubated at 37°C. The absorbance of the mixture was

recorded using a spectrophotometer (Model 722) at 650 nm within 48 hrs. The concentration of dye at equilibrium per gram of bacterial biomass and percentage biosorption of the dye by the cells was calculated using the expressions below[15]:

$$\text{Biosorption (\%)} = \frac{A - B}{A} \times 100 \quad (1)$$

$$Qe = A - B \times \frac{V}{M} \quad (2)$$

Where:

Qe = Concentration of dye at equilibrium
A = Initial concentration of dye in solution
B = Final concentration of dye in solution
V = Volume of solution (mL)
M = Quantity of biomass

The bio-decolourisation assay involved the use of supernatant of both species (expected to contain some amount of enzymes from the bacteria after centrifugation) from which the cells for the biosorption were removed was centrifuged (Centromix Selecta 540) at 10,000 rpm for 10 min. Nine millilitres of the supernatant was dispensed into sterilized test tubes and 1.0 mL of each dye wastewater was added and stirred. The initial absorbance of the test solution was measured at 650 nm and then, incubated at 37°C. Absorbance was subsequently recorded at an interval of 24 hrs for 2 days. Bio-decolourisation (%) of the dye by enzyme activity was then calculated using[16]:

$$\text{Biodecolourisation (\%)} = \frac{A - B}{A} \times 100$$

Where:

A = Initial concentration of the dye in solution
B = Final concentration of dye in solution after enzyme activity

Freundlich's isotherm was used to explain the pattern of biosorption employed by the species. A graph of log Qe against log B was plotted[17]. All experiments were performed in triplicates and the numerical values were expressed as Mean±Standard deviation and analysed by One-way Analysis of Variance (ANOVA) using Microsoft Excel 2007. Readings were considered significant when $p<0.05$.

RESULTS

Effective biosorption and bio-decolourisation potentials of dyes by the two bacterial species (*B. megaterium* and *B. velezensis*) were observed at varying levels (Fig. 1 and 2). The biosorption (%) of the dyes by *B. megaterium* from highest to least is as follows, RG19 (92.5%), RV1 (90.6%), RR198 (84.9%), RO122 (81.6%), RY176 (68.9%) and RR195 (49.3%). For *B. velezensis*, the percentage biosorption were RV1 (93.4%), RG19 (91.2%), RY176 (91.1%), RO122 (90.2%), RR198 (88.5%) and RR195 (73.2%) (Fig. 1). The results for bio-decolourisation (%) for *B. megaterium* are, RG19 (92.3%), RV1 (88.7%), RR198 (64.8%), RO122 (47.2%), RR195 (31.3%) and RY176 (26.5%), whereas, *B. velezensis* had the following percentages, RG19 (84.2%), RV1 (73.2%), RO122 (72.4%), RR198 (52.5%), RR195 (46.5%) and RY176 (29.4%) (Fig. 2). The results revealed biosorption using biomass to be more effective as both the two species had higher percentages interns of biosorption of the dyes.

Freundlich's isotherm constants for biosorption of dyes are presented in Table 1. Freundlich's isotherm explains the pattern of biosorption by the two species to be a multi-layered type. This is because the

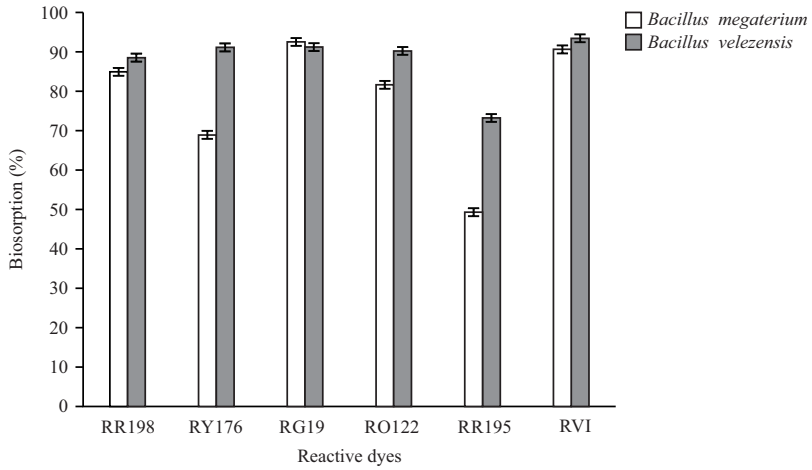

Fig. 1: Percentage of biosorption of the dyes by the bacterial species after 48 hrs of inoculation

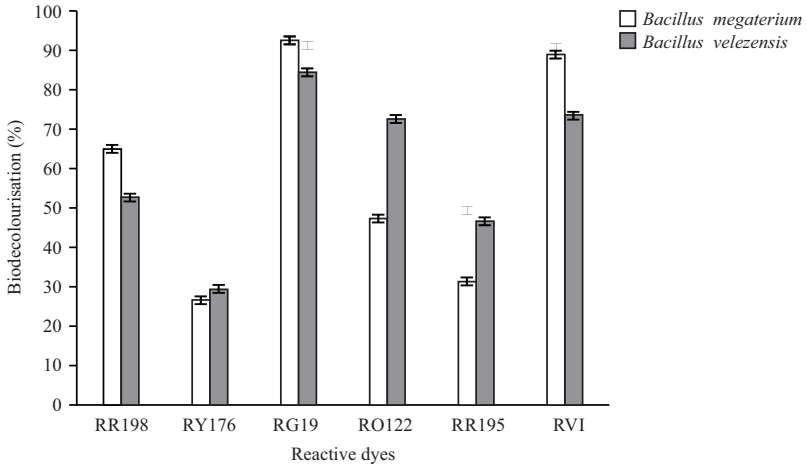

Fig. 2: Percentage bio-decolourisation of the dyes by the bacterial species after 48 hrs of inoculation

Table 1: Linear regression data for Freundlich's isotherm for biosorption of reactive dyes by the bacterial species isolated from dye-contaminated soil of Kofar Na'isa Dye Pit, Kano, Nigeria

		Reactive dyes					
Bacterial species	Freundlich's constant	RR198	RY176	RG19	R0122	RR195	RV1
Bacillus megaterium	K_f	25256	77500	8687	44333	437500	10694
	N	0.79	1.32	0.21	0.70	2.15	0.13
	R^2	0.999	0.998	0.999	0.999	0.999	0.923
Bacillus velezensis	K_f	22643	20500	8856	12618	80000	10592
	N	0.70	0.70	0.23	0.52	1.27	0.10
	R^2	0.999	0.993	1.00	0.999	0.999	0.909

Numerical values of Freundlich's model constants K_f were observed to be high in all the dyes

regression coefficients, R^2 were less than or equal to one, which also signifies a strong linear correlation. The Freundlich's constant, K_f were high, thus, showing the capability of the species in effective biomass biosorption.

As presented in Fig. 1, the highest biosorption ability (93.4%) was observed by *B. velezensis* on reactive violet 1 (RV1) and the least (49.3%) by *B. megaterium* on reactive red 195. Significant differences ($p<0.05$) were observed in the biosorption ability of *B. megaterium* on the different dyes.

The highest and least percentage of bio-decolourisation was by *B. megaterium* on reactive green 19 (92.3%) and reactive yellow 176 (26.5%).

DISCUSSION

The results of the study revealed the highest dye removal efficiency by enzymatic action and biomass biosorption to be achieved after 48 hrs, at pH 11.3 and a temperature of 37°C. The dye removal by biosorption and bio-decolourisation were within the ranges of 73.2-93.4 and 29.4-84.2% for *B. velezensis* and 49.3-92.5 and 26.5-92.3% for *B. megaterium*, respectively. Dye removal increased with an increase in contact time due to the growth of new bacterial cells.

Bacterial species with different morphological and physiological characteristics were reported to have been isolated from contaminated soils and effluents from textile industrial areas, some of which showed high efficiency in the removal of dyes[18]. The bacterial species isolated from the dye-contaminated soil in this study were identified as members of the *Bacillus* genus. All of the isolated species were observed to have remediated the dyes to certain levels. *Bacillus* species have been reported to have the ability to degrade different classes of dyes commonly used in the textile industry[19]. *Bacillus* species are extensively used in the degradation of dyes and other toxic effluents[20]. For example, *Bacillus* sp. VUS and *B. fusiformis* KMK5 decolourised navy blue 2GL, disperse blue 79 and acid orange 10 and within 48 hrs[21]. This also agrees with the findings of Karim *et al.*[22] revealing in their study that two *Bacilli* species were able to moderately decolourise reactive dye (Bezema red S2-B) at 37°C within 6 days when tested as individual monocultures. Colour removal for acid red 337 by *B. megaterium* was reported at 91% within 24 hrs at the optimum temperature of 30°C and pH of 7. It was also observed to have removed several red dyes from wastewater within 10 days[23]. In another study by Shah[24], *B. megaterium* removed 73% of acid orange dye and five additional azo dyes within 38 hrs under static conditions. Nair *et al.*[25], used *B. megaterium* to degrade and decolourise four azo dyes to 95% at neutral pH and temperature of 40°C.

Bacillus velezensis is known for its possession of azoreductases which are used to decolourise azo dyes of different molecular structures through enzymatic action[26,27]. It was also reported that *B. velezensis* contains a biopolymer that was isolated and used to decolourise azo dyes by bio-flocculation, with 91% efficiency[28].

Arora *et al.*[29] and Asad *et al.*[30] isolated Bacillus firmus and Halomonas species, respectively from textile effluent that had the potential to reduce textile azo dyes into simpler and less toxic compounds. Effective degradation of Reactive Blue 160 (RB160) by *B. firmus* isolated from dye-contaminated soil of the textile industry was reported by Barathi *et al.*[31]. Saranraj[32] also isolated *Bacillus subtilis* from the textile dye effluent sample and tested its remediating capability against some reactive dyes.

The results for remediation of RG19 differ greatly from the findings of Maheswar and Sivagami[33] that used *B. subtilis* and *B. cereus* to remediate malachite green (49 and 38.1%, respectively) but were in agreement with the findings of Vani *et al.*[34] that revealed *Bacillus* species can decolourise 92% of malachite green at 45°C after 72 hrs under shaking conditions[34]. In the remediation of RO122, all species were able to biosorp as well as bio-decolourise the dye to a certain level. The result was in line with the findings of Sriram *et al.*[35], who reported 77% decolourisation of reactive orange-M2R by *Bacillus* species. Karim *et al.*[22], Tripathi and Srivastava[36] also revealed in their study that *B. megaterium* and another *Bacilli* species were able to moderately decolourise novacron orange FN-R and orange G at 37°C within 6 days. The result differs from the findings of Modi *et al.*[37], who reported reactive red 195 with 97% decolourisation by *B. cereus* due to the addition of maltose and peptone as the ideal carbon and nitrogen sources during test preparation. Guadie *et al.*[38] and Maheswar and Sivagami[33] also studied the remediation of reactive pink MB, reactive purple and reactive red 239 dye using *B. subtilis*, *B. cereus* and *Bacillus* sp. strain CH12 and the results obtained were also in agreement with the findings of this research. Ito *et al.*[39], observed that during biosorption, decolourisation of dyes starts with the adsorption of the dyes on the bacterial cell surface and then the colour on the stained cells disappears within a period depending on the rate of

metabolic activity. For decades, *Bacillus subtilis* and *Staphylococcus aureus* have been used as biosorbent for the removal of reactive dyes like reactive blue, reactive red, reactive violet and reactive yellow[40,41]. Several types of research have revealed the effectiveness of *Bacillus* species (like *B. subtilis*, *B. cereus* and *B. megaterium*) in the remediation of a wide variety of synthetic dyes[22,33-36].

The numerical values of Freundlich's model constants K_f and N are presented in Table 1. The values explain the biosorption potentiality and intensity exhibited by the bacterial biomass[42,43]. The values of K_f and N reveal that the biosorption abides by the multilayer type that occurs at numerous sites on the bacterial cell surface occurring gradually until complete saturation is attained[43-45].

The discharge of untreated dye wastewater into the environment is undesirable as it causes serious environmental pollution due to its colour and toxicity. Due to the aforementioned reasons, dye-degrading bacterial species (like those used in this study) can be used in the remediation of such wastewater. Also, since previous research has shown that most of the dye remediation by these organisms is achieved through enzymatic action, thus, more strategies on how to extract and cultivate a high yield of dye-degrading enzymes contained in these organisms should be developed.

CONCLUSION

In conclusion, the two species, *B. megaterium* and *B. velezensis* displayed effective bioremediation potential on all the reactive dyes studied. Differences were also observed in the biosorption of dyes by *B. megaterium*.

SIGNIFICANCE STATEMENT

The increasing spread of re-dyeing processing activities in urban Kano is causing some concern to the population. The discharge of untreated wastewater from re-dyeing activities is unfavourable as it pollutes the environment with its persisting colour and formation of toxic carcinogenic intermediates such as aromatic amines that form as a result of dye degradation. Dye-contaminated environments are unsuitable for the survival of many ecologically important organisms (soil and aquatic) due to their toxicity. However, some organisms may adapt to the dye-contaminated environments consequently resulting in the accumulation of toxic compounds in the tissues of these organisms. These toxic compounds could be transferred to humans via food chains, which in turn may cause severe health issues.

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