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DECOLOURATION OF DYE IN WASTEWATER BY SELECTED FUNGAL AND BACTERIAL SPECIES: A BIOREMEDIATION APPROACH

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ABSTRACT

The objective of this investigation was to ascertain the dye decolouration ability of selected bacterial (Escherichia coli and Pseudomonas aeruginosa) and (Aspergillus niger and Aspergillus flavus) species in wastewater. The study was carried out under shake flasks conditions. After inoculation with the respective isolates, aliquot wastewater samples were aseptically removed from each flask, prior inoculation and every 24 h for the next 96 h for the estimation of dye concentration, using standard procedures. The results revealed optimum temperature for dye decolouration in the presence of the test isolates to range from 25 °C to 35 °C. A pH of 6 and 10 was observed to enhance dye by the bacterial species while 8 and 10 were observed to be optimum for the fungal species. At the different concentrations of sodium acetate used for investigation, 5 g/L was observed to be optimum, a trend that was observed in the presence of all the isolates. Among the different external carbon sources used for investigation, the study revealed methanol as the ideal external carbon source that enhanced dye decolouration by the isolates. With respect to nitrogen source, maximum decolouration was observed with yeast extract source, in the presence of the Aspergillus niger, Aspergillus flavus *and* Pseudomonas aeruginosa. *None of the concentrations of peptone used for investigation enhanced decolouration by the test isolates. This trend was also irrespective of the isolate used for investigation. The study was able to reveal the effect of temperature the optimum temperature, pH, sodium acetate and peptone concentrations and carbon and nitrogen sources in the declaration of decolouration of the test dye in wastewater by the microbial species used for the investigation.*

Keywords: Dye decoloration, Bacteria, Fungi, Wastewater.

1. INTRODUCTION

Textile effluent is among the most difficult to treat wastewater due to their considerable amount of toxic substances [1]. Globally, an estimate of 280,000 tons of textile dye is discharged in textile effluent every year [2]. The textile industry has the largest amount of aqueous waste and dye effluents which are discharged from dyeing process having a strong persistent color and high biological oxygen demand both making it aesthetically and environmentally unacceptable

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[3]. The dyestuff discharged into water bodies; reduce transparency, therefore reducing the dissolved oxygen concentration affecting aerobic organisms [4].Treatment of dyeing wastewater is difficult due to the complex aromatic structure and synthetic origin of most dyes.

Generally, synthetic dyes can be classified as anionic (direct, acid and reactive dyes), cationic (basic) and non-ionic (disperse) [5]. Color results from the presence of dye in wastewater and can affect the photosynthetic activity in the water body it is perceived by the public as the recognition of water quality and the presence of very small amounts of dye in water even less than 1ppm for some dyes is highly visible [6].

The methods used for decolorisation of dye include physical and chemical methods that include: chemical coagulation/flocculation, ozonation, oxidation, ion exchange, irradiation, precipitation and adsorption [7]. As these methods are very expensive, biological method is preferred as it is more economical and eco-friendly when compared [8]. Microbial decolorisation methods, such as bioremoval by growing cultures in the medium and biosorption by living or dead microbial mass are commonly applied, to the treatment of effluents from the textile industry. This is because several microorganisms such as bacteria, yeast, algae and fungi are able to remove different classes of dye [9, 10].The use of bacteria for dye removal has been extensively reviewed [11]. Some algae have also been used [12]. The role of fungi has also been extensively researched and reviewed [13, 14].

In previous studies, various bacterial species have been implicated in the decolorization of the textile effluent. Some bacteria have also been investigated for their potential in the reduction and stabilization of textile effluents [15-17]. Also, fungi have been reported to be suitable microorganisms for the treatment of textile effluents and dye removal [18]. This study was aimed at investigating the dye decolouration ability of selected bacterial (*Escherichia coli* and *Pseudomonas aeruginosa*) and (*Aspergillus niger* and *Aspergillus flavus*) species in wastewater.

2. MATERIALS AND METHODS

A total of four microbial isolates consisting, of two bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and two fungi (*Aspergillus niger* and *Aspergillus flavus*) were used for this study. The isolates were part of the laboratory stock at the Department of Biological Sciences, Landmark University, Omu-Aran, Kwara State, Nigeria.

Before the isolates were used in the study, the bacteria and fungi were first streaked on sterile nutrient agar (at 35 ^{o}C for 24 h) and sabourand dextrose agar (at 30 ^{o}C for 48 h) plates, respectively to first ascertain their purity. The pure cultures were then subcultured into agar slants before storing in the refrigerator at 4 ^{o}C until when needed. Prior to use, each bacterial and fungal isolate was first suspended in sterile normal saline (0.85 % w/v NaCl in distilled water), after which the population of each of the microbes per mL in the normal saline was estimated and expressed as colony-forming and spore-forming units, for the bacteria and fungi, respectively.

The wastewater used for the study was obtained from the Landmark University Commercial Farm located in Omu-Aran, Kwara State, Nigeria. The wastewater, which was collected in five litre containers, was first filtered, using Whatman No 1 filter paper and then supplemented with

the a known quantity of the test dye, used for the study and the required carbon and nitrogen source. After filtering and supplementing with sodium acetate (5 g/L) and yeast extract (5 g/L) to serve as external carbon and nitrogen source, respectively, and a known quantity of the test dye, the wastewater was dispensed in 200 mL quantities into 250 mL capacity conical flasks and sterilized in an autoclave at 121 °C for 15 min at 15 psi. Before sterilization, the pH of the supplemented wastewater was adjusted to 7, except when pH variation study was carried out.

The dye used for the investigation was a neutral dye (Trade name: G & W Color Dye) obtained from the Department of Biological Sciences, Landmark University, Omu-Aran, Kwara State, Nigeria. For the dye decolouration study, to the sterilized wastewater in a conical flask, a known inoculum size of the respective test bacterial and fungal isolate was inoculated and incubated at a temperature of 30 °C (except for the temperature variation investigation), in a shaker incubator at a speed of 150 rpm. Prior to inoculation and every 24 h, for 96 h duration, aliquot wastewater sample was aseptically collected from each flask for the estimation of the dye concentration. For the estimation of dye concentration, a calibration curve was first obtained by preparing different concentrations of the dye and their respective absorbance measured at a wavelength of 620 nm. During each sampling, the absorbance of the respective samples was estimated from the calibration curve, using the equation of a line obtained from the curve.

The different parameters investigated for in this study were temperature (25 °C, 35 °C and 45 °C), pH (6, 8 and 10), sodium acetate concentration (5 g/L, 10 g/L and 15 g/L), peptone concentration (5 g/L, 10 g/L, 15 g/L and 20 g/L), external carbon source (glucose, lactose, sucrose, methanol and sodium acetate) and external nitrogen sources (peptone, yeast extract and meat extract). All experimental procedures were carried out in triplicates. The reagents used were all of analytical grades.

3. RESULTS

As shown in Fig.1, the variation in dye concentration in the wastewater in the presence of *Aspergillus niger* after 96 h incubation period change from 191.12 mg/L to 199.62 mg/L, from 181.01 mg/L to 181.81 mg/L and from 181.63 mg/L to 198.19 mg/L at 25°C, 35°C and 45°C, respectively. Similarly, in the presence of *Aspergillus flavus*, the variation in the dye concentration at the end of 96 h was observed to change from 191.85 mg/L to 196.97 mg/L from 180.26 mg/L to 168.94 mg/L and from 194.74 mg/L to 219.05mg/L, at incubation temperatures of 25 °C, 35 °C and 45 °C, respectively. No remarkable change in the dye concentration in the wastewater was observed throughout the expiration of incubation. This trend was irrespective of the different temperatures. In the presence of the test fungal species, minimum decreases in dye concentrations were observed after 72 h, after incubation temperatures of 25 °C and 35 °C, for *Aspergillus niger* and *Aspergillus flavus*, respectively (Fig. 1). In the presence of *E. coli*, the variation in dye concentration in the wastewater at the end of the 96 h incubation time was observed to vary from 193.89 mg/L to 154.63 mg/L, from 180.42 mg/L to 199.60 mg/L and from 195.62 mg/L to 2308.09 mg/L at incubation temperatures of 25 °C, 35 °C and 45 °C, respectively. Similarly, in the presence of *Pseudomonas aeruginosa*, the variation in dye concentration at the end of the 96 h

incubation period varied from 191.56 mg/L to 186.53 mg/L, at 25 °C, from 185.59 mg/L to 193.74 mg/L at 35 °C and from 197.17 mg/L to 217.01 mg/L at 45 °C (Fig. 1).

With respect to pH, in the presence of *Aspergillus niger*, the dye concentration in the wastewater at the end of 96 h incubation period was observed to vary from 352.51 mg/L to 193.59 mg/L, from 279.82 mg/L to 140.84 mg/L and from 371.84 mg/L to 240.84mg/L at pH 6, 8 and 10, respectively. In the presence of *Aspergillus flavus*, the concentration of dye in the wastewater varied from 342.39 mg/L to 414.49 mg/L at pH 6, from 276.73 mg/L to 244.50 mg/L at pH 8 and from 388.70 mg/L to 244.50 mg/L at pH 10 (Fig. 2). After 96 h incubation time, dye concentrations in the wastewater at the different pH were observed to vary from 346.71 mg/L to 288.70 mg/L, from 254.64 mg/L to 296.37 mg/L and from 369.08 mg/L to 299.44 mg/L at pH 6, 8 and 10 respectively in the presence of *E. coli*. In the presence of *Pseudomonas aeruginosa*, the dye concentration at the end of 96 h incubation varied from 347.07 mg/L to 344.23 mg/L, from 267.07 mg/L to 344.23mg/L and from 364.48mg/L to 239.92mg/L at pH 6, 8 and 10, respectively (Fig. 2).

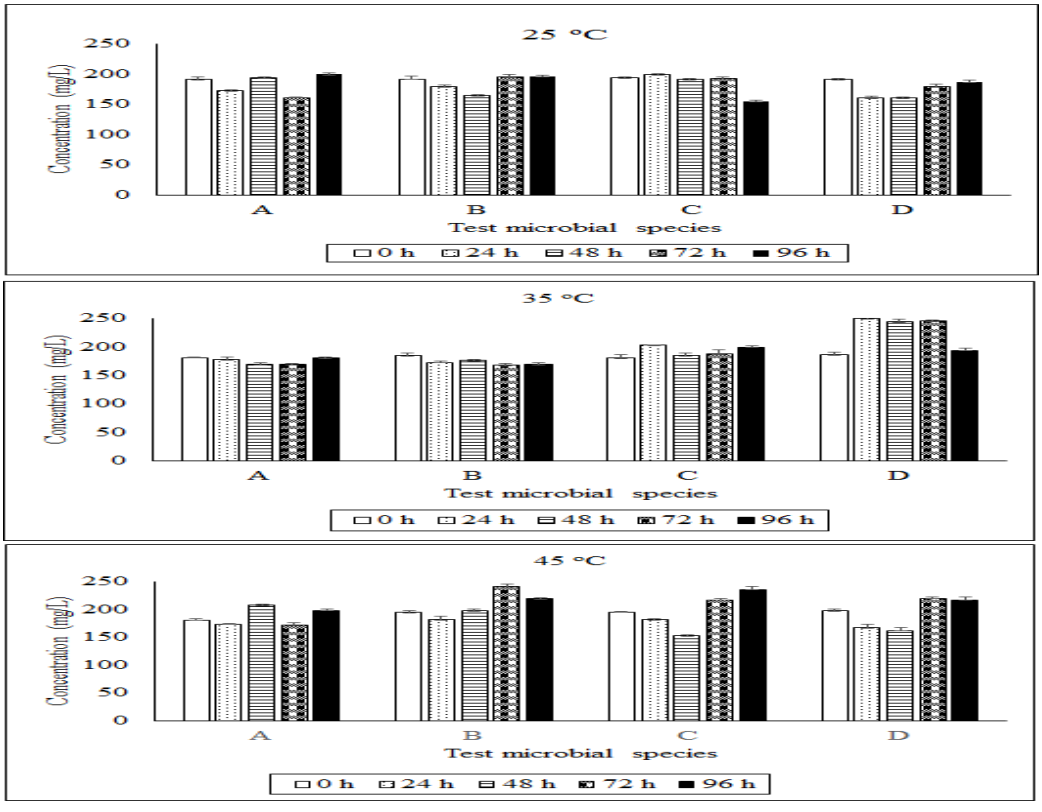


Fig-1. Concentrations of dye in the wastewater at the different incubation temperatures in presence of the test microbial species. A, B, C and D represent *Aspergillus niger*, *Aspergillus flavus*, *Escherichia coli* and *Pseudomonas aeruginosa*, respectively

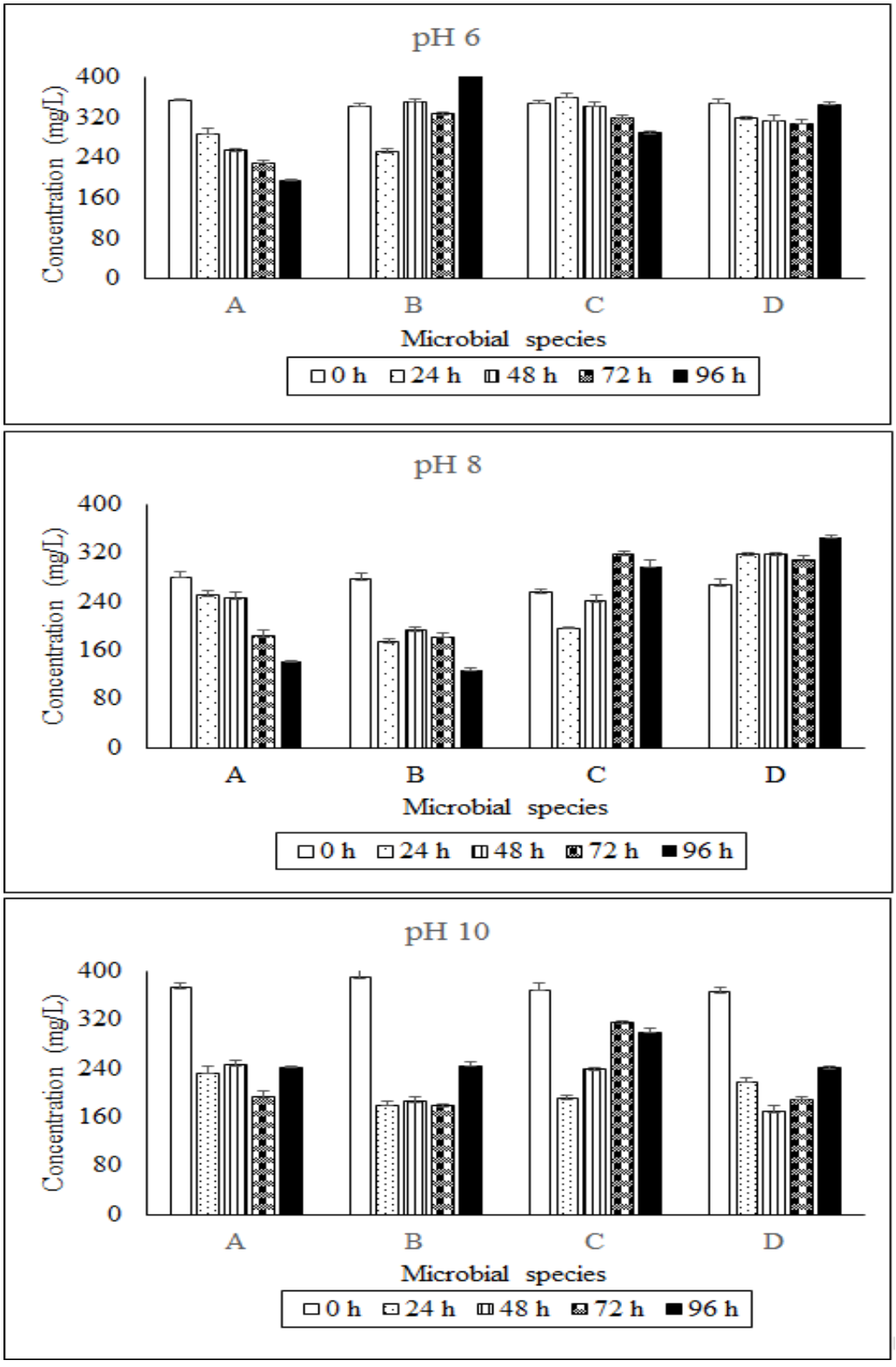


Fig-2. Concentrations of dye in the wastewater at the different pH in presence of the test microbial species. A, B, C and D represent *Aspergillus niger*, *Aspergillus flavus*, *Escherichia coli* and *Pseudomonas aeruginosa*, respectively

At the different sodium acetate concentrations of 5 g/L, 10 g/L and 15 g/L in the wastewater, dye concentration in the presence of *Aspergillus niger* was observed to vary from initial levels of 240.59mg/L, 274.79 mg/L and 350.05 mg/L to final levels after 96 h incubation of 366.32 mg/L, 254.95 mg/L and 437.50 mg/L, respectively. Similarly, in the presence of *Aspergillus flavus*, concentrations of dye in the wastewater after 96 h incubation period varied from 240.84 mg/L to 176.41mg/L, from 274.79 mg/L to 254.95 mg/L and from 364.49 mg/L to 385.07mg/L at sodium acetate concentration of 5 g/L, 10 g/L and 15 g/L, respectively (Fig. 3). When inoculated with *E. coli*, dye concentration after 96 h incubation time was observed to change from 242.07mg/L to 314.83 mg/L, from 289.92mg/L to 308.33mg/L and from 370.23mg/L to 447.62mg/L at sodium acetate concentrations of 5g/L, 10g/L and 15g/L, respectively. In the presence of *Pseudomonas aeruginosa*, at the expiration of incubation, dye concentration in the wastewater varied from initial levels of 344.60 mg/L, 284.64 mg/L and 360.79 mg/L, to final levels of 296.69 mg/L, 253.73 mg/L and 370.00 mg/L, at sodium acetate concentrations of 5g/L, 10g/L and 15g/L, respectively (Fig. 3). A general observation in the presence of the different test isolates was a remarkable decrease in dye concentration at sodium acetate concentration of 5 mg/L. At higher sodium acetate concentrations there either extreme low decreases in dye concentrations or sharp increases.

When different substrates were used as external carbon sources in the wastewater in the presence of *Aspergillus niger*, the concentration of dye in the wastewater after the expiration of incubation was observed to vary from 246.46 mg/L to 322.45 mg/L, from 288.41 mg/L to 468.79 mg/L, from 266.93 mg/L to 268.45 mg/L, from 276.14mg//L to 128.55 mg/L and from 262.02 mg/L to 382.82mg/L in the presence of glucose, lactose, sucrose, methanol and sodium acetate, respectively. In the presence of *Aspergillus flavus*, dye concentration after 96 h incubation varied from 287.70 mg/L to 406.85 mg/L, from 281.64 mg/L to 408.04 mg/L, from 284.09 mg/L to 469.42 mg/L, from 283.92 mg/L to 290.37 mg/L and from 290.37mg/L to 486.28 mg/L, in the presence of glucose, lactose, sucrose, methanol and sodium acetate respectively (Fig. 4). In the presence of *Escherichia coli*, dye concentration in the wastewater at the end of 96 h incubation showed a variation from 274.28 mg/L to 153.09 mg/L, from 269.98 mg/L to 381.66 mg/L, from 270.61 mg/L to 487.51mg/L, from 296.08 mg/L to 220.28 mg/L and from 282.14 mg/L to 465.42 mg/L in the presence of glucose, lactose, sucrose, methanol and sodium acetate as different carbon source respectively. Also, when *Pseudomonas aeruginosa* was used for inoculation, variation in dye concentration at the end of incubation was observed to vary from 256.48 mg/L to 226.73mg/L, from 266.30 mg/L to468.79 mg/L, from 263.87 mg/L to 409.32mg/L, from 258.96 mg/L to 169.97 mg/L and from 241.78 mg/L to 439.03 mg/L in glucose, lactose, sucrose, methanol and sodium acetate, respectively (Fig. 4).

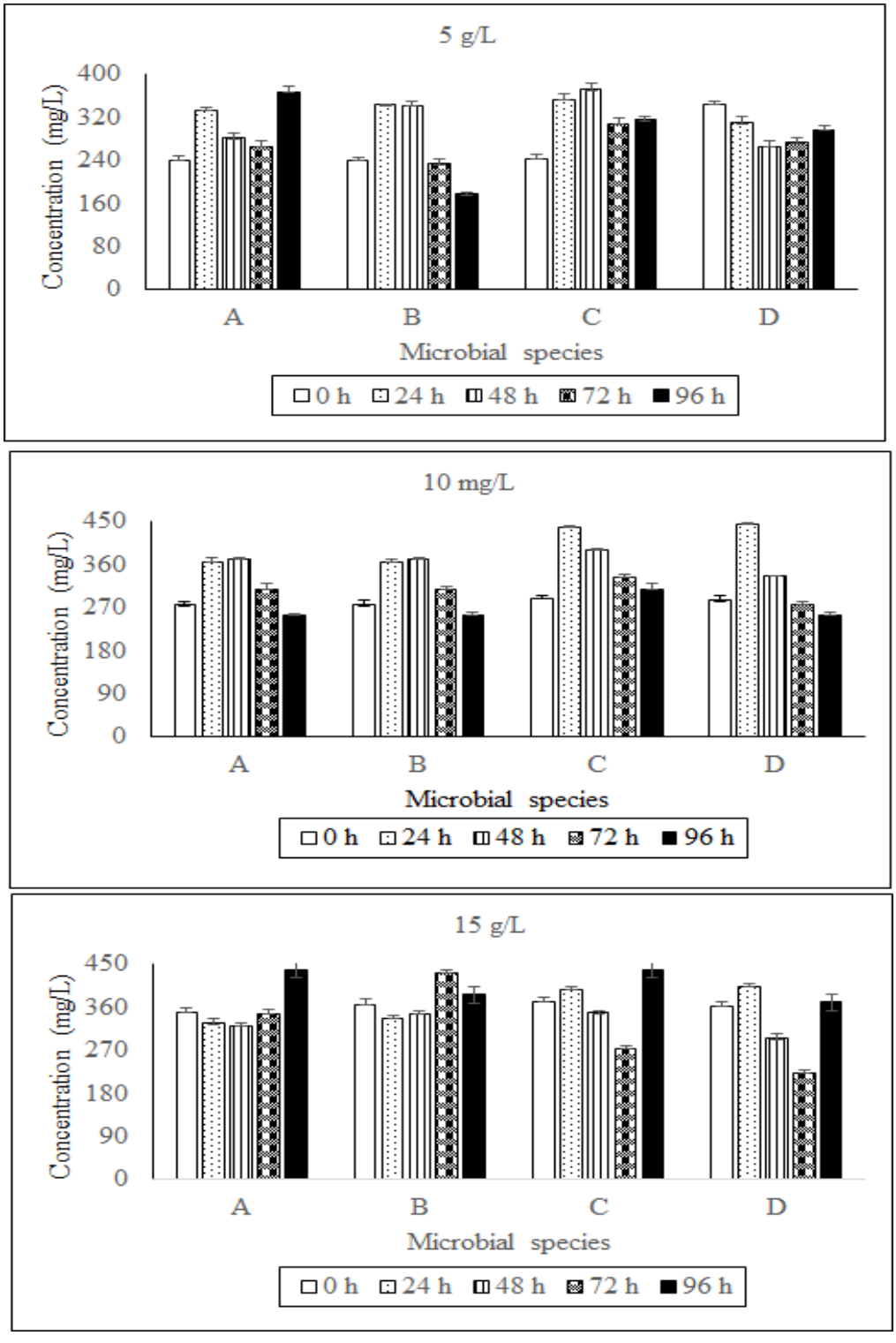
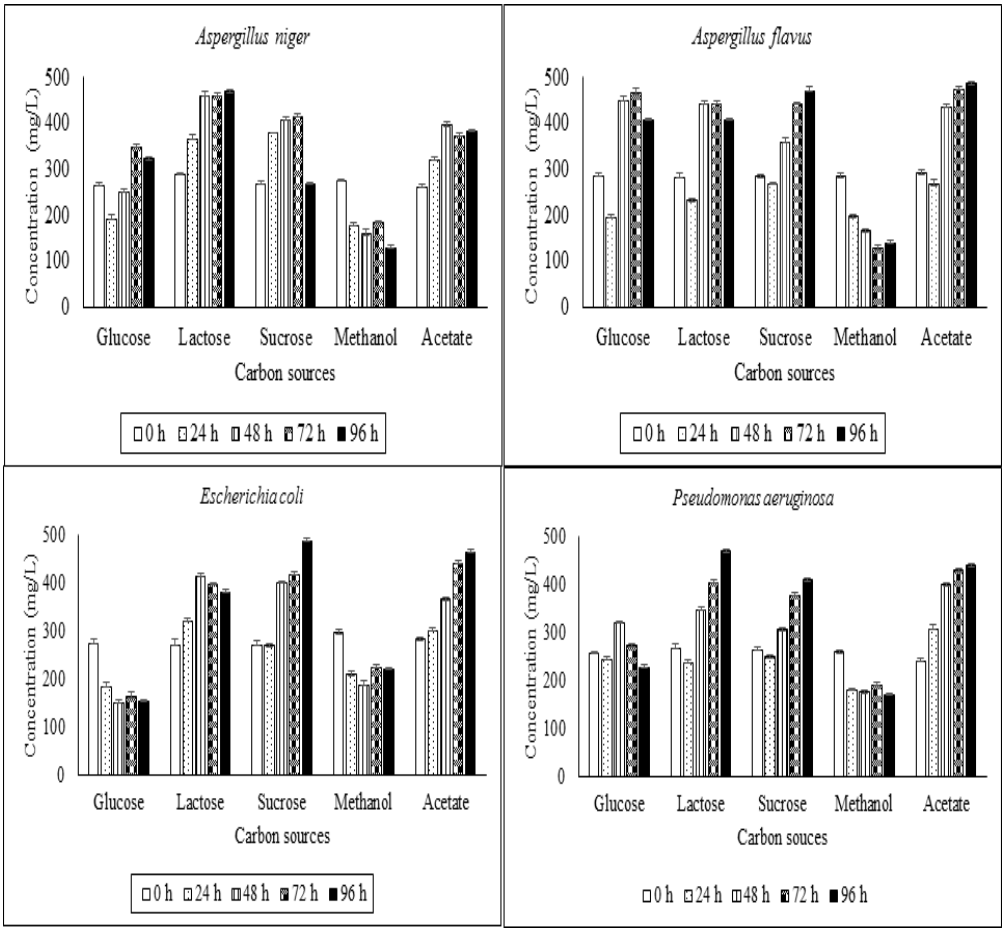


Fig-3. Concentrations of dye in the wastewater at the different sodium acetate concentrations in presence of the test microbial species. A, B, C and D represent *Aspergillus niger*, *Aspergillus flavus*, *Escherichia coli* and *Pseudomonas aeruginosa*, respectively



Fig–4. Concentrations of dye in the wastewater at the different carbon sources in presence of the test microbial species

In the presence *Aspergillus niger* at the different of concentrations of peptone in the wastewater, dye concentrations after 96 h varied from 147.71 mg/L to 243.59 mg/L, from 141.74mg/L to 182.55mg/L, from 139.96 mg/L to 319.07 mg/L and from 132.23 mg/L to 487.19mg/L at 5g/L, 10g/L, 15g/L and 20g/L of peptone, respectively. When inoculated with *Aspergillus flavus*, concentration of dye in the wastewater showed a variation after 96 h incubation from 140.53 mg/L to 280.42 mg/L, from 150.58 mg/L to 203.72 mg/L, from 137.45 mg/L to 441.79 mg/L and from 139.29mg/L to 463.57mg/L at 5g/L, 10 g/L, 15g/L and 20g/L, respectively (Fig. 5). Dye levels in the wastewater at the different concentrations of peptone dye showed variations after the 96 h incubation period in the presence of *E. coli* from 140.04 mg/L to 293.91mg/L, from 130.69 mg/L to 313.55 mg/L, from 127.02 mg/L to 470.14mg/L and from 131.92 mg/L to 489.06mg/L at 5g/L, 10g/L, 15g/L and 20g/L, respectively. In the presence of *Pseudomonas aeruginosa*, dye levels were observed to change at the end of the incubation period from 155.80 mg/L to 317.84 mg/L, from 154.32 mg/L to 255.87 mg/L, from 158.00 mg/L to 477.99mg/L and from 151.25 mg/L to 457.49mg/L at 5g/L, 10g/L, 15g/L and 20g/L, respectively (Fig. 5).

As shown in Fig. 6, in the presence of *Aspergillus niger* at the different nitrogen sources, dye concentration in the wastewater varied after 96 h incubation from 274.28 mg/L to 189.60 mg/L, from 242.99 mg/L to 260.47 mg/L and from 192.67mg/L to 138.98mg/L in the presence of yeast extract, peptone and meat extract as source of nitrogen respectively. Similarly, variation dye variation in the presence of *Aspergillus flavus* was observed to varied from 256.79 mg/L to 234.19 mg/L, from 200.03 mg/L to 277.96 mg/L and from 185.61 mg/L to 202.18 mg/L in the presence of yeast extract, peptone and meat extract, respectively (Fig. 6). Similarly, in the presence of *Escherichia coli*, dye concentration after 96 h showed a variation from 283.79 mg/L to 223.35 mg/L, from 207.09 mg/L to 353.43 mg/L and from 200.65 mg/L to 140.51 mg/L, for yeast extract, peptone and meat extract, respectively. In the presence of *Pseudomonas aeruginosa*, at the end of the 96 h incubation period, dye concentration was observed to change from 255.56 mg/L to 250.04 mg/L, from 205.19 mg/L to 316.62 mg/L and from 201.87 mg/L to 322.45 mg/L in the presence of yeast extract, peptone and meat extract, respectively (Fig. 6).

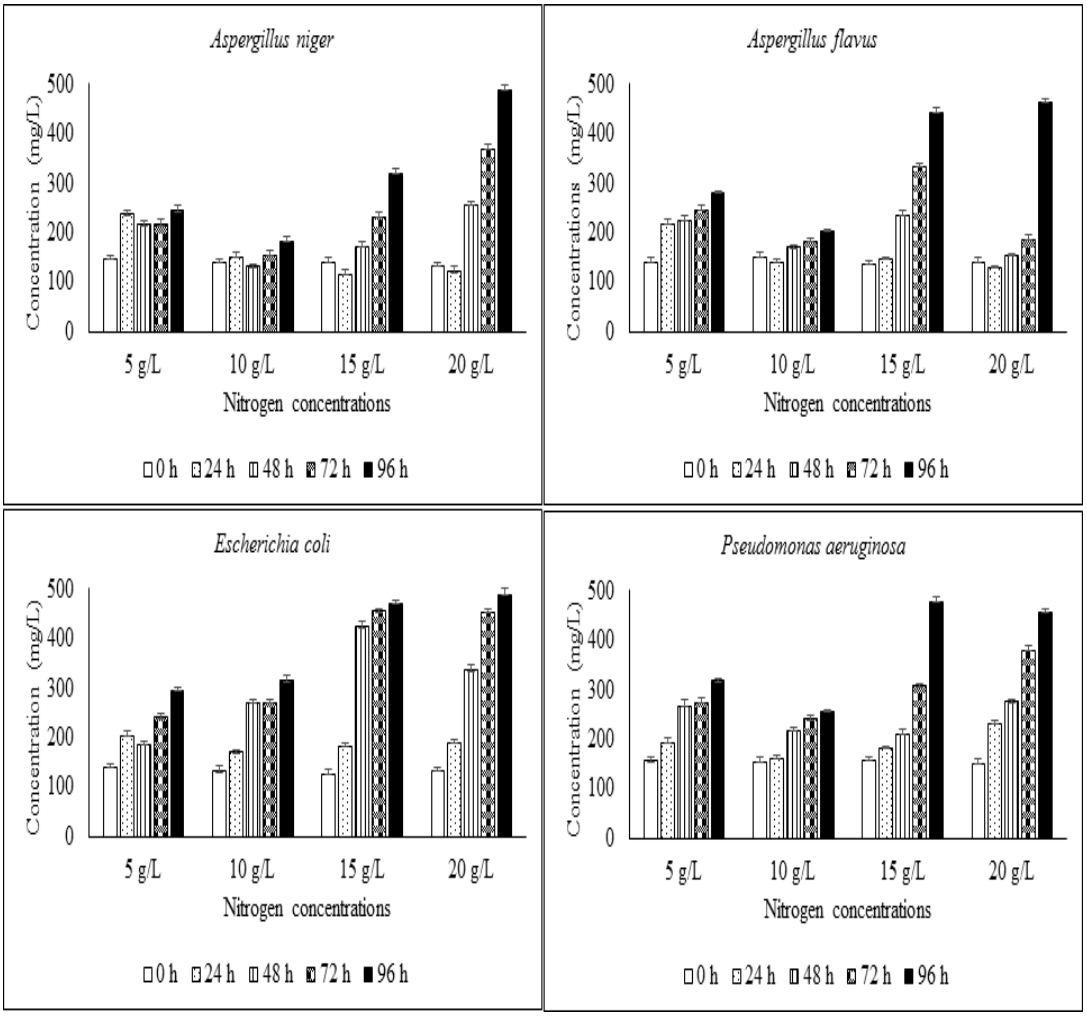


Fig-5. Concentrations of dye in the wastewater at the different peptone concentrations in presence of the test microbial species

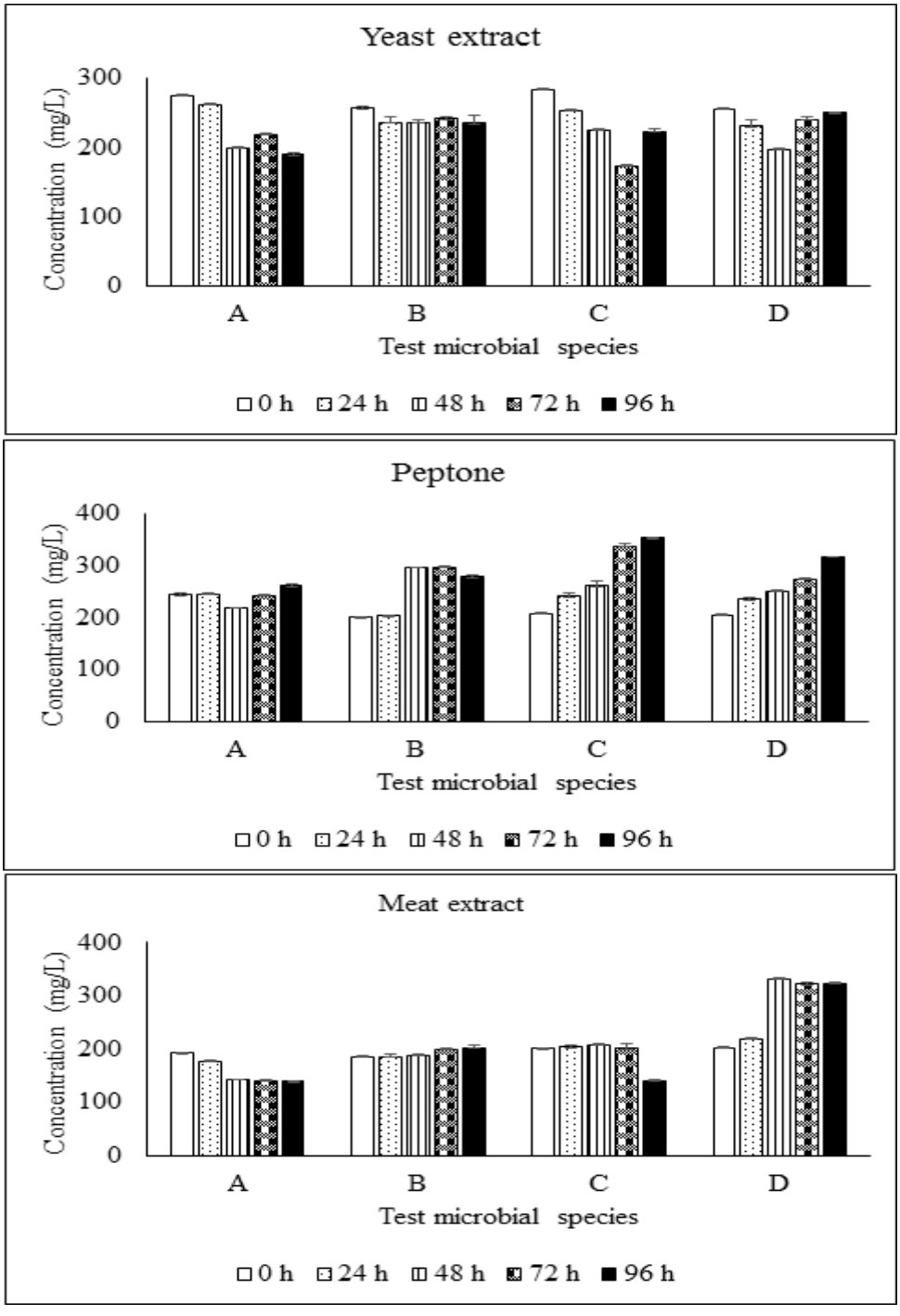


Fig-6. Concentrations of dye in the wastewater at the different nitrogen sources in presence of the test microbial species. A, B, C and D represent *Aspergillus niger*, *Aspergillus flavus*, *Escherichia coli* and *Pseudomonas aeruginosa*, respectively

4. DISCUSSION

The present study used of four test isolates that consisted of two bacterial species (*Pseudomonas aeruginosa* and *Escherichia coli*) and two fungal species (*Aspergillus niger* and

Aspergillus flavus). The bacterial isolates have been implicated in the decolorization and degradation of direct azo dyes and biodegradation of textile dye effluent. Some studies have indicated that fungi, such as *Aspergillus niger* are suitable microorganisms for the treatment of textile effluents and dye removal from wastewaters [17, 18].

In the present study, the bacterial species showed remarkable reduction in dye concentration at the end of incubation at incubation temperature of 25 °C and 35 °C was. The ability of *Pseudomonas aeruginosa* in color removal has been reported by earlier workers. Saranraj, et al. [17] have indicated that *Pseudomonas aeruginosa* as a good decolourizer of Congo dye. Species of *Pseudomonas* has also been reported to display remarkable dye removal ability at temperatures between 30 ºC–40 ºC [19]. Temperature is indicated to have effects on microbial growth, and enzyme production and consequently on rate of dye decolourisation. Also, *Micrococcus* has been reported to decolourise 60% of 300 ppm of Orange MR at 30 °C, 80% at 35 °C and 42% at 45 °C [20]. In a study by Jadhav, et al. [21], the ability of *Pseudomonas aeruginosa* in the degradation of 50 ppm of Remazol Red at different temperature was indicated. In the report, 97 %, 72 % and 82 % of the dye were degraded at 40 °C, 10 °C and 30 °C, respectively.

Similarly, adsorption and enzymatic activity and extent of decolourisation of dye in water are dependent on pH. The color of a solution and the solubility of a dye is also said to be affected by pH. In the present study, *Aspergillus niger* and *Aspergillus flavus* for investigation showed remarkable decrease in dye concentration at pH 8 and 10, respectively. *Aspergillus fumigatus* have been implicated in the decolourisation of 90% of textile effluent containing reactive textile dyes, such as Reactive Black RC, Reactive Yellow HF2-GL, Reactive Blue BGFN, Reactive Black B-150 and Reactive Red A-6BF at pH 3. At a pH of 5 and 8, 78% and 55% decolourisation of the textile dyes were achieved, respectively by the *Aspergillus fumigatus* [2]. The present study showed an optimum for decolourisation of the tested dye to be 10 and 8 in the presence of *Pseudomonas aeruginosa* and *Escherichia coli*, respectively. In a previous study with *Pseudomonas aeruginosa*, effective decolourisation was achieved at pH 7 [22]. Other investigators have indicated that optimum pH for dye decolourisation by *Pseudomonas* sp. ranges from pH 6-10 [19].

In the investigation on effect of different sodium acetate concentrations on dye removal ability of the isolates revealed varying removal rates at the different concentrations used among the different test isolates. In the presence of *Aspergillus niger*, no remarkable decolourisation in the dye was observed at the different sodium acetate concentrations while in the presence of the *Aspergillus flavus*, maximum decolourisation was achieved at sodium acetate concentration of 5 g/L. It is hypothesized that the presence of a high carbon concentration could lead to low decolouration. This is indicated to be due to the fact that the microorganisms responsible for the dye decolouration may utilize the carbon source preferentially to the dye [23, 24]. It is also reported that for optimum decolouration of dye, the concentration of carbon in the wastewater must be sufficiently low to limit the growth of the biomass and allow for metabolic activity without enhancing the biosorption process. In this study, several external carbon sources (glucose, sodium acetate, lactose, sucrose and methanol) were investigated for . The idea

of a carbon source was deliberate. This is because, dyes are indicated to be deficient in carbon and biodegradation without an external carbon source is difficult. From the results of this study, the entire test isolates showed remarkable dye decolouration when methanol was used as carbon source. In some studies on dye removal using bacteria, starch has been indicated as an ideal carbon source [19]. In a study on the decolouration of True Blue by *Aspergillus flavus*, fructose was indicated as an ideal carbon source [25]. Although this was not the observation in this study, some investigators have reported that microbial decolouration of dyes or a textile effluent is increased in the presence of glucose [26]. Similarly, Jin, et al. [2] have indicated improved enhanced decolouration of dyes that included Reactive Black RC, Reactive Yellow HF2-GL, Reactive Blue BGFN, Reactive Black B-150 and Reactive Red A-6BF with potato starch and sucrose in the presence of *Aspergillus fumigatus*.

At the different external nitrogen sources used for the investigation, the present study revealed the optimum removal when yeast extract was used as the nitrogen source. Maximum decolouration was observed at dye concentration of 5 g/L. This observation was irrespective of the test isolate used for investigation. The use of yeast extract as a nitrogen source has been indicated by earlier investigators in similar studies [25, 26].

5. CONCLUSION

This study, which was aimed at investigating the dye decolouration ability of the test microbial species in wastewater at the different temperature, pH, external carbon and nitrogen sources revealed optimum temperature for dye decolouration by the isolates to be 25 ^{o}C and 35 ^{o}C, for the fungi and 25 ^{o}C for the bacteria species. At the different pH, the optimum pH for dye decolouration was observed to range from 8 to 10. At the different concentrations of sodium acetate, optimum decolouration was observed at 5 g/L. Among the different external carbon sources that were used for investigation, maximum removal was observed when methanol was used. This observation was irrespective of the bacteria and fungi species used for investigation. With respect to the nitrogen sources, optimum removal was observed with yeast extract in presence of the test isolates. This trend was also irrespective of the isolate used for investigation. At the different concentrations of peptone used for investigation, no dye decolouration was observed in presence of any of the test isolate.

The present study was able to reveal the role of the test microbial species in the decolouration of dye in wastewater and the effects of the parameters investigated on their dye decolouration ability.

6. ACKNOWLEDGEMENT

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