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Profiling the Effects of pH and Temperature on Azo Dye-Decolourisation by *Aspergillus quadrilineatus* Strain BUK_BCH_BTE1 Isolated from Textile Effluents

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ABSTRACT

Wastewater from textile industries contains azo dye residues that negatively affect most environmental systems. The biological treatment of these wastes is one of the best option due to safety and cost concerns. This research was therefore aimed to isolate and identify fungus capable of decolorizing and utilizing azo dye (dimethyl yellow) as a sole carbon source using one-factor-at-a-time (OFAT). Pour plating method was used to isolate the fungus on mineral salt media (MSM) following serial dilution. The isolate was then morphologically and molecularly identified as *Aspergillus quadrilineatus* strain BUK_BCH_BTE1 with the accession number OK178927 based on ITS1 and ITS2 rRNA gene sequence and molecular phylogenetic analysis. Characterization was conducted by studying the effect of temperature and pH, in MSM media containing dimethyl yellow as sole carbon sources. The growth of this fungal isolate with dimethyl yellow as substrate was optimal at, temperature of 30 °C, and pH of 5.0. At these optimum conditions the isolate was able to decolourised up to 74.3% of dimethyl yellow dye. The isolate could be a suitable candidate in bioremediation of coloured effluents particularly containing these compounds.

Keywords: *Aspergillus quadrilineatus*, Characterization, Dimethyl yellow, Wastewater

INTRODUCTION

Textile and Clothing (T and C) is one of the largest and oldest industries present globally (Ghaly *et al.*, 2014). Industrial wastewater released from textile plants often contains residual azo-dyes, which are considered as environmental hazardous materials. These dyes are difficult to treat biologically, mainly due to their synthetic origin and complex aromatic structures. Chemical methods remain the major wastewater treatment procedures used in these factories, and often result in the production of other forms of chemical pollutants. Consequently, a lack of remediation technologies in industrial dye plants results in the discharge of wastewater into the environment, which affects plant, animal and human health (Ghaly *et al.*, 2014) . One major problem in the treatment of textile industrial wastewater is due to the variation in the types and chemistry of textile dyes. This is in part due to the staining requirements of different fiber types:1) cellulose (i.e. cotton, rayon, linen, ramie, and lyocell), protein (i.e. wool, angora, mohair, cashmere and silk) and synthetic fibers polyester, nylon, spandex, acetate, acrylic, ingeo and polypropylene (El-rahim *et al.*, 2017).

Azo dyes represent the largest production volume of dye chemistry today, and their relative importance may even increase in the future. They play a crucial role in the governance of the dye and printing market. These dyes are synthesized from a simple method of diazotization and coupling. Different routes and modifications are made to obtain the desired color properties, yield and particle size of the dye for improved dispersibility (Benkhaya *et al.*,2020).

High amount of water used during dyeing and washing textiles, ultimately finds their way to the surface water system carrying a considerable amount of organic dye. Also, from the dyes wasted (10 – 25% of the total dyes produced) during the textile processes, 2 – 20% are directly released to the surface water system (Karim *et al.*, 2018). These high colours renders the water unfit for use at the downstream of the disposal point and may hinder light penetration thereby affecting aquatic life and continuously threatening the biodiversity (Karim *et al.*, 2018).

Variation in textile dye types and chemistries prevent the use of a single microorganism to treat all wastewaters containing industrial dyes. Moreover, since there are many types of azo dyes, only relatively few isolated microorganism have been tested for decolourisation of a limited number of azo dye or textile effluents (El-rahim *et al.*, 2017).

Several primary, secondary and tertiary treatment processes have been used to treat these

effluents, which include flocculation, chemical coagulation, simple sedimentation, aerated lagoons, aerobic activated sludge, trickling filters, reverse osmosis and electrodialysis. However, these treatments are not found effective against the removal of all dyes and chemicals used in the industry (Ghaly *et al*., 2014). These effluents do not only contain high concentration of dyes, but also contain the chemicals used in the various processing stages. Some trace metals such as chromium, arsenic, copper and zinc are present in these effluent and are capable of causing several health problems including haemorrhage, ulceration of skin, nausea, severe irritation of skin and dermatitis (Ghaly *et al*., 2014).

Bioremediation of textile dyes can be achieved using microorganism and biocatalyst each of which has their pros and cons in terms of decolorizing efficiency, suitability and working capability (pH, concentration of dye and temperature). Microorganisms have the capability of releasing enzymes that can neutralize harmful pollutants from the contaminated site (Ghosh *etal*., 2016).

The role of fungi in the wastewater treatment has proved them suitable and effective for dyes degradation/ decolourisation and the removal of colorants from the textile effluents (Singh, 2017). Until present, the use of bacteria for dye decolourisation has been well exploited, though the search for a suitable degrader/ decolouriser/ reducer microbe is still on going with hope of getting a multi-degrader organism that could simultaneously dealt with many contaminants in the environment. This research was therefore, aimed to isolate, and identify and fungus with potential to decolorize azo dye (dimethyl yellow).

MATERIALS AND METHODS

Azo dye

Dimethyl yellow (N,N-Dimethyl-4-(phenyldiazenyl)aniline, is an organic compounds with the formula($C_{14}H_{15}N_3$)molecular weight of 225.3 g/mol and was purchased from Qualikems Fine Chemicals Pvt. Ltd Plot No.68 / 69, GIDC, Industrial Estate Nandesari, Vadodara, (India). It is 99.9 % pure. It is a yellow azo dye derived from dimethyl aniline. The dye was supplied by Department of Pure and Industrial Chemistry in Bayero University Kano, Nigeria and a working concentration of 100 µg/mL.

Samples collection

Wastewater and dye contaminated soil samples were collected from discharge site of Angels spinners and dyers located on latitude 11˚56-56.49”N and longitude 8˚29 -23.04”E, Plot 8 Sharada Industrial Estate Phase III, of Kano State, Nigeria in the month of August 2019. All samples were collected in sterile 1 L bottles and transferred to the laboratory and stored at 4 ℃ during 24 hours before fungal isolation.

Microbiological media

Mineral salts medium (MSM) was prepared as described by Yang *et al*.(2006) with some modifications. The medium contained: NH_4NO_3 (0.5g/L), K_2HPO_4 (1.5 g/L), KH_2PO_4 (0.5g/L), $MgSO_4{\cdot}7H_2O$ (0.2 g/L), $FeSO_4$ (0.02 g/L), $CaCl_2$ (0.05g/L) and $CuSO_4$ (0.02 g/L). The medium was supplemented with (0.5 g/L) yeast extract as a source of growth factors, then Dimethyl yellow (100 µg/mL) the pH was adjusted 5.0 using 0.5M phosphate buffer. This medium was solidified by the addition of 1.7% agar as solidifying agent. Liquid medium was used for testing the decolourisation activity of all fungi.

Isolation of azo dye-degrading microorganisms

A 10 g of each soil samples was separately suspended in 100 ml sterilized saline solution (0.85% NaCl) and shaken for 1 h at 150 rpm. A 1 mL aliquot of each soil suspension was separately added into a petri dish and 25 mL of molten cooled MSM agar medium, containing dimethyl yellow azo-dye at100 µg/mL, was poured into the plate and the contents was thoroughly mixed. Plates were incubated at 28 °C for 5 days. Colonies appearing after incubation were picked, purified by streaking multiple times on the same initial isolation medium, and maintained on the same medium. The medium was adjusted to pH 5.0 prior to use (El-rahim *et al*., 2017).

Morphological Identification of the dye decolorizing fungi

Fungi isolated was identified through macroscopic observation of their subculture colonies, microscopic examination of their spores and hyphal appendages using wet mount techniques. Distilled water and lacto phenol cotton blue were utilized as mountants(Ologbosere *et al*., 2016). The result of the microscopy was compared with illustrations contain in colour atlas of mycology.

Inoculum preparation

Spore suspension was prepared by washing 3 day-old fungus slants with sterilized saline solution (0.85% NaCl). The optical density was measured at 600 nm and adjusted to A 600 nm= 1.0 prior to inoculation(El-rahim *et al*., 2017).

Characterization of Fungal Growth

Factors influencing growth by the fungus were characterized using one factor at a time (OFAT). Experiments were performed in McCartney bottles containing 50 mL sterile MSM medium inoculated with 100 µL of the fungal isolates.

Effect of initial pH on fungal growth

pH was varied over a range of 5.0 – 7.0 in order to determine the optimum pH that support

fungal growth. Into 50 mL of MSM medium supplemented with 100µg/mL of the tested azo dye (dimethyl yellow) 100 µL (OD_{600} = 1.0) of spore suspension was inoculated and incubated at 28 °C for 96 h. Three replicates cultures were used. After every 24 h of incubation at 28 °C for the period of 96 h, aliquot (1 mL) from the culture media was removed while the optical density was measured at 600 nm (El-rahim *et al*., 2017).

Effect of temperature on fungal growth

Temperature was varied over a range of 25 ºC – 50 ºC in order to determine the optimum temperature that support fungal growth. Into 50 mL of MSM medium supplemented with 100µg/mL of the tested azo dye (dimethyl yellow) 100 µL (OD_{600} = 1.0) of spore suspension was inoculated and incubated at 28 °C for 96 h. Three replicates cultures were used.After every 24 h of incubation at 28 °C for the period of 120 h, aliquot (1mL) from the culture media was removed while the optical density was measured at 600 nm(El-rahim *et al*., 2017).

RESULTS AND DISCUSSION

Isolation and Identification of Dimethyl yellow Decolorizing-Fungi

a) Isolation of dimethyl yellow-decolorizing fungi

A total of 10 fungal isolates labelled (C1, C2, C3, C4, C5, Y1, Y2, Y3, Y5 and Y6) were isolated from dye contaminated soil and wastewater samples. The azo dye (dimethyl yellow)was separately used as sole carbon sources on MSM media. Only isolate with potential of metabolizing the dye as energy source grew on the media, only isolate C3 was able to grow on the dye Thus, this isolate was selected as candidate isolate for the characterization work.

b) Identification of dimethyl yellow-decolorizing fungal isolate

i) Wet Mounts Techniques

The results as observed using lacto phenol cotton blue and distilled water revealed the fungus to be filamentous, spore forming, and also containing smooth and colourless conidiophores (Ologbosere *et al*., 2016).

ii) Molecular identification and phylogenetic analysis

Identification of the fungus was carried out based on the ITS1 and ITS2 gene sequence of the fungus. A total of 1000 bases of the ribosomal gene were obtained and compared with GenBank database using the Blast server at (National centre for Biotechnology information) NCBI (http://www.ncbi.nlm.nih.gov/BLAST/). This analysis showed that the DNA sequences obtained from the ITS1 and ITS2 analysis were closely related to the partial sequences of several *Aspergillus* species with over 96.33% similarity. Molecular phylogenetic studies using the neighbour joining method linked the identity of the obtained sequence to *Aspergillus quadrilineatus*. Thus, this fungus was assigned tentatively as *Aspergillus quadrilineatus* strain BUK_BCH_BTE1. Details of the molecular phylogenetic studies is presented in Figure 1.

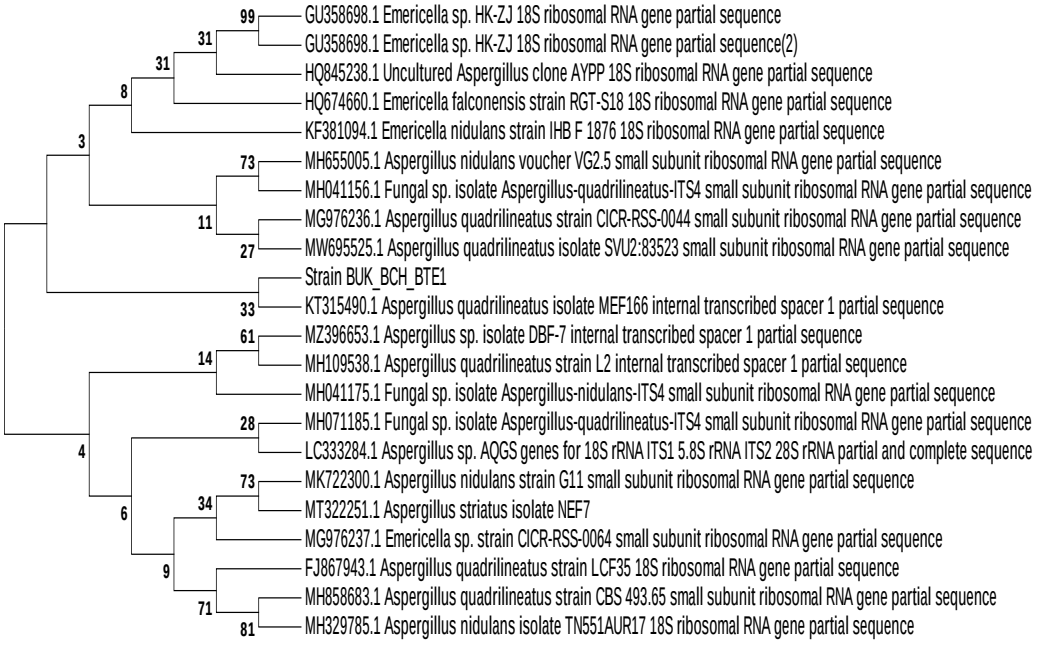

Figure 1: Cladogram (neighbour-joining method) indicating the genetic relationship between unknown and referenced related microorganisms based ITS1 and ITS2 gene sequence analysis. Accession numbers are accompanied by the specie names of their ITS1 and ITS2 rRNA sequences

Characterization of Growth Potentials of the Isolated Fungus

Effect of initial pH on fungal growth

The effect of various initial pH ranging from 4.5 to 7.0 on the growth of the *Aspergillus quadrilineatus* strain BUK_BCH_BTE1 on MSM media was presented on Figure 2.It was found that the growth of this isolate was optimum at pH 5.0, a significant decrease ($p<0.05$) in growth was observed at pH higher than 5.0 following 72 h incubation .This result was in agreement with the work of (Wang *et al*., 2017) who reported that tolerance of fungi to acidic condition is one of the important factors which should be considered in the large scale application. These results suggested that better fungal growth usually occurs at low pH values (Arti, 2019), during the adsorption process pH value affect the surface charges of mycelia and the structure of dye molecules. Mycelia surface shows negative charges when the pH value is higher than the isoelectric point of mycelia whereas surface shows positive charges. When the pH value is equal to the isoelectric point, surface charges of mycelia are in stable state in the dye solution. Congo red decolourisation is also affected by medium pH (Asses *et al*., 2018).

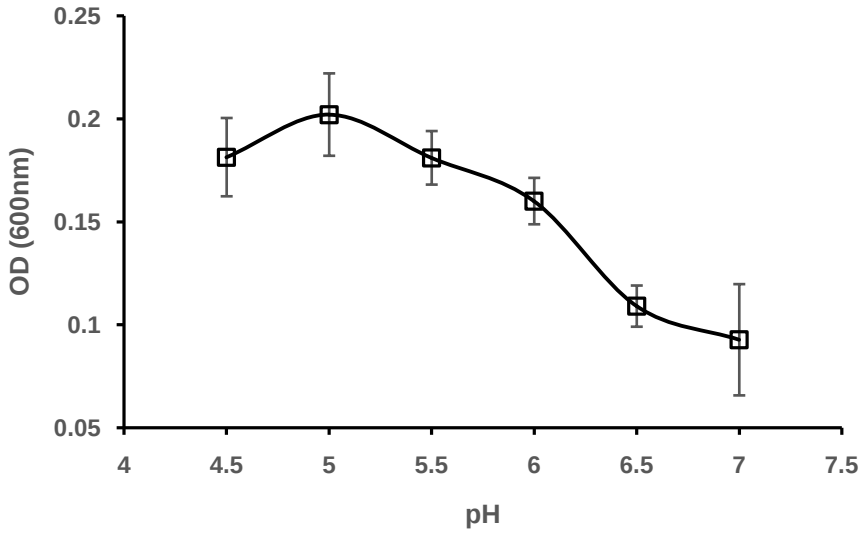

Figure 2: Effect of initial pH on the growth of *Aspergillus quadrilineatus* strain BUK_BCH_BTE1 on MSM media incubated for 72 h. Error bars represent mean ± standard deviation (n=3)

Effect of temperature on fungal growth

The effect of various range of temperatures 25 ℃ to 50 ºC on the growth of the *Aspergillus quadrilineatus* strain BUK_BCH_BTE1 on MSM media was presented in Figure 3. It was found that the growth of this isolate was optimum at 30 ºC. A significant decrease ($p < 0.05$) in growth was observed at temperature higher than30 ºC following 72 h incubation. Temperature is the most influencing factor that can affect the rate of dye decolourisation/degradation and biomass production (Arunprasath *et al*., 2019). Optimum temperature for respective organism induce growth and metabolic activities, all metabolic activities are based on performance of enzymes and optimum temperature provides favourable support for activation of enzymes (Arunprasath *et al*., 2019). Initially the growth of *Aspergillus quadrilineatus* strain BUK_BCH_BTE1 were found to increase with increasing incubation temperature for the organism. Further increase in temperature above 30 ºC resulted in decrease in growth activity. This could be attributed to the loss of cell viability (Hefnawy *et al*., 2017) or might be due to the denaturation of ligninolytic enzymes. Similar results were obtained by (Asses *et al*. 2018) who found that the maximum decolourisation activity of *Aspergillus niger* to Congo red dye was at 30℃ º and a significant decrease was observed at higher temperature.. The optimum temperature of 30 ºC was also previously obtained (Wang *et al*., 2017), who reported that temperature affects mycelia growth, enzyme amount and enzyme activities, then affected decolourisation ability indirectly. The results of this findings are also in agreement with those reported by Erum and Ahmed, (2011) (*Aspergillus* species and Acid red 151 dye); Tan *et al*. (2016) (*Scheffersomyces spartinae* TLHS-SF1 and Acid scarlet 3R azo dye): Omar, (2016) (*Aspergillus niger* and Reactive red 98, reactive orange 122, reactive yellow 160, reactive blue 21, and reactive blue 19 reactive dyes); Hefnawy *et al*. (2017) (*Aspergillus flavus Penicillium canescens* andDirec blue dye); Arunprasath *et al*. (2019) (*Lasidiplodia* spp and Triphenylmethane dye malachite green); Guo *et al*. (2019) (*Galactomyces geotricum* and Acid scarlet GR dye); and Singh

and Dwivedi (2020) (*Aspergillus terreus* GS28 and Direct blue-1 dye). Similar findings were observed by El-rahim *et al*. (2017) (*Aspergillus* spp and azo dyes); and Ilyas and Rehman, (2013) (*Aspergillus niger* and *Nigrospora* spp and Synozol red HF-6BN dye) which reported that *Aspergillus* species decolorizes best at 28 ºC. The result of the findings contradicts the works of Akdogan *et al*., (2014) (*Coprinus plicatilis* and Reactive blue 19 dye); Sumandono *et al*. (2015) (White rot fungus and Remazol brilliant blue R dye); Adnan *et al*., (2016) (*Armillaria* spp.sF022 and Acid red dye); Olufunke *et al*. (2016) (*Aspergillus* spp. *Cryptococcus* spp. *Candida albicans and* Malachite green, methyleneblue, Eosin Y and Carbolfuchsin dyes); and Fetyan *et al*. (2016) (*Saccharomyces cerevisiae* and Direct blues 71 dye) who reported temperature lower than 30ºC.

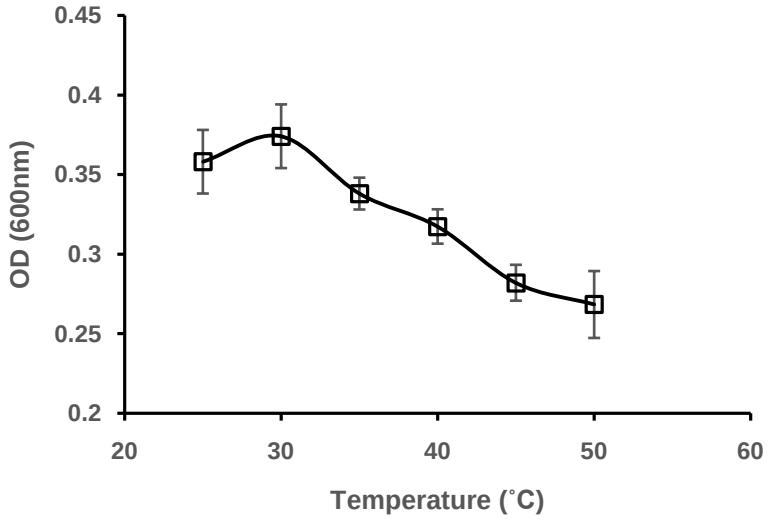

Figure 3: Effect of temperature on the growth of *Aspergillus quadrilineatus* strain BUK_BCH_BTE1 on MSM media incubated for 72 h . Error bars represent mean ± standard deviation (n=3)

CONCLUSION

The bioremediation of textile azo dye residues represents the most efficient approach to remove these pollutants from water before reaching the surrounding environment. This study isolated, molecularly identify and characterized fungus with azo dye-decolorizing potentials from soil and wastewater samples from Angel's spinners and dyers, Kano state Nigeria. The fungus belonged to genus *Aspergillus*. It was identified as filamentous, spore forming, and also containing smooth and colourless conidiophores based on morphology. Based on ITS1 and ITS2 rRNA sequencing and subsequent molecular phylogenetic analysis, the isolate was successfully identified as *Aspergillus quadrilineatus* strain BUK_BCH_BTE1.The isolate grew optimally in Dimethyl yellow at 30 ℃, pH 5.0 in 72 h. *A. quadrilineatus* is generally tolerant and can metabolized dimethyl yellow azo dye. These findings suggest this isolate as potentially suitable candidate for bioremediation of azo dyes in the environment.

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