

Biosorption of Crystal Violet Dye Solution by *Aspergillus striatus*, *Bacillus megaterium*, *Chlorella vulgaris* and *Fusarium equiseti****Sani, Z.M.¹, Garba, I.¹, Maigari, A.K.¹, Umar, S.A.¹, Kabir, A.² and Bala, I.²**¹Biological Sciences Department, Faculty of Life Sciences, Bayero University, Kano, Nigeria²Microbiology Department, Faculty of Life Sciences, Bayero University, Kano, Nigeria*** Corresponding Author: Email: zmsani.bio@buk.edu.ng, ORCID ID: 0000-0003-1980-2516, Phone no.: +2348027988187**

Abstract: Crystal violet is a synthetic triphenylmethane dye that serves as a biological stain which can also be used in dyeing textile materials. It is toxic and remains in the environment for longer periods, as such needs to be treated before discharge. This study was carried out to assess the biosorption potentials of *Aspergillus striatus*, *Bacillus megaterium*, *Chlorella vulgaris* and *Fusarium equiseti* on crystal violet dye. This was achieved through inoculation of pure cultures of the organisms into the dye solution. The highest percentage biosorption for all the test organisms was obtained at 24 hours after inoculation, with *Fusarium equiseti* recording 94.7%, *Aspergillus striatus*, 89.5%, *Chlorella vulgaris*, 77.1% and *Bacillus megaterium*, 68.8%. The results indicated no significant difference in dye removal among the four species with *Fusarium equiseti* having the highest biosorption potential and *Bacillus megaterium* the least. A multilayer biosorption pattern was predicted as the biosorption process fitted with the Freundlich's isotherm. To avoid further environmental contamination more eco-friendly strategies for generating dye-degrading organisms that can detoxify dyes should be introduced.

Keywords: *Aspergillus striatus*, *Bacillus megaterium*, *Chlorella vulgaris*, Crystal violet, *Fusarium equiseti*

INTRODUCTION

One of the main sources of pollution worldwide is the textile industry and its dye-containing wastewater. Many of the dyes released into the environment and their breakdown products are undesirable due to their persistent colour, toxicity, carcinogenicity or mutagenicity to life forms (Zaharia et al., 2009; Mesania et al., 2014; Bafana et al., 2020). The persistent colour of dye in wastewater which is stable and fast makes it difficult to degrade, rendering receiving water bodies unfit for their intended use. Cases of long-term exposure to synthetic dyes resulting in tumor, cancer, liver and kidney malfunctions have been reported by many investigators (Sajjala et al., 2008). These dyes can remain in the environment for longer periods of time without proper treatment. The removal of such dyes from industrial wastewater/effluents can be achieved through physical, chemical or biological treatments, of which, the latter is cheaper, effective and eco-friendly (Robinson et al., 2001; Roy et al., 2018). Physical and chemical effluent treatments are often unable to completely remove dyes, are exorbitantly expensive and they tend to generate other toxic substances that may elevate pollution in the receiving

environments (Shah, 2013; Bhattacharya et al., 2018). Due to aforementioned reasons, the use of microbial biomass to biosorb and/or degrade pollutants contained in effluents was employed (Bumpus and Brock, 1988; Yang et al., 2009; Lavanya et al., 2014; Ledakowicz and Pazdzior, 2021; Singh et al., 2021). Microorganisms remove dyes from the environment mainly through biomass sorption or enzymatic degradation (Chen et al., 2019). Apart from microorganisms, other high aquatic plants (macrophytes) such as water hyacinth, lettuce, ferns, penny worts, duck weeds and macroalgae (*Chara* and *Scenedesmus* species) have been reported in successful degradation and precipitation of some synthetic dyes (Mesania et al., 2014; Patil et al., 2015).

Microorganisms such as bacteria, fungi and algae have been proven to be very effective in treatment of textile wastewater containing a wide variety of toxicants such as dyes (Gupta et al., 2015). Bacterial species from the genera *Bacillus*, *Alcaligenes*, *Aeromonas*, *Pseudomonas*, *Sphingomonas*, *Rhodococcus*, *Corynebacterium*, *Escherichia*, *Agrobacterium* and *Mycobacterium* were reported with high remediation potentials as such are used in treatment of dye effluents/wastewater and soils of industrial

sites (Vidali, 2001; Parshetti *et al.*, 2011; Pokharia and Ahluwalia, 2013; Osama *et al.*, 2014).

Also, researches have shown various fungal species belonging to the genera, *Aspergillus*, *Phanerochaeta*, *Hirschioporus*, *Inonotus*, *Phlebia*, *Ceriporia*, *Coriolus*, *Candida*, *Fusarium* and *Rhizopus* with the ability to remediate synthetic dyes (Jebapriya and Gnanadosa, 2013; Rani *et al.*, 2014; Singh *et al.*, 2015; Hefnawy *et al.*, 2017; Wang *et al.*, 2017; Yucel, 2018; Ortiz-Monsalve *et al.*, 2019).

Algae are regarded as efficient bioremediators due to their photosynthetic capabilities aiding them in metabolic processes like absorbing carbon dioxide from the atmosphere, converting solar energy into useful biomasses, incorporating nutrients and other pollutants through bioaccumulation and biosorption. They have also been proven to remove dyes through various mechanisms such as biosorption, bioconversion and bioagulation (Khalaf, 2008; Ayele *et al.*, 2021). It was also stated that algal dye remediation may be either by enzymatic action or biosorption with live or dead biomass, which is dependent on optimized pH, temperature, biosorbent concentration and agitation (Paul *et al.*, 2011).

Crystal violet is a synthetic triphenylmethane dye commonly used in human and veterinary medicine as a biological stain, and as well for dyeing fabric in textile processing industries (Au *et al.*, 1978; Azmi *et al.*, 1998). It is known for being recalcitrant, toxic and remains in the environment for longer periods. It is also referred to as a mitotic poisoning agent and/or biohazard substance as it promotes tumor growth in some aquatic organisms, as such termed also as a potent carcinogen/clastogen (Au *et al.*, 1978; Fan *et al.*, 2009). Various chemical and physical methods have been employed in remediation of Crystal violet which have proven to be effective for smaller volumes generating toxic compounds as end products (Azmi *et al.*, 1998; Robinson *et al.*, 2001; Cheng *et al.*, 2008). Other researches have also proven that

remediation of Crystal violet dye can be achieved through membrane bound fraction and air bubble bioreactor packed with *Pseudomonas aeruginosa* (Jones and Falkinham, 2003; El-Naggar *et al.*, 2004). The study aimed at assessing the potentials of *A. striatus*, *B. megaterium*, *F. equiseti* and *C. vulgaris* in remediation of Crystal violet dye.

MATERIALS AND METHODS

Microorganisms

Microorganisms used in this study were *Aspergillus striatus*, *Bacillus megaterium*, *Chlorella vulgaris* and *Fusarium equiseti*. Pure cultures of the above mentioned species were collected from the staff research laboratory, Faculty of Life Sciences, Bayero University, Kano and further sub-cultured using streak culture and direct isolation methods on potato dextrose broth (fungi), nutrient broth (bacteria) and bold basal (algae) media (Benson, 1998; Lee *et al.*, 2013; Al-Mohanna, 2016). Crystal violet dye solution was prepared by dissolving 1 gram of its powder in 2 L of distilled water, from which 1 ml of the dye solution was subsequently added to a test-tube containing 5 ml of normal saline serving as the control.

Biosorption Assay

Aspergillus striatus and *Fusarium equiseti* were cultivated on potato dextrose broth for 5 days at room temperature (37 °C) as described by Al-Mohanna (2016) and 0.1 grams mycelia were harvested for the biosorption assay. *Bacillus megaterium* biomass was generated according to the method of Ngui *et al.* (2013), which involved sub-culturing on nutrient broth 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), and 2.39×10^3 cells/ml was used for the assay. Bold basal medium was used for cultivation of *C. vulgaris* at 30 °C with proper aeration (with an air pump - Shining beach SB660) and 2.24×10^4 cells/ml was generated with the aid of a cytometer.

The biomass for each of the species was separately placed in individual test-tubes containing 1 ml of dye solution and 5 ml of The absorbance of inoculated dye solution was recorded using a spectrophotometer (model 722) at wavelength 650 nm after 8, 16 and 24 hours. The amount of dye adsorbed

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

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

Where, Q_e = 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).

Preparation of assays was in triplicates and the numerical values recorded were expressed as mean $\pm$ standard error and further analyzed by one-way analysis of variance (ANOVA) using Microsoft Excel

normal saline (Verma *et al.*, 2015). The solution was mixed with an auto-vortex mixer and incubated at 37 °C.

per gram of the biomass and percentage biosorption of the dye by the biomass of individual species were calculated using equations 1 and 2 respectively.

2007. Readings were considered significant when P is less than 0.05. Freundlich's isotherm was used to further explain the pattern of the biosorption process (Mahmoud *et al.*, 2016).

Freundlich's equation is given by;

$$Q_e = K_f \times B^N$$

Where, Q_e = Concentration of dye at equilibrium, K_f = Freundlich's constant and N = Slope of graph (log Q_e versus log B) (Abel *et al.*, 2020).

RESULTS

The colonial and microscopic view of the organisms and the results for their biosorption of crystal violet dye are presented in Figures 1-3. The lower case alphabets 'a' and 'b' in Figure 1 represent the colonial and microscopic appearance of the species respectively. Figure 2 shows the percentage biosorption taken at an interval of 8 hours, to which the highest percentage was at 24 hours

for all species. The visual decrease in dye coloration is presented in Figure 3, where, the test-tube inoculated with *F. equiseti* (b) had a clearer solution than that of the other three species. There was no statistical significant difference in the biosorption of dye by the four species as $P > 0.05$ ($P = 0.12$). Table 1, provides regression data for crystal violet biosorption by the four species in accordance with Freundlich's isotherm

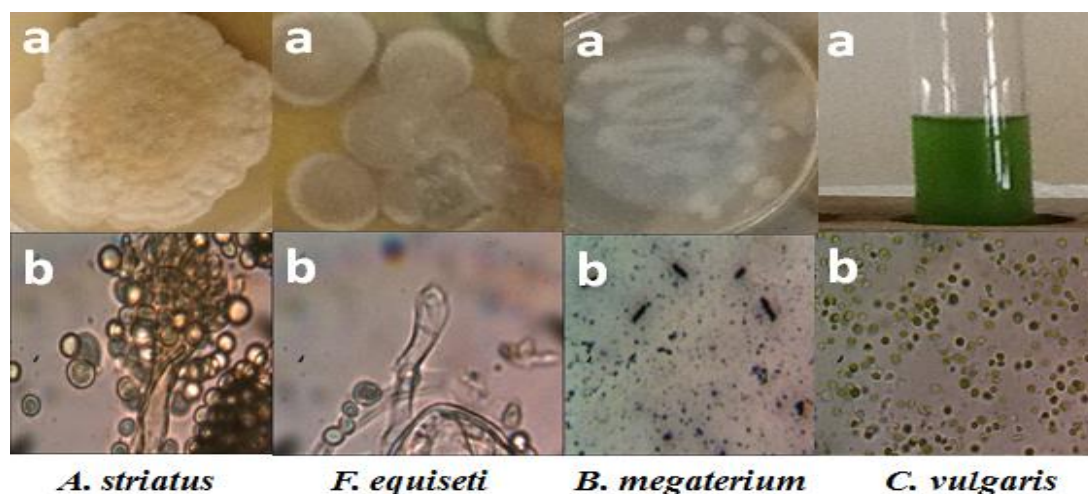


Fig. 1: Colony (a) and microscopic view (b) of the four species (mg. $\times 1/3$ for colonies and mg. $\times 100$ for microscopy)

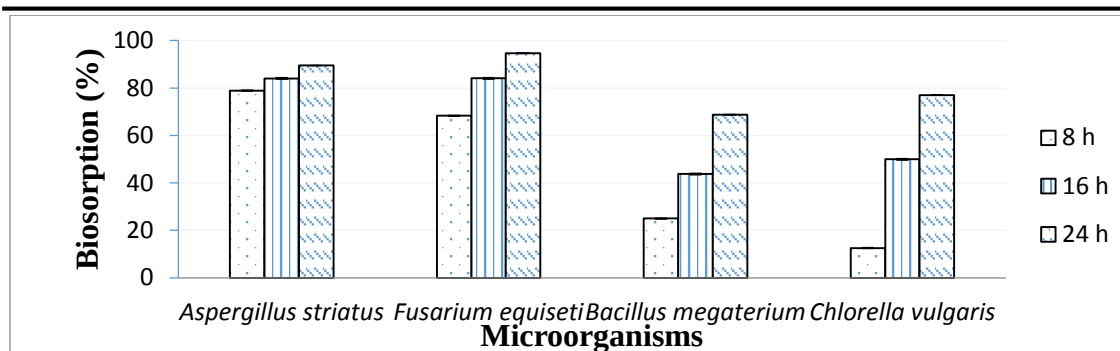


Fig. 2: Percentage Biosorption of Crystal Violet Dye within 24 Hours by the Four Species
In Figure 2 above, *F. equiseti* had the highest percentage (94.7 %) and *B. megaterium* the least (68.8 %).

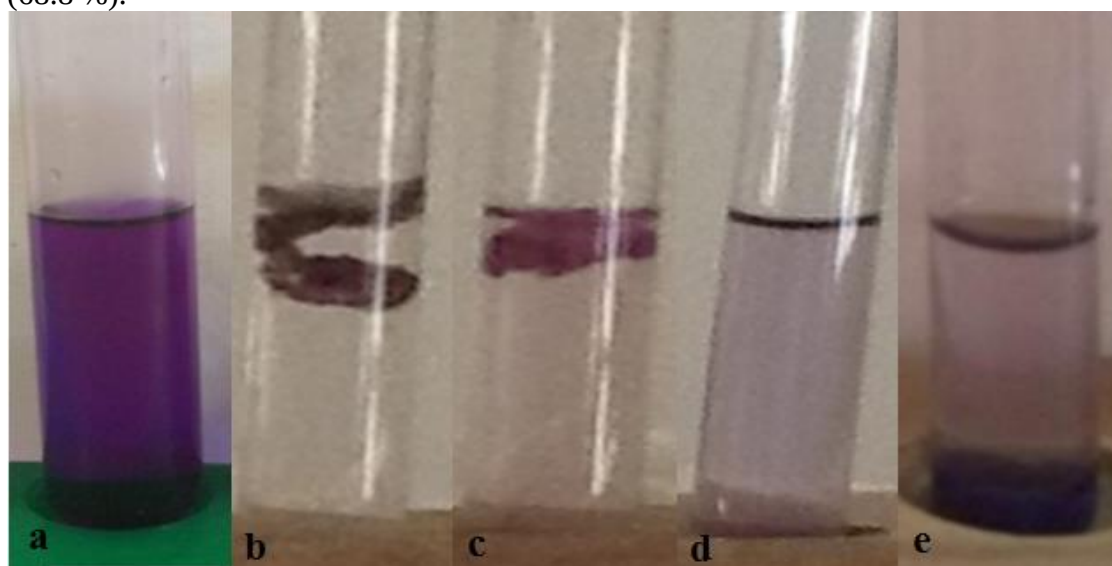


Fig. 3: Visual Decrease in Dye Colouration at 24 Hours after Inoculation
(a) Crystal violet dye solution (Control), (b) Crystal violet dye solution inoculated with *F. equiseti* (c) Crystal violet dye solution inoculated with *A. striatus* (d) Crystal violet dye solution inoculated with *C. vulgaris* (e) Crystal violet dye solution inoculated with *B. megaterium*

Table 1: Linear Regression Data for Freundlich's Isotherm for Crystal Violet Dye Biosorption by the Four Species

Microorganism	K_f (L/mg)	N	R^2
<i>A. striatus</i>	2.59E-01	0.18	0.9859
<i>F. equiseti</i>	3.13E-01	0.18	0.9192
<i>B. megaterium</i>	5.50E+04	1.2	0.9362
<i>C. vulgaris</i>	1.85E+04	1.36	0.8563

In Table 1, the K_f values especially that of *B. megaterium* and *C. vulgaris* indicate high biosorption capacity. All R^2 values were less than one, thus, showing the occurrence of a multilayer biosorption pattern.

DISCUSSION

The study determined the biosorption potential of 4 species (*A. striatus*, *B. megaterium*, *F. equiseti* and *C. vulgaris*) on Crystal violet dye under static conditions, pH

6.98 and temperature 37 °C for 24 hours. The results are presented in Figures 1-3. The highest percentage dye removal for all the species was recorded at 24 hours, with *F. equiseti* revealing the highest percentage

(94.7%), *A. striatus* (89.5%), *C. vulgaris* (77.1%) and *B. megaterium* (68.8%) (Figures 2 and 3).

The findings of this study is in line with several researches in this field, for example, Asad et al. (2007) observed that bacterial species from different genera can completely degrade and mineralize synthetic dyes such as crystal violet under optimized conditions (Jones and Falkinham, 2003; Al-Garni et al., 2013; Pokharia and Ahluwalia, 2013). In another study, *Bacillus* species was reported to have decolourized crystal violet dye within 2.5 hours of incubation (Ayed et al., 2009). *Bacillus subtilis* decolourized crystal violet dye at pH 8, temperature 35 °C under static condition in the presence of carbon and nitrogen sources (Kochher et al., 2011). *Bacillus* species are known for high effectiveness in degradation of dyes and other pollutants in industrial effluents and soils which is due to possession of various enzymes such as lignin peroxidase, laccase, azo reductase and biotransformation enzymes (Azmi et al., 1998; Telke et al., 2008; Parshetti et al., 2010; Akhtar et al., 2019).

A number of fungal species belonging to different genera have been proven to be effective bioremediators of dyes and other contaminants in aquatic and terrestrial habitats (Azmi et al., 1998; Shahid et al., 2013; Marcharchand and Ting, 2017; Ortiz-Monsalve et al., 2019). Remediation of crystal violet dye by a variety fungal species (like *Phanerochaete chrysosporium*, *Pleuroteus ostreatus*, *Piptoporus betulinus*, *Funalia trogii*, *Coriolus vesicolor*, *Cyathus stariatus*, *Irpex lacteus*, *Bjerkandera adusta*, *Cunninghamella elegans*, *Mucor mucedo*, *Lenzites betulina*, *Polyporus elegans*, species of *Aspergillus*, *Fusarium*, *Penicillium*, *Trametes* and *Trichoderma*) have been reported by several researchers (Bumpus and Brock, 1988; Azmi et al., 1998; Moturi and Singara, 2009; Yang et al., 2009; Shahid et al., 2013; Al-Jawhari, 2015; Gupta et al., 2015; Ali et al., 2016; Chen et al., 2019; Gao et al., 2020), which is in agreement with

findings of this research. Another study revealed *Diaporthe schini* and *Candida krusei* to have biosorp and degraded Crystal violet and Basic violet 3 respectively (Deivasigamani and Das, 2011; Grassi et al., 2019; Slama et al., 2021).

Many researchers have reported positive degradation of synthetic dyes by *Chlorella vulgaris* (Chu et al., 2009; Kosha et al., 2013; Arteaga et al., 2018; Naji and Salman, 2019; Raymond and Omo, 2019; Chin et al., 2020; Ishchi and Sibi, 2020). Species from the genera *Chlorella* and *Oscillatoria* have been reported to possess potentials for remediating Azo Dyes to aromatic amines which are further metabolized to simple organic compounds (Wen-Tung and Ming-Der, 2011). Algae and cyanobacteria species are capable of utilizing some Azo dyes to obtain carbon and nitrogen (El-Sheekh et al., 2018; Hussein et al., 2018). In a study conducted by El-Sheekh et al. (2018), an algal species (*Chlorella vulgaris*) and cyanobacterium (*Aphanocapsa elachista* and *Nostoc carneum*) were able to degrade and mineralize synthetic dyes within 7 days (Hussein et al., 2018). Another study revealed the ability of *Hydrocoleum oligotrichum* and *Oscillatoria limnetica* to decolourize and degrade certain synthetic dyes (Abou-El-Souod and El-Sheekh, 2016). Srashti (2013) reported *Spirulina platensis* with effective degradation of dyes.

CONCLUSION

In conclusion, the results of the study revealed that all the four species had the capacity to biosorp crystal violet dye as there was significant decrease in dye colouration visually and spectrophotometrically. The highest percentage biosorption was recorded after 24 hours of inoculation, with *F. equiseti* having the highest value, then, *A. striatus*, *C. vulgaris* and *B. megaterium*. The results revealed no significant difference in dye biosorption by the species. The biosorption process indicated a multilayer biosorption pattern, as it fitted with the Freundlich's isotherm.

RECOMMENDATION

Introduction of adequate strategies for extraction of dye-remediating enzymes from organisms which could be used to detoxify dyes in effluents/wastewater before discharging into the environment to avoid further contamination is strongly recommended.

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