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Monoculture Solid Phase Degradative Potential of Congo red by *Aspergillus Niger* and *Aspergillus Flavus*

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ABSTRACT

Mycoremediation is a great technology and can be exploited for the bioremediation of dyes contaminated soil and also to reclaim wastewater. Fungal strain isolated from local Tie and Dye factory in Awka was examined for their dye-degrading potentialsand it was found to be capable of degrading Congo red under its optimized medium and growth conditions using solid (agar) phase biodegradation analysis, it is an employable approach for textile effluent degradation to less toxic components. The effect of independent variables such as time, temperature, and pH on decolorization efficiency was constant and put into perspectives. The biodegradation of Congo Red was demonstrated by decolorization of solid state dye-minimal culture medium, the extent of degradation was measured to be directly proportional to fungal growth which was determined by monitoring the growth of the 2 fungal isolates(*Aspergillus niger* and *Aspergillus flavus*) diameterin of this dye-minimal containing various concentration (0.1%, 0.01%, and 0.001%) of dyes formulation. The biodegradation of the dye was related to its decolorization during growth of fungi and *Apergillus niger* showed a degradation potential of about 81% while *Aspegillus flavus* showed a potential of 62%. There was increase in decolorization diameter along with increase in incubation time. While performing the process optimization studies for the decolorization of the dye-maximum decolorization was observed at room temperature and under static conditions. It was found that the isolated fungal strain was considered to be well adapted, resistant and highly acclimatized to dye contaminated soils showing the decolorization of Congo red dye.

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KEYWORDS: *Aspergillus niger, Aspergillus flavus* and Congo red

INTRODUCTION

Congo red histologic stain is still of fundamental importance in the laboratory. ‘‘Congophilic’’ staining of fixed tissue and the detection of apple-green birefringence when viewed under polarized light remain essential for the diagnosis of amyloidosis The first produced aniline dyes were limited by the need to use a substance known as a *mordant* to fix the dye permanently to the textile fiber, a requirement that added an extra step to the dyeing process. Congo red dye brilliant red dye was the first aniline dye that did not require a mordant to stain textile fibers- *direct dye* (David, 2001).

Since dyes are typically stable to light and oxidation and cannot be treated by traditional methods of aerobic digestion, the traditional methods of removing dye using alum, ferric chloride, activated carbon, lime, among others for the treatment of dyes in industrial wastewater create a number of problems (EI-Said, 2012). Hence, the need for more environmental friendly approach through the use of autochthonous fungi isolates from Tie and dye effluent that have acclimatized with a variety of basic dyes to curb the abyss associated with toxicity caused from dye pollution in both aquatic and terrestrial habitat through erosion. Nigeria Textile Industry was the second largest in Africa after Egypt with above 250 vibrant factories and over 50 percent capacity utilization (Semshak Gompil, 2004) contributing a large proportion of the quantity of dyes that makes it into water bodies and terrestrial habitat- It is against

this backdrop of degrading the untreated dyes effluent this research was borne.

Microorganisms provide a potential wealth in biodegradation (Agu *et al*., 2015; Anaukwu *et al.,* 2016; Okafor *et al.,* 2016). Bioremediation *per se,* embodies a host of other processes including biodegradation. Biodegradation is the biologically catalyzed break down in the structural and molecular complexity of compounds into smaller components such as carbondioxide and minerals by enzymatic or metabolic processes in the environment (Agu *et al.,* 2014; Mbachu *et al.,* 2014; Orji *et al.,* 2014; Ifediegwu *et al.,* 2015; Okafor *et al.,* 2016; Agu *et al.,* 2022; Orji *et al.,* 2022. Microorganisms have the ability to interact, and utilize substances leading to structural changes or complete degradation of the target molecules (Raymond *et al.,* 2001; Anaukwu *et al.,* 2016, Ojiagu *et al.,* 2018; Agu and Odibo, 2021; Agu *et al.,* 2022).

The aim of the present study was to screen and isolate a potent fungus capable of biodegrading the azo dye Congo red using solid phase degradation analysis though optimize the medium conditions and factors for maximum dye degradation.

METHODOLOGY

Sample collection

30grams of soil samples were randomly collected from three (3) different local tie and dye factories in Awka, Anambra state, Nigeria. The soil samples were collected aseptically using a sterile spatula and placed in a sterile container.

Sample analysis

One gram (1g) of the soil sample was weighed out aseptically and introduced into 9ml of sterile water. It was properly shaken to homogenize the sample. A 10- fold serial dilution of each of the sample was carried out using peptone water as the diluent. 0.1ml of appropriate dilution (10^{-3}) of the sample were pour plated in sterile plate of Sabouraud dextrose Agar(SDA) and Mineral Salt Medium for the culture of fungi. The culture plate was incubated at 37^{o}C aerobically for 48-72 hours for fungi. Developing colonies on SDA were counted to obtain total fungi count. Discrete colonies were obtained by sub-culturing into SDA plates and were subsequently identified using standard methods (Agu and Chidozie, 2021).

Total fungi count was calculated as thus:

$$TFC/\ TDDFC = \frac{N}{VxD}$$

TFC: Total Fungi Count
TDDFC: Total Dye Degrading Fungi Count
N: Number of Colonies
V: Volume Plated
D: Dilution Factor

DYE AND MEDIA PREPARATION

Congo red was obtained from Trust Chemicals Limited (Bangalore, India) and other fine chemicals were from SRL Chemicals, India. To study the potency of pure culture of fungal species for the degradation of the azo dye Congo red, a modified Sourav *et al.*(2011)semi synthetic media was used with the following composition (g/L) along with Congo red of 0.05 g/L, soluble starch, 10; $(NH_4)_2SO_4$, 0.28; KH_2PO_4, 0.0067; NaCl, 0.015, $MgSO4.7H_2O$, 0.04; beef extract, 0.2 and Agar agar, 20g at pH of 7.0

FUNGI IDENTIFICATION AND CHARACTERIZATION

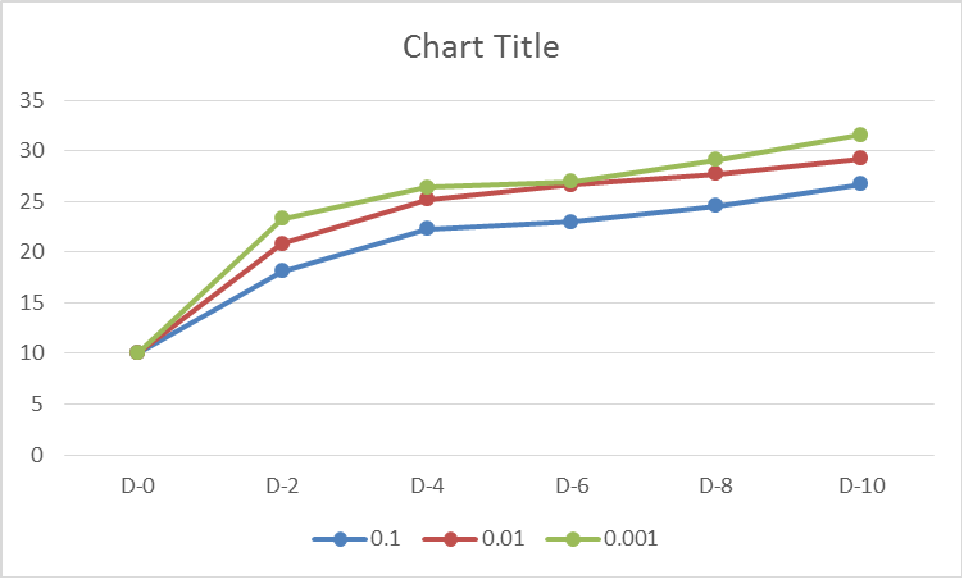
The fungal morphology was studied macroscopically by observing the colony features (color, shape, size and hyphae) on Sabouraud dextrose agar, and microscopically by a compound microscope using a lactophenol cotton blue stained slide mounted with a small portion of the mycelium with x10 and x40 lens microscopic lens(Gaddeyya *et al.,* 2012).

CONGO RED DECOLOURIZATION ANALYSIS

Solid phase (Agar plate) dye biosorption analysis was used for this assay, varying concentration of the azo dyes (0.1%, 0.01%, and 0.001%) was used in a solid phase medium. Fungi spotting using a diameter 8.00mm was placed on agar plate and the underside of the petri-dish was observed for clear zones and the diameter of the zones was measured for a period of 12 days in a 2 day interval

Table 1: Total fungi count and total dye degrading fungi count of the soil sample.

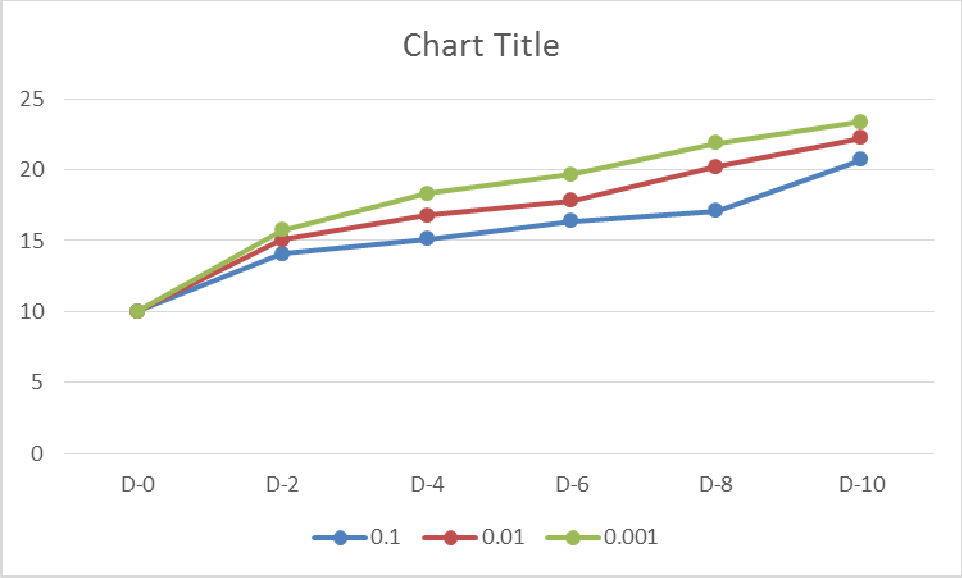
SAMPLE	TOTAL FUNGI COUNT(cfu/ml)			TOTAL DYE DEGRADING FUNGI COUNT(cfu/ml)		
	X	y	Mean count	x	y	Mean count
SITE A	85	87	8.6 X 10^5	31	34	3.3 X 10^5
SITE B	105	101	1.03 X 10^6	58	52	5.5 X10^5
SITE C	43	47	4.5 X 10^5	30	34	3.2 X 10^5



Graph 1: Graph showing the biodegradation curve of *Aspergillus niger*

Table 2*: Aspergillus niger* degradation rate of Congo red

	D0	D0	x	D2	D2	x	D4	D4	x	D6	D6	x	D8	D8	x	D10	D10	X
0.1	10	10	10±0.00	18.00	18.30	18.15 ±0.15	22.50	22.10	22.30 ±0.20	23.20	22.80	23.00 ±0.20	24.30	24.80	24.55 ±0.25	26.80	26.60	26.70 ±0.10
0.01	10	10	10±0.00	21.20	20.50	20.85 ±0.35	25.40	25.00	25.20 ±0.20	26.50	26.80	26.65 ±0.15	27.50	27.95	27.73 ±0.23	28.95	29.60	29.23 ±0.33
0.001	10	10	10±0.00	23.10	23.55	23.33 ±0.23	26.70	26.15	26.43 ±0.28	26.80	27.10	26.95 ±0.15	29.40	28.85	29.13 ±0.28	31.40	31.75	31.58 ±0.18



Graph 2: Graph showing the biodegradation curve of *Aspergillus flavus*

Table3: *Aspergillus flavus* degradation rate of Congo rate

	D0	D0	x	D2	D2	x	D4	D4	X	D6	D6	x	D8	D8	x	D10	D10	X
0.1	10	10	10±0.00	14.20	13.90	14.05 ±0.15	15.50	14.75	15.13 ±0.38	16.85	15.90	16.38 ±0.48	17.30	16.90	17.10 ±0.20	20.10	21.30	20.70 ±0.60
0.01	10	10	10±0.00	15.20	14.90	15.05 ±0.15	17.48	16.10	16.79 ±0.69	18.50	17.10	17.80 ±0.70	20.50	19.95	20.23 ±0.28	22.75	21.70	22.23 ±0.53
0.001	10	10	10±0.00	16.10	15.35	15.73 ±0.38	18.80	17.85	18.33 ±0.48	20.40	18.90	19.65 ±0.7	22.30	21.50	21.90 ±0.40	23.80	22.95	23.38 ±0.43

Table 4: Table showing the microscopic and macroscopic features of the isolates

SN	Colonial morphology	Microscopic features	Probable Isolate
1	On SDA, colonies had rapid growth rate. However, colonies were flat and compact with yellow basal felt covered by a dense layer of black conidial heads with powdery texture. The color on the reverse side was pale yellow. Colonies were incubated at 30°C for 5 days.	Septate hyphae with Conidiophores were hyaline or pale-brown to black, erect, simple, with foot cells basally, inflated at the apex forming globose vesicles, bearing conidial heads split into over 4 loose conidial columns with over 4 fragments apically composed of catenulate conidia.	*Aspergillusniger*
2	On SDA, colonies had rapid growth rate. However, colonies were flat and compact with yellow basal felt covered by a dense layer of green conidial heads with powdery texture. The color on the reverse side was pale yellow. Colonies were incubated at 30°C for 5 days.	Septate hyphae with Conidiophores were hyaline or pale-brown to black, erect, simple, with foot cells basally, inflated at the apex forming globose vesicles, bearing conidial heads split into over 4 loose conidial columns with over 4 fragments apically composed of catenulate conidia.	*Aspergillusflavus*

DISCUSSSION

The study showed that several fungal isolates found in the soil possess the capability to utilize certain dyes through biosorption and bioaccumulation processes which can be an important biotechnological tool for harmful waste effluent treatment associated with threats to both aquatic and terrestrial life.

Assessment ofcongo red dye utilization through bioaccumulation capacity of the retrieved isolates showed that *Aspergillus niger*as compared to *Aspergillus flavus*has an increased degradability ratefor the coingo red dye to be degraded, as seen and compared to the by the control set up values in the control experiment in Table 3 and 4 . It was also observed that monoculture degradation with *Aspergillus niger* had a degradative potential of about 81% and *Aspergillus flavus* had a degradative potential of about62 %. However, a bi-culture dye degradation has been proposed with increased degradative potential for the same monitoring time which agrees with the wok of Sourav *et al.*(2011).

CONCLUSION

A significant cause of water contamination across the world is due to textile industry effluents. Because of their xenobiotic nature, they pose a threat to both people and aquatic life. Bioremediation in the textile industry may make use of fungi and dead cells as adsorbents through bioaccumulation and biosorption. Bioremediation has evolved as an environmentally benign, low-cost, and efficient method for treating textile effluents. Microorganisms such as fungi isolated from textile dye contaminated environments can be effectively taken for bioremediation due to their well-developed mechanisms such as oxidative and reductive enzymes that assist in cleaving the chemical molecules of dye. In addition, fungi may effectively treat dye polluted wastewaters due to their several additional benefits, including their great capacity for biosorption due to their huge surface area, and the presence of chemical groups on their cell walls, which gives numerous sites for electrostatic adsorption. Although various issues require urgent attention, a future study in this field may be able to overcome the majority of the existing obstacles. The existing bioremediation strategies are primarily laboratory-scale. Integration of these systems for large-scale commercial applications is a major technological problem. There are several factors to consider when using bioremediation, including the kind of effluent, the toxicity of the metabolites, the costs involved, and the intended use of the treated water.

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