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DECOLOURIZATION OF TEXTILE DYES, DISPERSE BLUE 56 AND CIBACRON DEEP RED DYES BY FUNGAL ISOLATES

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ABSTRACT

The presence of a very small amount of dyes in water (less than 1 ppm for some dyes) is highly visible and affect the aesthetic merit, water transparency and gas solubility in lakes, rivers and other water bodies and as a result of this, the removal of the colour and the chemicals present in water is necessary. This research work is therefore aimed at the decolourization and degradation of dyes by fungi isolated from industrial effluents. Nine fungi out of twenty isolates that were isolated from the effluent and soil samples collected from the premises of textile manufacturing and dyeing industries were used for the biodecolourization experiments. These fungi includes Aspergillus terreus, Fusarium flocciferum, Neurospora sitophila, Candida valida, Candida valida, Aspergillus melleus, Saccharomyces uvarum, Penicillium expansum and Penicillium atrovenetum. The decolourization and degradation studies were carried out using UV visible spectrophotometer. The results obtained showed that all the isolates used had potential of being used for decolourisation experiment. Six out of the nine isolates used can decolourise disperse blue 56 to about 60% and eight out of nine can decolourise cibacron deep red to about 60% and above within four and six days respectively while other demonstrated less than 60% decolourization for both dyes within four and six days. The highest decolourization of 72.02% was recorded for Aspergillus melleus on day six for Disperse blue 56, while Neurospora sitophila had the highest biodecolourization of 83.6%on day 2 for Cibacron deep red dye. Thus, the potentials of these isolates can be employed industrially for the treatment of dye effluent.

KEYWORDS: Biodecolourization, Cibacron, Disperse Blue, Fungal Isolates, Textile Effluent

INTRODUCTION

Population expansion, rapid urbanization, industrial and technological expansion often lead to generation of untreated or partially treated wastes which are indiscriminately discharged directly or indirectly into the environment, rendering the water resources and soil unwholesome and hazardous to man and other living systems (Banat et al., 1996; Singh and Singh, 2010). Textile industries are found in most countries and have shown a significant increase in the use of synthetic complex organic dyes as the colouring materials. Large numbers of chemicals, including dyes are used in textile industries and the annual world production of textiles is about 30 million tonnes requiring 700,000 tonnes of different dyes which cause considerable environmental pollution problems (Cripps et al., 1990). About 10-15% dyes are released into processing water during this procedure (Selvam et al., 2003). Dyes may also significantly affect photosynthetic activity in aquatic life by reducing light penetration intensity and toxic to some aquatic fauna and flora due to the presence of aromatic, metals, chlorides e.t.c (Dhaneshavar et al., 2007). It is therefore important to monitor and control the effluents from these industries.

The treatment of textile effluent is of interest due to their toxic and aesthetic impact on receiving water. Though, physical and chemical treatment techniques are costly and lack effective colour reduction and sensitivity to many variables waste water input (Banat et al., 1996). However, microbial decolourization is seen as cost effective method of removing these pollutants from the environment (Ogunjobi et al., 2012) and studies have shown that some microorganisms (bacteria, fungi, algae and yeasts) are capable of effectively decolourizing wide range of dyes (Fu and Viraghan, 2001).Many studies have shown that fungi have the potential of treating wastewater from textile industries (Ramyaet al., 2007).

Therefore the major objective of this study is to determine the microbial degradation / decolourization of textile dyes, Disperse blue 56 and Cibacron deep red by using fungi isolated from textile effluents.

MATERIALS AND METHODS

Collection of Samples

Textile effluents and soil samples were collected from discharging sites of a textile manufacturing company at Ikorodu in Lagos State and also from a local textile dyeing centre in Abeokuta, Nigeria by random sampling from a depth of 5 to 10 cm into a sterile glass bottles and kept on ice for further study.

Determination of Physico-Chemical Parameters of the Effluent

The digital pH meter and thermometer were used to determine the pH and temperature of the effluents respectively. The Biological Oxygen Demand (BOD) was determined using BOD Trak instrument and the Chemical Oxygen Demand (COD) was also determined by using the standard titration method of potassium dichromate with ferrous ammonium sulphate solution.

Isolation of Microorganisms

Isolation of microorganisms from the textile effluents and soil samples was carried out by using serial dilution method and pour plate techniques on Nutrient agar and Potato Dextrose agar for bacteria and fungi respectively. The morphologically distinct colonies were sub cultured to obtain pure isolates and stored on agar slants.

Screening for Isolatesdye Decolourization Potential and Characterization

The pure microbial isolates were tested for their dye decolourization properties by culturing them on dye-agar medium with the composition: $MgSO_4.2H_2O$ 0.1%, KH_2PO_4 0.1%, $CaCl_2$ 0.1%, $FeSO_4$ 0.05%, NH_4NO_3 0.1%, $(NH_4)_2SO_4$, K_2HPO_4, Glucose 0.05% and Agar 2% according to Sumathi and Phatak, (1999). Those microorganisms capable of growth on this medium were further purified. They were later characterized and identified using their morphological, physiological and biochemical properties as earlier described byDomsch et al. (1980).

Preparation of Dye Stock Solution

The stock solutions were prepared by dissolving 5 mg of each dye in 0.09% (w/v) NaOH. The preparations were filter sterilized using membrane filtration method. Dyes absorbance was determined spectro photo metrically from the prepared stock solution using Scanning UV spectrophotometer (GENESYS 10 UV). The maximum absorbance of Disperse blue 56 was 230nm while that of Cibacron deep red was 224nm

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Decolourization Experiment

The decolourization experiment was set up in 50 ml conical flasks corked with cotton wool, containing 20 ml of dye decolourizing medium and the 13 ml of the dye stock solution, inoculated with 7 ml of the 24 hour old broth culture of the isolated microorganisms, to make up 40 ml. Control flasks without any culture were also set up for each of the dyes to serve as control. The flasks were incubated aerobically at room temperature for six days; samples were withdrawn on the second, fourth and six day to determine the degree of decolourization. The residual dye content was determined by taking the absorbance reading of the supernatant of the samples after centrifugation at 4,000 rpm for 20 minutes to remove microbial cells. The fungi count for yeast and fungal mycelia weight were also determined.

Analytical Method

The degree of dye decolourization was measured spectrophoto metrically from the residual dye content and calculated from the absorbance value obtained against the uninoculated control % Decolourization= (Absorbance of uninoculated – Absorbance of inoculated dye broth)/ Absorbance of uninoculated dye broth

Determination of Fungal Mycelia Weight and Yeast Count

This was carried out by taking out 5 ml of the decolourization experiment samples and their subsequent filtration through apre-weighed filter paper. The filtrate was later dried in oven at 40^0C until a constant weight was obtained. To determine the yeast count, 0.5mL of the sample was cultured on already prepared yeast agar plates using spread plate method, incubated for 24 hours at 25^0C after which plate counting was done.

RESULTS

The physicochemical properties of textile dye effluents (Table 1) showed that the effluent from textile industry in Ikorodu had the highest BOD of 270 mg/l and the lowest COD of 1200 mg/l with pH and temperature of 10.8 and 39 ^{0}C respectively. The effluent from local textile dyeing centre in Abeokuta had the lowest BOD of 106 mg/l and COD of 3200 mg/l. The effluent from Lagos textile industry had BOD of 125 mg/l and had the highest COD of 4000 mg/l with a P^H of 11.3 and temperature of 41 ^{0}C.

A total of nine isolates (Table 2) obtained from the screening on dye decolourizing medium were fungi. Out of nine fungi isolated, two were Candida valida while the rest include Aspergillus terreus, Fusarium flocciferum, Neurospora sitophila, Aspergillus melleus, Saccharomyces uvarumand Penicillium expansum.

Table 1: Physico-Chemical Properties of the Textile Dye Effluents

Parameter	ITI	ABK	UTI
BOD (mg/l)	270	106	125
COD (mg/l)	1200	3200	4000
pH	108	-	11.3
Temperature	39^0C	-	41^0C

Key:
ITI- Effluents from textile industry Ikorodu UTI- Effluent from textile industry, Lagos
ABK- Effluents from local textile dyeing centre in Abeokuta

Table 2: Fungal Isolates Used for Dye Decolourizing Experiment

Isolate	Type	Source(s)
Aspergillus terreus	Moulds	SSA
Fusarium flocciferum		SS1
Neurospora sitophila		SS12
Aspergillus melleus		SSU2
Penicillium expansum		SSU4
Penicillium atrovenetum		SSU5
Saccharomyces uvarum	Yeast	SSU3
Candida valida		ESA, SSU

Key:
SSA: Soil sample from Abeokuta ESA: C- Effluent sample from Abeokuta
SSI: Soil sample from textile industry, Ikorodu SSU: D- Soil sample from textile industry, Lagos

In Table 3, the percentage decolourization of disperse blue 56 by the fungal isolates showed that at 48 hours, all the isolates had percentage decolourization less than 50%. However, at 96 hours, there was an increase in the colour reduction as all the isolates were found to have decolourization greater than 50%. In addition, after 144 hours, all the isolates had percentage greater than 50% with A. melleus (SSU) showingthe highest percentage of 72.0% followed by C. valida (ESA) 70.0%, P. atrovenetum (SSU) 64.0%, P. expansum (SSU4) 63.8%, S. uvarum (SSU) 63.7%, F. flocciferum (SSI) 63.3%, A. terreus (SSA) 58.5% while the lowest percentage of 55.0% was observed in both N. sitophila(SSI2) and C. valida (SSU).

Table 3: The Percentage Decolourization of Disperse Blue 56 Dye by the Fungal Isolates

Duration of Treatment / Fungal Isolates	48 Hours	96 Hours	144 Hours
A. terreus (SSA)	34.6	57.8	58.5
F. flocciferum (SSI)	25.9	62.3	63.3
N. sitophila (SSI)	35.6	57.0	55.0
C. valida (ESA)	29.0	63.6	70.0
C. valida (SSU)	33.6	66.9	55.0
A. melleus (SSU)	39.5	64.0	72.0
S. uvarum (SSU)	36.4	65.1	63.7
P. expansum (SSU)	28.4	61.3	63.8
P. atrovenetum (SSU)	27.2	55.4	64.0

Key:
SSA: Soil sample from Abeokuta ESA: Effluent sample from Abeokuta
SSI: Soil sample from textile industry, Ikorodu SSU: Soil sample from textile industry, Lagos

The pH of experimental set up for disperse blue 56 dye as the decolourization proceeds (Table 4) showed that the pH for all the fungal isolates ranged from 6.6 to 10.8.

Table 4: The pH **of Experimental set up for Disperse Blue 56 as the Decolourization Proceeds**

Duration of Treatment / Fungal Isolates	48 Hours	96 Hours	144 Hours
A. terreus (SSA)	7.9	8.6	7.9
F. flocciferum (SSI)	6.9	6.9	6.9
N. sitophila (SSI)	7.9	8.5	8.2
C. valida (ESA)	6.7	6.7	6.7
C. valida (SSU)	6.7	7.2	6.9
A. melleus (SSU)	6.6	7.1	6.3

S. uvarum (SSU)	7.9	8.6	8.1
P. expansum (SSU)	7.9	8.6	8.0
P. atrovenetum (SSU)	7.1	8.6	8.2
Control	**10.7**	**10.7**	**10.8**

Key:
SSA: Soil sample from AbeokutaESA: Effluent sample from Abeokuta
SSI: Soil sample from textile industry, IkoroduSSU:Soil sample from textile industry, Lagos

The percentage decolourization of cibacron deep red dye by fungal isolates (Table 5) revealed that all the isolates decolourized cibacron dye to about 60% after 48 hours except F. flocciferum (SS1) which was 59.7%. The highest percentage (83.6%) was observed with A. melleus (SSU) while the lowest (28.9%) was observed with S. uvarum(SSU). At 144 hours of fungal decolourization, S. uvarum (SSU) had the highest percentage of 51.6% while the lowest 37.9% was observed in P. atrovenetum (SSU). Generally, the decolourization capability of the fungal isolates decreases as incubation period increases with the exception of N. sitophila, C. valida(SSU), S. uvarumand P. expansum which increased after 144 hours.

Table 5: The Percentage Decolourization of Cibacron Deep Red Dye by the Fungal Isolates

Duration of Treatment / Fungal Isolates	48 Hours	96 Hours	144 Hours
A. terreus (SSA)	66.9	50.3	44.6
F. flocciferum (SSI)	59.7	44.8	41.4
N. sitophila (SSI)	83.6	39.7	45.0
C. valida (ESA)	69.4	46.6	45.5
C. valida (SSU)	62.6	46.7	51.2
A. melleus (SSU)	69.6	50.9	47.1
S. uvarum (SSU)	65.4	28.9	51.6
P. expansum (SSU)	69.4	33.2	51.2
P. atrovenetum (SSU)	67.3	45.8	37.9

Key:
SSA: Soil sample from Abeokuta ESA: Effluent sample from Abeokuta
SSI: Soil sample from textile industry, Ikorodu SSU: Soil sample from textile industry, Lagos

Table 6: The pH of the Experimental set up for Cibacron Deep Red Dye as the Decolourization Proceeds

Duration of Treatment / Fungal Isolates	48 Hours	96 Hours	144 Hours
A. terreus (SSA)	8.0	8.4	8.2
F flocciferum (SSI)	7.0	7.5	7.2
N. sitophila (SSI)	7.7	8.6	8.4
C. valida (ESA)	6.7	7.3	6.8
C. valida (SSU)	6.7	7.0	6.9
A. melleus (SSU)	6.6	7.4	6.9
S. uvarum (SSU)	7.8	8.6	8.0
P. expansum (SSU)	8.6	8.6	7.8
P. atrovenetum (SSU)	7.9	4.5	7.8
Control	**9.6**	**6.7**	**9.7**

Table 6 shows the pHof the experimental set up for Cibacron deep red dye during decolourization process. The result showed that the pH of the decolourization process with the fungal isolates at 48 hours, 96 hours and 144 hours ranges from 6.6-8.6, 4.5-8.6 and 6.8-8.4 respectively.

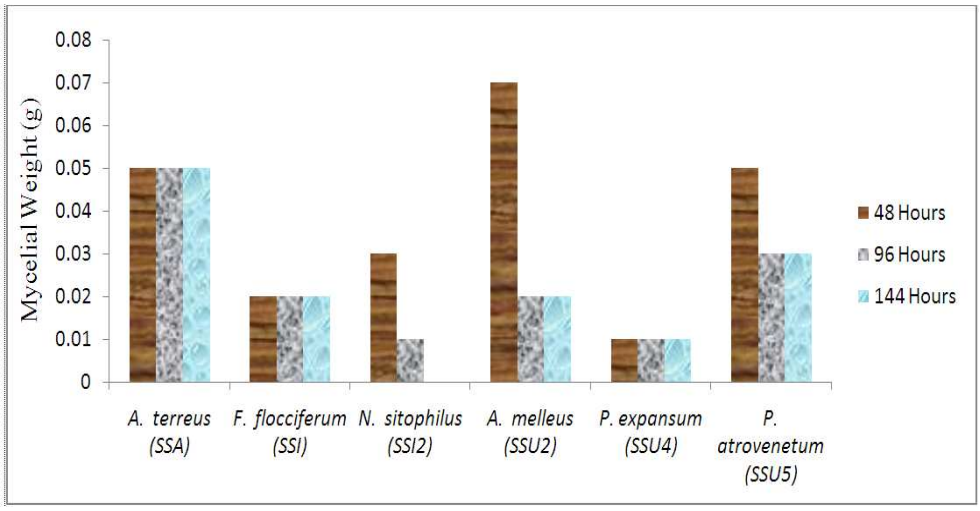

Figure 1: The Fungi Mycelia Weight Per 20 Ml of the Decolourization Experiment set up for Disperse Blue 56 Dye

Fungal growth during disperse blue 56 dye decolourization showed increase in mycelia weight as the decolourization progresses at 48 hours. This was also observed at 96 and 144 hours (figure 1). A. melleus had the highest mycelia weight of 0.07g and 0.06g respectively while P. expansum had the lowest mycelia weight of 0.01g.

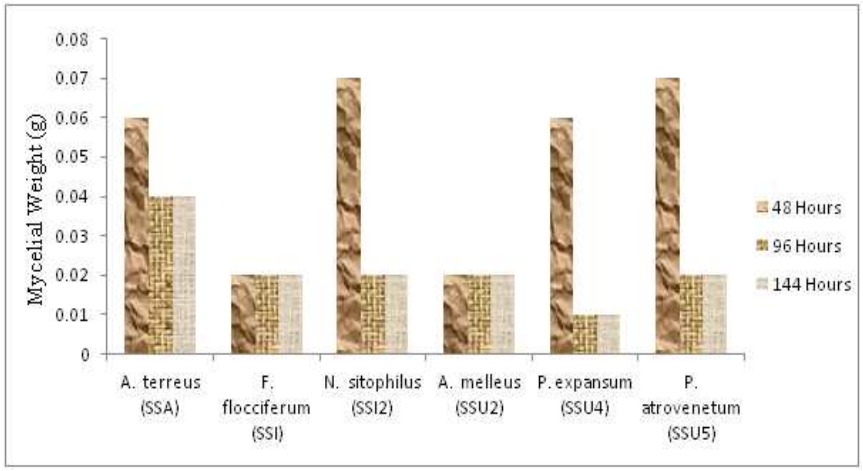

Figure 2: The Fungi Mycelia Weight in Gram (Per 20 Ml) of the Decolourization Experimental set Up for Cibacron Deep Red Dye

Figure 2 shows the fungal mycelia weight in gram per 20 ml of the decolourization experiment setup for Cibacron deep red. *N. sitophila* (SSI2) and *P. atrovenetum* (SSU5) had the highest microbial weight at 48 hours while *P. expansum* (SSU4) had the lowest mycelia weight at both 96 hours and 144 hours.

The fungal counts for the yeasts (*Candida valida* and *Saccharomyces uvarum*) used in this work showed high counts at 96 hours while they continued to reduce as incubation period increases in the biodecolourization of Disperse blue 56 experiment while the same thing was also observed with Cibacron deep red dye (results not shown).

DISCUSSIONS

The result of Physico-chemical parameters especially BOD and the COD of the three textile effluents obtained from this study varies greatly from each other. The BOD of the effluent from Ikorodu was above the 50 mg/l interim effluent limitation guideline in Nigeria by FEPA (1991) confirming the high polluted nature of the effluent which upon

release into the water body will have a significant environmental and health impact. The high BOD and COD recorded with Abeokuta effluent, which is from a local textile-dyeing centre confirmed the fact that effluent from local textile industries are always discharge into the environment without treatment. The pH of the effluent indicates that the wastewater is at high alkaline level while the temperatures were not too high.

The fungal isolates used in this work were able to biodecolourize the two dyes used in this work. The results obtained from the biodecolourization of the textile dyes by the isolates have shown the ability of *Candida* species to metabolize dyes as reported by De Angelis and Rodrigues (1987).Yang *et al*. (2003); Polman and Breckenridge (1996) also reported the decolourization capability of *Penicillium* and *Aspergillus* species respectively. Biodecolourization of Bromophenol blue and Congo red by *Aspergillus flavus* has also been reported by Singh and Singh, (2010). Ayandele and Ademola, (2012) also reported the biodecolourization of Cibacron scarlet and Remazol blue dyes by *Candida*, *Penicillium* and *Rhizopus* species.

The slower rate of biodecolourization of disperse blue 56 by the fungi may be attributed to the high molecular weight of the dye, structural complexity or those organism have not been able to adapt to the new environment but when incubation period was extended, the fungal isolates were able to adjust and actively degrade or absorb the dye. The high rate of absorption of the cibacron deep red dye by the organism might be due to structure of the dye, its molecular weight or the treatment with NaOH making the absorption process very fast while its gradual decrease after an extension of incubation might be due to the production of inhibitory substance by the organism. The ability of the isolates to remove the colour to a larger extent after 48 hours is of great interest. Sarnack and Kaneker, (1995) reported that the incubation period of five to seven days is quite long and unsuitable for any industry for actual application purpose, thus some of the isolates used in this study can therefore be used for industrial application because they were able to decolourize greater percentage within 96 hours. The greater percentage of decolourization observed after 96 hours might be due to the adsorption of dyes to the microbial cells surface because changes in the colour of the fungi mycelia was observed, Knapp *et al*., (1995) and Singh and Singh, (2010) also reported that adsorption of dyes to the microbial cell surface is the primary mechanism of decolourization by fungi. Likewise the study showed that the degradation of each dye by the isolates is different, the extension of incubation periods favour some isolates in disperse blue 56dye solution and does not favour those isolates in theCibacron deep red dye solution probably because of the secretion of toxic or inhibitory substance by the organisms or the structures of those dyes.

The decolourization in relation to the pH from this study partly confirmed the report by Chang and Kuo (2000) that increase in pHcan lead to an increase in the specific decolourization rate which was also observed in this study because the pH range was between 7.0 to 9.0. This showed that neutral and basic pH value would be more favourable for decolourization of dye by the fungi using these types of dyes. Thah *et al*. (2013) also reported that neutral pH supported bacterial activity to decolourize Remazol Black-B in liquid medium.

The increase in the fungal mycelia weight observed during the decolourization process may be due to either the growth of the fungi or the absorption of the dye to the fungal mycelia which can also contribute to their increased weight. Decrease or absence in the weights of mycelia after an extension in the incubation period might be due to the secretion of toxic or inhibitory substance produced within the decolourizing medium

CONCLUSIONS

This study has confirmed the potential of microorganism to decolourize and degrade different types of dyes and thus, this method can be employed as a cost effective method in the treatment of dye effluents.

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