



Ecological And Environmental Aspects Of Dye Usage Decolourisation By *Aspergillus Flavus*

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ABSTRACT

The textile industry generates a significant amount of wastewater not only as a result of the operations that take place in the textile mills but also as a consequence of the chemicals that are used for the processing effluents. The main aim of this work to study the **Ecological** and Environmental Aspects of Dye Usage Decolorization by *Aspergillus flavus*. The irradiation process was carried out in order to determine the effect that radiation has on the degradation of dye. The element content of the four Isolan dyes on the mycelial wall and ECF was tested by Energy Dispersive X-ray Spectroscopy (EDS), and the results detect that the elements were below the limit in dried mycelia.

Keywords- *Aspergillus flavus*, Isolan

1. INTRODUCTION

1.1 Ecological and Environmental Aspects of Dye Usage-

The textile industry generates a significant amount of wastewater not only as a result of the operations that take place in the textile mills but also as a consequence of the chemicals that are used for the processing effluents. Because of the insufficient absorption of colours by the fibres and the nature of the application, a significant quantity of dyes is lost in the effluent. The treatment of the additional dyes that are flown in the effluent is a challenge due to the fact that dyes are developed to be stable against photolytic and chemical degradation. Therefore, there are ecotoxic concerns associated with the buildup of dyes in the environment, which leads to the bioaccumulation of these colours in the food chain. Therefore, it is necessary to develop effective technologies that would allow for increased dye-fibre binding and lower dye house losses in order to control the presence of dyes and high levels of nitrogen (due to the widespread use of azo dyes) in the textile effluents. This is the only way to successfully control the problem. Because of the diversity in the structures of dyes, it is impossible to employ any one treatment approach to remove such a wide variety of dyestuff. This is because of the complexity of the dyes. In most cases, the quantity of dyes found in wastewater from the textile industry is not large in comparison to the other chemicals; nevertheless, the bright hue of these colours presents an aesthetic difficulty during disposal. Understanding the chemical structures and formulating a plan to treat a particular kind of effluent using a particular chemical and biological technology is the most probable alternative that textile producers have at their disposal.

1.1.1 Conventional Physicochemical Decolorization Processes

In the past, a variety of different physical, chemical, and biological therapies were used, but only one technique of application was employed. The strategy is used, which results in a poor amount of dye removal, a low amount of mineralization, and a low biodegradability, in addition to a high cost and the need of post-treatment in order to fulfil the discharge criteria. In the combined approach, physical, chemical, and biological approaches are used in sequence, which ultimately results in effluent that is of higher quality at the site of disposal. It is possible to treat the wastewater from a textile mill using a combination of activated carbons, the use of nanomaterials, the use of metal ions and composites, and natural materials catalysed ozonation. This results in a higher percentage of dye removal, a lower cost, higher mineralization and biodegradability, higher COD removal percentage, and higher TOC removal percentage. The effluent from the textile mill may be completely treated using this method, which also allows for some degree of reusability and a significant amount of regeneration.

2. LITERATURE REVIEW

Ashar, Ambreen & Shoaib (2022) The industries that employ synthetic colours most often include those in the textile, leather, paper, food, and pharmaceutical sectors. One of them uses a lot of these colours is in textiles. A total of 10,000 colours are used in the textile process, with an annual output of around 7×10^5 t. Azo dyes are the biggest class of synthetic dyes used in the textile industry and released into the environment as waste, making them one of the main causes of water pollution issues on a global scale. Azo dyes are mostly produced and used in the textile sector. They have aromatic functional groups with one azo group ($-N=N-$) and are classified as electron-deficient heterologous biological molecules.

Pinheiro, Lucas & Gradíssimo (2022) Since the beginning of time, people have used colours, and as population and industrial expansion have expanded, synthetic dyes have become more popular. Due to their extensive usage and difficult degradability, azo dyes need particular consideration because of their significant environmental effect. The versatility of bacteria, which can be used as single organism cultures, microbial consortia, in bioreactors, acting in the detoxification of azo dyes breakage by-products, and having the potential to combine biodegradation with the production of economically valuable products, makes them a standout among the biological solutions created to address this problem. These traits go hand in hand with the capacity of different strains to function in a variety of chemical and physical circumstances, including a broad range of pH, salinity, and temperature, with strong performance under conditions important to industry and the environment.

Okeke, Chidi & Kenechi, Chukwu & Ndukwe (2022) Since no one treatment approach can reliably remove both the dye components and the intermediate metabolites that are often produced during the treatment process, treating dye waste effluent has proven to be a persistent challenge. Hybrid therapy methods have also been used; however, they are not without drawbacks. Although efficient, this method does not entirely mineralize the pollutant or intermediates produced by the wastewater treatment process.

Mishra, Arti & Takkar (2022) Synthetic dyes offer a serious risk to the environment, which has a negative impact on human health. Numerous dyes are employed in the textile, cosmetics, and pharmaceutical sectors and are untreatably dumped into the environment, harming both the natural environment and nearby human populations. While numerous physical and chemical approaches for dye degradation are already available, they have several limitations.

Guddi, Kumari & Sarkar, Angana (2022) Dye is a significant component of the wastewater produced by numerous businesses. Nowadays, synthetic dyes are commonly employed. The majority of these colours are harmful to the environment. They are carcinogenic to people in addition to killing aquatic vegetation and wildlife. Water environments may suffer as a result of sunlight being blocked by strongly coloured effluent. When people come into touch with the dye effluents, the dyes induce allergies and skin irritations.

3. RESAERCH METHODOLOGY

The ECF of a liquid culture of *Aspergillus flavus* in modified BSM IV was analyzed using a Shimadzu UV-2100 spectrophotometer both before and after decolorization of the four Isolan dyes. The analysis was performed before and after the dyes were removed. After an incubation period of 72 hours, this step was taken. This was done in order to determine whether or not the mode of decolorization occurred via degradation. A graph showing the absorbance vs the wave length was created. The Indian Gamma Chamber with 4000 A activities and 9100 CU was used, and the dose rate was set at 5 kGy/h. Isolan dyes viz. Isolan Yellow, Isolan Green, Isolan Red and Isolan Navy were placed in separate tubes and subjected to radiation doses of 0.5, 1.0, 1.5, 2, 2.5, 3, 6, 10, 15, and 20 kGy. The percentage of decolorization and the change in pH value were then measured. In addition to that, FTIR was carried out. The samples were dried at 50 degrees Celsius overnight, ground, then combined with potassium bromide (KBr), and lastly pressed into discs before being examined using an FTIR-6300 Jasco instrument (Japan). After and before the decolorization process, results were displayed as transmittance so that changes could be monitored. In addition, pure powder of dyes was combined with KBr, as was described before, and the results were assessed.

4. RESULTS

The conventional aerobic biological process, such as the activated sludge process, is unable to readily treat textile wastewater because the majority of commercial dyes are toxic to the microorganisms, and they result in sludge bulk. On the other hand, gamma radiation has been considered as a potentially fruitful process for the treatment of textile wastewater. According to the findings in Table -1, gamma rays may cause a rise in the colour intensity of I.Y, which may be the consequence of the dimerization of the parent chemical, which then leads to polymerization. The other three Isolan dyes, on the other hand, exhibited a negative decolorization efficiency until 2.5 kGy, after which a steady rise in the decolorization was noticed. This remained the case for as long as the experiment lasted.

Table -1 Effect of gamma radiation on dyes decolorization.

Dyes	Percentage of increase in color intensity	Percentage of decolorization			
		I.Y	I.R	I.N	I.G
Radiation dose (kGy)					
0	0	0	0	0	0
0.5	0	0	0	0	0
1.0	0	0	0	0	0
1.5	0	0	0	0	0
2.0	0	0	0	0	0
2.5	0	0	0	0	0
3.0	52	21.5	7.7	6.58	
6.0	54	37.3	17.7	14	
10.0	54	47.8	23.8	21.9	
15.0	55	53.1	36.2	28.1	
20.0	56	59.4	43.4	32.9	

We determined the pH levels of irradiated and non-irradiated dye solutions via our experiments. According to the findings in Table-2, the pH of the solution had a slow but steady decline as the irradiation dosage increased.

Table-2 Effect of gamma radiation on the dye's pH for tested Isolan dyes.

Dyes	pH values			
	I.Y	I.R	I.N	I.G
0	7.9	7.97	7.94	7.98
0.5	7.8	7.9	7.9	7.8
1.0	7.8	7.87	7.89	7.8
1.5	7.7	7.85	7.8	7.7
2.0	7.6	7.80	7.75	7.6
2.5	7.5	7.79	7.75	7.6
3.0	7.3	7.72	7.7	7.54
6.0	7.4	7.64	7.7	7.5
10.0	7.0	7.2	7.48	7.21
15.0	7.1	7.13	7.45	7.03
20.0	7.1	7.11	7.03	6.91

4.1. Combined physical/ biological treatment:

The application of radiation as a pretreatment for biological oxidation to convert initially non-biodegradable compounds to more easily biodegradable intermediates is a promising alternative to a complete oxidation of bio refractory wastewater. The results shown in Table -3 showed that combined treatment gave approximately the same value as decolorization by biological treatment only.

Table -3 Effect of combined treatment on dyes decolorization by *Aspergillus flavus*

Dyes	Dose (k Gy)					
	Control	0.5	1	1.5	2	2.5
I.Y	93.8%	93.7%	93.5%	93.8%	93.3%	93.8%
I.R	99.8%	99.5%	99.3%	99.3%	99.3%	99.5%
I.N	98.0%	97.5%	97.1%	97.0%	98.0%	98.0%
I.G	95.5%	95.4%	95.0%	95.2%	94.0%	95.1%

4.1.2. Application of *Aspergillus flavus* as decolorizing strain on mixture of the four Isolan dyes:

In most cases, fungi are able to remove the colour from a single dye; however, the wastewater from textile factories often contains a combination of many colours that are the consequence of the printing step. Prior to this, *Aspergillus flavus* was put through a series of tests to determine whether or not it possessed the capacity to decolorize various azo dyes. The strain was found to be effective in decolorizing various textile dyes when exposed to glucose oxidase conditions and a total dye concentration of 200 mg/l. The application of these findings to the decolorization of a combination of the four Isolan dyes showed good results. It was possible to see, from the section on co-metabolism, that BSM I and IV supplied the greatest amount of decolorization. Because of this, it is important to investigate how well *Aspergillus flavus* decolorizes dye mixtures in both medium. At the conclusion of 72 hours, the level of decolorization that was accomplished by BSM I and BSM IV was rated at 86.3 and 84% respectively, as shown in Table 4.51. Additionally, it was observable that

the combination did not have a detrimental impact on the glucose oxidase activity generated by *Aspergillus flavus*. Instead, the activity reached 7 and 2 U/ml in BSM I and BSM IV, respectively, in addition to the expansion of the fungal colony.

Table -4 Effect of mixture of the four Isolan dyes on percentage of decolorization, extracellular GOD and fungal growth in two different BSM medium by *A. flavus*.

Time (h)	Percentage of decolorization		Extracellular GOD(U/ml)		Dry weight (g/100ml)	
	BSM I	BSM IV	BSM I	BSM IV	BSM I	BSM IV
0	0.0	0.0	0.0	0.0	0.0	0.0
72	86.3	84.0	7.0	2.0	2.5	0.7
168	86.0	84.0	2.5	0.3	2.6	0.8

4.1.3. Application of *Aspergillus flavus* on textile effluents:

(i) Decolorization of textile effluents under various conditions:

It is well known that the parameters of the culture impact the physiology of the fungus as well as the expression and activity of the enzymes that break down lignin. Because of this, the settings for decolorization should be tuned in order to get the best possible outcomes. As a consequence of this, the findings of the tests that were conducted in order to assess the capability of *Aspergillus flavus* to decolorize cotton textile wastewater under four distinct settings are shown in Table 4.3. The findings shown in Table 4.5 and Figure (33) demonstrated that, for the greatest amount of decolorization to take place, the addition of the components of the medium and the pH adjustment were both necessary processes. This was the case for each of the four culture conditions that were selected. The use of a single parameter by itself did not make a significant contribution to the decolorization process. This was most likely due to bioaccumulation, which was seen using a microscope by viewing the bluish-green tint inside the fungal mycelia, as shown in Image 1. (4.7)

Table -5 Effect of different conditions on decolorization, extracellular GOD activity and dry weight by *Aspergillus flavus* of textile dye effluent.

Condition	Percentage of decolorization	Extracellular GOD activity (U/ml)	Dry weight (g/100ml)
1	75	0.5	2.14
2	7.5	Zero	0.15
3	2.1	Zero	0.87
4	2.2	Zero	0.93

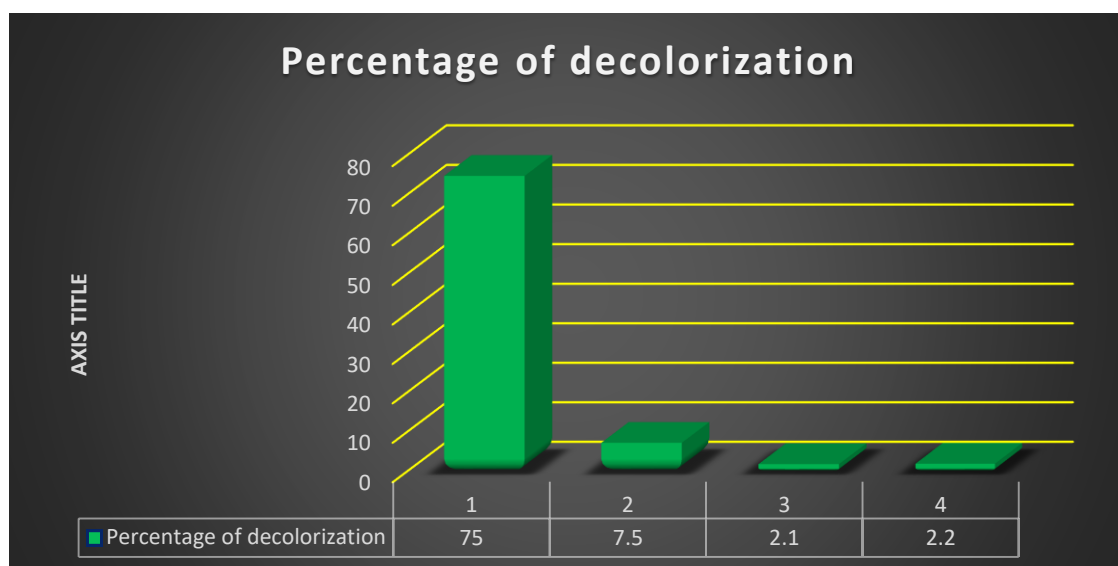


Fig. -1 Decolorization of textile effluent under different conditions after 72 h incubation.



Image (1): Showing wastewater uptake by *A. flavus* mycelia after 24, 48 and 72 h of incubation.

The level of maximal decolorization that could be accomplished in textile effluents by utilising *Aspergillus flavus* was lower than that which could be accomplished by using Isolan dyes individually. The action of salts, inhibitory molecules (sulphur compounds, surfactants, heavy metals, and bleaching agents), carbon, and nutrients found within these solutions may be responsible for the mild decolorization of these effluents. The pH of the textile effluent was 10 when it was first measured; because of this, the process of decolorization was inhibited; as a result, the pH should be lowered to 5.5. Additionally, nutritional inefficiency had an effect on the decolorization process; hence, nutrients had to be provided in order to start the decolorization process efficiently in *A. flavus*. As a result, the first criterion in Table -3 was chosen to fulfil our requirements. Only under circumstances that were ideal for GOD creation was it possible to see both the production of GOD and the expansion of mycelial networks (Figure -2)

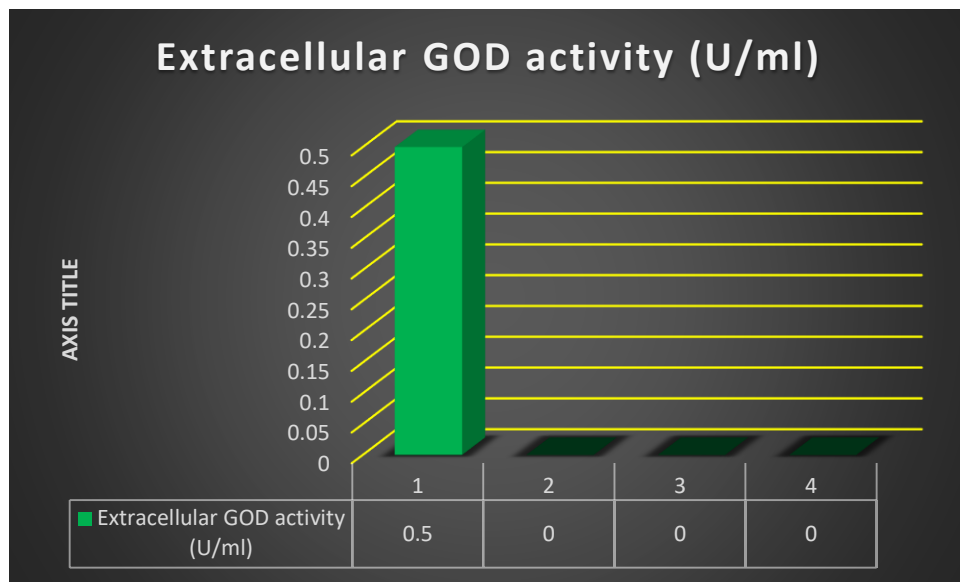


Fig. -2 GOD production of *A. flavus* after 72 h incubation with textile effluent under different conditions.

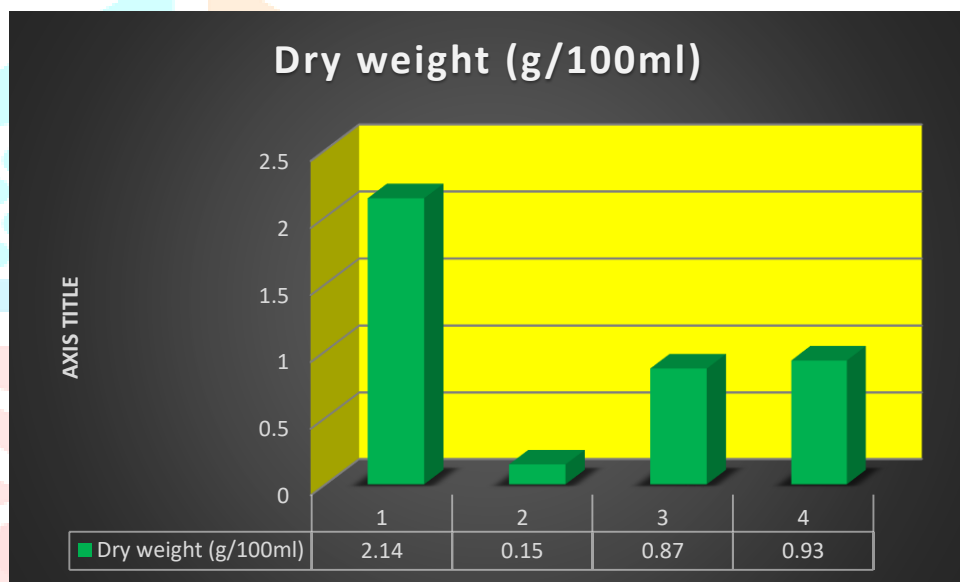


Fig. -3 Dry weight of *A. flavus* after 72 h incubation with textile effluent under different conditions.

(ii) Measurement of some chemical parameters and elements assay:

Although the presence of dyes is considered to be the primary reason for increasing the COD and BOD in textile effluents, the results in Table 4.6 showed that the removal of 75% of the dyes from the textile wastewater effluent by the strain under study did not induce the expected decrease in the COD and BOD levels. This was the case despite the fact that the presence of dyes is considered to be the primary reason for increasing these levels.

Table -6 Some tested parameters and elements measured after decolorization by *Aspergillus flavus* in textile effluents.

Parameters	Percentage of reduction
COD	7.8
BOD	12.9
Na	13.8
Al	60.7
Cl	0.6
Ca	1.5
Co	100
Nitrate	ND
Nitrite	ND
Phosphate	ND
TSS	46.3

(iii) Fourier Transform Infrared spectroscopy analysis (FTIR) of textile wastewater:

The FTIR analysis that was performed on textile wastewater (Fig. -4) revealed that in the region of aromatic rings (800-400 cm^{-1}), two peaks had gone, while the remaining peaks had moved and reduced in strength. This was seen in the area. As well, in the azo bond area (1500-1400 cm^{-1}), two peaks vanished, which indicates that colour was removed from the wastewater. Also, in the range of 1650–1560 cm^{-1} , one peak in the aniline spectrum had migrated and experienced a loss in strength, while the other peak had gone, which suggested that additional degradation had taken place.

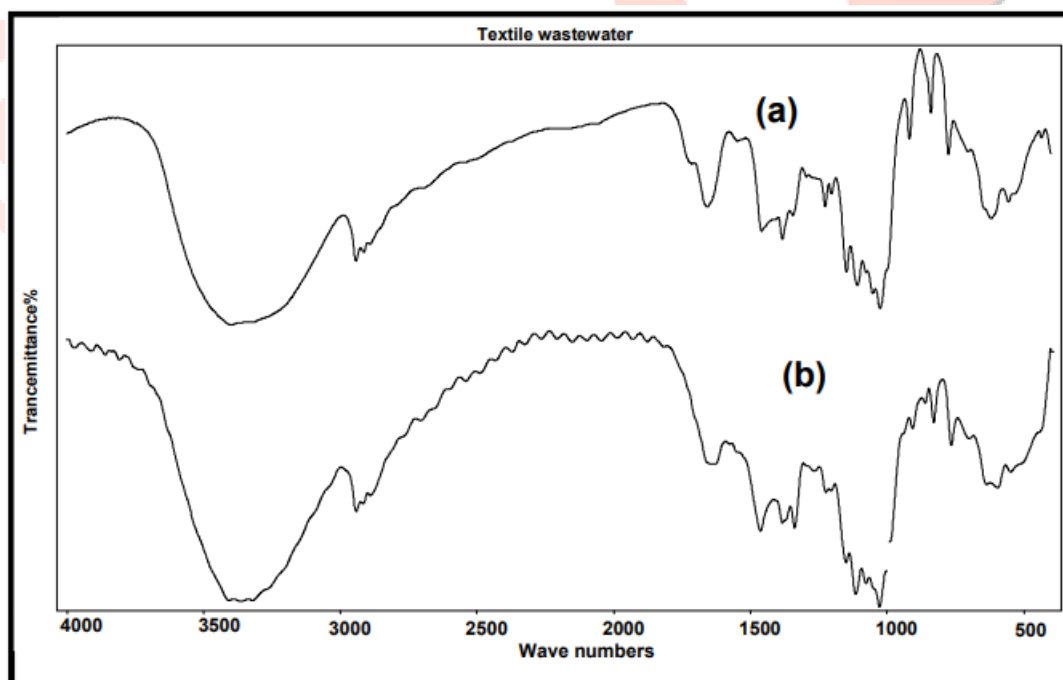


Fig. - 4 FTIR of textile wastewater (a) before and (b) after fungal treatment.

4.2. Physical treatment of textile effluents:

(i) Irradiation:

Due to the fact that a radiation dosage of 20 kGy did not provide the desired results as described in the section on the physical treatment of the four Isolan dyes, a higher radiation dose of 25 kGy was used. The results of Table 4.7 demonstrate that gamma rays are not very effective in decolorizing textile wastewater.

Table -7 Effect of gamma radiation on textile wastewater decolorization.

Radiation dose (kGy)	Percentage of decolorization
25.0	41.6

(ii) Pre and Post treatment of textile effluents with H₂O₂:

The application of hydrogen peroxide prior to the inoculation of *Aspergillus flavus* exhibited a lag time for decolorization that reached 4 days, as shown in Table -8 and Fig. (5). This is in contrast to the control cultures, which showed 82.7% elimination after the same amount of time. In spite of this, after 10 days of incubation, both approaches finally showed roughly the same percentage of elimination, and the post treatment prevented any additional decolorization from occurring. Additionally, the visual observation of the cultures indicated that the presence of hydrogen peroxide in the culture is not a contributor for enhancement of decolorization. This is because the presence of hydrogen peroxide in the culture has a negative effect on the growth of *Aspergillus flavus*, as evidenced by the fact that there was only a minimal increase in fungal dry weight for each culture in comparison to that of the control.

Table -8 The effect of hydrogen peroxide (H₂O₂) inoculated at the beginning of the incubation period (referred to as pre-treatment) and again after 72 hours of incubation (referred to as post-treatment) on the decolorization of textile dyes effluent caused by *Aspergillus flavus* under condition 1 was investigated.

Time (h)	Percentage of decolorization				
	0	72	96	168	240
Control	0	73.0	82.7	93.2	94.2
Pretreated	0	0.0	3.6	59.5	92.8
Post treated	0	63.8	75.4	88.0	88.0

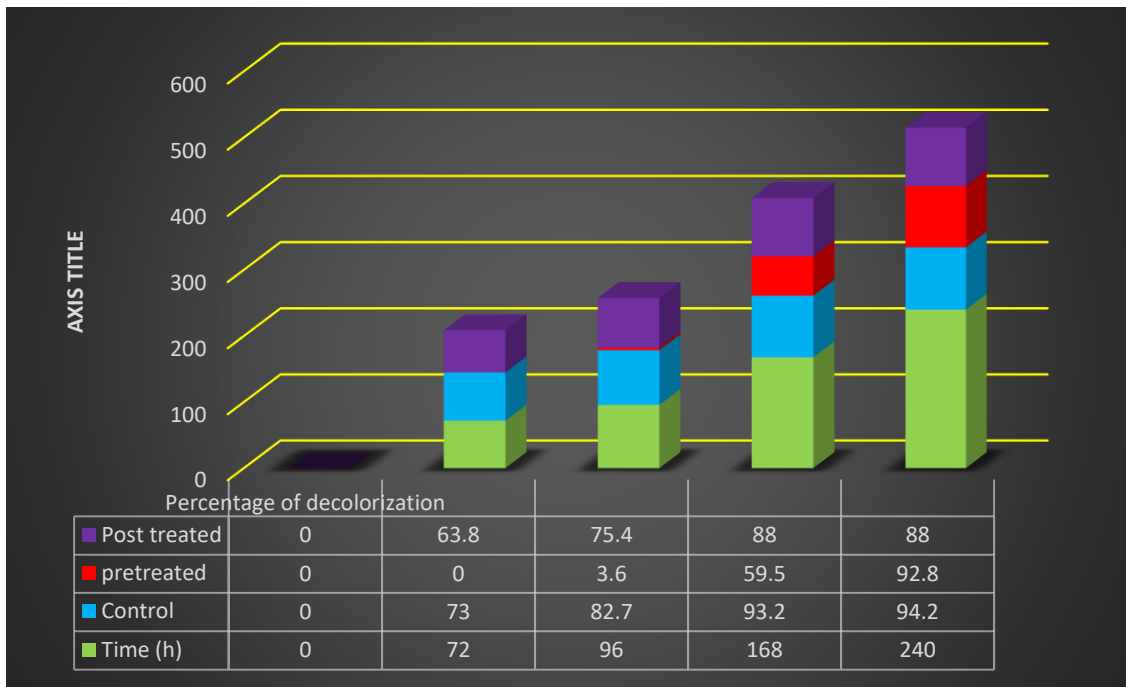


Fig.-6 The use of pre and post oxidation treatment of the dye cultures combined with biological treatment using *Aspergillus flavus* and compared to control dye cultures.

The findings that were obtained following UV-Vis spectrophotometric examinations, which are shown in Fig. -6, revealed that there was a drop in the visible range that is distinctive for the dye colour. This was shown by the fact that the visible range was represented lower. After 168 hours of incubation, the fungal growth was examined, and the degree to which it had been decoloured (93.2% of the original colour) was measured. It was evident that the cells had not maintained their natural colour, but rather obtained the colour of the wastewater; this suggests that adsorption may be a mechanism that is involved in the process of decolorization.

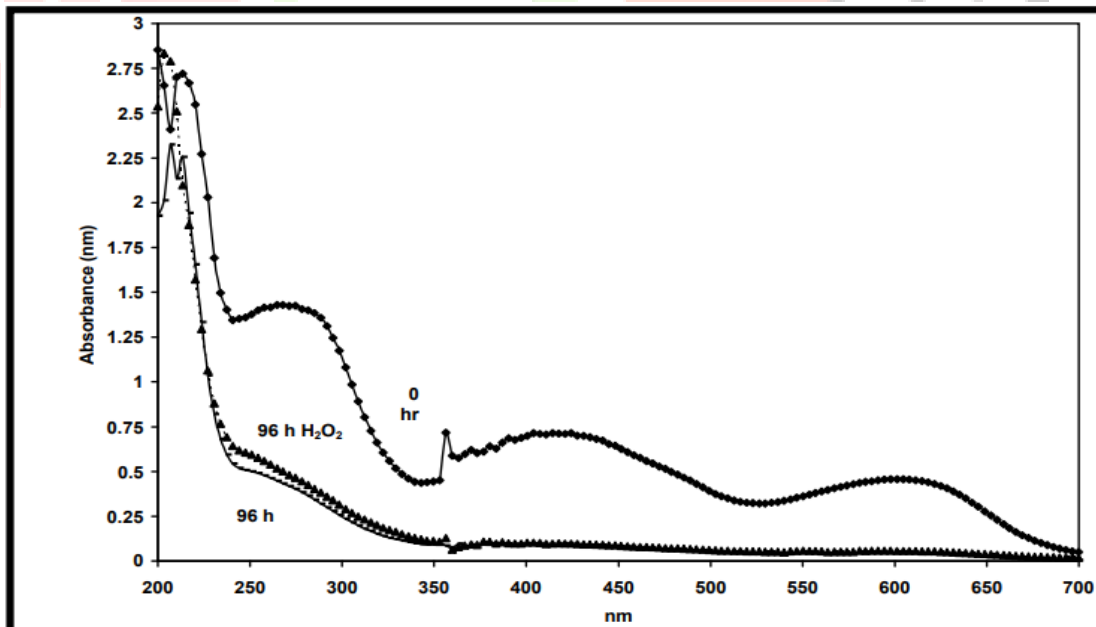


Fig. -7 UV-Visible spectrum of dye cultures before (0 h) and after biological treatment (96 h) and in combination with hydrogen peroxide post-treatment (96 h H₂O₂).

4.3.Application: retrieve of textile dyes and reuse in dyeing of cotton and polyester:

Following this, effort was done to extract the dyes and utilise them for dyeing cotton and polyester swatches. These were then compared to those swatches that had been coloured using the real colours before the bioremediation process was carried out. The results can be seen in both Images. which represent the swatches after the dyeing process for cotton (a) and polyester (b). The colour of the swatches is nearly identical for cotton and polyester that were dyed with the original dye powder, as well as for those that were dyed with extracted dyes after intracellular accumulation in *A. flavus* mycelia.

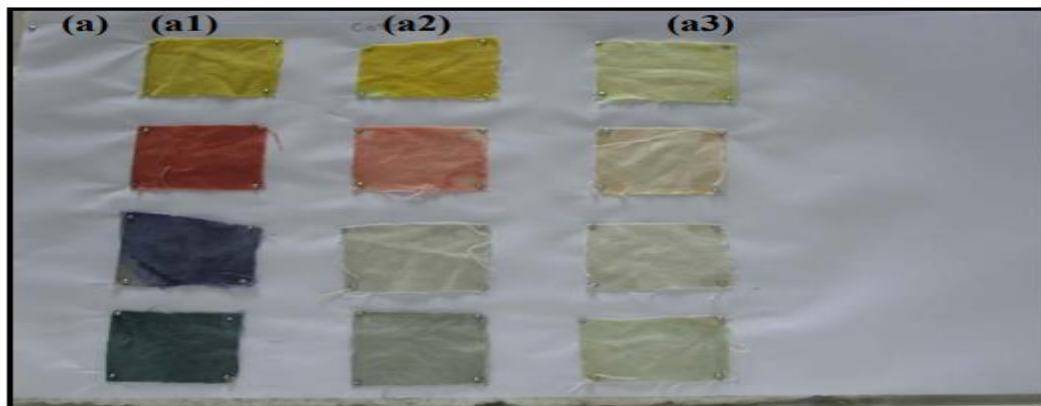


Image (2): Cotton swatches dyed with (a1) initial dye solution, (a2) intracellular extraction of grinded fungal mycelia and (a3) ECF after treatment.

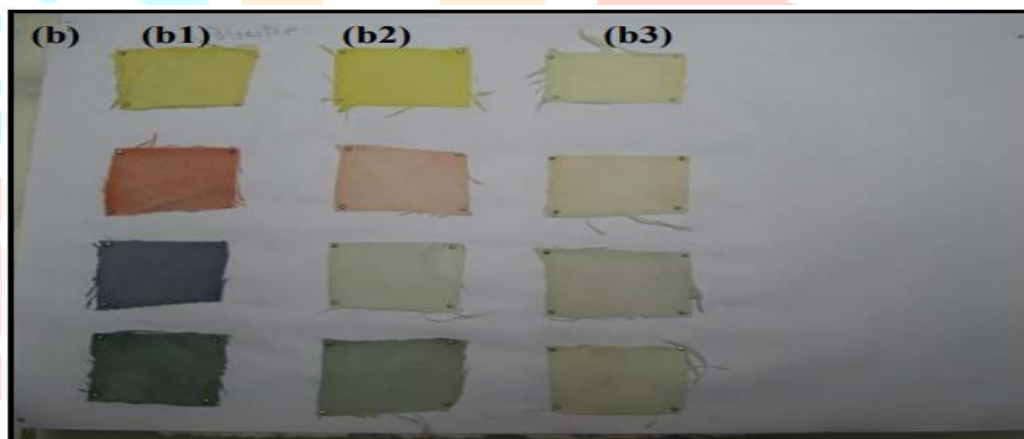


Image (3): Polyester swatches dyed with (b1) initial dye solution, (b2) intracellular extraction of grinded fungal mycelia and (b3) ECF after treatment.

5. CONCLUSION

The element content of the four Isolan dyes on the mycelial wall and ECF was tested by Energy Dispersive X-ray Spectroscopy (EDS), and the results detect that the elements were below the limit in dried mycelia. This indicated that dyes entrapped inside fungal biomass, and this suggestion was confirmed by photos that revealed that the dyes were bioaccumulated rather than bio transformed. After conducting FTIR, UV, and HPLC analysis, the researchers concluded that *Aspergillus flavus* could break down the azo bond and convert it to aniline and sulphanilic acid. Additionally, the researchers found confirmation that *Aspergillus flavus* could decolorize the Isolan dyes but could not reach complete degradation of the dyes. It was possible to draw the following conclusion from all of the previous experiments: the decolorization of the Isolan dyes by *Aspergillus flavus* appears to be a complex process comprising different removal mechanisms. These removal mechanisms include bioaccumulation as the primary mode of decolorization, biodegradation, physico-chemical biosorption by whole fungal biomass as the subsidiary mode of removal, and so on.

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