

## The potentials of white-rot fungi to decolorizing azo dyes and organic components of textile effluents

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### Abstract

Synthetic dyes released by various textile industries are a source of concern to environmental safety. Existing physicochemical methods of dye removal from effluents suffer setbacks like high operational cost, low efficiency and large amount of sludge generation. Over the last two decades considerable work has been done with the goal of using microorganisms as remediation agents in the treatment of dyes containing wastewaters. Microorganisms are capable of removing dyes due to their high metabolic potentials and one of the best organisms that is known to display these wonderful features are the white-rot fungi. In this present study we demonstrate successfully using white-rot fungi (*Phanerochaete chrysosporium*) for this purpose and the role of each culture in the decolorization process was elucidated. The effect of temperature, pH, concentration, mean weight (g) and optical density were studied after every 48hrs in 12 days period. The enhancement of the degradation was effected by UV-radiation. The adsorption capacity of textile waste effluent and reactive dyes i.e. Congo red and Direct blue 80 was determined spectrophotometrically by monitoring absorbance of different dyes at constant wavelengths ( $\lambda_{max}$ ). The shift in the absorption maxima in decolorizing samples indicated the ability of the fungus to degrade dyes and this can be exploited for bioremediation of dyes and their derivatives containing wastes.

**Keywords:** Decolourization, degradation, dyes, *Phanerochaete chrysosporium*, textile effluents.

### 1. Introduction

The basidiomycete, *P. chrysosporium* belongs to the white rot class of wood-rotting fungi (reviewed by Kües, 2015) [16]. White-rot fungi are those organisms that are capable of degrading lignin, the structural polymer found in woody plants (reviewed by Mathews *et al.*, 2015) [18]. The most widely studied white-rot fungus with regards to xenobiotic degradation is *Phanerochaete chrysosporium* (Singh *et al.*, 2015) [24]. This fungus is capable of degrading dioxins, polychlorinated biphenyls (PCBs) and other chloro-organics (see reviews by Gowri *et al.*, 2014 [12]; Marco-Urrea *et al.*, 2015) [17].

White-rot fungi are able to degrade dyes using enzymes such as lignin peroxidases (LiP), manganese dependent peroxidases (MnP). Other enzymes used for this purpose include H<sub>2</sub>O<sub>2</sub>-producing enzymes such as glucose-1-oxidase and glucose-2-oxidase with laccase and a phenoloxidase enzyme (Archibald & Roy, 1992 [1]; Thurston, 1994 [25]; Schliephake & Lonergan, 1996) [23]. Azo dyes, the largest class of commercially produced dyes are not readily degraded by micro-organisms but can be degraded by *P. chrysosporium* (Robinson *et al.*, 2001) [22]. Other fungi such as *Hirschioporus larincinus*, *Inonotus hispidus*, *Phlebia tremellosa* and *Coriolus versicolor* have also been reported to decolorize dye-containing effluent (Banat *et al.*, 1996 [4]; Kirby, 1997 [15]; Jebapriya & Gnanadoss, 2013). *P. chrysosporium* has also been shown to mineralize a range of obstinate aromatic pollutants (Asamodu *et al.*, 2005 [3]; Dafale, 2011) [8]. It has also been reported that white rot fungi can efficiently degrade various organic pollutants other than lignin including polycyclic aromatic hydrocarbons and persistent environmental pollutants such as DDT (Bumpus *et al.*, 1985) [5], alkyl halide insecticides (Kennedy *et al.*, 1990) [14] and xenobiotic compounds (Paszczyński & Crawford, 1995) [19].

One distinct advantage these fungi have over bacterial systems is that they do not require preconditioning to the particular pollutant. Bacteria usually must be pre-exposed to a pollutant to allow the enzymes that degrade the pollutant to be induced and the pollutant must also be in a significant concentration for the enzyme synthesis to follow. Thus, there is a finite level to which bacteria can degrade pollutants. But this is not the case with fungi, because the induction of the degrading enzyme is not dependent on the pollutants, therefore, the pollutant can be degraded to a near non-detectable level. In contrast to the bacterial system, the degradative enzymes of white rot fungi are induced by nutrient limitation. Thus, cultivation of the white rot fungi on a nutrient-limited substrate will initiate the process (Tuomela, 2002 [26]; Piggott *et al.*, 2015) [20]. The basidiomycete produce different other extracellular enzymes involved in pollutant degradation (Wesenberg *et al.*, 2003; Ghani *et al.*, 2015) [11, 27].

The objective of this paper is to examine the decolorization activities of the white-rot fungi for the treatment of textile waste effluents and two most widely used reactive dye in cultures of *Phanerochaete chrysosporium* and also to monitor and optimize the kinetic parameters for remediation of the organic effluent in different conditions of pH, mean weight, concentration and optical density in a biotreatment system.

### 2. Materials and Methods

#### 2.1 Collection of textile wastewater sample

The textile waste water was collected in the premises of effluent treatment plant of textile industry in Kano, Nigeria. The waste water sampling site is situated behind the wax and super production confluent units. This point contains a mixture of all effluents from the various units of the production, and is capable of holding the effluent volume of about 1550 m<sup>3</sup>

generated daily. The samples were collected in clean 5 litre plastic containers with screw caps and placed in cold boxes containing ice pack immediately after collection. These were then transported to laboratory for immediate analysis.

## 2.2 Preparation of the treated sample

A filter paper was folded and inserted on the mouth of the Soxhlet extractor unto which some quantity of XAD-2 resin was carefully dispensed. 2.5 litres of textile wastewater was measured using graduated cylinder and carefully dispensed in the XAD-2 resin. The organic components of textile waste effluent were retained within the organic resin as residues and the filtered were collected and discarded. The resulting residues are the desired component needed for the analyses, and were allowed to dry for 2 hrs within in the Soxhlet extractor. After drying, 50 cm<sup>3</sup> of ether was passed through the residues of XAD-2 resin contained in the Soxhlet extractor, and all the organic components in the XAD-2 were miscible with ether as organic solvent, which are collected as filtered and warmed gently in a hot air oven at 180 °C to allow the ether to evaporate in 48 minutes. The solid deposit was allowed to cool to 35 °C and weighed, thereafter; 30 g was diluted to 1000 cm<sup>3</sup> with sterile de-ionized water and properly labelled as stock solution (1000 mg/dm<sup>3</sup>).

## 2.3 Chemicals

Two reactive dyes of relatively simple structures were chosen for this work, apart from the textile waste effluent collected directly from confluent unit of the textile wastewater discharge of African Textile Industry Kano, Nigeria. Congo red (% purity - 60) and Direct blue 80 (% purity- 40), was obtained from Aldrich Chemical Company (Milwaukee, Wis.), as 90 percent pure form of Congo red. The organic solvents employed in the experiments were of reagent grade and used as purchased.

## 2.4 Microorganism

*Phanerochaete chrysosporium* fungus used as discussed herein was isolated from the Fungi Identification Centre, Department of Crop Protection, Institute of Agricultural Research, Samaru Zaria. The culture was maintained on malt agar plates at 30 °C, with subcultures routinely made every two weeks.

## 2.5 Isolation and enumeration of fungal cultures

The white-rot fungi were isolated from the textile waste samples using Czapek dox agar unto which sterile streptomycin (50 mg/ml) was added to suppress bacterial growth. Pure cultures of the fungi isolates were made and transferred using Czapek dox agar slants as stock cultures. The microscopic and macroscopic features of the hyphal mass, morphology of cells and spores and nature of the fruiting bodies were used for identification.

## 2.6 Cells-mass measurement (dry weight)

Direct approached method was used to determine the microbial dry weight. Cells growing in liquid medium were collected by centrifugation, washed, dried in an oven and then weighed using Mettler Toledo as described earlier (Cheesbrough *et al.*, 2005) [7].

## 2.7 Media, growth conditions and irradiation

After 70 - 80% confluent hyphal mass were cultured in mineral salt medium overnight, UV-irradiation was performed on the

hyphal mass according to the method described by Dhanwada *et al.* (1995) [10]. UV-irradiation was carried out in a chamber safe for UV-B exposure. The UV-dose was quantified in joules per square meter with the use of a microvolt ammeter. The organisms were grown in mineral salt medium on Luria Bertani (LB plates). In all experiments, fungi were grown from a single colony in mineral salt medium at 30 °C until they reached an optical density at 600 nm (Dhanwada *et al.*, 1995) [10]. For UV-experiments, serial dilutions of fungal cultures were plated on LB plates and irradiated with a dose of UV-28 nm light of 10 J/m<sup>2</sup>, with a constant dose rate of 0.25 J/m<sup>2</sup>/s. Colonies of survivors were scored after 24 to 48 hrs of incubation at 30 °C and later sub-cultured on nutrient agar ready to prepare broth formation (Cheesbrough *et al.*, 2005) [7].

## 2.8 Mineral salt broth for wild and mutant types of *Phanerochaete chrysosporium* isolates

Measured volume, 99.9 cm<sup>3</sup> of mineral salt medium was dispensed into 250 ml Erlenmeyer flask and distinct isolates from nutrient agar slants were picked gently using a sterile wire loop and inoculated into the mineral salt medium. The mixture was shaken and incubated at room temperature of 30 °C for 24 hrs. The mineral salt broth for fungal isolates were kept near freezing point in order to have a control of microbial load (inoculum size) and this has been prepared to measure the mean weight, pH and optical density at different wavelengths until the high peak was attained using UV- visible spectrophotometer.

## 2.9 Decolourization of textile effluent and two commercial dyes

Mineral salt broth of fungal isolate 99.0 cm<sup>3</sup> was dispensed into nine 250 ml Erlenmeyer flasks arranged sub sequentially to contain two (commercial dyes) and seven (organic components of textile effluents), in order of 48 hrs interval at pH 5.0. To each reaction flask was added 1 cm<sup>3</sup> of the effluent sample and cultures were placed in shaken incubator at 200 rpm and allowed to grow for twelve days (commercial dyes) and twenty four days (organic components of textile effluents). At the end of the days the two commercial azo dyes and organic component of textile dyes effluents were dissolved in methanol at 1.0 mg/l concentration. Controls were run with the same reaction conditions but excluding fungal strains. These experiments were conducted in replicates of five. The optical density (absorbance) at different wavelengths, cell mass, concentration and pH of the cultures were determined (Cheesbrough *et al.*, 2005) [7].

## 2.10 Degradation and decolourization assay

Aliquots of 2 cm<sup>3</sup> of a clear dye solution were taken from each of the reaction flasks at time intervals and measured immediately using a UV-visible recording double beam spectrophotometer. Diluted cultures were used prior to the measurement to change the absorbance value to below 1.0 absorbance units per centimeter of path length. Due to low water solubility of the organic dyes, an equal volume of methanol was mixed with the analytical solution to ensure complete solubilisation prior to measurement and decolourization was assessed in two ways; Firstly, by monitoring absorbance spectrophotometrically at maximum wavelength for each cultured solution and secondly by observing the reduction of the major peak area in the visible

region for each cultured solution. Both approaches were used because colour change may occur during biodegradation with a concomitant wavelength shift. Therefore, the maximum wavelength of the cultured solution would not be sufficient to accurately reflect the full degree of decolourization. To obtain additional information regarding these changes, the area under the curve in the visible regions (400 - 800 nm) was incorporated.

### 3. Results and Discussion

The absorption rate is an intrinsic property of a colorant. Measuring the spectral absorption curves of dyes during biodegradation provided a means to determine the reaction rate and to give evidence of structural changes in dyes during biodegradation. Previous experience showed that fading of the original colour of the dyes is sometimes accompanied by a colour change (wavelength shift) in the solution. As a result of this, both the ultraviolet and visible regions were monitored by a UV-visible spectrophotometer to provide more information about the biodegradation. The decrease of the maximum absorbance of disperse dyes occurred primarily in the first several days of treatment with no substantial additional decrease in absorbance as the treatment time increased.

White-rot fungi are known to degrade textile effluents and dyes using many different enzymes. The wild type *Phanerochaete chrysosporium* was able to present an excellent result by decolorizing and mineralizing textile waste effluent up to 97.5% and 97.7%, while the mutant type up to 84.2%, and 87.9% respectively in 12 days period of treatment (Fig. 1) and

the weight of the dried culture after a 12 days period of treatment increases from 0.31 g to 0.62 g for wild type and for the mutant type decreases from 0.26 g to 0.22 g (Fig. 4). There was a variable change in pH as a result of reaction (redox reaction) that took place in the reaction flask, which seems to appear in a straight line in most of the figures below and this can easily be deduced clearly as shown in Fig. 5, that demonstrate a remarkable change in pH values in all the figures. This results obtained corroborate with the findings of Chao & Lee, 1994 [6]; Reddy, 1995 [21]; Davis *et al.*, 1993 [9] and also agree with findings of Archibald & Roy, 1992 [1]; Thurston, 1994 [25]; Schliephake & Lonergan, 1996 [23]; Kirby, 1997 [15]. *Phanerochaete chrysosporium* was used for biotreatment of direct dyes, Congo red and direct blue '80' and in the case of the wild type of the fungal isolates, it was wonderfully able to decolorizes and mineralizes Congo red dye up to 100% and 99.8%, while for a mutant type it decolorizes and mineralizes up to 77.9% and 87.5% after 12 days period of treatment (Fig. 2). Hence, there was an increase in the dry weight from 0.18 g to 0.42 g for wild type, and for the mutant type, there is a decrease in dry weight from 0.21 g to 0.17 g at a constant wavelength of 525 nm and 515 nm in 12 days period treatment (Fig. 4). From the result obtained it shows that when the fungal isolates are left in their natural form (wild types) are effectively more potential to decolorizes and degrades textile waste effluents than the mutant form (genetically altered) because of their inability to withstand the adverse conditions (UV- irradiation), hence were unable to repair and reform its cells been damaged. This result agrees with the findings of Reddy, 1995 [21].

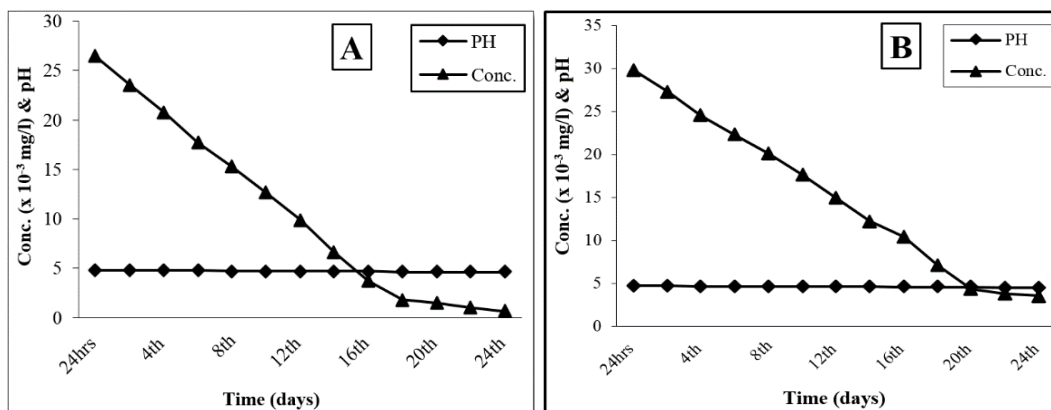


Fig 1: Growth profile of a wild (A) and mutant (B) type of *Phanaerochaete chrysosporium* in mineral salt medium containing organic textile waste effluent as the sole carbon source and energy.

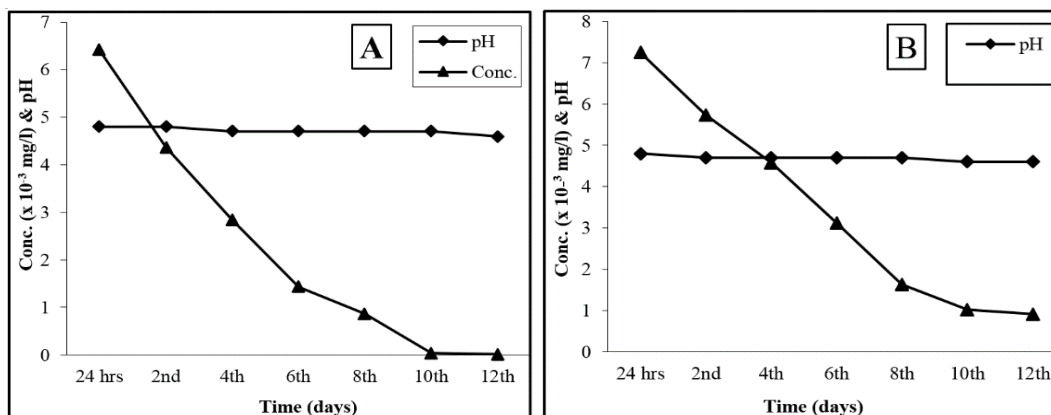
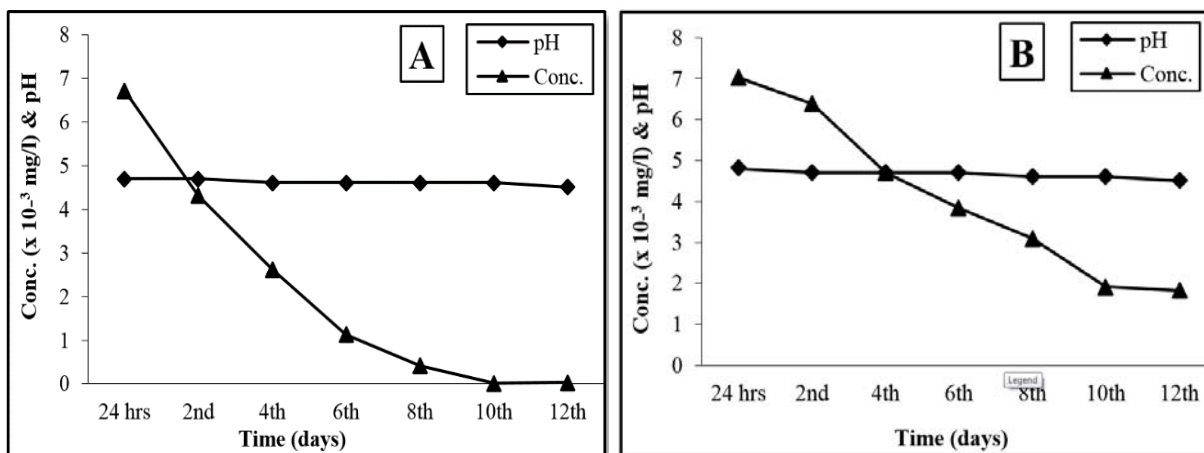


Fig 2: Growth profile of a wild (A) and mutant (B) type of *Phanaerochaete chrysosporium* in mineral salt medium containing Congo red dye as the sole carbon source and energy.

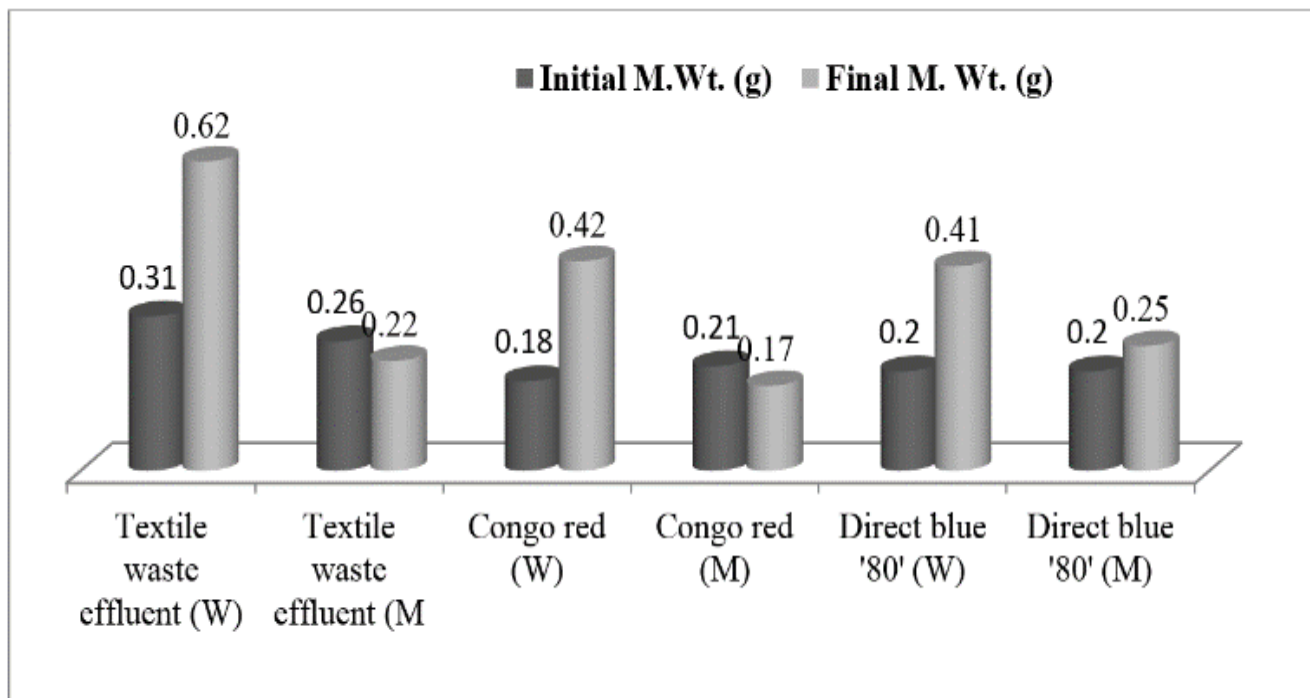
The wild type of *Phanaerochaete chrysosporium* was able to decolorize and mineralize Direct blue '80' dye up to 99.6% and 99.96% with an increase mean weight from 0.20 g to 0.41 g in 12 days period of treatment (Fig. 4), while for the mutant type, there is a negligible increase in mean weight after 12 days period of treatment from 0.20 g to 0.25 g. However, the mutant organism exhibits sharp ability to decolorize and mineralizes Direct blue '80' dye up to 77.6% and 74.1% respectively (Fig. 3).

The overall analysis the results obtained showed high decolorization efficiency (i.e. biosorption) was recorded from an augmented wild-type of *Phanerochaete chrysosporium* (Fig.

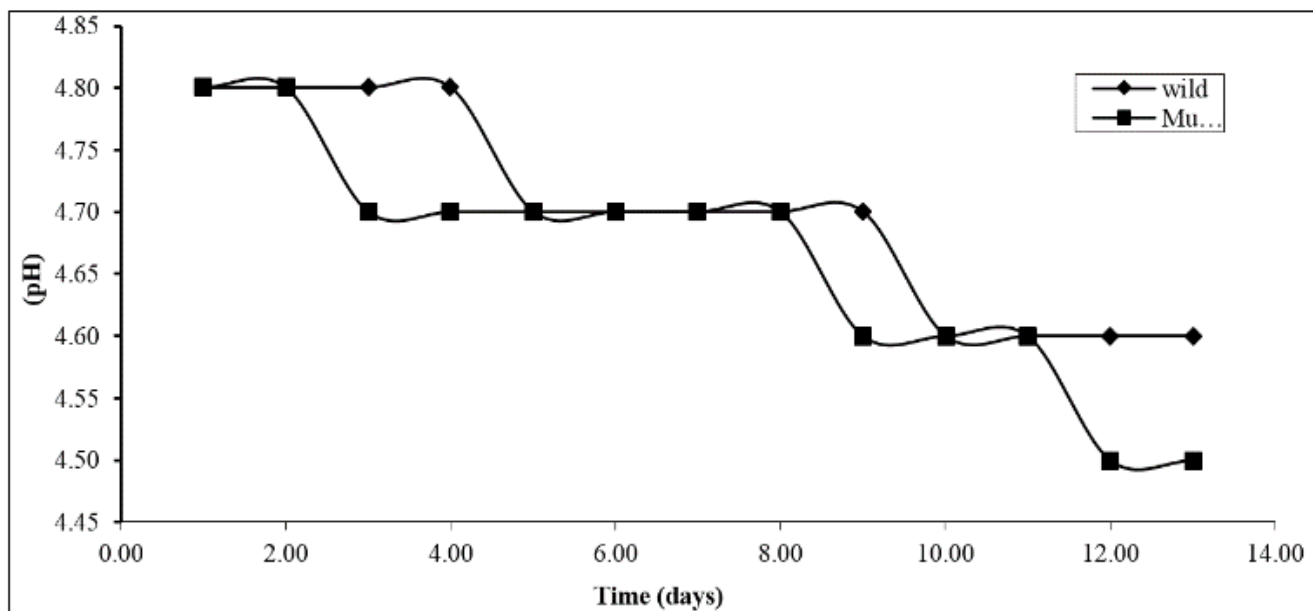
1), determined by the optical density in which the visible region of the dye effluent showed a major peak at 515 nm. After 24 days of treatment by monitoring the  $\lambda_{max}$ , this peak shifted to 310 nm and the absorbance decrease by 97.5% (percentage absorbance) which has a direct relationship with decrease in concentration from  $26.43 \times 10^{-3}$  mg/l to  $0.6 \times 10^{-3}$  mg/l with an excellent increase in the mean weight value from 0.31 - 0.62 g, which indicated substantial decolorization and substantiated by the decrease of absorption at maximum wavelength and decrease in area under the absorption curve in visible region (results not shown). No new peaks appeared in the UV region after decolorization.



**Fig 3:** Growth profile of wild (A) and mutant (B) types of *Phanaerochaete chrysosporium* in mineral salt medium containing Direct blue dye '80' as the sole carbon source and energy.



**Fig 4:** Growth performance of wild (w) and mutant (m) types of *Phanerochaete chrysosporium* in mineral salt medium containing textile waste effluent in 24 days and two commercial dyes in 12 days treatment period.



**Fig 5:** pH profile of wild and mutant types of *Phanerochaete chrysosporium* for the decolourization of organic component of textile waste effluent.

The monitoring of the UV- visible spectra supplied information on the nature of the decolorization, particularly with the shifts in the absorption maxima. These could only be the result of chemical change in the conjugated double bond structure, rather than just removal of the unchanged dye molecules. Biosorption shown by *Phanerochaete chrysosporium* demonstrated very promising result for decolorizing textile effluents. These result

obtained corroborates the findings of Aririatu *et al.*, (1999) [2], which indicates decolorization up to 78.9% of Azo dyes in 7 days' time and the result also agrees with that of Davis *et al.*, 1993 [9]; Chao & Lee, 1994 [6]; Reddy, 1995 [21]. An increase in biomass of isolates after 24 days period was also observed (Fig. 4), as an indication of that cell growth occurs and can be represented by this simple reaction equation;



where,  $\text{O}_2$  is the terminal electron acceptor.

This showed that the organisms were able to utilize and degrade the textile effluent constituents and those textile effluents degraders could be isolated from non-textile effluent polluted environment and could show remarkable degradation potentiality. Although, those from textile polluted environments have high degradation potentials.

#### 4. Conclusion

From the results of this work, it can be concluded that the isolate has the potentiality to decolorize and mineralize textile dyes and other toxic effluents based on the fact that; the wild type proved to be the most active strain in this investigation and degraded dyes to the maximum level similar to the potentialities exhibited by *P. chrysosporium* species. It also indicates that the treatment of textile dyes by *P. chrysosporium* is proficient, cost effective and environmentally friendly. Lignin is the single most important activity in the biological circle of carbon. The multitude of inter-unit bonds and functional group as well as the heterogeneity of the dyes is the main reason for the resistance of lignin to microbial attack. Biological treatment of textile dyes effluent using this fungus to decolorize effluents has yielded remarkable results similar to some of the best decolorizing activities reported in the literature. The results obtained will be used in the development of a comprehensive analytical method for investigating the degradation of dyes by white rot fungi.

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