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## ENVIRONMENTAL CHEMISTRY, POLLUTION & WASTE MANAGEMENT | RESEARCH ARTICLE

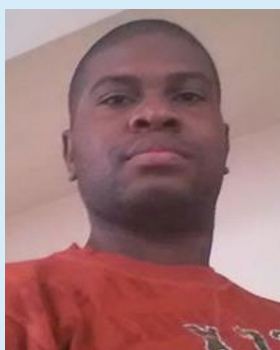
# Mycodecolorization of Reactive Red HE7B dye by *Achaetomium strumarium* and *Aspergillus flavus* and shelf life determination

Paul Olusegun Bankole<sup>1,2\*</sup>, Adedotun Adeyinka Adekunle<sup>2</sup> and Olayide Folashade Obidi<sup>3</sup>

**Abstract:** The decolorization of Reactive Red HE7B dye, a sulphonated reactive azo dye, was achieved under static condition with *Achaetomium strumarium* (KR262716) and *Aspergillus flavus* (KJ880096). The growth profile of isolated organisms and physicochemical parameters from the dye was fitted into a multiple linear regression model to predict the shelf life. The dye has an estimated shelf life of 36 months. The fungi showed complete decolorization of over 95% within 15 days. While *A. flavus* achieved maximum decolorization at a neutral pH, maximum decolorization was achieved by *A. strumarium* at pH of 6. The fungi optimally decolorized the dye at a temperature of 35°C. The UV-Visible and FTIR spectrometric analyses were used to study decolorization. The decrease, shifts and disappearance of peaks in UV and FTIR spectra of treated samples indicated myco-decolorization. The formation of aromatic amine was supported by Fourier Transform Infrared spectrometry (FTIR), which revealed the disappearance of certain peaks, particularly those of the aromatic C-H bending at 600–800 cm<sup>-1</sup>. In phytotoxicity studies, *A. strumarium* showed higher detoxification efficiency on Reactive Red HE7B dye than *A. flavus*. The results conclusively showed that *A. strumarium* exhibited greater decolorization efficiency on Reactive Red HE7B dye than *A. flavus*.

**Subjects:** Engineering & Technology; Technology; Clean Tech

**Keywords:** decolorization; shelf life; Reactive Red HE7B dye; *Achaetomium strumarium*; *Aspergillus flavus*



Paul Olusegun Bankole

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### PUBLIC INTEREST STATEMENT

The present study focused on the need to develop an eco-friendly bioremediation technique using fungi. Dyes, dye effluents and dye wastewaters indiscriminately discharged into our environment pose great environmental and health challenges. Production of extensive hyphal and different enzymes systems by filamentous fungi has proven to be very effective and efficient in the clean-up of dye pollutants. This study also predicted the shelf life of an azo dye, Reactive Red HE7B used by most textile industries.

## 1. Introduction

Reactive dyes are formed by the combination of azo-based chromophores with different types of reactive groups such as vinyl sulphone, chlorotriazine, trichloropyrimidine and difluorochloropyrimidine (Aksu & Dönmez, 2005). Reactive dyes are commonly used in textile industries because of their favourable characteristics of bright colour, water-fastness and simple application techniques with low-energy consumption (Aksu & Dönmez, 2005). Azo dyes are resistant to degradation and remain persistent for long time due to its fused aromatic structure (Xu, Guo, Zeng, Zhong, & Sun, 2006). The textile industry is estimated to consume as much as two-third of the total annual production of dyes (Melgoza, Cruz, & Bultron, 2004). The dyestuff usage has increased day by day because of the tremendous increase in industrialization and man's urge for colour (Venkata Mohan, Chandrasekhar Rao, Krishna Prasad, & Karthikeyan, 2002). It is estimated that 280,000 tons of textile dyes are discharged every year as industrial effluents worldwide (Maas & Chaudhari, 2005). The toxicity of most of the azo dyes is one of the serious environmental concerns (Wang et al., 2009) as the effluents coming from dye processing and manufacturing industries are known to be carcinogenic as well as mutagenic to various organisms (Chen, 2006; Mathur & Bhatnagar, 2007; Mathur, Bhatnagar, & Bakre, 2005; Novotný et al., 2006). In many Nigerian cities, the textile factories daily discharge millions of litres of untreated effluents in the forms of wastewater into public drains that eventually empty into rivers (Olayinka & Alo, 2004).

The environmental challenges posed as a result of indiscriminate release of textile dye wastewater by two foremost textile companies; Nichemtex and Sunflag at Ikorodu and Surulere, Lagos State, respectively, is unquantifiable. This increasing toxicity of discharged wastewater affects the human beings in a number of ways making dye contamination both an environmental as well as public health issues. Most synthetic dyes are recalcitrant and are not easily degraded by conventional physical and chemical processes. Physical and chemical methods include adsorption, chemical precipitation, flocculation, photolysis, chemical oxidation and reduction, electro-chemical treatment and ion-pair extraction (Moreira, Mielgo, Feijoo, & Lema, 2000; Rajeshkannan, Rajasimman, & Rajamohan, 2010, 2011). Fungi or their oxidative enzymes can decolorize textile wastewater either by adsorption of dye on fungal mycelium or oxidative degradation of dye molecule (Singh, Prakash, & Shahi, 2013). Fungal enzymes are non-specific towards different structures of dyes and thus oxidize a wide range of them (Aust, 1990). Fungi have been extensively studied to degrade textile dyes due to their extracellular oxido-reductive and non-stereo-selective enzyme system, including lignin peroxidase, laccase, manganese peroxidase and tyrosinase (Hofrichter, 2002; Kaushik & Malik, 2009). Fungal systems appear to be most appropriate biological agent in the treatment of coloured and metallic effluents. Important fungal biosorbents include *Aspergillus* sp. (Fu & Viraraghavan, 2002), *Penicillium* sp. (Isen & Kiran, 2007) and *Rhizopus* sp. (Kumari & Abraham, 2007). Shelf life of dye is the maximum time the dye colour fastness, light fastness, wash fastness and perspiration fastness quality is guaranteed on shelf. Shelf life testing and prediction consists basically of selecting the quality characteristics which deteriorate most rapidly in time and the mathematical modelling of the change.

However, fewer reports are available on the decolorization of Reactive Red HE7B dye by indigenous filamentous fungi isolated from dye wastewater and the dye itself. There is also paucity of information on the shelf life of reactive dyes used by textile industries. Hence, this study is aimed at isolating fungi from the dye itself/dye wastewater and evaluating the myco-decolorization efficiency of the isolates on a sulphonated azo dye, Reactive Red HE7B used by textile industries and ultimately predicts the shelf life of Reactive Red HE7B dye.

## 2. Materials and methods

### 2.1. Dye collection

Reactive Red HE7B dye and dye wastewater were kindly donated by Sunflag Nigeria Limited, textile manufacturing industry in Surulere, Lagos State, Nigeria. The dye collected in sterile air tight plastic can and stoppered was of high purity, best analytically grade and used without prior purification. UV-Visible spectrophotometer—Shimadzu UV spectrophotometer (Shimadzu UV 1600, Japan) and Fourier Transform Infrared spectrometry (FTIR) model 8400S spectrophotometer (Shimadzu, Japan) were used for spectral characterization.

### 2.2. Description of the dye

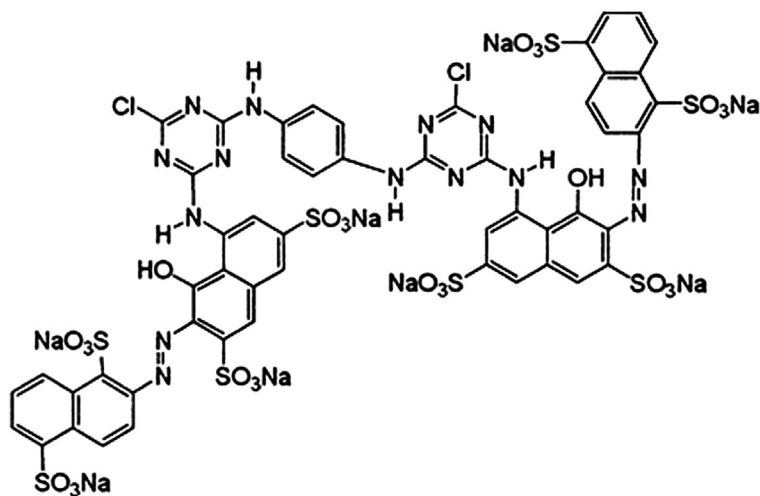
Reactive Red HE7B dye is a complex di-azo dye with the chemical formula  $C_{52}H_{26}Cl_2N_{14}Na_8O_{26}S_8$ , molecular weight  $1,774.19 \text{ gmol}^{-1}$ , and chemical structure (Figure 1).

### 2.3. Isolation and identification of the dye decolorizing fungi

The fungi were isolated from the dye and wastewater from Sunflag Textile Nigeria Limited, Lagos State, Nigeria consistently for 6 months. The 23S Ribosomal Ribonucleic Acid (rRNA) analyses were performed using the full-length FUNGAL ID service by Laragen Inc., Los Angeles, USA. The 23S rRNA sequence was initially analysed at the National Centre for Biotechnology Information, USA (NCBI) database (<http://www.ncbi.nlm.nih.gov>) using BLAST (blastn) tool. The sequence data were then submitted at the GenBank using the BankIt tool and later made available by the GenBank at Deoxyribonucleic acid (DNA) Databank of Japan and European Molecular Biology Laboratory (EMBL). Highly similar sequences were later downloaded from the GenBank and evolutionary history was inferred using the Neighbour joining tree method by adopting the guideline of Felsenstein (1985). Using the method of Saitou and Nei (1987), the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) was placed next to the branches of the tree.

Phylogenetic analyses were conducted in Molecular Evolutionary Genetics Analysis (MEGA) Version 4 (Tamura, Dudley, Nei, & Kumar, 2007). The phylogenetic tree was linearized using the method of Takezaki, Rzhetsky, and Nei (1995) assuming equal evolutionary rates in all lineages. The clock calibration to convert distance to time was 0.01 (time/node height). The tree was then drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura, Nei, & Kumar, 2004) and are in the units of the number of base substitutions per site.

Figure 1. Chemical structure of Reactive Red HE7B dye.



## 2.4. Micro-organisms and culture conditions

The organisms used in this study were isolated from dye samples and dye wastewaters. It was then maintained on Potato Dextrose Agar slants at 4°C. The pure culture was grown in 250 ml Erlenmeyer flask containing 100 ml fresh basal medium (gl<sup>-1</sup>): Glucose 10 g, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 0.5 g, Yeast extract 2.5 g, KH<sub>2</sub>PO<sub>4</sub> 5 g, MgSO<sub>4</sub>·H<sub>2</sub>O 0.5 g, CaCl<sub>2</sub>·H<sub>2</sub>O 3 g at 37°C for 24 h. The 24 h culture was kept at 4°C.

## 2.5. Shelf life estimation

Multiple linear regression model of Obidi, Nwachukwu, Aboaba, and Nwalor (2010) and Almalik, Nijhuis, and van den Heuvel (2014) was employed in the shelf life study with slight modifications. 500 mg l<sup>-1</sup> concentration of Reactive Red HE7B dye solutions were prepared and stocked in 12 different well-corked, air tight Erlenmeyer flasks. There was no pre- or post-sterilization procedure carried out on the dye solution. The formulation of the dye solution was prepared in triplicates. Total viable spore count and physicochemical parameters (Absorbance, Hydrogen ion concentration-pH, Specific gravity, Transmittance and Viscosity) were then observed, monitored and recorded (in triplicates) in each flask on monthly basis for 12 months.

$$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_6 X_6 + U_T \quad (1)$$

where  $\beta_0$  is the regression intercept,  $\beta_1$ – $\beta_6$  are regression parameter estimates (at 0–360 days)  $X_1$ – $X_6$  (Absorbance, pH, Specific gravity, Total viable spore count, Transmittance and Viscosity) are the regression coefficients of the dye under shelf life study,  $Y_i$  (the Dependent Variable) is the time (Days) and  $U_T$  is the error factor.

$$Y_i = \beta_0 + \beta_1 (\text{Absorbance}) + \beta_2 (\text{pH}) + \beta_3 (\text{Specific gravity}) + \beta_4 (\text{Total viable spore count}) + \beta_5 (\text{Transmittance}) + \beta_6 (\text{Viscosity}) + U_T \quad (2)$$

## 2.6. Decolorization experiments

The maximum wavelength ( $\lambda_{\text{max}}$ –456 nm) of Reactive Red HE7B dye solution was determined and recorded using UV–Visible spectrophotometer. Spore suspensions of each isolates were made by adding 10 ml of sterile distilled water to the plate containing the 48-h growth culture. Five millilitre of the mixture in the plate was then transferred to 100 ml Erlenmeyer flasks containing 100 ml of fresh basal medium buffered to pH 7.2 supplemented with 100 mg l<sup>-1</sup> of the dye, in triplicates. The flasks were incubated in dark at 28°C for 15 days. Non-inoculated culture medium was used as control. Aliquots of the fungal culture after 0, 3, 6, 9, 12 and 15 days of incubation were centrifuged at 10,000 rpm for 10 min and then the supernatant was diluted to 1:10 ratio with sterile distilled water.

Dye decolorization was determined using a UV/Vis spectrophotometer by monitoring the decrease in the absorbance at the wavelength of maximum absorption for the dye (in triplicates) with the formula

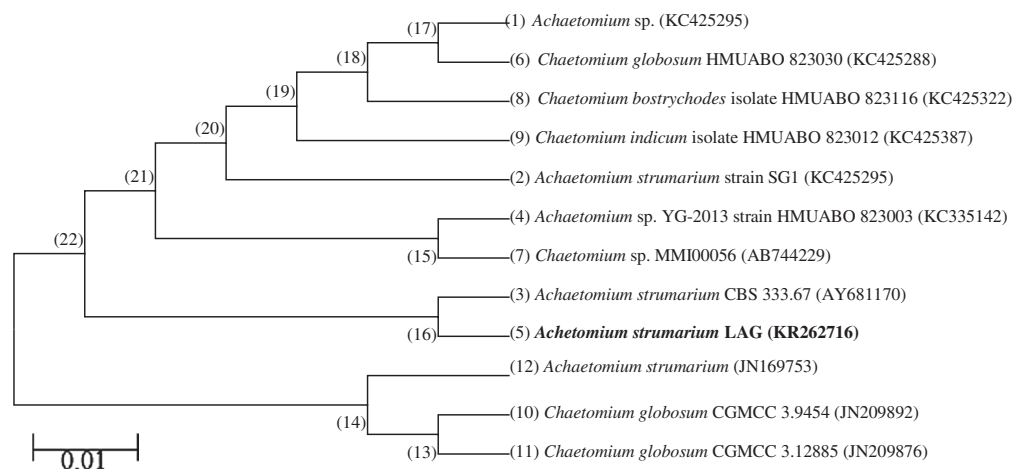
$$\% \text{Decolorization} = \frac{(A_o - A_t)}{A_o} \times 100$$

where  $A_o$  and  $A_t$  are initial and final absorbance units at specific time (t).

Further decolorization studies were carried out using the Fourier Transform Infrared (FT-IR)—(FTIR model 8400S spectrophotometer (Shimadzu, Japan) spectra analyses of the dye before and after decolourization of the dyestuff. The scans were done in the midIR region of 400–4,000 cm<sup>-1</sup> with 16 scan speed. The pure dye solid sample of 50 mg ml<sup>-1</sup> concentration was mixed with Infrared (IR) grade Potassium Bromide (KBr) in the ratio 8:92. On centrifuging at 10,000 rpm for 10 min, pellets of the supernatant were then fixed in the sample holder, scans and spectra were done and recorded, respectively (Olukanni, Osuntoki, Kalyani, Gbenle, & Govindwar, 2010; Saratale, Kalme, & Govindwar, 2006).



**Figure 2. Phylogenetic analysis of 23S rRNA sequence of fungus, *A. strumarium* LAG (KR262716).**



### 2.6.1. Optimization of physicochemical parameters during decolorization studies

Experiments were conducted in static cultures at various pH values ranging from 4 to 9 in a water bath with the temperature preset and maintained at 32°C. Approximately 0.1 g dry weight of each of the fungi was added to 50 ml of the solution (100 mg l<sup>-1</sup> of RR HE7B) in 100 ml conical flasks. The studies were equally repeated by varying the temperature between 20 and 40°C at pH of 7.0. Dry weights (g) were also determined within 84-h period at 12 h intervals. All experiments were done in triplicates and abiotic controls were used as 0% decolorization.

### 2.7. Phytotoxicity studies

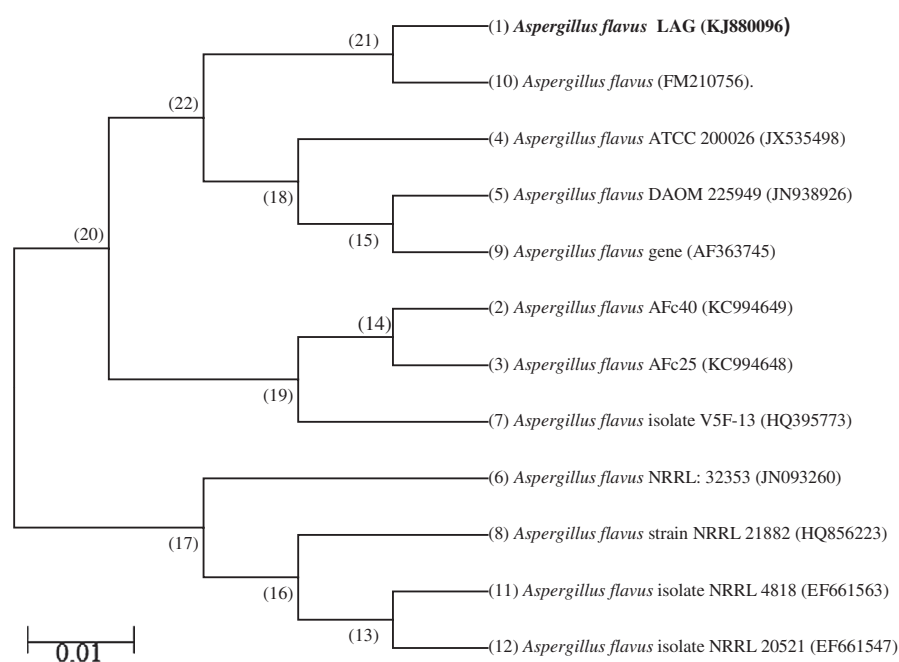
Phytotoxicity experiments were carried out to determine the toxic effects on plants of the dye, its 360 days shelf life products and dye metabolites after decolorization by the fungi. The method of Kalme, Ghodake, and Govindwar (2007) was adopted with slight modifications. Ethyl acetate extract of Reactive Red HE7B metabolic products was dried and dissolved in sterile distilled water to a final concentration of 1,000 ppm. The phytotoxicity studies were performed on seeds of *Pennisetum glaucum*, *Cucumeropsis manni* and *Vigna unguiculata*, three plants commonly found in Nigeria. The seeds (50 each) were wetted (20 ml per day) with dye metabolites and shelf life products of both *Achaetomium strumarium* and *Aspergillus flavus* (1,000 ppm) in separate Petri plates. Seeds wet with tap water were included as controls. Length of plumule (shoot), radicle (root) and germination (%) was recorded after 12 days.

## 3. Results and discussion

This study reported isolation of filamentous fungi, *A. strumarium* and *A. flavus* directly from dye effluents/dye wastewater. Further studies were carried out to determine the efficiency of the isolated filamentous fungi, *A. strumarium* and *A. flavus* in the removal of di-azo dye, Reactive Red HE7B. The 23S rRNA sequence data of *A. strumarium* and *A. flavus* deposited to GenBank were assigned accession numbers; KR262716 and KJ880096, respectively. The phylogenetic analyses conducted revealed that the organisms belonged to their respective genera (Figures 2 and 3).

On shelf, it was discovered that there were increases in the viable fungal count, absorbance and decreases in pH, specific gravity, viscosity and transmittance of the dye from day 0 to day 360. The residual error (or error term) which symbolizes other factors that can affect the shelf life of the dyes only exhibited 0–0.10% causality strength. Hence, the model has a strong goodness of fit since it is less than 0.50%. The model was statistically significant at 1 per cent significant level, since the *p*-value of *F*(0.0000) is less than 0.01. Therefore, the model was sufficient for this analysis. The shelf life (estimated) of 500 mg l<sup>-1</sup> of Reactive Red HE7B dye was 36 months on the average (Table 1).

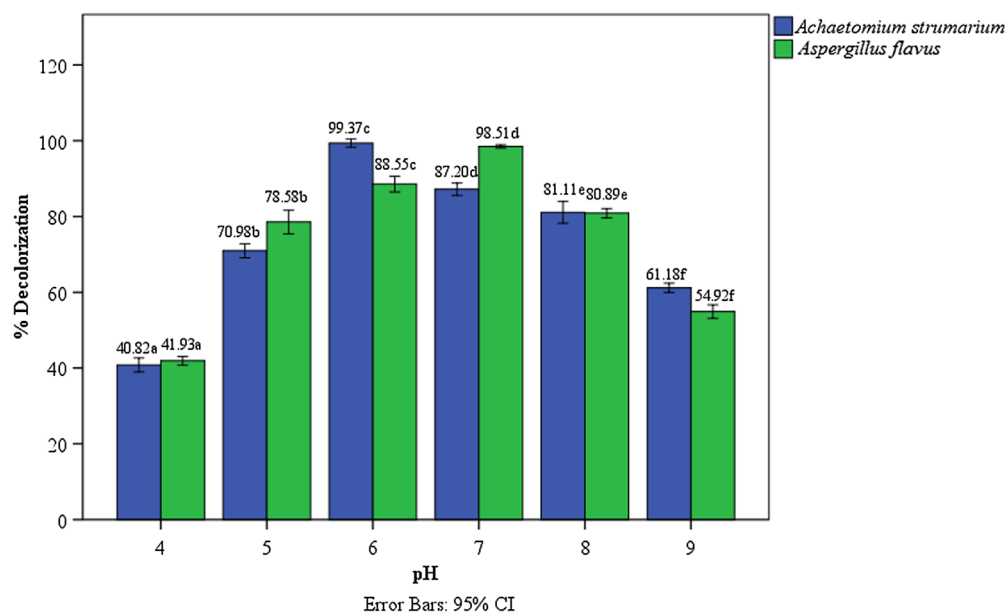
**Figure 3. Phylogenetic analysis of 23S rRNA sequence of fungus, *A. flavus* LAG (KJ880096).**



**Table 1. Shelf life (estimated) of the dye used in the study**

Dye	Concentration (mg l <sup>-1</sup> )	Shelf life (estimated)
Reactive Red HE7B	500	36 months 28 days

**Figure 4. Effect of pH at 30°C on the decolorization of the dye by the fungi.**



The  $\lambda_{max}$  of the Reactive Red HE7B dye was found to be 456 nm. Over 50 % decolorization rate were recorded by the fungi on the 9th day. Complete decolorization was recorded at the end of the 15 days. In decolorization experiments,  $99.37 \pm 1.44\%$  decolorization rate was achieved by *A. strumarium*, while  $98.51 \pm 3.21\%$  decolorization rate was achieved by *A. flavus*. *A. strumarium* and *A. flavus* achieved optimum decolorization of the dye at pH of 6 and 7 (Figure 4) respectively.



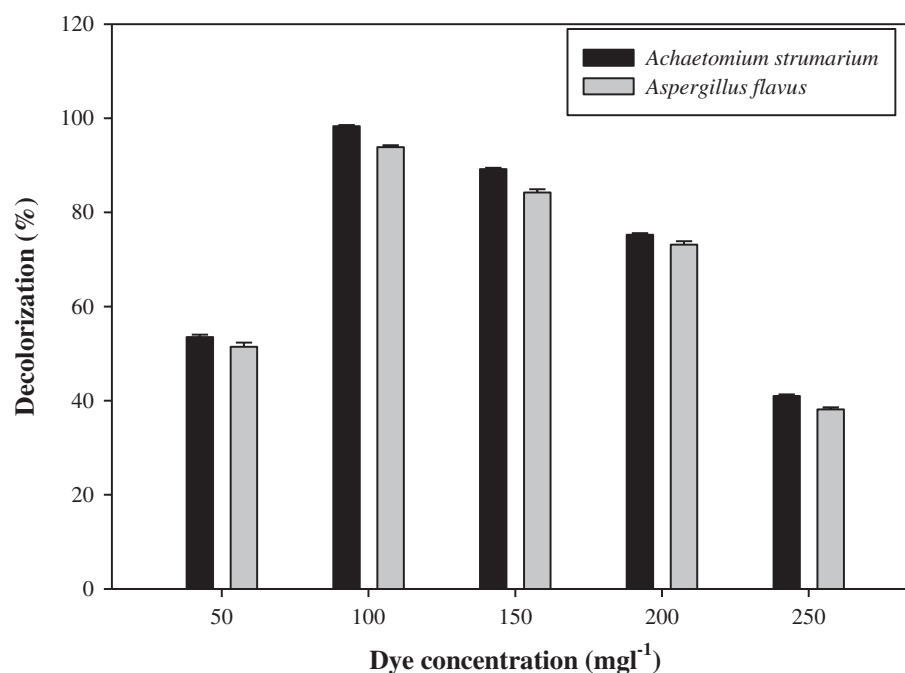
Filamentous fungi optimally decolorize dye at an optimal pH range of 6–7 (Ramesh, Subramanian, & Kalaiselvam, 2014). There was a steady increase in the decolorization rate with increasing pH for both fungi (Figure 4). This suggested that there was an increase in positively charged protons released by the fungi during decolorization process (Acemioglu, Kertmen, Digrak, & Hakki, 2010). The decolorization rate of the dye by both fungi increased tremendously with increasing concentration from 50 to 150 mg l<sup>-1</sup> (Ali & El-Mohamedy, 2010; Deveci, Unyayar, & Mazmanci, 2004; Palmieri, Cennamo, & Sannia, 2005). Peak decolorization percentage was however recorded at 150 mg l<sup>-1</sup> concentration (Figure 5). Steady decrease was observed in decolorization rate with increasing concentration from 150 to 250 mg l<sup>-1</sup> (Figure 5).

Greater decolorization percentage of the dye was observed at temperature range of 30–35°C (Figure 6) indicating the optimum range suitable for maximum decolorization efficiency of the Reactive Red HE7B dye by the fungi used in this study. Decreasing decolorization rate was however recorded when the temperature was set at 40°C indicating a thermal denaturation of enzymes (Rajalakshmi & Sudha, 2011).

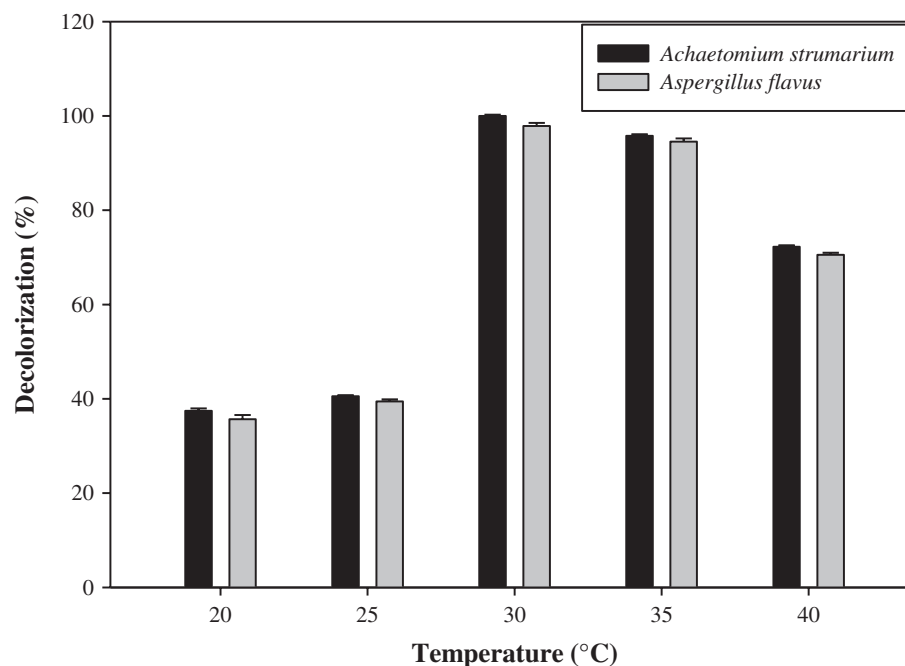
The increase in dry weight recorded (Figure 7) gave proportional rise to decolorization rate of Reactive Red HE7B dye which is indicative of the fact that the fungi produced extensive hyphal cells which in turn resulted in greater biomass production. This further suggested that filamentous fungi have high surface-to-cell ratio which makes them better degraders (Ashoka, Geetha, & Sullia, 2002).

The spectral analyses were performed using UV–Visible spectrophotometer and FTIR spectroscopy. The spectrum of control dye and the spectrum of the decolorized sample were overlaid and recorded. From spectral analyses by UV spectrophotometer, the peak present in the control dye was reduced drastically and the peaks almost disappeared at its  $\lambda_{\text{max}}$  after decolorization in the dye on treatment with the fungi (Figures 8 and 9). These results are in agreement with earlier work done by Ekambaram, Perumal, and Annamalai (2016). The spectrum of the control dye and decolorized samples was recorded with FT-IR spectroscopy. Figures 10 and 11 showed the spectra recorded from the control and the 15 days metabolites of Reactive Red HE7B on treatment with *A. strumarium* and *A. flavus*, respectively. Compared to the control dye spectrum, the FTIR spectrum of the decolorized sample showed a significant change in the position of peaks. In Figures 10 and 11, it is vivid that there were distortions in all the peaks in the control dye and their metabolites on decolorization by

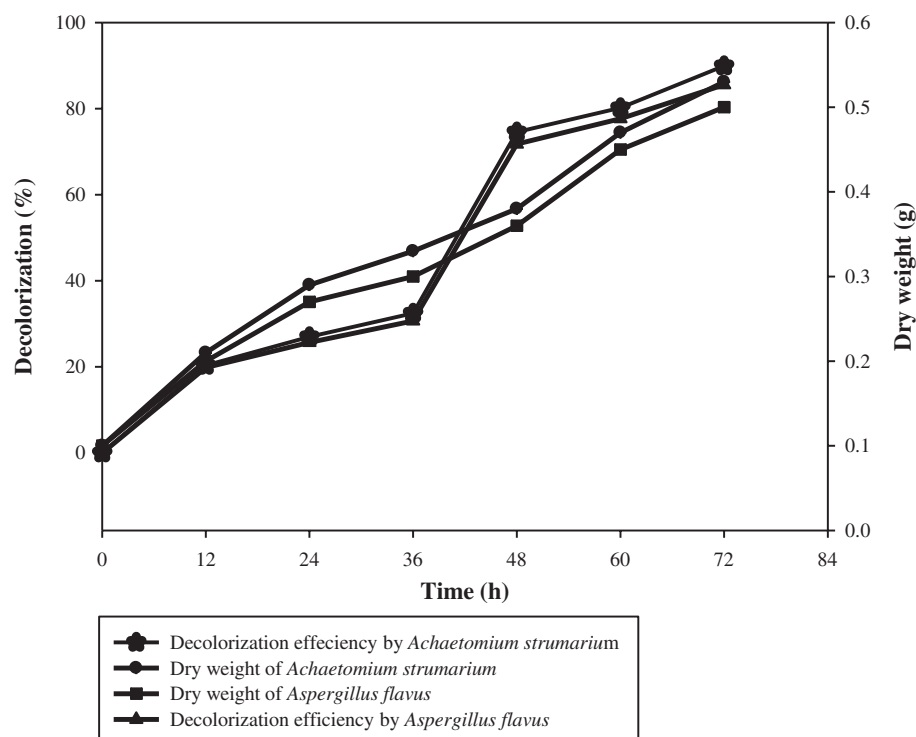
**Figure 5. Effect of the initial concentration on the decolorization of Reactive Red HE7B dye by the fungi.**



**Figure 6. Effect of initial temperature on the decolorization of Reactive Red HE7B dye by the fungi.**

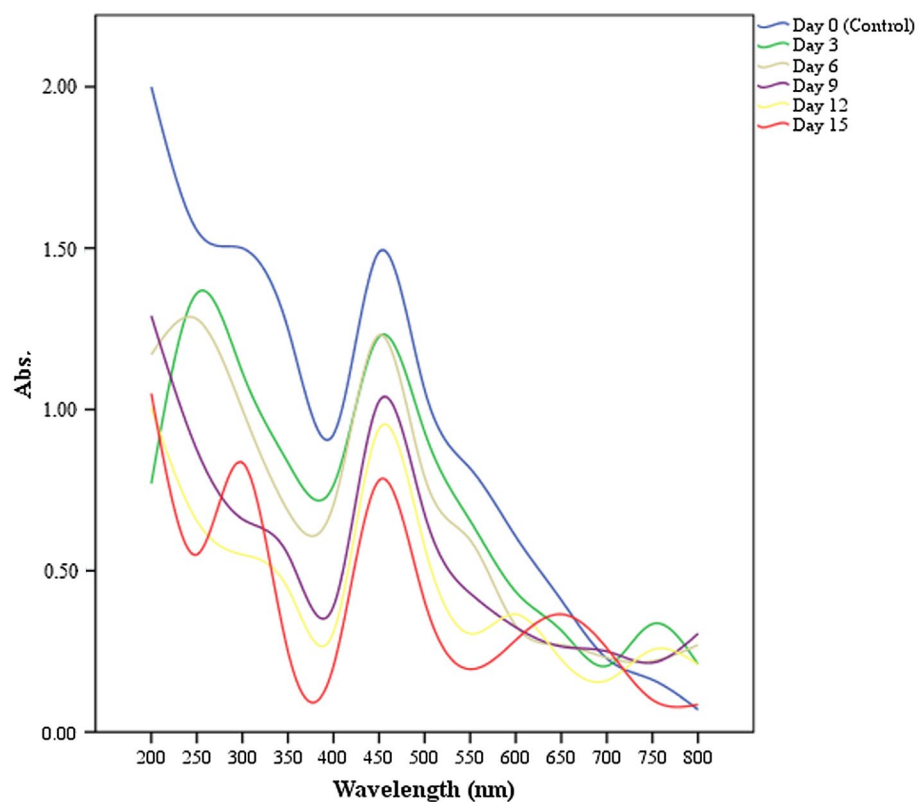


**Figure 7. Effect of the fungal biomass on the decolorization rate of Reactive Red HE7B dye.**

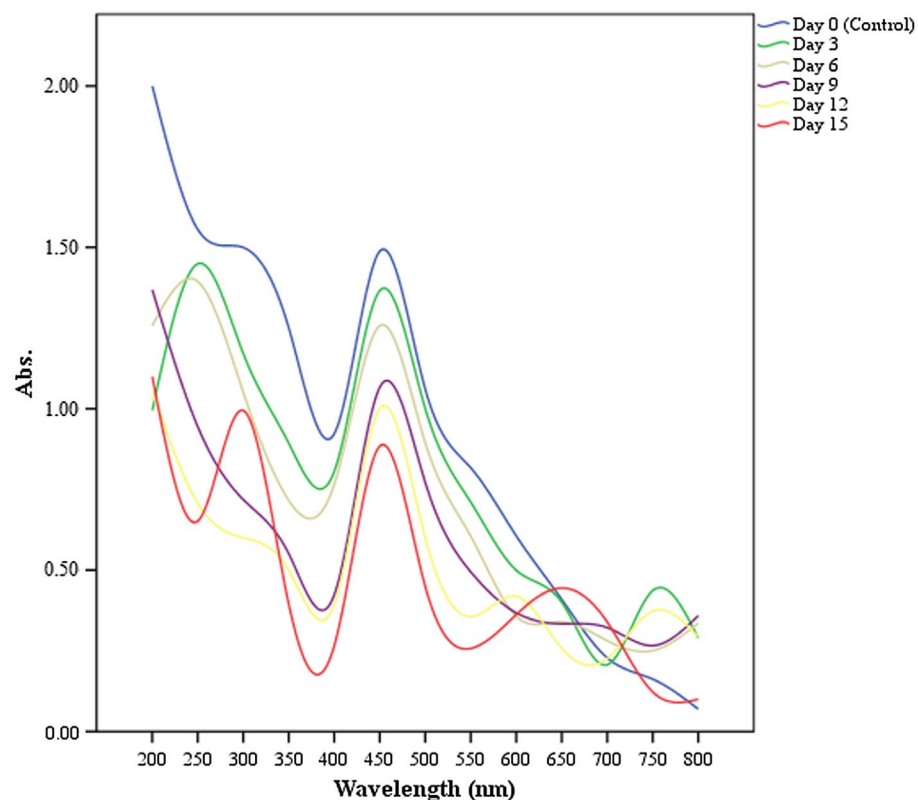


the fungi. The peaks in the control dye spectrum of Reactive Red HE7B represented the stretching vibrations of N-H bond (secondary amides) at  $3,419.90\text{ cm}^{-1}$ , C-H stretching of alkanes at  $2,920.32\text{ cm}^{-1}$ , N=N stretching of azo compounds at  $1,627.97\text{ cm}^{-1}$ , N-H trans stretching of secondary amides at  $1,464.02\text{ cm}^{-1}$ , S=O asymmetric stretching of sulfones at  $1,209.41\text{ cm}^{-1}$  and C-H out of plane bending at  $677.04\text{ cm}^{-1}$ . The FT-IR spectrum of treated Reactive Red HE7B dye by A.

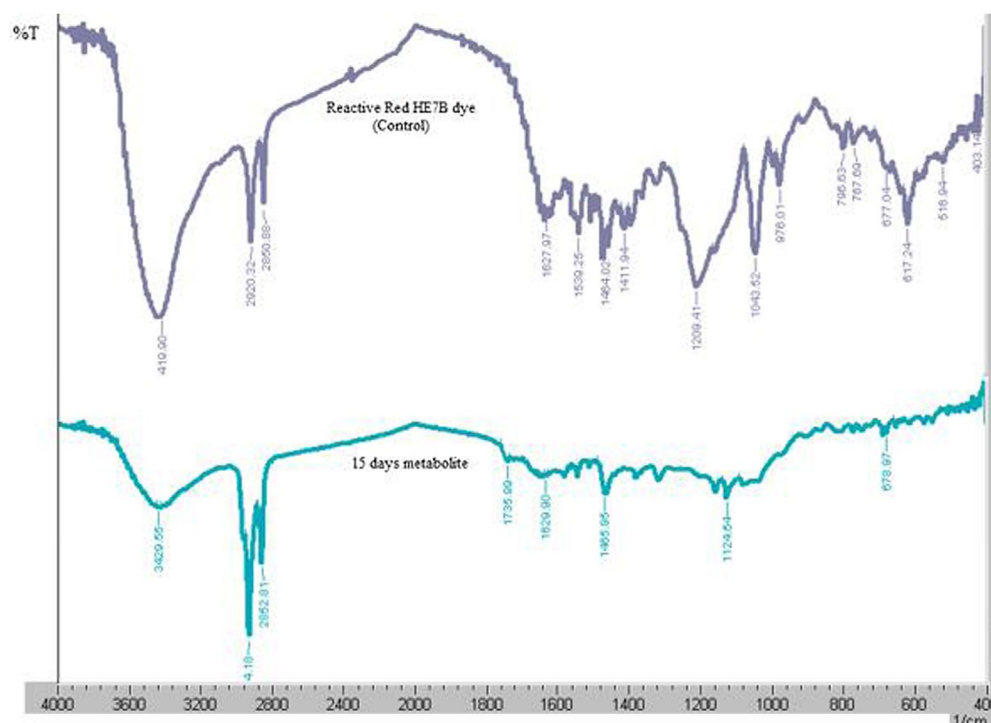
**Figure 8. UV-Vis spectra analyses of the Reactive Red HE7B dye (control and on treatment with *A. strumarium* at day 0, 3, 6, 9, 12 and 15).**



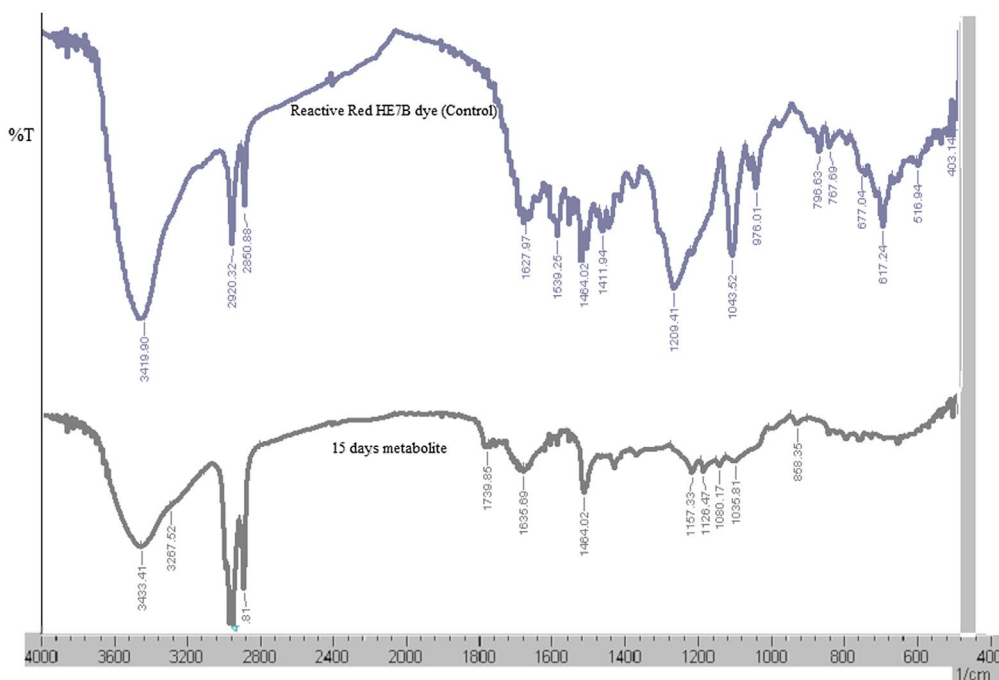
**Figure 9. UV-Vis spectra analyses of the Reactive Red HE7B dye (control and on treatment with *A. flavus* at day 0, 3, 6, 9, 12 and 15).**



**Figure 10.** FT-IR spectra of the control (untreated) dye and dye (metabolite) when treated with *A. strumarium*.



**Figure 11.** FT-IR spectra of the control (untreated) dye and dye (metabolite) when treated with *A. flavus*.



*strumarium* showed major peaks at 3,429.56  $\text{cm}^{-1}$  for N-H stretching, 2,418.69  $\text{cm}^{-1}$  for C-H stretching of alkanes, 1,629.90  $\text{cm}^{-1}$  for acetamide group and 679.97  $\text{cm}^{-1}$  for C-H out of plane bending. All other peaks disappeared in the dye metabolites. In all, the 16 peaks recorded in the control dye spectrum have been reduced to 8 peaks at the end of the 15 days treatment with *A. strumarium*. The dye metabolites (Figure 11) on treatment with *A. flavus* showed shift in peaks (3,433.41, 1,635.69

**Table 2. Phytotoxicity studies of Reactive Red HE7B dye and its 15 days decolorized metabolites**

Parameters	<i>Pennisetum glaucum</i>			<i>Cucumeropsis manni</i>			<i>Vigna uguiculata</i>		
	Water	Extracted metabolite (Af)	Extracted metabolite (As)	Water	Extracted metabolite (Af)	Extracted metabolite (As)	Water	Extracted metabolite (Af)	Extracted metabolite (As)
Germination %	100 ± 0.00	60 ± 0.01	92 ± 0.01	100 ± 0.00	70 ± 0.02	90 ± 0.01	100 ± 0.00	55 ± 0.01	95 ± 0.02
Radicle	6.54 ± 0.54	4.47 ± 1.59 <sup>a</sup>	6.41 ± 2.43	7.05 ± 1.23	4.32 ± 0.17 <sup>b</sup>	6.04 ± 0.67	5.54 ± 0.32	3.04 ± 0.14 <sup>c</sup>	5.05 ± 0.42
Plumule	9.35 ± 1.79	8.35 ± 0.23 <sup>a</sup>	8.87 ± 1.57	8.10 ± 1.21	7.22 ± 1.07 <sup>b</sup>	7.78 ± 1.32	8.35 ± 1.31	6.76 ± 1.21 <sup>c</sup>	8.21 ± 1.07

Notes: Values are presented as Mean ± S.E.M.

a, b, c show significant differences different from the control (seeds germinated in water) at  $p < 0.05$ , using a one-way analysis of variance (ANOVA) with Tukey's—b comparison test.

Extracted metabolite (Af)—Degraded products of Reactive Red HE7B dye on treatment with *A. flavus*.

Extracted metabolite (As)—Degraded products of Reactive Red HE7B dye on treatment with *A. strumarium*.

and 858.35  $\text{cm}^{-1}$ ) as well as disappearance of peaks. In all, the 16 peaks recorded in the control dye spectrum have been reduced to 12 peaks at the end of the 15 days treatment with *A. flavus*. The decrease or disappearance of peak(s) in the visible region indicates decolorization, while decrease or disappearance of peak(s) in the ultraviolet region further suggested decolorization (Ramesh et al., 2014). The methods have been used to indicate decolorization (Jadhav, Parshetti, Kalme, & Govindwar, 2007; Telke, Kalyani, Jadhav, & Govindwar, 2008)

The results showed that *A. strumarium* exhibited greater degradation activities on the Reactive Red HE7B dye than when the dye was treated with *A. flavus*. Similarly, changes in the peaks around 1,500 and 1,650  $\text{cm}^{-1}$  have been reported as the reduction of azo bond (Telke et al., 2008). The change in peak pattern as well as disappearance of few peaks in the treated samples after degradation when compared to the control dye spectrum showed that decolorization of the dye has occurred.

The phytotoxicity experiment results showed significant effect on the % germination and length of the plumule and radicle of the Reactive Red HE7B dye solution (1,000 ppm) wetted seeds. The germination percentage of *P. glaucum*, *C. manni* and *V. unguiculata* seeds was higher (100%) when treated with water than the dye 15 days decolorized metabolites on treatment with *A. strumarium* and *A. flavus*, respectively. The Reactive Red HE7B dye treatments affected the length of plumule and radicle significantly, while there were no significant effects with the decolorized products (Table 2). The detoxification effect on treatment of Reactive Red HE7B dye with *A. strumarium* was greater than on treatment with *A. flavus*. The non-inhibition of germination with viable plumule and radicle formation in the toxicity experiments of Reactive Red HE7B dye (at 1,000 ppm) suggested that the dye was not only decolorized but also detoxified. The results showed that *A. strumarium* exhibited greater detoxifying efficiency on the dye than on treatment with *A. flavus*. This may be due to the removal of aromatic amines by the fungi used in this study (Kalme et al., 2007).

#### 4. Conclusion

This research study showed that the filamentous fungi, *A. strumarium* and *A. flavus* decolorized the reactive azo dye and also the dye shelf life was determined through predictive modelling. The use of fungi would greatly help in the clean-up/remediation process of dye/dye wastewater polluted environment which is very economical, cheap and eco-friendly. The fungi showed maximum capacity in the biosorption of the azo dye. Contrary to the belief that dyes can last for an infinite number of years on shelf, the study revealed that Reactive Red HE7B dye fastness product quality could not be guaranteed beyond 36 months on shelf. However, due to the recalcitrant and toxic nature of reactive azo dyes, pragmatic research must be undertaken at discovering new fungi strains that are capable of decolorizing and detoxifying them. Further research work is on-going to determine the mechanism and kinetics of dye sorption/adsorption and biodegradation by the fungi used in this study.

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## Cover image

A piece of cloth dyed with Reactive Red HE7B dye.  
 Source: Paul Olusegun Bankole.

## References

- Acemioğlu, B., Kertmen, M., Digrak, M., & Hakki, A. (2010). Use of *Aspergillus wentii* for biosorption of methylene blue from aqueous solution. *African Journal of Biotechnology*, 9, 874–881.
- Aksu, Z., & Dönmez, G. (2005). Combined effects of molasses sucrose and reactive dye on the growth and dye bioaccumulation properties of *Candida tropicalis*. *Process Biochemistry*, 40, 2443–2454. <http://dx.doi.org/10.1016/j.procbio.2004.09.013>
- Ali, N. F., & El-Mohamedy, R. S. R. (2010). Microbial decolorization of textile waste water. *Journal of the Saudi Chemical Society*, 16, 117–123.
- Almalik, O., Nijhuis, M. B., & van den Heuvel, E. R. (2014). Combined statistical analyses for long-term stability data with multiple storage conditions: A simulation study. *Journal of Biopharmaceutical Statistics*, 24, 493–506. <http://dx.doi.org/10.1080/10543406.2014.888439>
- Ashoka, C., Geetha, M. S., & Sullia, S. B. (2002). Biodegradation of composite textile dye effluent using bacterial consortia. *Asian Journal of Microbial Biotechnology and Environmental Science*, 4, 65–68.
- Aust, S. D. (1990). Degradation of environmental pollutants by *Phanerochaete chrysosporium*. *Process Biochemistry*, 3, 89–94.
- Chen, H. (2006). Recent advances in azo dye degrading enzyme research. *Current Protein and Peptide Science*, 7, 101–111. <http://dx.doi.org/10.2174/138920306776359786>
- Deveci, T., Unyayar, A., & Mazmanci, M. A. (2004). Production of Remazol Brilliant Blue R decolorising oxygenase from the culture filtrate of *Funalia troglia* ATCC 200800. *Journal of Molecular Catalysis B: Enzymatic*, 30, 25–32. <http://dx.doi.org/10.1016/j.molcatb.2004.03.002>
- Ekambaram, P. S., Perumal, S. S., & Annamalai, U. (2016). Decolorization and biodegradation of remazol reactive dyes by *Clostridium* species. *3 Biotech*, 6(1), 1–8.
- Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, 39, 783–791. <http://dx.doi.org/10.2307/2408678>
- Fu, Y., & Viraraghavan, T. (2002). Dye biosorption sites in *Aspergillus niger*. *Bioresource Technology*, 82, 139–145. [http://dx.doi.org/10.1016/S0960-8524\(01\)00172-9](http://dx.doi.org/10.1016/S0960-8524(01)00172-9)
- Hofrichter, M. (2002). Review: Lignin conversion by manganese peroxidase (MnP). *Enzyme and Microbial Technology*, 30, 454–466. [http://dx.doi.org/10.1016/S0141-0229\(01\)00528-2](http://dx.doi.org/10.1016/S0141-0229(01)00528-2)
- Iscen, C. F., & Kiran, I. (2007). Biosorption of reactive black 5 dye by *Penicillium restrictum*: The kinetic study. *Journal of Hazardous Materials*, 143, 335–340. <http://dx.doi.org/10.1016/j.jhazmat.2006.09.028>
- Jadhav, J. P., Parshetti, G. K., Kalme, S. D., & Govindwar, S. P. (2007). Decolorization of azo dye methyl red by *Saccharomyces cerevisiae* MTCC 463. *Chemosphere*, 68, 394–400. <http://dx.doi.org/10.1016/j.chemosphere.2006.12.087>
- Kalme, S., Ghodake, G., & Govindwar, S. (2007). Red HE7B degradation using desulfonation by *Pseudomonas desmolyticum* NCIM 2112. *International Biodeterioration and Biodegradation*, 60, 327–333. <http://dx.doi.org/10.1016/j.ibiod.2007.05.006>
- Kaushik, P., & Malik, A. (2009). Fungal dye decolorization: Recent advances and future potential. *Environment International*, 35, 127–141. <http://dx.doi.org/10.1016/j.envint.2008.05.010>
- Kumari, K., & Abraham, T. E. (2007). Biosorption of anionic textile dyes by nonviable biomass of fungi and yeast. *Bioresource Technology*, 98, 1704–1710. <http://dx.doi.org/10.1016/j.biortech.2006.07.030>
- Maas, R., & Chaudhari, S. (2005). Adsorption and biological decolorization of azo dye Reactive Red 2 in semicontinuous anaerobic reactors. *Process Biochemistry*, 40, 699–705. <http://dx.doi.org/10.1016/j.procbio.2004.01.038>
- Mathur, N., & Bhatnagar, P. (2007). Mutagenicity assessment of textile dyes from Sangner (Rajasthan). *Journal of Environmental Biology*, 28, 123–126.
- Mathur, N., Bhatnagar, P., & Bakre, P. (2005). Assessing mutagenicity of textile dyes from Pali (Rajasthan) using Ames bioassay. *Applied Ecology and Environmental Research*, 4, 111–118.
- Melgoza, R. M., Cruz, A., & Bultron, G. (2004). Aerobic/Anaerobic treatments of colorants present in textile effluents. *Water Science Technology*, 50, 149–155.
- Moreira, M. T., Mielgo, I., Feijoo, G., & Lema, J. M. (2000). Evaluation of different fungi strains in the decolorisation of synthetic dyes. *Biotechnology Letters*, 22, 499–503.
- Novotný, C., Dias, N., Kapanen, A., Malachová, K., Vandrovcová, M., Itävaara, M., & Lima, N. (2006). Comparative use of bacterial, algal and protozoan tests to study toxicity of azo- and anthraquinone dyes. *Chemosphere*, 63, 1436–1442. <http://dx.doi.org/10.1016/j.chemosphere.2005.10.002>
- Obidi, O. F., Nwachukwu, S. C., Aboaba, O. O., & Nwalor, J. U. (2010). Estimation of shelf life for water-based paints using regression methods. *Journal of American Science*, 6, 123–127.
- Olayinka, K. O., & Alo, B. I. (2004). Studies on industrial pollution in Nigeria: The effect of the effects of textile effluents on the quality of groundwater in some parts of Lagos. *Nigerian Journal of Health and Biomedical Sciences*, 7, 22–25.
- Olukanni, O. D., Osuntoki, A. A., Kalyani, D. C., Gbenle, G. O., & Govindwar, S. P. (2010). Decolorization and biodegradation of Reactive Blue 13 by *Proteus mirabilis* LAG. *Journal of Hazardous Materials*, 184, 290–298. <http://dx.doi.org/10.1016/j.jhazmat.2010.08.035>



- Palmieri, G., Cennamo, G., & Sannia, G. (2005). Remazol Brilliant Blue R decolourisation by the fungus *Pleurotus ostreatus* and its oxidative enzymatic system. *Enzyme and Microbial Technology*, 36, 17–24.  
<http://dx.doi.org/10.1016/j.enzmictec.2004.03.026>
- Rajalakshmi, G., & Sudha, S. (2011). Decolourisation of methyl orange and methyl red by live and dead biomass of fungi. *Asian Journal of Experimental Biological Science*, 2, 569–574.
- Rajeshkannan, R., Rajasimman, M., & Rajamohan, N. (2010). Optimization, equilibrium and kinetics studies on sorption of Acid Blue 9 using brown marine algae *Turbinaria conoides*. *Biodegradation*, 21, 713–727.  
<http://dx.doi.org/10.1007/s10532-010-9337-0>
- Rajeshkannan, R., Rajasimman, M., & Rajamohan, N. (2011). Decolorization of malachite green using tamarind seed: Optimization, isotherm and kinetic studies. *Chemical Industry and Chemical Engineering Quarterly*, 17, 67–79.  
<http://dx.doi.org/10.2298/CICEQ100716056R>
- Ramesh, T., Subramanian, J., & Kalaiselvam, M. (2014). Degradation of triphenylmethane dye: Malachite green by *Aspergillus flavus*. *World Journal of Pharmacy and Pharmaceutical Sciences*, 3, 44–50.
- Saitou, N., & Nei, M. (1987). The neighbour-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 4, 406–425.
- Saratale, G. D., Kalme, S. D., & Govindwar, S. P. (2006). Decolorization of textile dyes by *Aspergillus ochraceus*. *Indian Journal of Biotechnology*, 5, 407–410.
- Singh, A. K., Prakash, D., & Shahi, S. K. (2013). Decolorization of the textile dye (Brown GR) by isolated *Aspergillus* strain from Meerut region. *International Research Journal of Environment Sciences*, 2, 25–29.
- Takezaki, N., Rzhetsky, A., & Nei, M. (1995). Phylogenetic test of the molecular clock and linearized trees. *Molecular Biology and Evolution*, 12, 823–833.
- Tamura, K., Dudley, J., Nei, M., & Kumar, S. (2007). MEGA4: Molecular evolutionary genetics analysis (MEGA) Software Version 4.0. *Molecular Biology and Evolution*, 24, 1596–1599.  
<http://dx.doi.org/10.1093/molbev/msm092>
- Tamura, K., Nei, M., & Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences*, 101, 11030–11035.  
<http://dx.doi.org/10.1073/pnas.0404206101>
- Telke, A., Kalyani, D., Jadhav, J., & Govindwar, S. (2008). Kinetics and mechanism of Reactive Red 141 degradation by a bacterial isolate *Rhizobium radiobacter* MTCC 8161. *Acta Chimica Slovenica*, 55, 320–329.
- Venkata Mohan, S. V., Chandrasekhar Rao, N., Krishna Prasad, K. K., & Karthikeyan, J. (2002). Treatment of simulated Reactive Yellow 22 (Azo) dye effluents using *Spirogyra* species. *Waste Management*, 22, 575–582.  
[http://dx.doi.org/10.1016/S0956-053X\(02\)00030-2](http://dx.doi.org/10.1016/S0956-053X(02)00030-2)
- Wang, J., Lu, H., Jin, R., Zhou, J., Liu, G., & Xing, L. (2009). Decolorization of 1-amino-4-bromoanthraquinone-2-sulfonic acid in bioaugmented membrane bioreactor. *Process Biochemistry*, 44, 812–816.  
<http://dx.doi.org/10.1016/j.procbio.2009.03.015>
- Xu, M., Guo, J., Zeng, G., Zhong, X., & Sun, G. (2006). Decolorization of anthraquinone dye by *Shewanella decolorationis* S12. *Applied Microbiology and Biotechnology*, 71, 246–251.  
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