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CO-METABOLIC BIODEGRADATION AND DETOXIFICATION OF REACTIVE ORANGE DYE BY LACCASE-PRODUCING PLEUROTUS PULMONARIUS KP826832

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ABSTRACT

The use of biological methods is a promising alternative to the harsh physico-chemical methods usually employed in textile industries. Laccase-producing Pleurotus pulmonarius mediated decolourization of Reactive Orange dye was investigated. The dye was degraded into various molecular fragments. The degradation products were identified by FTIR and GC-MS analysis and a tentative pathway for the oxidative degradation of Reactive Orange dye was postulated. Pleurotus pulmonarius showed promising efficiency for degradation of Reactive Orange dye and could possibly be used for the remediation of textile effluents.

Keywords: Laccase, Pleurotus pulmonarius, reactive orange, biodegradation, FTIR, GC-MS

1.0 INTRODUCTION

Dyes are widely used in cosmetics, textile, leather, food, paper and plastic industries and in view of toxicity of dyes; the environment is under the threat (Iqbal, 2016; Nouren *et al.,* 2017). Synthetic dyes are widely used in the textile, paper, cosmetics, leather, dyeing, colour photography, pharmaceutical and food industries. In textile industries, during dyeing process, about 10 to 30 per cent or more of the dyes are released into water bodies causing serious environmental problem in many parts of the world (Shanmuga Priya, 2013).

Water pollution has become one of the seriously environmental questions in the world. Most of The textile industries consume a large quantity of water, and generate a large amount of wastewater and effluents containing dyes that are very recalcitrant to treatment, then toxic and potentially carcinogenic compounds into receiving River (Nataraj *et al.,* 2007; Yuan *et al.,* 2016; Kurade *et al.,* 2016).

Coloured effluents from textile wastewater which are discharged from textile industries pose a significant environmental pollution problem. Even at low concentrations, textile wastewaters are

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intensely coloured (Ayed *et al.,* 2017). About 11 % of the wastewater entered natural water directly without treatments (Robinson *et al.*, 2001). Azo dyes contain (N=N) functional group, which make them persistent under natural environmental conditions (Nouren *et al.,* 2017).

The dye-containing wastewater discharged from industries can adversely affect the aquatic environment by impeding light penetration. Moreover, many of the dyes are toxic, carcinogenic and harmful to the human health. Even at low concentration, dyes could cause aesthetic pollution (Ayed *et al.,* 2017). Therefore, there is an increasing demand of efficient and economical technologies to remove dyes from water environment in the world (Ayed *et al.,* 2017). Conventional treatments are either ineffective or incur considerable costs (Santos *et al.*, 2015), hence, to develop a technique based on biological treatment seems to be an inspiring solution.

The biological methods have been paid more attention because of their strong adaptability, easy operation, low cost and mild reaction condition, lower sludge production, and ecological sociability (Kurade *et al.,* 2016; Wang *et al.,* 2017). The use of Laccase catalysis in different applications such as textile dye bleaching, pulp bleaching, bioremediation and biotransformation might be an environmentally friendly way that can replace the used chemical processes (Usha *et al.*,., 2014). A number of species of genus *Pleurotus* have been explained as manufacturers of laccase (Leonowicz *et al.,* 2001). However, few reports are available on the intermediate or products of biodegrdation of reactive orange dye so also the possible mechanism of biodegradation pathway of reactive orange by *Pleurotus pulmonarius.* Present study was conducted to affirm the decolorization, detoxification and laccase production potential utility of *Pleurotus pulmonarius* as the candidates for biodegradation of reactive orange dye.

2.0 MATERIALS AND METHODS

2.1 Microorganism and Culture Conditions

The culture of white rot fungal strain of *Pleurotus pulmonarius* was obtained from Federal Institute of Industrial Research Oshodi Collection Center in Lagos State, Nigeria. The culture mycelium was stored on malt extract agar slant at 4 ^{0}C.

2.2 Screening for Laccase Production

The screening for the production of laccase by the test organism was done using potato dextrose agar. The potato dextrose agar (PDA) plates were prepared in duplicate maintaining the pH at 6.5 with the addition of 0.02 % of guaiacol. The cultures were supplemented with 150mM copper sulfate ($CuSO_4$) sterile solution as laccase-inducer and incubated at 25 ^{0}C for 5days (Kiiskinen *et al.,* 2004).

2.3 Qualitative Analysis for Decolorizing Ability

The decolorizing ability of the isolated fungal strain was evaluated by inoculating the fungus on PDA plates containing 0.02 % reactive orange dye. Fungal culture about 1cm agar disc was then

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inoculated on the plates and incubated for 10 days at 25 ^{0}C. Formation of zone of clearance under and around the fungli colony indicated the decolorizing potential of the isolated culture (Dhouib *et al.,* 2005). Dye plates that were not inoculated served as control.

2.4 Decolorization and Detoxification Experiment

Two hundred milliliters of prepared Potato Dextrose broth supplemented with 0.02 % of reactive orange dye with 150mM of copper sulfate ($CuSO_4$) was used. The medium was sterilized at 121 ^{0}C for 15 minutes in the autoclave and allowed to cool and thereafter inoculated with fungal spore suspensions (10^5 spores/ml) and incubated in an orbital shaker at 120 rpm with a temperature of 25 ^{0}C for a period of 10 days (Shanmuga Priya *et al.,* 2013). Decolorizing activity was observed for the period of days and the preparation was done in duplicate. Aliquots of the fungal culture incubation were collected at an interval of 3 days; centrifugation of the aliquots were carried out using the centrifuge at 4,000rpm for 15 minutes, and then the supernatants were used to determine dye decolorization by monitoring the decrease in absorbance at the wavelength of maximum absorption for each dye using a spectrophotometer (Visible Spectrophotometer L1-722) (Da Silva *et al.,* 2009).

The color removal was recorded as percentage:

Decolorization = $\frac{Ao-At}{Ao} \times 100$

Ao = Absorbance of the initial dye solution (day 0)

At = Absorbance after incubation (Da Silva *et al.,*., 2009)

2.5 Biodegradation Analysis

Biodegradation was determined by comparing the Fourier Transformed Infrared Spectroscopy (FTIR) peak profiles of the metabolites of reactive orange dye. Attempt was also made to identify the dye metabolites using their Gas Chromatography-mass Spectroscopy (GC-MS) spectra. The decolorized reactive orange solution, withdrawn after 10 days of incubation and centrifuged at 8,944 ×g for 10 min, extracted using ethyl acetate. The extract was dried in a rotary evaporator and re-dissolved in High-Performance Liquid Chromatography grade methanol for GC-MS analyses. FTIR analysis of biodegraded reactive orange dye was carried out using a Shimadzu 800 spectrophotometer and compared with that of the control dye. The FTIR analysis was done in the IR region of 400-4,000 cm-1 with 10 scan speed. The identification of metabolites formed after degradation was done using a GCMS-QP2010 PLUS SHIMADZU,JAPAN.

3.0 RESULTS

The ability of the *Pleurotus pulmonarius* to utilize reactive orange dye and other synthetic dyes as source of carbon and energy using Bushnel-Haas medium was demonstrated. The findings

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show that the organism cannot utilize any of the synthetic dyes as source of carbon and energy. While tremendous growth was seen in the plate of PDB supplemented with each of the dye indicating co-metabolic method of degradation as shown in Plate 1.

Plate 1: Growth patterns of *Pleurotus pulmonarius* on PDB supplemented with different synthetic dyes

Guaiacol is an indicator which reported to be used for laccase production. Reddish brown color due to the oxidative polymerization of guaiacol in the presence of extracellular fungal laccase was demonstrated. Laccase production increases with increase in incubation as shown in Plate 2

Plate 2: Laccase production by *Pleurotus pulmonarius* after 5 days of incubation

Figure 1: Graph of % decolorization plotted against time (days) at 490nm wavelength. A_o is Absorbance of the initial dye solution (day 0) and A_T is the absorbance at cultivation time.

Fourier transform infrared spectroscopy (FTIR) was used to monitor the biodegradation and detoxification of reactive orange dye. FTIR spectra of non-degraded reactive orange dye showed the specific peaks in the region of fingerprint. The various peaks were shown which correspond to alkyne, alkene, alcohol, carboxylic acid and ester found to be dominant in in non-degraded reactive orange as presented in Figure 2 and Table1.

Figure 2: FTIR Spectrum of non-degraded Reactive Orange dye (control)

Table 1: FTIR analysis of non-degraded reactive orange dye (control)

Fig 3: GC-MS fingerprint of non-degraded Reactive Orange (control)

The FT-IR spectral of functional groups of degraded dye is presented in Fig. 3. Also, it was observed from the Table 3. The following functional groups Alcohol, carboxylic acids, Aromatic, Alkenes, Alkane and both primary and secondary amines were the most abundant in non-degraded dyes, and most of them possess stretching type of vibrations appearing at low peak of the spectrum interestingly, some functional groups were not seen in non-degraded dye, such as alkyne, Alkyl-halide, Thiol and phenol.

Fig 3: FTIR Spectrum of degraded Reactive Orange

Table 2: FTIR analysis of the degraded reactive orange

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The GC-MS profiling of the degraded reactive orange dye showed various peaks which correspond to various metabolites as shown in Fig. 4. The Table 4 the retention time and the corresponding metabolites such as 3-Benzylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione, 1-Ethyl-1,5-cyclooctadiene, Crodamide O, cis-13-Docosenamide, Caprylamide.

Figure 4: GC-MS fingerprint of degraded Reactive Orange dye

Table 4: GC-MS analyses of fragmentation products (reactive orange)

4.0 DISCUSSION

One of the parameters widely used in the detection of ligninolytic enzymes is the chromogen. In the present study, guaiacol was used as a chromogen. The reddish brown zone surrounding the mycelia of the culture on the plate supplemented with guaiacol was an indication of Bevandamm's reaction (Thakur and Gupte, 2014; Sara *et al.,* 2016).The fungus pattern of degradation was found to be co-metabolism due to its failure to utilize reactive orange dye as carbon source as demonstrated on Minimum basal medium, when co-substrate was excluded, this underscore the fact that the higher glucose consumption by the fungus in the presence of synthetic effluent might be evidence of increased metabolism due to the detoxification mechanism (Machado *et al.,* 2009). Also, glucose supplementation has been proved as an important factor in order to bioremediate textile effluents with ligninolytic fungi (Novotný *et al.,* 2011; Plácido *et al.,* 2016).

The chemical structural differences in textile dyes due to the substitution of various functional groups on aromatic base greatly influence their decolorization rates (Lade *et al.,* 2014).This clearly indicates that decolorization was due to degradation of dyes into intermediate products. The initial step in this degradation pathway of reactive orange was reductive cleavage of N=N (azo) bond leading to formation of colorless aromatic amines. These amines are further oxidized to simpler forms. FTIR analysis was done to characterize the metabolite produced. The results of FT-IR analysis of the dye and control sample obtained after decolorization showed various peaks. The FT-IR spectra of control reactive orange dye display peaks at 630, 683.5, 1048.9-1112, 1195.2, 2550, and 3410. The IR spectra of degradation products display peak for –C=C, =C-H, C-O, C-H, S-H and O-H all stretching. The peak characteristic of azo bond at 630,685.5, 1195.2,1655, 2550 which correspond to –C=C-H:C-H,=C-H,C-H,S-H. Also the following peaks (1018.45-111.03), (1411.94-1450.52), (2522.98-2839.31) and 3356.25 which correspond to C-O, C-C, O-H and both primary and secondary amine were found to be dominant in the degraded reactive dyes .The FTIR spectra of dye of Fig. 3 and reactive orange degradation products differed with number of peaks and their positions Fig. 4. The appearance of peak at 1,018.45 cm-1 confirmed the presence of carbonyl compound and azo group in dye whereas peaks at 1,411.94 and 1,450.52 cm^{-1} were related to aromatic compounds. The -C=C-stretching is indicated by peak at 1651.12 are due to the presence of the sulphonated dye compound. The peak at 3356.25 cm-1 indicates amines and amides. The sharp peak at 3356.25 cm-1 in azo compounds absent in the FTIR spectral analysis of degraded products confirms the cleavage of azo bonds. The peak at2839.31 cm-1 due to O–H stretching indicates hydroxylation of the product. A significant

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change in FTIR spectrum in degraded dye sample which displayed peaks at 2522.98 and 2839.31 cm^{-1} for–OH stretching and 3356.25 for N-H strecthing. Thus, the FTIR analysis confirms biotransformation of dye into other compounds. The IR spectrum for reactive orange shows that the vibrations between 3700-3100 cm-1 are associated with the –OH (carboxylic acids), -NH- (Amine) is almost changed after degradation by consortia as shown in Fig 1. The strong absorption at2550 is because of presence of Azo bond (S-H). Absence of these peaks after decolorization indicates cleavage of azo bond and also the disappearance of the peaks at the fingerprint region in the FTIR spectrum of extracted metabolites is clear evidence of biodegradation (Olukanni *et al.,* 2013).

In IR spectra of treated sample Fig.1, new peak at 3356.25 was observed which indicates that there may be formation of new compounds which originated from the fragmentation of parent dye molecule. The control IR spectrum of CF shows the following observation. The peaks in between 3700-3100 cm-1shows the presence of –OH,-NH-, =C-H (amides and amines).The peaks in between 2700-2000cm-1 responsible of Nitriles,azide compounds in the sample and 800-400cm-1 peaks are associated with Aromatic compounds in the sample Fig.3. The IR spectra obtained from treated sample shows the several variations in the region at 3700-3100 cm-1, 2700-2000cm-1 and 800-400cm-1as it is compared with control IR spectra of CF (Fig.2b). It may be the because of degradation of reactive orange by *Pleurotus pulmonarius*. The presence of aromatic amine in the degraded sample indicates the effect of laccase activity. Park *et al.,* (2006) reported that the biodegradation was more important than biosorption for decolorization because *F. trogii* ATCC 200800 was able to produce the laccase or MnP to mineralize synthetic dyes. It has been reported that the azo compounds with hydroxyl or amino groups were more likely to be degraded than those with methyl, methoxy, sulfo or nitro groups. In this study, the metabolites of reactive orange fractions were identified by comparing the mass spectra with data in the NIST98 library and independently by interpreting the fragmentation patterns. GC-MS analysis of the dye metabolite showed the presence of derivatives of reactive orange with clear evidence of ring cleavage. Based on the analytical profile analysis and the identified metabolites, a pathway was proposed for the biodegradation of reactive orange by *Pleuroteus pulmonarius* Enzymatic activities that resulted in intermediate products, some of which were identified as 2-Ethyl-4-methyl-1-pentanol with the retention time of 4.450min and a mass peak of 128; 2(2-ethylhexyloxy) ethanol with the retention time of 10.175min and a mass peak of 174; and Alpha-tridecene with the retention time 11.367min and a mass peak of 182.The dye that undergoes degradation process results in generating intermediate metabolites. These metabolites are identified with respect to their molecular weight and the resulting intermediates are presented in Table 4. It is seen from the Tables, that reactive orange is degraded into various intermediates the mechanism of degradation can be proposed as reactive orange being degraded into intermediates compounds such as n-Octyl phthalate, 13-Methyloxacyclotetradecane-2,11-dione, 3-Benzylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione, 3-Isobutylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione, 1-Ethyl-1,5-cyclooctadiene with respective mass of 390, 240, 244, 210 and 136.It is evident from the mass analyses report that the above dye in the presence of laccase undergoes degradation phenomenon and resulted in various intermediate metabolites (Pandey *et al.*,.,2007).White rot fungi posses different degradative capacity towards azo dyes may be

attributed to their structural differences in dyes. These isolates probably have acquired natural adaptation to survive in the presence of the dyes (Khadijah *et al.*, 2009).The complete degradation of dye could be achieved during study which was confirmed by FT-IR analysis. This suggests that *Pleuroteus pulmonarius* may have an efficient enzymatic system for the cleavage of parent dye. The hypothesis that could explain mechanism of biodegradation of reactive orange somewhat similar to ara operon through which certain metabolites produced from co-substrate may have induced synthesis of various enzymes involved in biodegradation of reactive orange. It could successfully be employed in the treatment of textile effluent. Gomare and Govindwar (2009), Yuan *et al*.,.(2016)reported that the metabolites of dyes can induce or inhibit biotransformation, so the metabolites generated during the degradation process of dye might cause different side effects on enzymes. However, further work is needed to identify the genes responsible for this kind of textile azo dyes decolorization.

5.0 CONCLUSION

The complete degradation of dye could be achieved during study which was confirmed by FT-IR and GC-MS analysis. This suggests that *Pleuroteus pulminorius* posses an efficient enzymatic capacity for the cleavage of reactive orange dye. It could successfully be employed in the treatment of textile effluent. However, further work is needed to identify the genes responsible for this kind of textile azo dyes decolorization. This study shows that laccase enzyme from fungus *Pleurotus pulmonarius* possesses a significant dye degradation capacity and further can be applied in biodegradation of toxic industrial dyes in the environment.

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REFERENCES

Ayed, L., Bekir, K., Achour, S., Cheref, A. and Bakhrouf, A. (2017). Exploring bioaugmentation strategies for azo dye CI Reactive Violet 5 decolourization using bacterial mixture: dye response surface methodology. *Water and Environment Journal*, **31**:80–89.

Chulhwan Park, C., Lee, M., Lee, B., Seung-Wook Kim, S-W., Chase, H.A., Jinwon Lee, J., and Sangyong Kim, S. (2007) Biodegradation and biosorption for decolorization of synthetic dyes by *Funalia trogii*. *Biochemical Engineering Journal*, **36**: 59–65.

Da Silva, M., Bergsten-Torralba, L.R., Nishikawa, M.M., Baptista, D.F. and MagalhAes, D.P. (2009). Decolorization of different textile dyes by *Penicillium simplicissimum* and toxicity evaluation after fungal treatment. *Brazilian Journal of Microbiology*, **40**(4): 808-17.

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Dhouib, A., Hamza, M., Zouari, H., Mechichi, T., H'midi, R., Labat, M., Martinez, M.J. and Sayadi, S. (2005). Autochthonous fungal strains with high ligninolytic activities from Tunisian biotopes. *Afric. J. Biotechnol.,* **4**: 431-436.

Gomare, S.S. and Govindwar, S.P. (2009) *Brevibacillus laterosporus* MTCC 2298: a potential azo dye degrader. *J. Appl. Microbiol*., 106: 993–1004. CrossRef PubMed

Iqbal, M., (2016). Vicia faba bioassay for environmental toxicity monitoring: a review. *Chemosphere*, **144:** 785-802.

Khadijah, O., Lee, K. K. and Abdullah, M.F. (2009). Isolation, screening and development of local bacterial consortia with azo dyes decolourising capability, *Malaysian Journal of Microbiology*, **5**(1): 25-32.

Kiiskinen, L.L., Ratto, M. and Kruus, K. (2004). Screening for novel laccase producing microbes. *J. Appl. Microbiol*., **97**: 640-646.

Kurade, M.B., Waghmode, T.R., Patil, S.M., Jeon, B-H. Govindwar, S.P. (2016) Monitoring the gradual biodegradation of dyes in a simulated textile effluent and development of a novel triple layered fixed bed reactor using a bacterium-yeast consortium, *Chemical Engineering Journal*. doi: http://dx.doi.org/10.1016/j.cej.2016.09.028.

Lade, H., Kadam, A., Paul, D. and Govindwar, S. (2014). Biodegradation and Detoxification of Textile Azo Dyes by Bacterial Consortium Under Sequential Microaerophilic/Aerobic Processes. *EXCLI Journal* , **14**: 158-174.

Leonowicz A, Cho NS, Luterek J, Wilkolazka A, Wotjas-Wasilewska M, Matuszewska A, Hofrichter M, Wesenberg D, Rogalski J. (2001). Fungal laccase: properties and activity on lignin. *J. Bas. Microbiol.*, **41**: 185–227.

Machado, K.M.G., Compart, L.C.A., Morais, R.O., Rosa, L.H. and Santos, M.H. (2006). Biodegradation of Reactive Textile Dyes by Basidiomycetous Fungi from Brazilian Ecosystems. *Brazilian Journal of Microbiology,* **37**: 481-487.

Nataraj, S.K. Sridhar, S. Shaikha, I.N. Reddy, D.S. Aminabhavi, T.M. (2007). Membrane-based microfiltration/electrodialysis hybrid process for the treatment of paper industry 25 wastewater, *Sep. Purif. Technol.*, **57**: 185-192.

Nouren, S., Bhatti,H-N, Iqbal, M , Bibi, I., Kamal, S., Sadaf, S., Sultan, M., Kausar, A. and Safa, Y. (2017). By-product identification and phytotoxicity of biodegraded Direct Yellow 4 dye. *Chemosphere*, **169**: 474-484.

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Novotný, Č., Svobodová, K., Benada, O., Kofroňová, O., Heissenberger, A. and Fuchs, W. (2011). Potential of combined fungal and bacterial treatment for color removal in textile wastewater. *Bioresour. Technol.,* **102**: 879–888.

Olukanni, O.D, Osuntoki, A.A., Awotula, A.O. Kalyani, D.C. Gbenle, G.O. and Govindwar, S.P. (2013). Decolorization of Dyehouse Effluent and Biodegradation of Congo Red by *Bacillus thuringiensis* RUN1. J. Microbiol. Biotechnol., **23**(6): 43–849 .http://dx.doi.org/10.4014/jmb.1211.11077.

Pandey, A, Singh, P. and Iyengar, L. (2007) Bacterial decolorization and degradation of azo dyes. *Int. Biodeter. Biodegr.*, **59**: 73–84.

Plácido, J., Chanagá, X., Ortiz-Monsalve, S., Yepes, M. and Mora, M. (2016). Degradation and detoxification of synthetic dyes and textile industry effluents by newly isolated *Leptosphaerulina* sp. from Colombia. *Bioresour. Bioprocess.*3: 6.DOI 10.1186/s40643-016-0084-x.

Robinson, T., McMullan, G., Marchant, R. and Nigam, P. (2001). Remediation of Dyes in Textile Effluent: A Critical Review on Current Treatment Technologies with a Proposed Alternative. *Bioresource Technology*, **77**(3): 247-255.

Santos, S.C.R. and Boaventura, R.A.R. (2015) Treatment of a simulated textile wastewater in a sequencing batch reactor (SBR) with addition of a low-cost adsorbent. *J. Hazard. Mater*., **291**: 74–82 CrossRef PubMed.

Sara, B., Noreddine, K. C. and Jacqueline, D. (2016). Production of laccase without inducer by *Chaetomium* species isolated from Chettaba forest situated in the East of Algeria. *Afr. J. Biotechnol.*, **15**(7): 207-213.

Shanmuga Priya, M., Divyashree, K., Chiranjeeb Goswami, M., Lakshmi Prabha, A.K. and Satheesh, Babu (2013). Bioremediation of Textile Dyes by White Rot Fungi Isolated From Western Ghats Area. *International Journal of Engineering and Advanced Technology,* **2**(4): 913-918.

Thakur, S. and Gupte, A. (2014). Optimization and hyper production of laccase from novel agaricomycete *Pseudolagarobasidium acaciicola* AGST3 and its application in *in vitro* decolorization of dyes. *Ann. Microbiol.*, **65**(1): 185-196.

Usha, K.Y., Praveen, K and Rajasekhar Reddy, B. (2014). Enhanced Production of Ligninolytic Enzymes by a Mushroom *Stereum ostrea. Biotechnology Research International*, Volume 2014, Article ID 815495,http://dx.doi.org/10.1155/2014/815495. 9 pages.

Wang, N., Chu, Y., Wu, F., Zhao, Z. and Xu, X. (2017). Decolorization and degradation of Congo red by a newly isolated white rot fungus, *Ceriporia lacerata*, from decayed mulberry branches. *International Biodeterioration and Biodegradation*, **117**: 236-244.

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ISSN: 2456-8643

Yuan, X., Tian, G., Zhao, Y., Zhao, L., Wang, H. and Bun, T. (2016). Degradation of dyes using crude extract and a thermostable and pH-stable laccase isolated from *Pleurotus nebrodensis.* Bioscience Reports (2016) 36, e00365, doi:10.1042/BSR20160163.

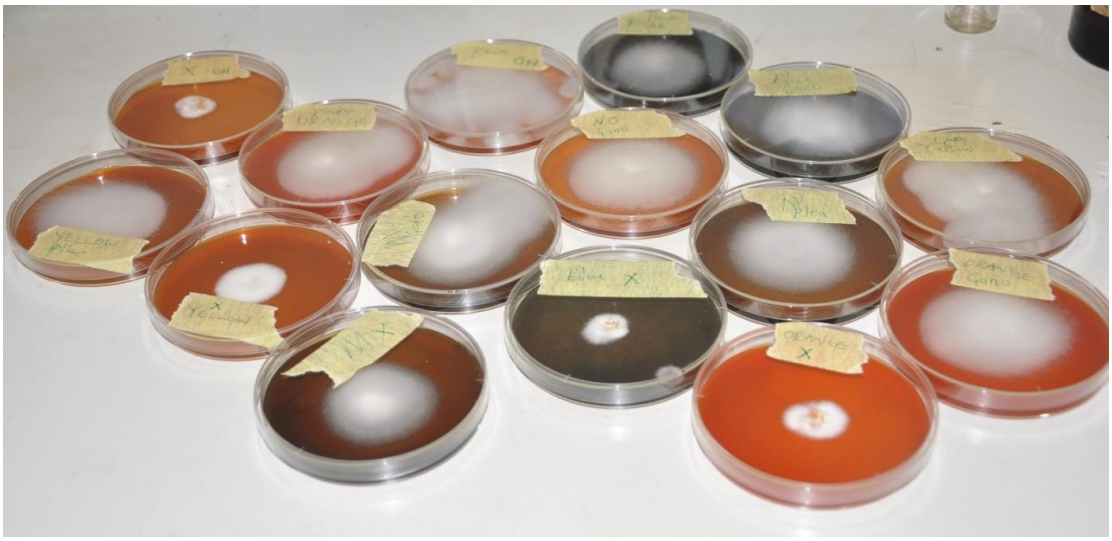

Plate 1: Growth patterns of Pleurotus pulmonarius on PDB supplemented with different synthetic dye

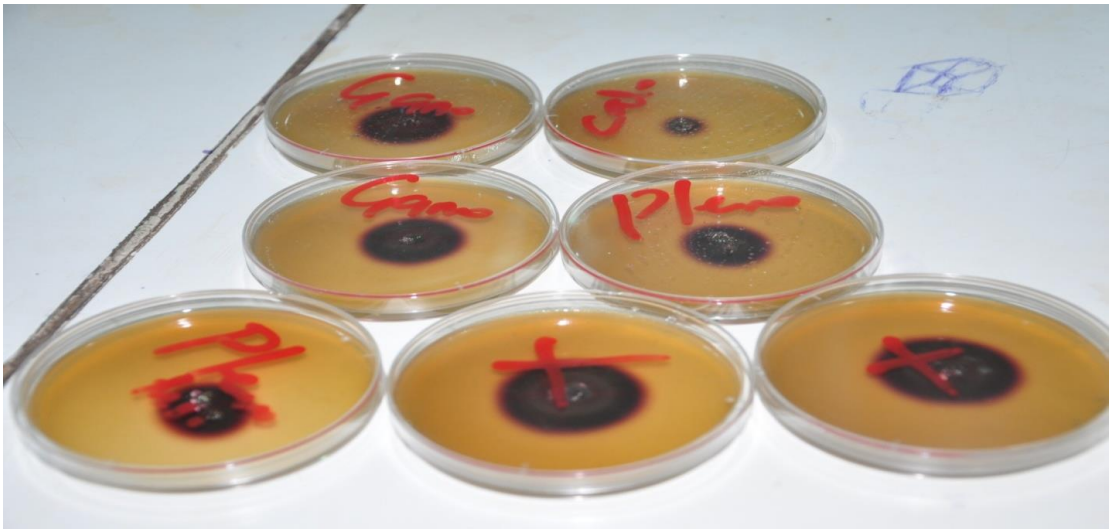

Plate 2: Laccase production by Pleurotus pulmonarius after 5 days of incubation

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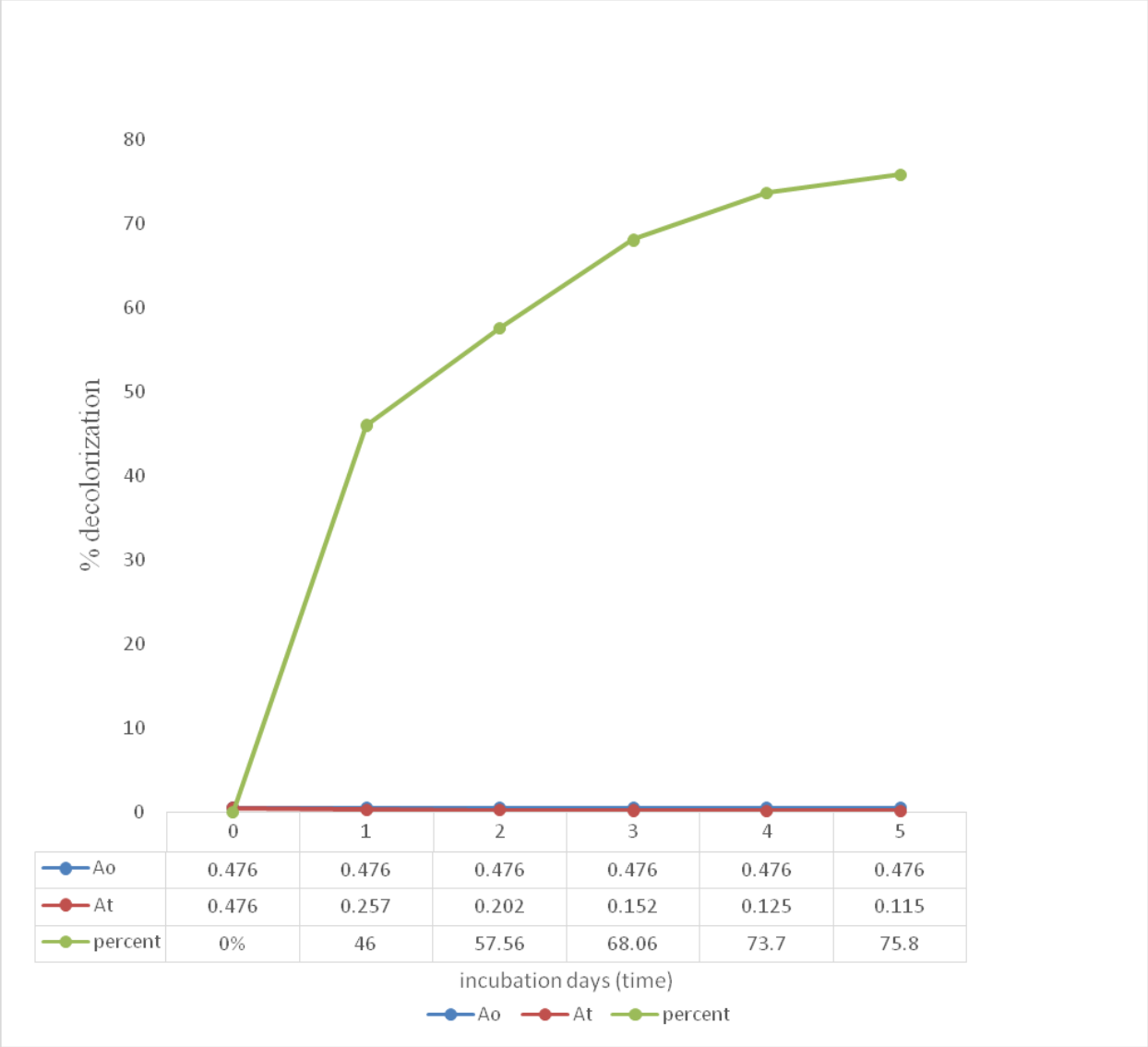

Figure 1: Graph of % decolorization plotted against time (days) at 490nm wavelength. Ao is Absorbance of the initial dye solution (day 0) and AT is the absorbance at cultivation time.

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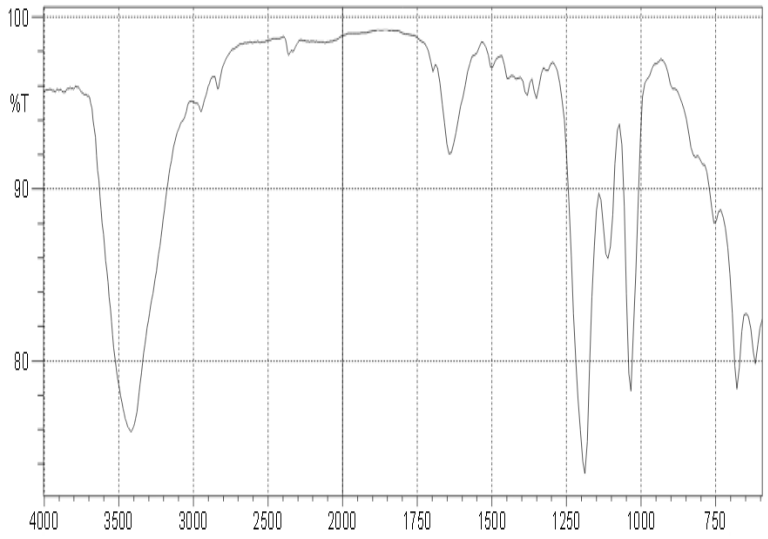

Figure 2: FTIR Spectrum of non-degraded Reactive Orange dye (control)

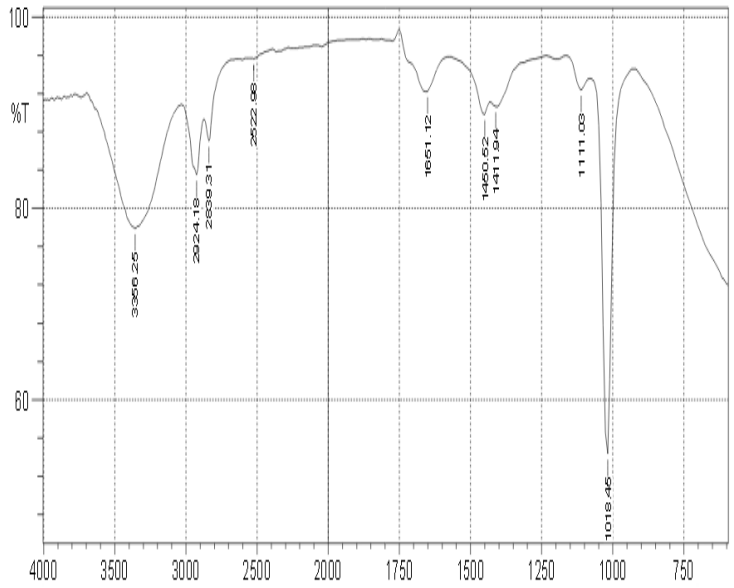

Fig 3: FTIR Spectrum of degraded Reactive Orange

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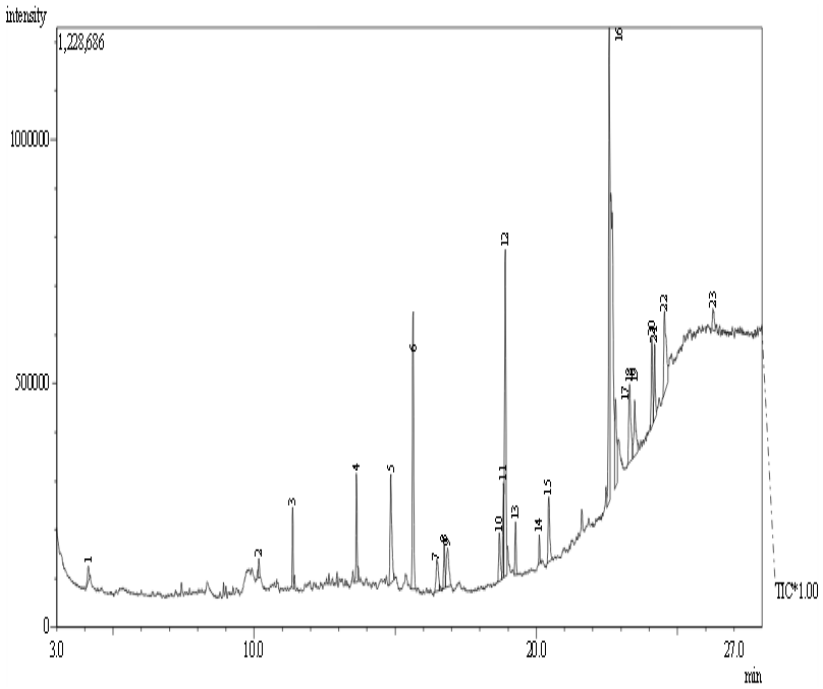

Figure 4: GC-MS fingerprint of degraded Reactive Orange dye

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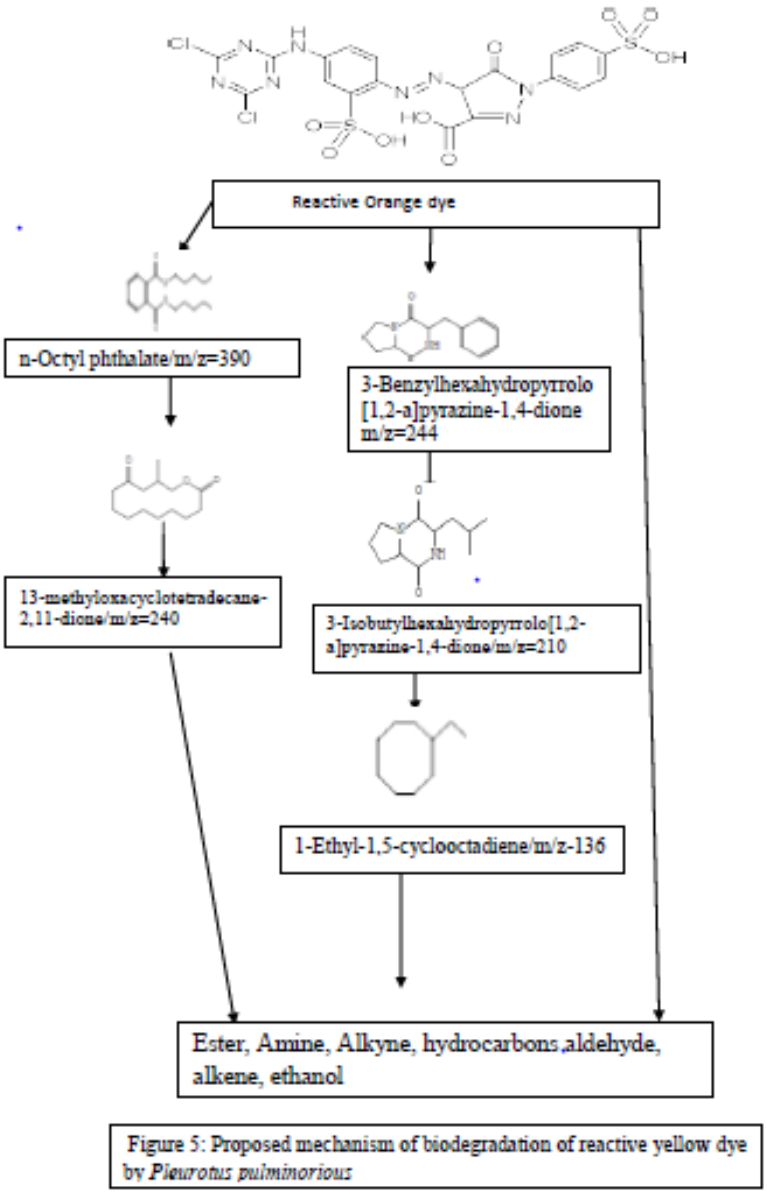

Figure 5: Proposed mechanism of biodegradation of reactive yellow dye by *Pleurotus pulminorious*

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Table 1: FTIR analysis of non-degraded reactive orange dye (control)

Peak	Frequency cm^{-1}	Type of vibration	Type of bond	Functional group
630	650-600	bend	-C=C-H:C-H	Alkynes
683.5	700-650	Bend	=C-H	Alkenes
1048.9	1050-1000	Stretch	C-O	Alcohols, carboxylic acids, esters, ethers
1112	1150-1100	Stretch	C-O	Alcohols, carboxylic acids, esters, ethers
1195.2	1200-1150	Wag	C-H	Alkyl halides
1655	1700-1650	Stretch	-C=C-	Alkynes
2550	2600-2550	Stretch	S-H	Thiol
2965	3000-2900	Stretch	C-H	Alkanes
3410	3500-3400	Stretch	O-H	Alcohols, phenols

Table 2: FTIR analysis of the degraded reactive orange

Peak	Frequency cm^{-1}	Type of vibration	Type of bond	Functional group
1018.45	1050-1000	Stretch	C-O	Alcohols, carboxylic acids, esters, ethers
1111.03	1150-1100	Stretch	C-O	Alcohols, carboxylic acids, esters, ethers
1411.94	1450-1400	Stretch (in-ring)	C-C	Aromatics
1450.52	1500-1450	Stretch	C-C	Aromatics

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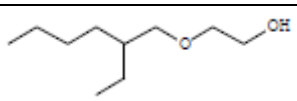
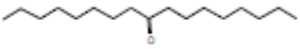
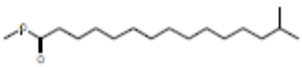
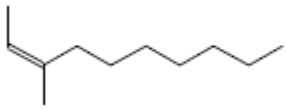
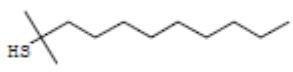
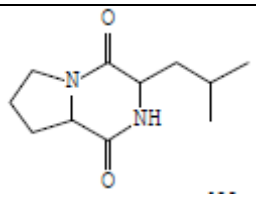
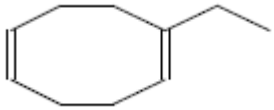
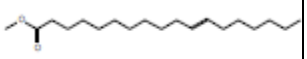
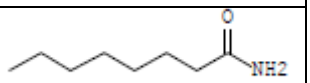
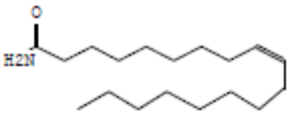
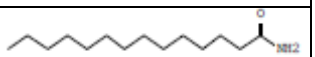
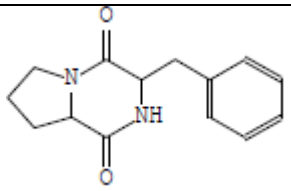
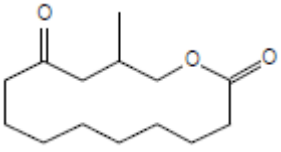
		(in-ring)		
1651.12	1700-1650	Stretch	-C=C-	Alkenes
2522.98	2550-2500	Stretch	O-H	Carboxylic acids
2839.31	2850-2800	Stretch	O-H	Carboxylic acids
2924.18	3000-2950	Stretch	C-H	Alkanes
3356.25	3400-3350	Stretch	N-H	1°, 2° amines, amides

Table: GC-MS analyses of fragmentation products (reactive orange)

Peak	Retention Time	Compound name	Molar Mass	Chemical Formula	Structure
1	4.133	Octaldehyde	128	$C_8H_{16}O$	
2	10.175	2(2-ethylhexyloxy)ethanol	174	$C_{10}H_{22}O_2$	
3	11.367	Alpha-tridecene	182	$C_{13}H_{26}$	
4	13.625	Hexadecene-1	224	$C_{16}H_{32}$	
5	14.842	Pelargone	254	$C_{17}H_{34}O$	
6	15.642	Methyl 14-methylpentadecanoate	270	$C_{17}H_{34}O_2$	
7	16.467	(2Z)-3-methyl-2-decene	154	$C_{11}H_{22}$	
8	16.733	1,1-dimethyldecylhydrosulfide	202	$C_{12}H_{26}S$	

9	16.858	3-Isobutylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione	210	$C_{11}H_{18}N_2O_2$	
10	18.683	(14E)-14-Hexadecenal	238	$C_{16}H_{30}O$	
11	18.833	1-Ethyl-1,5-cyclooctadiene	136	$C_{10}H_{16}$	
12	18.900	`Methyl (11E)-11-octadecenoate	296	$C_{19}H_{36}O_2$	
13	19.258	Methyl 15-methylhexadecanoate	284	$C_{18}H_{36}O_2$	
14	20.108	Hexadecene-1	224	$C_{16}H_{32}$	
15	20.433	Caprylamide	143	$C_8H_{17}NO$	
16	22.583	Crodamide O	281	$C_{18}H_{35}NO$	
17	22.817	Tetradecylamide	227	$C_{14}H_{29}NO$	
18	23.308	3-Benzylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione	244	$C_{14}H_{16}N_2O_2$	
19	23.492	13-Methyloxacyclotetradecane-2,11-dione	240	$C_{14}H_{24}O_3$	

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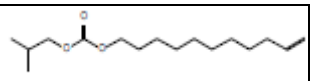
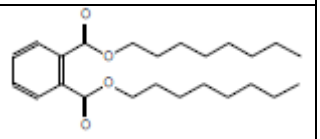
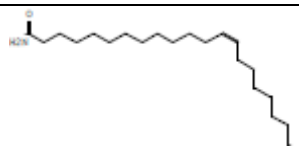
20	24.092	isobutyl undec-10-enyl ester	270	$C_{16}H_{30}O_3$	
21	24.192	n-Octyl phthalate	390	$C_{24}H_{38}O_4$	
22	24.533	cis-13-Docosenamide	337	$C_{22}H_{43}NO$	
23	26.267	Z-3-Hexadecen-7-yne	220	$C_{16}H_{28}$