

# Food Dyes Decolourized by *pleurotus ostreatus*

Anthony Au, Fazreen Dzulkaffli

Faculty of Biotechnology & Life Sciences, UNISEL, Malaysia

**Abstract-** This study employed a comprehensive approach to testing the capability of *P. ostreatus* in decolourizing four different food dyes and to investigate the bio-adsorption properties of *P. ostreatus* under the equilibrium state. Decolourization of Carmoisine Red, Tartrazine Yellow, Brilliant Blue FCF and Fast Green FCF were determined by UV-visible spectrophotometric method. Present study had confirmed that *P. ostreatus* provided almost complete decolourization of 10ppm food dyes. Dried *P. ostreatus* along with agitation have a higher efficiency, efficacy and capability during the dye decolourization. As high as 81.34% for Carmoisine, 45.36% for Tartrazine, 19.82% for Brilliant Blue FCF and up to 28.99% for Fast Green FCF are removed by 5g/L of dried *P. ostreatus* in 150rpm agitation speed. The interpretation of results had shown the maximum capacity ( $Q_m$ ) of *P. ostreatus* which range from 0.05 to 0.43mg/L in dyes decolourization. Our data were adequately fitted to Langmuir isotherm and subordinate to Pseudo second kinetic order. Colourless solution of Carmoisine Red was visually transformed by *P. ostreatus* within 5 days of dye treatment.

**Index Terms-** *Pleurotus ostreatus*, food dye, decolourization, fresh biomass, dried biomass

## I. INTRODUCTION

Dyes have been increasingly applied in the industries of cosmetics, textile, manufacturing, and food. With the high stability to light, temperature and resistance to microbial attacks, a range of cost-effective and colourful synthetic food dyes can be manufactured in pilot scale to sustain the limited choice of natural food dyes (Kiseleva *et al.* 2002).

The synthetic food dyes are made up of complex aromatic molecular structures of azo and phenylmethane groups, which are recalcitrant xenobiotic compounds, that resistance to environmental decolourization process (Fu and Viraraghavan 2001). Thus, leaving the unsolved environmental problem to biologists and public.

Chemical and physical wastewater treatments have been widely practiced for the past decades compared to biological approach. Most of the physical approach, however, simply accumulate and concentrate wastewater, left over carcinogenic solid waste (food dyes), that further impede disposal problem. Expensive chemical approach using peroxide or ozone is inapplicable to schedule food industrial wastewater treatment.

To fill in the gap, biological approach using fungi to treat wastewater has received much attention recently. The white rot fungi have proven its capability to degrade synthetic chemicals, such as azo dyes from textile industry (Zhao and Hardin, 2007). The plausibility was this fungi secretes ligninolytic enzyme to degrade complex molecular structure of the synthetic dyes; thus,

it was an alternative to the existing approach (Selvam *et al.*, 2003).

To date, vast majority of fungal strains have involved in biological decolourization approach. There were *Aspergillus spp.*, *Fusarium*, *Phanerochaete chrysosporium*, *Trametes versicolor*, *Coriolus versicolor*, *Cunninghamella polymorpha*, *Funalia trogii* and *Rhizopus arrhizus*, *Rhizopus oryzae*, *Cyathus bulleri*, *Laetiporus sulphureus*, *Streptomyces sp.*, *Trametes versicolor*, *Polyporus elegans*, *Trametes versicolor*, *Lenzites betulina*, *Mucor mucedo*, *Phanerochaete sordida*, *Pycnoporus sanguineus*, and *Trametes elegans* (Da-Re and Papinutti, 2011; Erum and Ahmed, 2010; Moturi and Singara-Charya, 2009; Seyis and Subasioglu, 2008; Erkut *et al.*, 2007; Nigam *et al.*, 2000)

*Pleurotus ostreatus* (*P. ostreatus*), is widely available, an edible mushroom for food industry. However, not much attention has been raised for its decolourization ability. This research is to source out the capability, efficacy and efficiency of *P. ostreatus* in decolourizing the food dyes, indirectly contributing knowledge to wastewater treatment in food industries.

## II. MATERIALS AND METHODOLOGY

### A. Microorganism and Dye Preparation

Fresh *P. ostreatus* bodies were purchased from TESCO Shah Alam, Malaysia. The unlive *P. ostreatus* biomass was dried in the incubator (30°C, overnight) after removed its stipe. Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB) (Fluka, Sigma-Aldrich USA) were prepared followed by autoclaving (121°C, 20mins).

*P. ostreatus* was cultured on PDA. Active mycelium was cut and was transferred to Universal bottles containing 10ml of PDB and incubated at 28°C. Prior to sterilization by autoclaving, the medium was adjusted to pH 6.5 with 20mM acetate buffer. After 3-5 days, the inoculum of each bottle was homogenized.

Four different colour dyes (Nona, Malaysia) were selected and each of their unique properties was further described in Table I.

### B. Decolourization Assay

Dye decolourization assay was started on Petri dishes (3 parallel) with different parameter of dyes after inoculated with mycelial plugs cut from actively growing mycelia. Another 3 parallel of *P. ostreatus* that cultivated in the liquid medium was decolourized with the respective dyes. Inoculated universal bottles with the same medium without dye served as control.

Table I: Properties and Characteristic of Food Dyes

Commercial Name	Colour	Chemical Formula	$\lambda$ max (nm)	Mole-Weight (g/mol)	Group
Carmoisine	Red	C <sub>20</sub> H <sub>12</sub> N <sub>2</sub> Na <sub>2</sub> O <sub>7</sub> S <sub>2</sub>	608	502.44	Monoazo
Tartrazine	Yellow	C <sub>16</sub> H <sub>9</sub> N <sub>4</sub> Na <sub>3</sub> O <sub>9</sub> S <sub>2</sub>	510	534.4	Monoazo
Brilliant Blue FCF	Blue	C <sub>37</sub> H <sub>34</sub> N <sub>2</sub> Na <sub>2</sub> O <sub>9</sub> S <sub>3</sub>	698	792.84	Triphenyl methane
Fast Green FCF	Green	C <sub>37</sub> H <sub>34</sub> N <sub>2</sub> Na <sub>2</sub> O <sub>10</sub>	664	808.85	Triphenyl methane

C. Adsorption Isotherm and Kinetics Studies

Adsorption studies were carried out by dissolving 10ppm (part per million) of dye. The 5, 10, 50g/L of *P. ostreatus* gills (fresh and dried) were xthoroughly with different concentration of dyes and the suspensions were shaken (150rpm) at room temperature.

D. Spectrophotometric Analysis

Samples of 1.0 mL were collected from the duplicate flasks and were centrifuged for 10,000rp for 5 min. The residual dyes concentration in the solutions were measured by Hitachi UV-vis U1900 spectrophotometer at required time intervals 1, 6, 24, 48, 72, 96 and 120 hours. Decolourization was determined by monitoring the absorbance at the wavelength maximum for each dye as shown in Table 1.

III. RESULTS

A. Fungal Morphology and Decolourization Ability

For the spore decolourization studies, the decolourization rate of respective strains was not as efficient as compared to the bio-adsorption studies. Fresh and dried gills of *P. ostreatus* showed its excellence capability in both decolourization and adsorption. However, cultured media condition may be the pitfall for the effectiveness of the cultured *P. ostreatus* dye adsorption activity (Seyis and Subasioglu, 2008). Dye degradation is improved by the active enzymes encoded in *P. ostreatus* biomass (Neelamegam et al., 2004). Therefore, we will emphasis on the biomass usage that is more advantageous in decolourization studies.

Decolourozation assay was chosen on 4 commercially important dyes, with a wide range of applications across the household and food industries. Decolourization in dyes solution was assessed by visual disappearance of colour and reduced in absorbance reading after 5 days. Carmoisine, Tartazine Yellow, Brilliant Blue FCF and Fast Green FCF were decolourized by 0.1g of fresh *P. ostreatus* with a percentage of 30.49±1.3, 12.66±1.2, 5.11±0.2 and 10.43±0.3% respectively (Figure 1). Meanwhile, dried *P. ostreatus* was significantly decolourized the Carmoisine (77.83%±0.9) and red colour was visually disappeared on the fifth days. Tartzine showed 36.45±1.1 % of dye decolourization its appearance was transformed to light

yellow. Decolourization of Brilliant Blue FCF (16.21±0.3%) and Fast Green FCF (21.85±0.5%) were also achieved by 5g/L of dried *P. ostreatus*. Figure 1 illustrated the greater decolourization efficacy for dried biomass than those of fresh biomass in our study.

Percentage for dye decolourization was calculated according to the formula:

$$D(\%) = ((C-S)/C) \times 100\%; \text{ where}$$

C -- absorbance of dye in a control sample,  
S -- absorbance of dye in samples

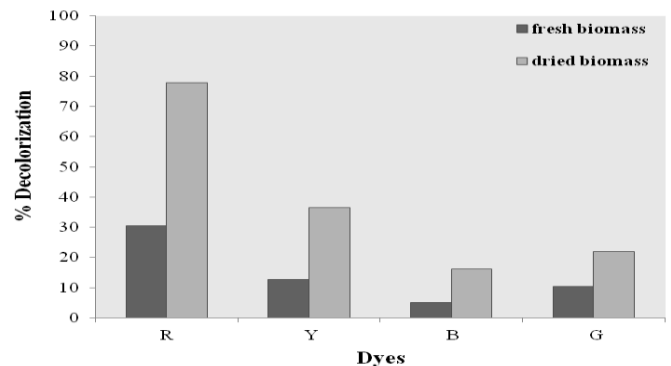


Fig. 1 Total percentage of dyes (R= Carmoisine Red, Y= Tartazine Yellow, B= Brilliant Blue FCF, G= Fast Green FCF) at 10ppm decolourized by 5g/L dried and fresh *P. ostreatus* biomass after 120 hours treatment

B. Effect of Agitation

Shaking at 150rpm, all dyes decolourization was enhanced gradually with the passage of time. After 5 days of treatment, 5g/L of fresh *P. ostreatus* biomass has decolourized 5.72%, 3.8%, 2.1% and 1.59% with respect to Carmoisine, Tartazine Yellow, Brilliant Blue FCF and Fast Green FCF. Agitation has an effect on the dried *P. ostreatus* decolourization efficiency, the percentage of dye adsorption increased to the maximum of 3.51% (Carmoisine), 8.91% (Tartazine), 3.07% (Brilliant Blue FCF) and 7.15% (Fast Green FCF).

C. Langmuir Equilibrium and Adsorption Isotherm

The capability of *P. ostreatus* in colour dyes absorption was demonstrated in Table II. The amount of adsorption at equilibrium,  $q_e$  (mg/g), was calculated using following formula (Santhi et al., 2010; Hameed et al., 2008; Islek et al., 2008):

$$q_e = \frac{(C_0 - C_e)V}{W}; \text{ where}$$

$q_e$  (mg/g) -- amount of dye adsorbed by *P. ostreatus* biomass at equilibrium state,  
 $C_0$  and  $C_e$  (mg/L) -- the initial and equilibrium liquid phase concentration of dye  
 $V$  (L) -- total volume of dye solution,  
 $W$  (g) -- weight of the biomass

Table II Total bio-adsorption  $q_e$  of *P. ostreatus* (Sample Dosage: 5g/L, Dye Concentration: 10mg/L, Speed of Stirring: 150rpm, room temperature)

<i>P. ostreatus</i>	$q_e$ (mg/g)			
	C	Y	B	G

Fresh	7.28	3.16	1.46	2.32
Dried	16.24	8.64	3.98	5.74

Adsorption Isotherms is to investigate the association between the adsorbate concentration and the amount adsorbed on the adsorbent at the constant rate. Langmuir Isotherm equation value was given in Table III. The equation may be expressed as (Vijayakumar *et al.*, 2012; Santhi and Manonmani, 2009):

$$C_e/q_e = \frac{1}{kQ_m} + \frac{C_e}{Q_m}, \text{ where}$$

$Q_m$ (mg/g). -- maximum adsorption capacity, slopes

$k$  -- Langmuir constant

From the graph of  $C_e/q_e$  vs  $C_e$ , we can calculated where  $Q_m$  equal to slope ( $1/Q_m$ ) and value of  $k$  is intercepts ( $1/kQ_m$ ) of linear plots

Table III Adsorption Isotherm of Food Dyes on Dried *P. ostreatus* (Sample Dosage: 5g/L, Dye Concentration: 10mg/L, Speed of Stirring: 150rpm, room temperature)

Dyes	$Q_m$ (mg/g)	$k$
Carmoisine	0.43	0.739
Tartrazine	0.33	0.1779
Brilliant Blue FCF	0.05	0.1313
Fast Green FCF	0.16	0.1355

#### D. Lagergren's Pseudo-First Order Kinetics Analysis

Kinetic studies of adsorption were carried out in different contact time to the surface of *P. ostreatus*. The amount of dye absorbed were increase with the passage of time. Referring Vijayakumar *et al.* (2012), the amount of adsorption  $qt$  (mg/g) was calculated by

$$qt = \frac{(C_0 - C_t)V}{W}$$

Figure 2 and 3 represent the bio-adsorption model for Pseudo first and second kinetics order respectively. The high values of correlation coefficients in our data (Table IV) proved the kinetics of bioadsorption are obeying pseudo-first-order rate kinetic model.

The kinetic data were acquired from the Lagergren's pseudo-first order rate equation (Kumar *et al.*, 2010; Santhi and Manonmani, 2009):

$$\log(q_e - qt) = \log q_e - \frac{k_1 t}{2.303}; \text{ where}$$

$k_1$  -- first order rate constant, obtained from the slopes of the linear plots of  $\log(q_e - qt)$  vs  $t$

The linear form of pseudo-second-order kinetics described as (Kumar *et al.*, 2008; Hameed *et al.*, 2008):

$$\frac{t}{qt} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e(t)}, \text{ where}$$

$k_2$  -- second order constants rate in (g/mg h), obtained from the slope and intercept of plot  $t/qt$  vs  $t$

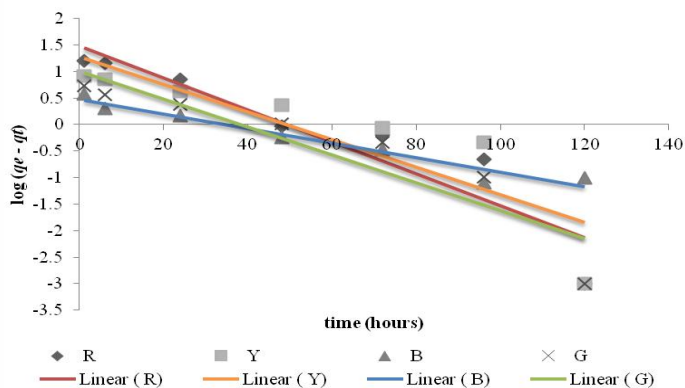


Fig. 2 Lagergren's Pseudo-First Order Graph Plot for Bio-adsorption of *P. ostreatus* (Sample Dosage: 5g/L, Dye Concentration: 10mg/L, Speed of Stirring: 150rpm, room temperature)

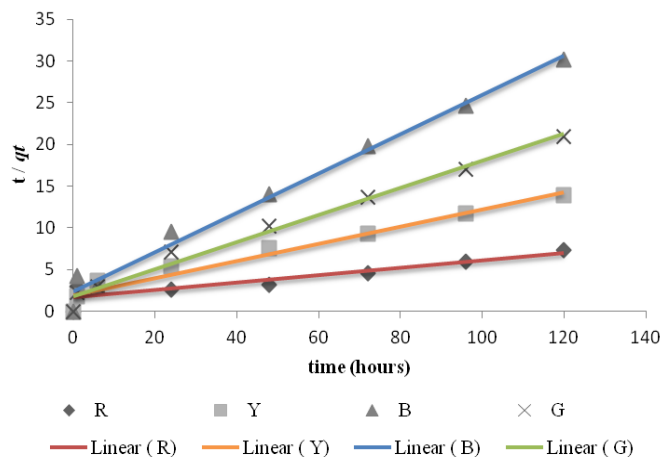


Fig. 3 Lagergren's Pseudo-Second Order Graph Plot for Bio-adsorption of *P. ostreatus* (Sample Dosage: 5g/L, Dye Concentration: 10mg/L, Speed of Stirring: 150rpm, room temperature)

Table IV Slope and  $R^2$  Values Generated by Lagergren Plot

Dyes	Pseudo first order kinetic model		Pseudo second order kinetic model	
	$k_1$	$R^2$	$k_2$	$R^2$
Carmoisine	-0.0301	0.8721	0.0449	0.8838
Tartrazine	-0.0205	0.8529	0.1207	0.9591
Brilliant Blue FCF	-0.0137	0.9519	0.2351	0.9861
Fast Green FCF	-0.0212	0.8496	0.1616	0.9845

#### IV. DISCUSSION

Dead *P. ostreatus* with the amount of 20g/L showed an excellent dye decolourization characteristic with an increasing surface area for adsorption as a consequence of cell rupture upon death. Increase dyes exposure on fungal cell wall that covers with chitin or chitosan is the major site of biosorption, for example the pileus (Vetter, 2007). Apart, researchers have also reported that dead biomass was better choices that can effectively adsorbing organic pollutants and dyes compare to live biomass (Maurya *et al.*, 2006; Seyis and Subasioglu, 2008). The

disappearing of dyes (measure by visual appearance) was due to the chromophoric group transformation either by metabolism and adsorption of *P. ostreatus*. (Zhao, 2004) *P. ostreatus* biomass shown its highest capability and efficiency to adsorb and decolourize Carmoisine which contains monoazo group. Phenols structure in Carmoisine is ease to oxidize to phenoxyl radicals by laccase in the presence of O<sub>2</sub> cofactor (Couto, 2009). Similarly, monoazo group of Tartrazine, which owned a simple molecular structure and light molecular weights has successfully been decolourized by *P. ostreatus* biomass. High laccase activity was reported in the decolourization of Direct Dye Solar Golden Yellow R under optimized condition (Jilani *et al.*, 2011). The Naphthyl compounds can be decolourized to Naphthoquinone by *P. ostreatus* (Zhao, 2004). All the bonding and structure (azo, phenolic and naphthalene) can react by *P. ostreatus* and produce uncoloured compound after metabolism thus it appeared translucent (Yiping *et al.*, 2008). However, Brilliant Blue FCF with the high molecular weight and complex molecular structure are more resistant to be decolourized by *P. ostreatus*. Fast Green FCF also has similar instance with Brilliant Blue FCF, thus representing the Triphenylmethane dye group that contained complex molecular structure and large molecular weight is steric hindrance and resist food dyes to bind. The steric hindrance occurs due to the large molecular size and consequently caused it resistant to be transformed or degraded into simpler structure. Faraco *et al.* (2009) has reported in the presence of complex poly-azo and a stilbene structure in dye wastewater was unable to be degraded by the laccases of *P. ostreatus*. The chromophoric group did not transformed by *P. ostreatus*, thus the colour still can be visualized. All of these four dyes contain sulfonate groups can be decolourized by *P. ostreatus* (Lu *et al.*, 2008).

## V. CONCLUSION

As the result, the low cost edible *P. osteratus* can act effectively toward monoazo group of food dyes for Camoisine and Tartrazine in aqueous solution. The isothermal data fitted to Langmuir equation and Q<sub>m</sub> was accounted as 0.45mg/g. The bioadsorption processes followed the pseudo- second order rate kinetics. Thus, these results suggested that chemisorption was the underlying mechanism rather than physiosorption for dye in order to adsorb on the intersurface of *P. ostreatus*.

## APPENDIX



*P. ostreatus* mycelium culutred in PDA



*P. ostreatus* culutred in PDB added with dyes



Decolourization of Carmoisine on 3<sup>rd</sup> day (middle), 5<sup>th</sup> day (right) and control (left)



Decolourization of Tartrazine on 3<sup>rd</sup> day (middle), 5<sup>th</sup> day (right) and control (left)



Decolourization of Brilliant Blue FCF on 3<sup>rd</sup> day (middle), 5<sup>th</sup> day (right) and control (left)





Decolorization of Fast Green FCF on 3<sup>rd</sup> day (middle), 5<sup>th</sup> day (right) and control (left) (right) and control (left)

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**Correspondence Author** – Anthony Au, email: auzl@live.com