



Absorption efficiency of Bromophenol Blue and Congo Red using King oyster mushroom (*Pleurotus eryngii*)

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Abstract

King oyster mushroom or its scientific name *Pleurotus eryngii* is an edible mushroom and it is widely available at local market. The potential of the expired King oyster mushroom as an alternative low-cost material for the removal of textile dyes in industrial effluents is explored. Absorption technique is used to optimize and to evaluate the absorption of Bromophenol Blue and Congo Red by the King oyster mushroom. The outcome of this study concludes that the optimum parameters for the absorption of Bromophenol Blue are 5 hours, pH 5 and 10 g of King oyster mushroom. On the contrary, the optimum parameters for the absorption of Congo Red by King oyster mushroom are 5 hours, pH 3 and 12 g of King oyster mushroom. The performance is attained by using heated King oyster mushroom as it achieves high degree of hydration of 0.4 at 5 hours. The King oyster mushroom portrays higher absorption of Congo Red than Bromophenol Blue at optimum conditions.

Key words – absorption – Bromophenol Blue – Congo Red – King oyster mushroom

Introduction

These days, excess dyes used to colour their products were derived out from numerous manufacturing industries in Malaysia such as dye, paper, textile and furniture (Kant 2012). The process of dyeing utilizing significantly volume of liquid, hence it generates sizeable amount of coloured wastewater which led to long-term toxic effect (Alam et al. 2015). Also, it damages the biodiversity of aquatic eco-system. Consequently, the dissolved pollutants increasing annually and it is estimated that 280 000 tons of dyes were released into the nearby rivers, drains, stagnant ponds or lagoons space as industrial liquid waste (Abdurrahman et al. 2013, Ali 2010). Nevertheless, it is difficult to remove the dyes from the industrial waste, because dyes are not easily degradable (Alam et al. 2015).

Dyes are categorized based on their chemical structure. According to Jain & Shrivastava (2013), dye is an organic compound having a strong and complex aromatic molecular structure, obviously toxic and harmful. Bromophenol Blue and Congo Red dyes are among the regularly used industrial dyes in manufacturing industries such as textile, paper and food processing (El-Gamal et al. 2015). Bromophenol Blue dyes, a triphenylmethane structure, is an anionic dye (Koyuncu 2009) which is also known as Albutest Tetrabromophenol blue (El-Zahhar et al. 2014). A Bromophenol

Blue has a chemical molecular formula of $C_{19}H_{10}Br_4O_5S$ with the molar mass of 669.97 g/mol, acidity level of pH 3 to 4.6 and it is blueish violet color. While, Congo Red is an anionic and a red colloidal solution, which is soluble in water and organic solvents (Swan & Zaini 2019). It has molar mass of 696.65 g/mol with the chemical molecular formula of $C_{32}H_{22}N_6Na_2O_6S_2$. Fig. 1 depicts the chemical structure for Bromophenol Blue and Congo Red.

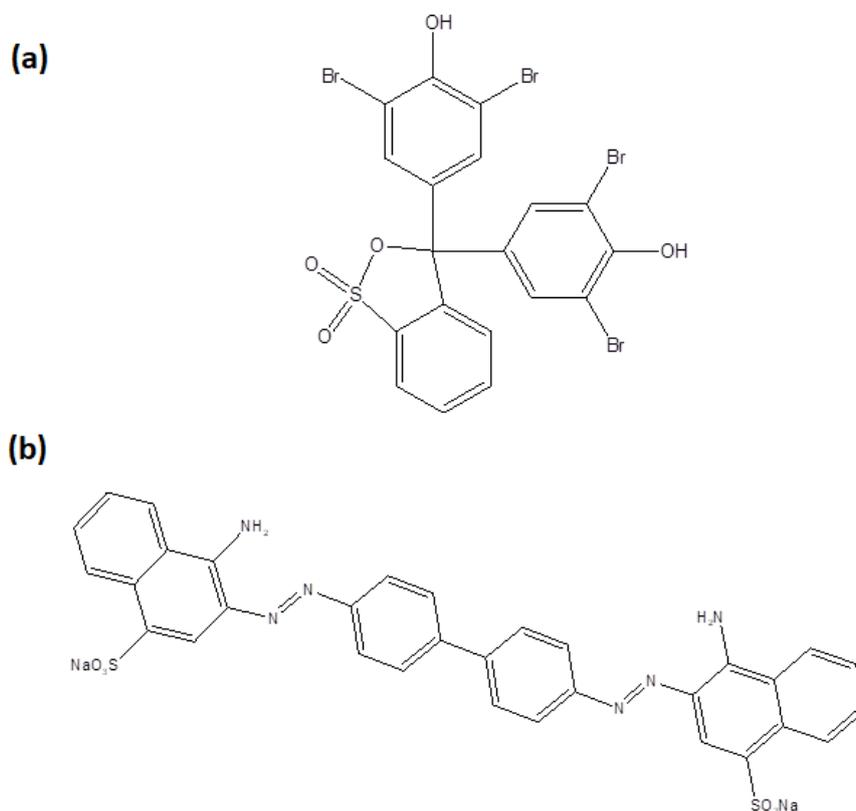


Fig. 1 – Chemical structure. a Bromophenol Blue. b Congo Red.

To date, many methods such as absorption, chemical transformation, incineration, photo catalysis or ozonation and complex filtration systems have been investigated to remove dyes pollutants. However, the cost of maintaining the materials and the filtration systems are expensive as well as the release of toxic gaseous as side-products via ozonation (Bello et al. 2013, Deaconu et al. 2016, Robinson et al. 2001). Hence, absorption technique is still in favour as the technique is easy, flexible and fast.

Many studies were carried out by different mushroom species on the biodegradation of various dyes such as Congo Red dye by the mushroom *Tricholoma species* (Aghizioninbakani et al. 2015), malachite green by wild mushroom of Chhatisgrah (Yogita et al. 2011) and textile azo dyes by using fungi (Dharshini & Sumathy 2014). Additionally, the use of mushroom in removal of dyes is able to mitigate the huge volume of unnecessary wastage particularly from a large-scale of mushroom production, notably not grown well or expired mushrooms (Grimm & Wösten 2018, Nicolcioiu et al. 2016). Hence, there is a necessity to explore the potential of expired King oyster mushroom as an alternative material for the removal of dye solution via absorption technique.

The King oyster mushroom or scientific name is *Pleurotus eryngii* is an edible mushroom from the family of pleurotaceae (Fu & Liu 2016). This fungus can be found on soil, open areas, cultivate lands and wood. King oyster mushroom is developed around the world, particularly in Southeast Asia, India and Europe. *Pleurotus eryngii* has ligninolytic enzymes (Patil et al. 2011) system that has been greatly investigated for innumerable applications such as transformation of edible biomass into agro waste, which could be used for animal feed, food, or a source of drugs as

well as for the biodegradation of organic pollutants and different industrial contaminants (Akpınar & Urek 2012).

The objectives of the present study are to compare the optimum parameters for the removal of Bromophenol Blue and Congo Red using expired King oyster mushroom via absorption technique in order to create an eco-friendly environment.

Materials & Methods

Sample preparation

The expired King oyster mushroom, which were provided by HeveaGro Sdn. Bhd. in Gemas, Negeri Sembilan were sliced into two. Then, the sliced mushrooms were dried at 60°C for 24 hours to remove the moisture content. Fig. 2 illustrates the fresh and dried King oyster mushroom from HeveaGro Sdn. Bhd.

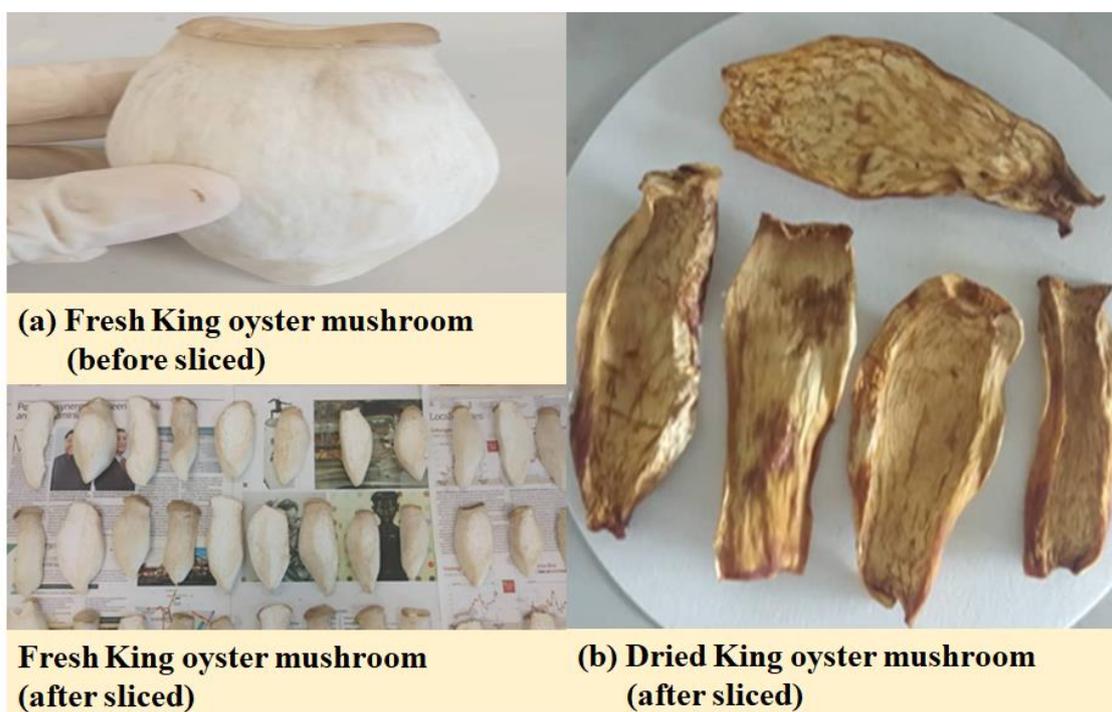


Fig. 2 – King oyster mushroom. a fresh. b dried from HeveaGro Sdn. Bhd.

Preparation of stock solutions for Bromophenol Blue and Congo Red dye

The stock solution of Bromophenol Blue (1000 mg/L) were prepared by dissolving 1 g of dye, procured from Sigma Aldrich, in 1 liter of distilled water. It was mixed thoroughly until a homogeneous solution was formed. The volumetric flask containing the stock solution was wrapped with aluminium foil to avoid the light. The steps were repeated to prepare the stock solution of Congo Red, purchased from Sigma Aldrich.

Preparation of calibration curve for Bromophenol Blue and Congo Red

A series of diluted standard solution (5, 30, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500 and 550 mg/L) of Bromophenol Blue was prepared from the stock solution. Following this, a series of diluted standard solution (5, 10, 30, 50, 100, 150, 200, 250, 300 and 350 mg/L) of Congo Red was prepared from the stock solution. The test tubes were labelled and filled with 10 ml of diluted standard solutions. Each of the test tube was mixed by using Vortex mixer for 5 seconds before it was measured using the spectrophotometer at wavelength 595 nm and 497 nm for Bromophenol Blue and Congo Red, respectively. The absorbance reading for each set of dye was triplicated.

Water-holding Capacity

A 10 g of non-heated King oyster mushroom was added into a 100 mL of deionized water. The mixture was stirred under room temperature at 150 rpm for 5, 24 and 48 hours. These steps were repeated for heated King oyster mushroom. A bar chart of degree of hydration against contact time was plotted for non-heated and heated King oyster mushroom. Water-holding capacity (Y_H), which is also known as degree of hydration is expressed as the weight of the heat-treated sample (W_T) compared to their fresh weight (W_F) before biodegradation process. The formula for water-holding capacity is as follows (Paudel et al. 2016):

$$Y_H = \frac{W_T}{W_F} \quad \text{Eqn. 1}$$

Absorption Experiments

The absorption of Bromophenol Blue and Congo Red by the expired dried King oyster mushroom were conducted in Erlenmeyer flask. The flask containing the samples at each parameter were withdrawn from the orbital shaker at predetermined time intervals, filtered and the final concentrations of dyes in the supernatant solutions were analysed using the UV-visible spectrophotometer (Model SECOMAM-3904). The mixtures were agitated on the orbital shaker at constant stirring speed of 150 rpm. The absorbance reading for each parameter was triplicated with different batches of King oyster mushrooms. The percentage of dye removal for each parameter was calculated using the following formula as follows (Jeeva et al. 2019):

$$\text{The percentage of dye removal} = \frac{(C_o - C)}{C_o} \times 100 \% \quad \text{Eqn. 2}$$

where C_o is the initial concentration of dye, which is 500 mg/L, and C is the final concentration of the residual.

Effect of contact time

A 10 g of dried King oyster mushroom was added into a 100 mL of 500 mg/L dye solution. The mixture was stirred under room temperature at 150 rpm for 1, 2, 3, 4 and 5 hours. A graph of percentage of dye removal against contact time was plotted.

Effect of pH

A 10 g of dried King oyster mushroom was added into a 100 mL of 500 mg/L dye solution. The mixture was stirred for 5 hours under room temperature at 150 rpm. These steps were repeated for pH 3, 5, 7, 9 and 11. The pH of the dye solution was adjusted by using 0.1 M HCl and 0.1 M NaOH solution. A graph of percentage of dye removal against pH was plotted.

Effect of the amount of King oyster mushroom

A 6 g of dried King oyster mushroom was added into a 100 mL of 500 mg/L dye solution. The mixture was stirred for 5 hours under room temperature at 150 rpm. These steps were repeated with 8, 10 and 12 g of King oyster mushroom. A graph of percentage of dye removal against the amount of King oyster mushroom was plotted.

Results & discussion

Standard curve of Bromophenol Blue and Congo Red dyes

UV-Visible spectrophotometer is an analytical tool to determine the concentration of dye solutions. It was observed that the statistical means of the absorbance reading were proportionate to the concentration level of the Bromophenol Blue and Congo Red. Figs 3, 4 show the calibration curves for Bromophenol Blue and Congo Red, respectively. The regression linear (R^2) values for

Bromophenol Blue and Congo Red were 0.9779 and 0.9998, respectively. These values were close to unity which indicates that the concentration of the dyes increased with the absorbance values.

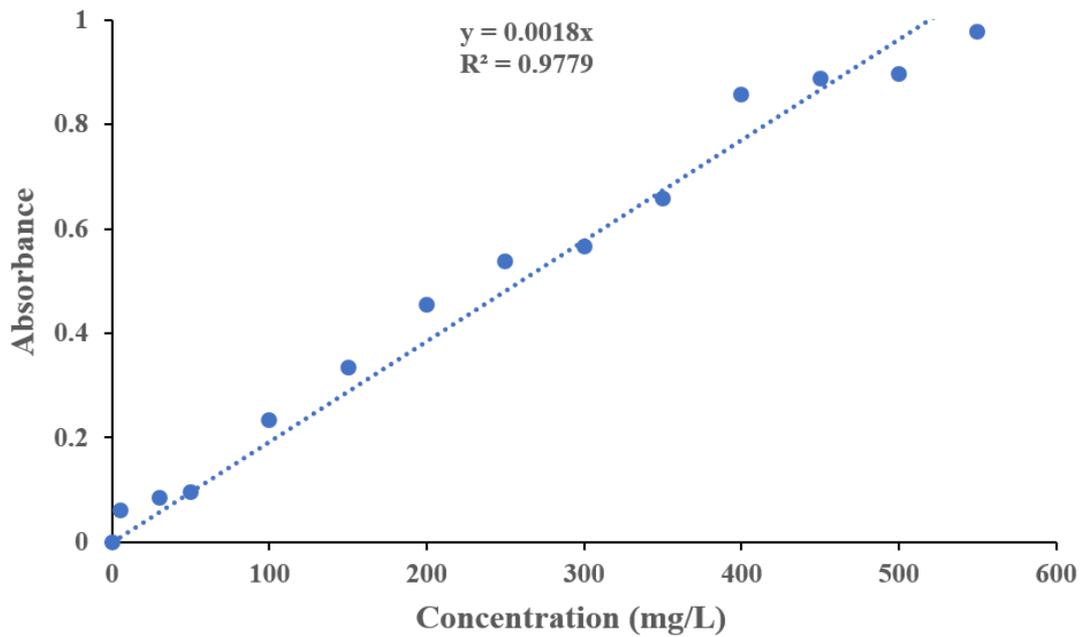


Fig. 3 – Standard curve for Bromophenol Blue at 595 nm.

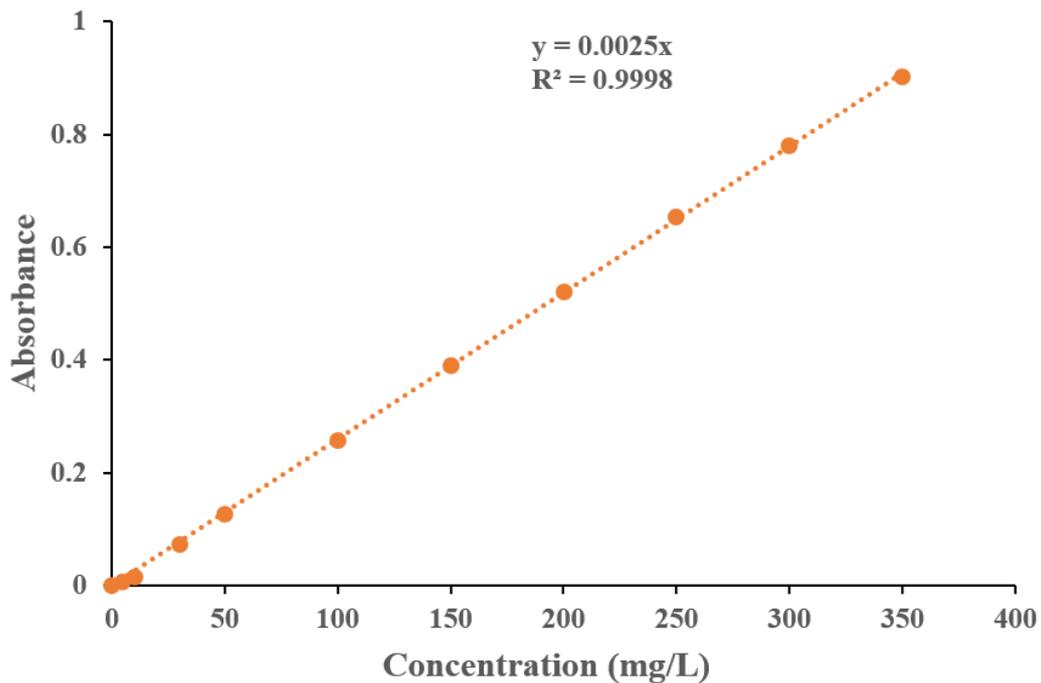


Fig. 4 – Standard curve for Congo Red at 497 nm.

Water-holding capacity

Water-holding capacity was calculated to compare the performance of heated and non-heated King oyster mushroom towards the absorption of dyes. Generally, the pore space in the mushroom naturally well open to the environment and easily filled with water via agitation. The variation in the porosity of mushrooms, that ranges between 0.25 to 0.50, were due to nature and maturation (Paudel et al. 2016). Fig. 5 depicts the degree of hydration by non-heated and heated King oyster mushroom towards the absorption of dyes for 5, 24 and 48 hours. At short time (5 hours), the

heated King oyster mushroom performed better in absorbing water because it achieved 0.4 degree of hydration in comparison to 0.3 degree of hydration attained by non-heated King oyster mushroom. This was because heat vaporizes the water trapped in the pore and hence it absorbed water readily until an equilibrium state was achieved owing to the space created. Subsequently, the degree of hydration for heated King oyster mushroom dropped to 0.3 at 24 hours and it became constant at 48 hours due to saturation. This phenomenon was not observed in the non-heated King oyster mushroom as the degree of hydration increased to 0.4 at 24 hours and then it became constant at 48 hours. It indicates that the non-heated mushroom requires longer time for water to penetrate the pore which was initially contains water. In short, heated King oyster mushroom absorbs the dyes faster than non-heated King oyster mushroom. As a result, the contact time of 5 hours was used consistently in this study for the removal of dyes by heated King oyster mushroom.

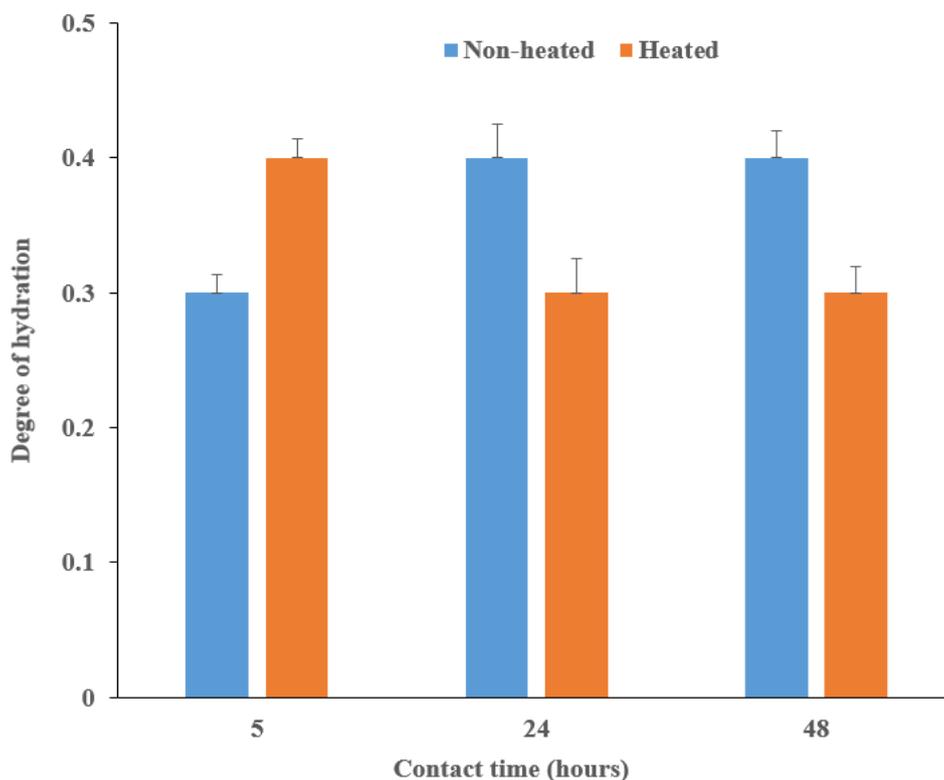


Fig. 5 – Degree of hydration of non-heated and heated King oyster mushroom for 5, 24 and 48 hours.

Effect of contact time

Fig. 6 describes the percentage of dyes removal by King oyster mushroom at various contact time. At lower contact time, King oyster mushroom was more efficient in removing Bromophenol Blue as the percentage of removal in 1 hour and 2 hours were 1.80% and 2.90%, respectively. For Congo Red, the percentage of dye removal in 1 hour and 2 hours were 0.72% and 0.96%, respectively. On the contrary, King oyster mushroom was more efficient in removing Congo Red at long contact time as the percentage of removal at 3, 4 and 5 hours were 4.48%, 12.48% and 15.68%, respectively. On the other hand, the percentage removal for Bromophenol Blue by King oyster mushroom at 3, 4 and 5 hours were 3.8%, 6.7% and 8.6%, respectively. Generally, the percentage removal of both dyes by King oyster mushroom increased with the contact time. This phenomenon could be potentially due to accessibility of unoccupied pores in the mushrooms during the removal process (Maurya et al. 2006). In all, King oyster mushroom removes Congo Red better than Bromophenol blue because the percentage of removal for Congo Red increased exponentially. The increase was not observed in the removal of Bromophenol Blue by the King oyster mushroom

because the unoccupied sites get utilize by the dye molecules that led to create a repulsive force between the dye molecules. (Knapp et al. 1995). Fig. 7 shows the appearance of King oyster mushroom upon removal of (a) Bromophenol Blue and (b) Congo Red after 5 hours.

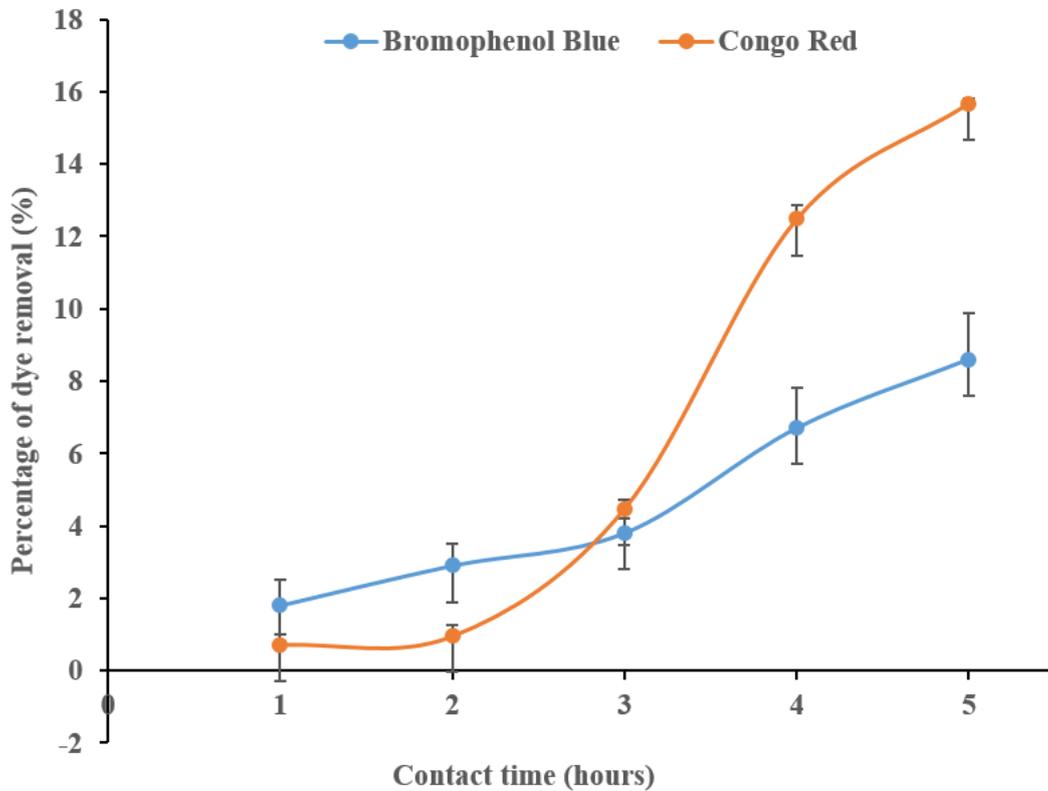


Fig. 6 – The percentage of dye removal by King oyster mushroom at various contact time.

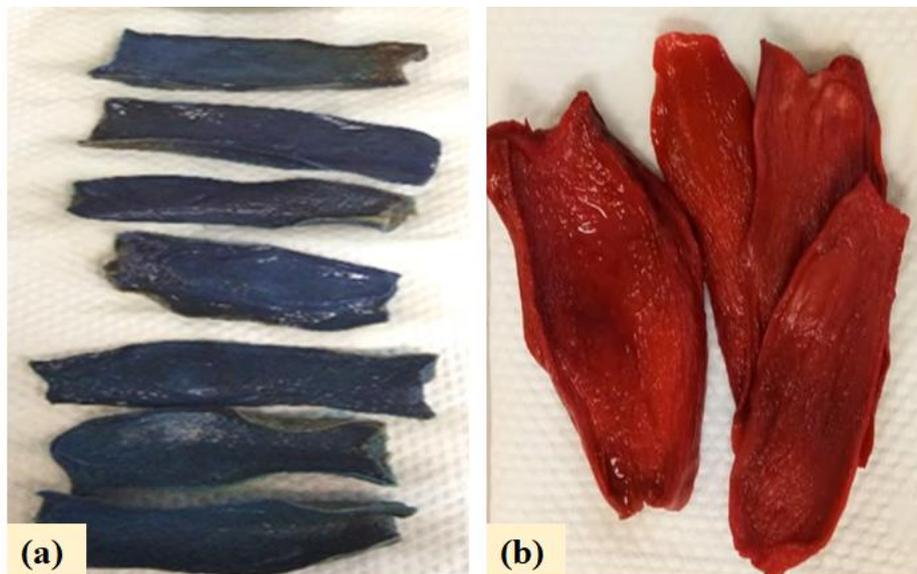


Fig. 7 – The apperance of King oyster mushroom. a Bromophenol Blue. b Congo Red for 5 hours

Effect of pH

The pH of the dye solution plays a significant role as it defines the surface charge of the King oyster mushroom and the ionization of the dye solution (Banerjee & Chattopadhyaya 2017). Also, according to Boumediene et al. (2018), the pH affects the interaction between the King oyster

mushroom and dye molecules. Fig. 8 portrays the percentage removal of dyes by King oyster mushroom at various pH. The King oyster mushroom attained the highest percentage removal of Bromophenol Blue and Congo Red, that were 16% and 55.04% at pH 5 and 3, respectively. At low pH (3 and 5), both anionic dyes created electrostatic attraction with the positively charged acid which resulted in greater attraction due to the functionalized surface which led to the increase penetration of dye molecules into the pores (Antunes et al. 2020, De Sá et al. 2013). However, the removal efficiency decreased at too low pH due to the fast corrosion of King oyster mushroom (Bellettini et al. 2019).

The King oyster mushroom experienced a dropped in the absorption of both dyes at high pH (9 and 11) due to the decrease in the percentage of removal. The percentage removal of Bromophenol Blue at pH 9 and 11 were 3.74% and 1.96%, respectively. On the other hand, the percentage removal of Congo red at pH 9 and 11 were 40.64% and 37.60%, respectively. At high pH, the binding sites of the King oyster mushroom becomes negatively charged which caused repulsion with the anionic dyes used that are Bromophenol Blue and Congo Red. Thus, the penetration of dyes into the pores were less effective (Reddy et al. 2017).

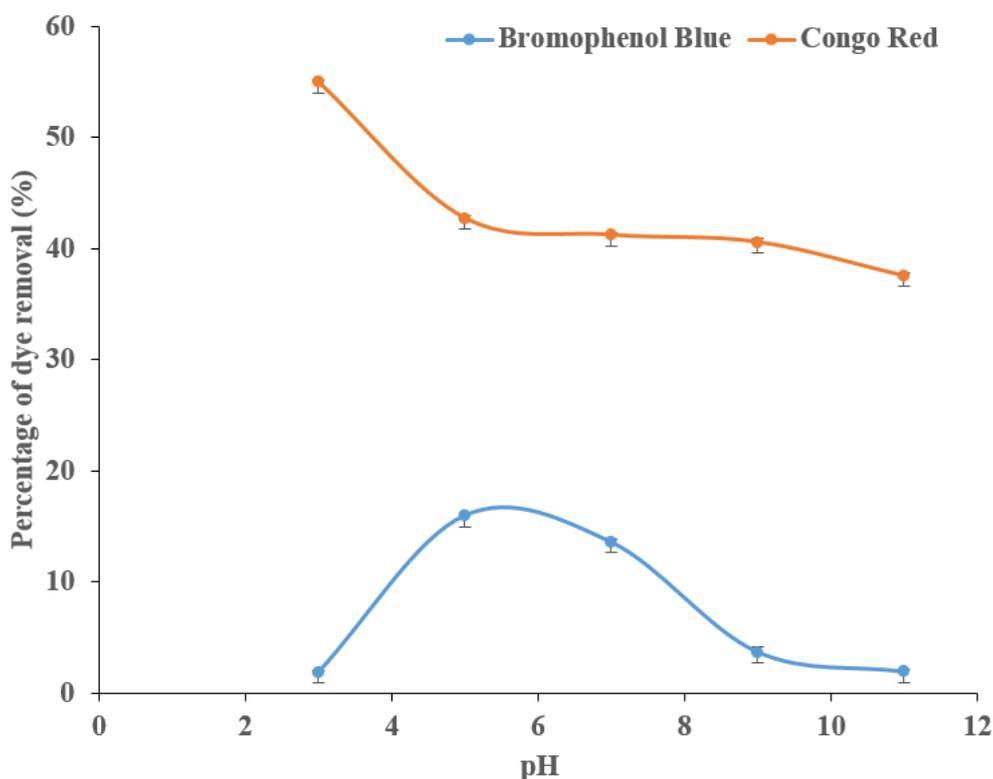


Fig. 8 – The percentage removal of dyes by King oyster mushroom at various pH.

Effect of the amount of King oyster mushroom

The amount of King oyster mushroom used in the removal process is important to describe the absorption capability for a particular dye concentration. Theoretically, the amount of King oyster mushroom increased proportionally with the percentage of dye removal. Fig. 9 shows the percentage of dyes removal at various amount of King oyster mushroom. Both Bromophenol Blue and Congo Red attained the maximum percentage removal of dye that were 31.19% and 56.88% when 10 g and 12 g of King oyster mushroom were used, respectively. At low amount of King oyster mushroom (6 and 8 g), both dyes experienced small percentage of dye removal because provides less pores for the absorption process (Radha et al. 2005). The percentage of Bromophenol Blue removal by 6 g and 8 g of King oyster mushroom were 6.26% and 9.96%, respectively whereas the percentage of Congo Red by 6 g, 8 g and 10 g of King oyster mushroom were 49.6%, 54.08% and 56.88%, respectively. At high amount of King oyster mushroom (12 g), Bromophenol

Blue experienced a dropped of percentage of dye removal. The percentage of Bromophenol Blue removal by 12 g of King oyster mushroom is 25.63% because the passage of dye molecules to the pores of the King oyster mushroom is bounded and thus the absorption process dropped (Nagai et al. 2002). On the contrary, the absorption of Congo Red was experiencing an equilibrium state when 12 g of King oyster mushroom is used, which was due to the saturation in the pore of the King oyster mushroom. The equilibrium state was indicated by the small difference in percentage of dye removal upon addition of 8 g, 10 g and 12 g of King oyster mushroom, respectively.

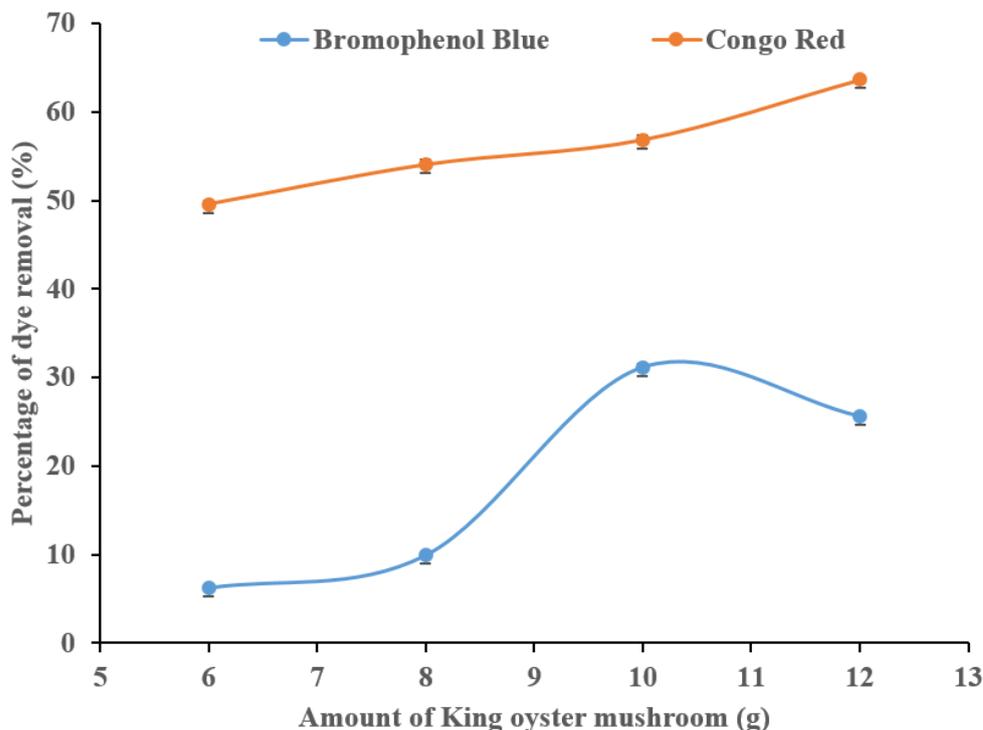


Fig. 9 – The percentage removal of dyes by King oyster mushroom at various amount of King oyster mushroom.

Conclusion

In conclusion, dried King oyster mushroom is used in the removal of Bromophenol Blue and Congo Red dyes because it achieves high degree of hydration of 0.4 at shorter period of time. The optimum parameters for the absorption of Bromophenol Blue are 5 hours, pH 5 and 10 g of King oyster mushroom. On the contrary, the optimum parameters for the absorption of Congo Red are 5 hours, pH 3 and 12 g of King oyster mushroom. It is worth to mention that the Congo Red absorbed more readily than Bromophenol Blue on the King oyster mushroom owing to the size of the Bromophenol Blue which limits its penetration into the pore.

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