Biosorption of Remazol Brilliant Blue R Dye onto Chemically Modified and Unmodified Yarrowia lipolytica Biomass

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Biosorption of Remazol Brilliant Blue R Dye onto Chemically Modified and Unmodified *Yarrowia lipolytica* Biomass

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Abstract

Remazol Brilliant Blue R (RBBR) is a widely used carcinogenic and toxic dye. This study focused on RBBR dye from aqueous solution using potassium permanganate, cetyltrimethylammonium bromide (CTAB) modified, and unmodified *Yarrowia lipolytica* biomass as biosorbent. RBBR dye biosorption studies were carried out as a function of pH, initial dye concentration, biosorbent dose, contact time, and temperature. The pH of the aqueous solution strongly influenced the biosorption percent of RBBR dye. The highest dye biosorption capacity yield was obtained at pH 2-3 as well as above pH 3, very low yield biosorption of RBBR was observed. No differences were found between chemically modified and unmodified biomass in terms of RBBR dye biosorption capacity. In the first 15 min, almost 50% RBBR dye was removed from the solution and reached equilibrium within,180 min at pH 2. Biosorption isotherm obeyed Langmuir isotherm model and pseudo-second-order kinetic model.

Keywords: Biosorption, Remazol brilliant blue R, Dye removal, Biomass

1. Introduction

Thousands of synthetic dyes have been developed and used in industrial applications since the first discovery in 1856 by William Henry Perkin(Jianlong 2002; Saratale and Chang 2011). Dyes are widely used in many industrial applications such as manufacturing, plastics, food processing, printing, and cosmetics. Discharge of dye-containing industrial effluents into aquatic environments causes serious health problems and pollution of water sources. Some dyes have detrimental effects on human health such as dysfunction of the reproductive system, liver, brain, kidney, and central nervous system (Yagub et al. 2014). Remazol Brilliant Blue R (RBBR) also known as Reactive Blue 19 is an anthraquinone dye (Trivedi et al. 2009). Remazol Brilliant Blue R used in the textile industry and as the starting agent for the manufacture of polymeric materials is highly toxic, carcinogenic, and hazardous to aquatic organisms (Mate and Mishra 2020) The chemical structure of the reactive dyes leads to low biodegradability and recalcitrant characteristics. Therefore, it is crucial to treat reactive dyes containing effluents before discharge to surrounding environments (Yagub et al. 2014).

Several physical and chemical methods are used to remove dyes from water bodies such as ozonation, electrochemical oxidation, photocatalytic oxidation, and adsorption (Novotnýeněk et al. 2001; Liu et al. 2015). Adsorption refers to a process in which a gas or substance dissolved in liquid accumulates on the surface of a solid or liquid to form a molecular or atomic film. (El-Naas and Alhaija 2013). Adsorption is an effective method for the removal of heavy metals, dyes, pharmaceuticals, and other toxic substances (Rao and Viraraghavan 2002; Kumar et al. 2006; Fan et al. 2008; Hasan et al. 2012; Jung et al. 2013). Adsorption is a useful technique due to its ease of application, low cost, and no secondary waste generation (Zheng et al. 2021).

The use of activated carbon in adsorption-based removal of organic compounds is the first choice due to its excellent adsorption efficiency, however, the use of commercial activated carbon is limited due to its high cost (Gad and El-Sayed 2009). Consequently, researchers have focused on finding novel and efficient adsorbents. In this regard, previous studies showed that agro-based materials, chitin, peanut hull, algal, and fungal biomass can be used as alternative adsorbents for removing unwanted substances from aqueous solutions (GONG et al. 2005; Luo 2006; Xiong et al. 2010; Mohamed et al. 2015; Ayub et al. 2019). Fungal biomass is used to remove dyes from aqueous solutions (Xiong et al. 2010; Salvi and Chattopadhyay 2016). A significant amount of biomass can be obtained inexpensively from yeasts (Jianlong 2002). *Yarrowia lipolytica* (Fig. 1) is an aerobic, dimorphic yeast is used in various industrial applications such as the production of citric and isocitric acid, proteases, lipases, biosurfactants, and fruit aroma (Gonçalves et al. 2014). We think it is important to demonstrate the use of biomass obtained from this yeast, which has a wide range of industrial applications, in removing environmental pollutants. Therefore, in this study, chemically modified and unmodified yeast biomass was investigated in terms of RBBR biosorption capabilities.

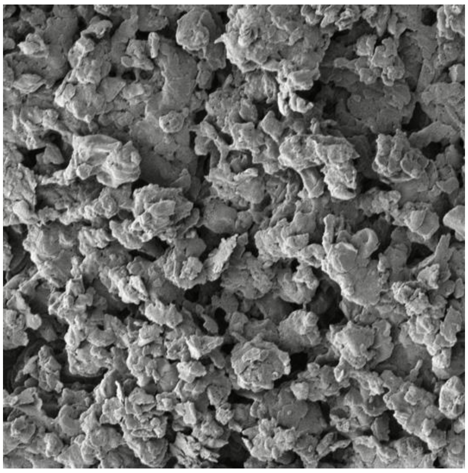

Figure 1. Scanning electron micrograph of the *Yarrowia lipolytica* cell surface

2. Materials and Methods

2.1. Chemicals and microorganism

Remazol Brilliant Blue R (CAS Number: 2580-78-1), cetyltrimethylammonium bromide (synonym: CTAB, CAS Number: 57-09-0), and potassium permanganate (CAS Number: 7722-64-7) were obtained from Sigma-Aldrich. *Yarrowia lipolytica* NBRC 1658 strain was used to obtain biomass. Fungal species were kept on sabroud dextrose agar for up to 20 days at 4 °C in the refrigerator and transferred to a fresh solid media, periodically.

2.2. Production of biomass

Yarrowia lipolytica cells were inoculated into 1000 ml Erlenmeyer flasks containing 500 ml malt extract broth medium and incubated for 2 days at 30°C (pH 4.8, 150 rpm). At the end of the incubation period, biomass was filtrated by filter paper and washed three times with distilled water. After filtration, fungal biomass was autoclaved at 110 °C for 25 min and left to dry for 2 days at 40°C. Dried and heat-inactivated biomass ground into a powder and sieved through a screen (0,15mm).

2.3. Chemical modification methods

CTAB treatment:

Three hundred milligrams of heat-inactivated biomass were added into 25 ml C TAB solution (1.5% w/v) and incubated at 30°C for 24 h (150 rpm) (Huang et al. 2016). After 24h incubation period biomass was separated by filtration, the filtrate was washed with 1 L distilled water. After the washing process modified biomass left to dry 48 h at 40°C and sieved through a 0.15 mm screen.

Potassium permanganate treatment:

Heat-inactivated yeast biomass (300 mg) was oxidized with 10 mM solution of potassium permanganate at 30 °C for 30 min (Luo 2006). At the end of 30 min modified biomass was separated by filter paper, washed with distilled water (1L), and left to dry (48h, 40°C). After that sieved through a 0,15 mm screen.

2. 4 Characterization

The surface morphology of *Yarrowia lipolytica* was monitored with GAIA3 + Oxford XMax 150 EDS scanning electron microscope.

Unmodified biomass and modified biomass samples were characterized spectroscopically by Nicolet IS50 FTIR spectrophotometer (Thermo Scientific). All spectra were recorded between 4000 and 400 cm−1 wavenumber regions.

The change in the surface areas of the obtained biomass and modified biomass was followed with the Tristar II (Micromeritics) model device.

2.5. Biosorption experiments

Unless otherwise stated, 10 mg of biomass modified by different methods and unmodified biomass were placed in 15 ml tubes and treated with 10 ml of dye solution.

The tubes containing the dye solution and adsorbent were shaken horizontally at 150 rpm (25°C). To determine suitable conditions for dye removal, the effect of pH (2-7), amount of adsorbent (250-2500 mg/L), initial dye concentration (25-175 mg/L), temperature(+4-30°C), and contact time were investigated.

The biomasses were separated via a syringe filter and the change in the RBRR concentration was measured with a spectrophotometer (Shimadzu UV-1700) at 592 nm wavelength.

The formulas of the dye RBBR adsorption capacity (q) and removal rate (r) as follows:

$$q = \frac{(C_i - C_f)}{m\ (g)} x V(L) \qquad \textit{eq. (1)}$$

$$r = \frac{(C_i - C_f)}{C_i} \times 100 \qquad \textit{eq. (2)}$$

where C_i is the RBRR concentration before adsorption and C_f is the final RBRR concentration after adsorption, m is the amount of fungal biomass (g) and V is the reaction medium (L).

2.6. Isotherms

In this study, two widespread used adsorption models, i.e., Freundlich and Langmuir isotherms, were employed to describe RBBR adsorption. Isotherm plots were drawn via R studio with a PUPAIM adsorption package.Linearized Langmuir and Freundlich models are expressed with following equations (Freundlich 1907; Langmuir 1918).

$$Langmuir: \; Q_e = Q_{max}K_LC_e/(1+K_L\,C_e) \qquad eq.(3)$$

$$Freundlich: log_{Qe} = logK_F + 1/nlogC_e \qquad eq.(4)$$

Where C_e (mg/L) is the concentration at equilibrium, q_e (mg/g) is the adsorption capacity at equilibrium, K_L (L/g) is the adsorption strength, q_m (mg/g) is the maximum adsorption capacity, K_F is the adsorption coefficient, and 1/n is the adsorption index.

Langmuir isotherm dimensionless constant separation factor (R_L) was used to determine the favorability of the adsorption. The values of dimensionless separation factor indicate the type of adsorption to be favorable ($0 < R_L < 1$), unfavorable ($1 > R_L$) or linear ($R_L = 0$).

$$R_L = 1/(1+K_LC) \qquad eq.\ (5)$$

2.7. Kinetics

The pseudo-first-order and pseudo-second-order kinetic models were tested for the biosorption of Remazol Brilliant Blue R dye on biomass. The pseudo-first-order model was calculated according to the Lagergren equation (Lagergren 1898).

$$ln(q_e - q_t) = ln(qe) - K_1t \qquad eq.\ (6)$$

The pseudo-second order kinetic model that is proposed as (Ho and McKay 1999):

$$t / q_t = 1 / (K_2\,q_e^2) + t / q_e \qquad eq.\ (7)$$

3. Results and discussion

3.1. Characterization

BET surface areas unmodified, CTAB modified, and potassium permanganate modified were found to be 0,3630 m^2/g, 0,3947 m^2/g, 3,5686 m^2/g, respectively. A previous study showed that modification of fungal biomass with CTAB, increases bet surface area 1.5 to 2.03 m^2/g which means CTAB modification caused 26% increase in BET surface area (Huang et al. 2016). . In our study, modification with CTAB increased by only 8%.

FTIR spectra of unmodified (A), CTAB modified (B) and KMNO$_4$(C) modified biomass are shown in Fig. 2. For all sample spectra, the strong broadband ranging from 3600 to 3100 cm $^{-1}$ may cause by the overlap of NH and OH stretching vibrations. The bands between 3000 and 2800 cm^{-1} are the CH stretching vibrations of the –CH3 and >CH2 functional groups of fatty acids found in membrane phospholipids. Peaks at 1744 cm $^{-1}$ (A), 1743 cm $^{-1}$ (B), and 1743 cm $^{-1}$(C) may be attributed to C═O stretching vibrations of lipids. The IR peak at 1715 cm^{-1} of potassium permanganate modified biomass is more intense than unmodified biomass. This peak belongs to the group of carboxylic acids (Jeon et al. 2002). Carboxyl groups of potassium permanganate modified biomass are increased. The sharp peaks at 1646 (A), 1650 (B), and 1652 cm $^{-1}$ (C) can be attributed to C═O stretching vibrations of primary amides. The bands present at 1539 (A), 1550 (B), and 1538 cm $^{-1}$ (C) indicate the presence of secondary amides (Silverstein, RM. Webster X. F. 2005). The peaks at 1394 (A) cm $^{-1}$, 1375cm $^{-1}$(B), and 1378 cm $^{-1}$ may represent –CH$_3$ wagging (Kumar and Min 2011). Stretching vibration peaks of C-N were observed at 1241 cm^{-1}(A), 1250 cm^{-1} (B), and 1242 cm^{-1}(C) (Silverstein, RM. Webster X. F. 2005). The strong peaks at 1026 cm $^{-1}$, 1031 cm $^{-1}$, and 1038 cm $^{-1}$ could be assigned to -CN stretching vibration (Bai and Abraham 2002). Except for the shifting in wavenumbers, no difference was observed between unmodified biomass and CTAB modified biomass

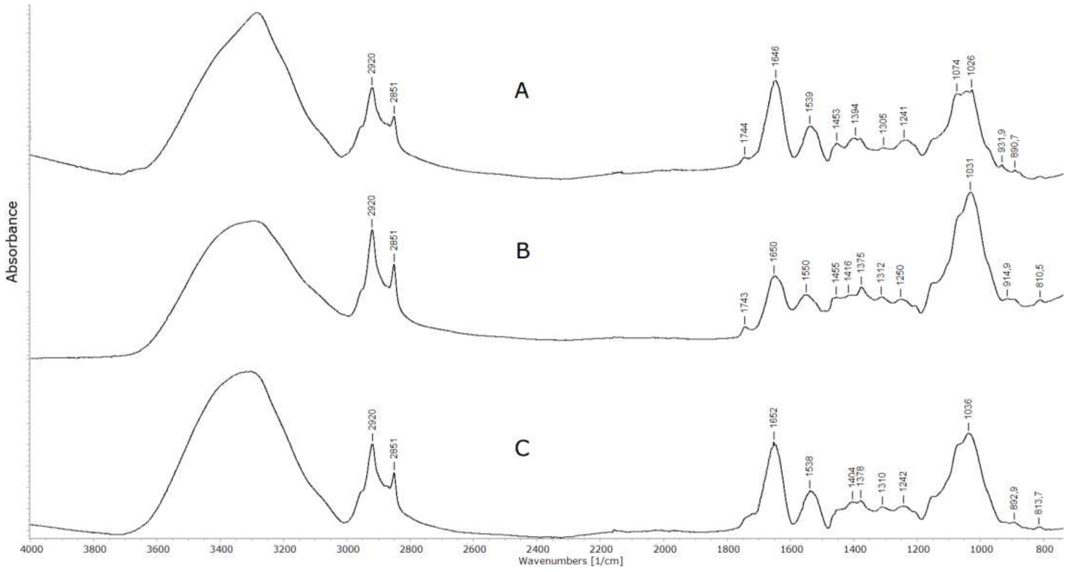

Figure 2. FTIR spectra of Unmodified (A), CTAB modified (B), and KMNO$_4$(C) modified biomass

3.2. Effect of pH and modification methods

The pH value of the medium is one of the important parameters in adsorption. In this study, the effect of pH on adsorption of RBBR was investigated in the range from 2-7 using unmodified, CTAB and potassium permanganate modified yeast biomass. As depicted in Figure 3, all three adsorbents showed maximum Remazol Brilliant Blue R adsorption at pH 2-3 (CTAB modified biomass:103 mg/g, potassium permanganate modified biomass: 102 mg/g, unmodified biomass: 102.89 mg/g. RBBR adsorption capacity of all adsorbents decreased significantly when the pH value increased from 3 to 4.

In water, RBBR dye dissociates as X-SO3Na ⇄ X-SO_3^- +Na^+ (Mate and Mishra 2020).

At low pH values, functional groups on the cell protonated and attain a positive charge (Ergene et al. 2009). As a result, the negatively charged RBBR dye is adsorbed by the positively charged modified and unmodified biomass due to electrostatic interactions. At high pH values, RBBR adsorption has decreased due to excess OH^- ions competing with anionic dye and electrostatic repulsion (Silva et al. 2016).

In this study, where the effect of two different chemical modification methods on RBBR biosorption was examined, it was found that modified and unmodified yeast biomass did not cause a difference in terms of RBBR biosorption at pH 2 and 3 values at which maximum adsorption was observed. Therefore, further studies will be continued with unmodified biomass.

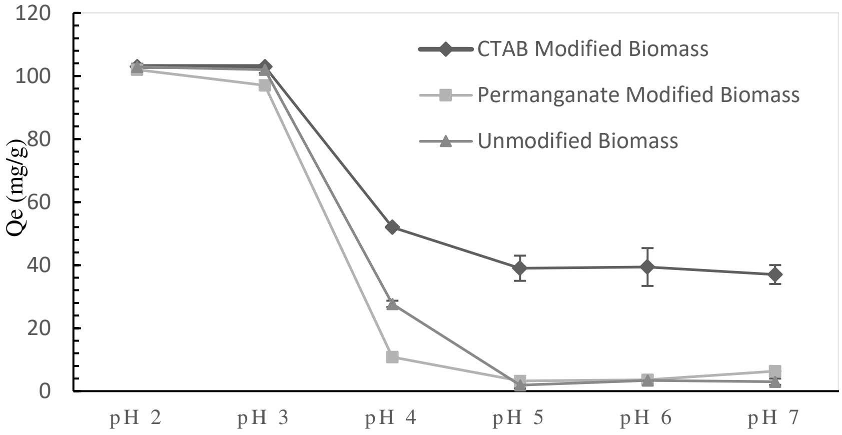

Figure 3. Effect of pH on biosorption of RBBR dye (C_i: 100 mg/ mg/L, 25°C, 150 rpm)

3. 3. Effect of initial dye concentration on adsorption

The effect of initial RBBR dye concentration on biosorption was studied by altering dye concentration from 25 to 175 mg/L. The dye adsorption capacity was increased with increasing dye concentration up to 150 mg/L. The maximum adsorption value (110.96 mg/g) was observed at a concentration of 150 mg/L (Fig. 4). The concentration of RBBR dye at higher than 150 mg/L, adsorption efficiency was decreased. At higher RBBR concentration availability of active adsorption sites decreased, reducing dye adsorption efficiency (Mate and Mishra 2020).

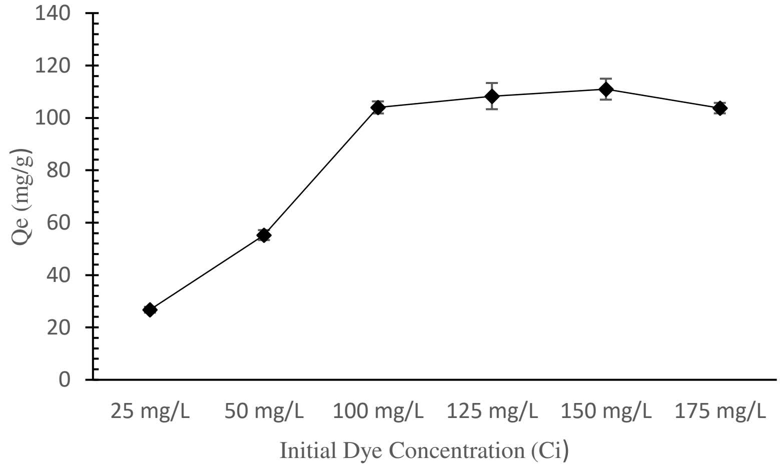

Figure 4. Effect of initial RBBR dye concentration on biosorption (pH:2, 25°C, 150 rpm)

3.4. Effect of biosorbent dosage on adsorption

Dosage of biosorbent is an important variable to determine the effective removal of pollutants. With increasing biomass dosage, the removal efficiency of RBBR dye increases up to a certain limit (Fig. 5). The maximum removal rate, for RBBR dye (99.43%) dye was attained at 1500 mg/L biosorbent dosage. As the biomass dose increases, dye removal increases due to the adsorbent surface area, and active sites to which RBBR dye can bind will increase (Ratnamala et al. 2012).

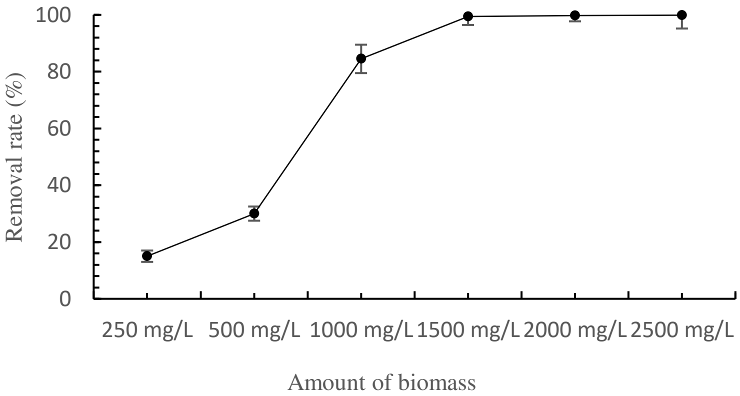

Figure 5. Effect of biomass dose on the removal of RBBR dye (C_i:150 mg/L, pH:2, 25°C,150 rpm)

3.5. Effect of temperature and contact time

Temperature is an important factor affecting the adsorption capacity. If adsorption capacity increases with increasing temperature indicate that the adsorption is endothermic. Conversely, if the adsorption capacity decreases with increasing temperature, the adsorption is exothermic (Yagub et al. 2014). As depicted in figure 6, the biosorption capacity of unmodified biomass increases with the increasing temperature that corresponds to an endothermic process.

The diffusion rate of dye in the inner pores of the adsorbent increases with temperature (Ratnamala et al. 2012). Additionally, at higher temperatures, more RBBR dye molecules have enough energy to interact with the active sites of the adsorbent (Ahmad et al. 2014). In previous studies, adsorption of RBBR dye was defined as an endothermic process (Ada et al. 2009; Mafra et al. 2013).

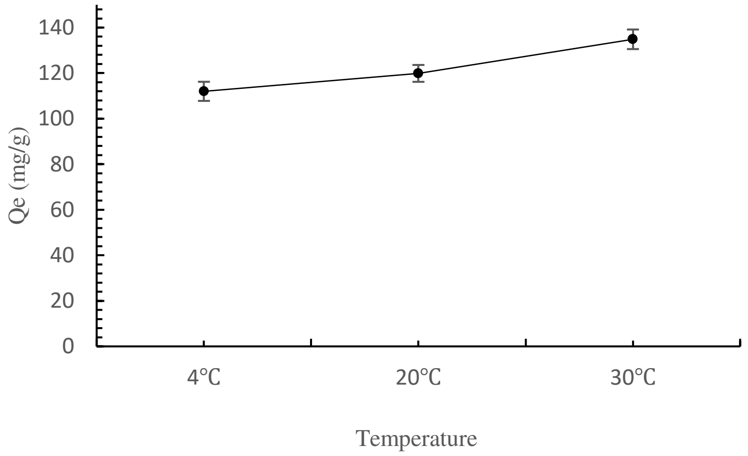

Figure 6. Effect of temperature on biosorption of Remazol Brilliant Blue R (C_i:150 mg/L, pH:2,150 rpm)

When the biosorption of RBBR against time is considered, it is seen that almost 50% of the dye is absorbed within the first 15 minutes (Fig. 7). It is seen that biosorption takes place in two stages: in the first stage, dye molecules are adsorbed rapidly due to the abundant active binding sites of the biosorbent, in the second stage the rate of biosorption decreases, and the removal efficiency decreases.

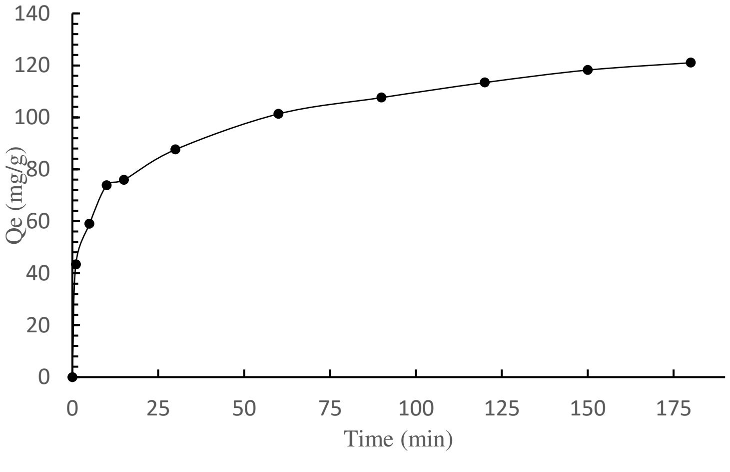

Figure 7. Effect of contact time on biosorption of RBBR dye (C_i:150 mg/L, pH:2,150 rpm)

3.6. Isotherms

The Langmuir isotherm model explained the monolayer and homogenous adsorption with limited active sorption sites on the sorbent. Thus, sorption sites are covered once by adsorbate and no adsorption will occur thereafter (Hussain et al. 2021). As depicted in figure 8, the plot of experimental $1/Q_e$ vs $1/C_e$ fits well linear Langmuir adsorption model ($R^2 = 0.991$ and adjusted $R^2 = 0.989$, $K_L = 0.00027$).

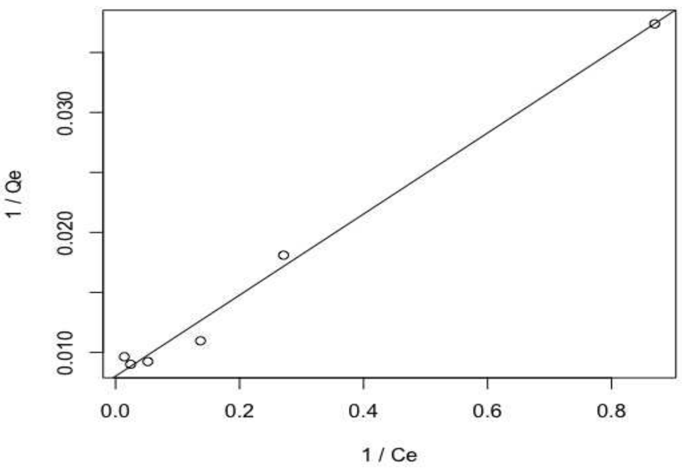

Figure 8. Linearized Langmuir plots of RBBR biosorption (pH:2, 25°C,150 rpm)

The theoretically calculated Qmax value (124.68 mg/g) was found to be higher than the experimental values. R_L values for all initial dye concentrations in the range of 0 <RL <1 which indicates that the adsorption model is favorable for dye (Table 1).

A previous study of zinc oxide powders, Z075 and Z300 showed theoretical maximum adsorption capacity values 38.9 mg/g and 89.3 mg/g, respectively (Ada et al. 2009). Immobilized green algae *Scenedesmus quadricauda* biomass obeyed Langmuir isotherm, as well (Ergene et al. 2009)

Table 1. R_L values of the Langmuir isotherm at different initial dye concentrations at 25°C.

Initial Dye Concentration (C_i)	R_L
25 mg/L	0,993
50 mg/L	0.987
100 mg/L	0.974
125 mg/L	0.967
150 mg/L	0.961
175 mg/L	0.955

The Freundlich isotherm is a commonly used model for adsorption studies. The Freundlich adsorption isotherm is valid for heterogeneous adsorption surface and interaction between adsorbate molecules (Alver and Metin 2012). The experimental data of this study is better for the Langmuir adsorption model than the Freundlich adsorption model (R^2=0.804, Adjusted R^2= 0.752, Fig. 9).

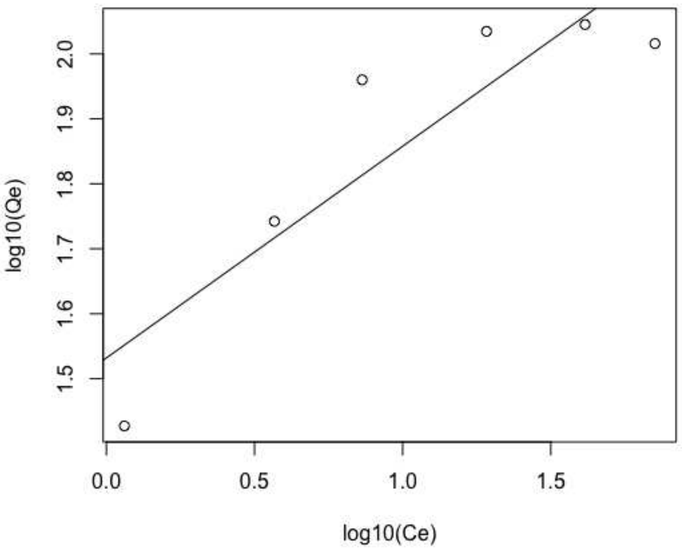

Figure 9. Freundlich isotherm of RBBR dye biosorption

Adsorption of RBBR dye with activated carbon prepared from pinang frond better fit Freundlich adsorption model than Langmuir adsorption model [34]. A previous study reported that adsorption of RBBR obeyed both Langmuir and Freundlich models (Aksu and Dönmez 2003). In this study adsorption RBBR dye onto unmodified *Yarrowia lipolytica* biomass better fit Langmuir isotherm ($R^2 = 0.991$) than Freundlich isotherm (R^2=0.804).

3.7. Kinetics

Kinetic adsorption data of Reactive Brilliant Blue R on biomass were considered using two kinetic models: pseudo-first order and pseudo-second-order. Pseudo-second-order kinetic model gives a higher R^2 *(0.995)* value than the first-order kinetic model *(R^2:0.943)*. The low correlation coefficient value (R2 = 0.8911–0.9478) indicates that the pseudo-first-order kinetic model does not fit the experimental value well. Figure 10 shows the assumed pseudo-second-order model for adsorption of Reactive Brilliant Blue R (RBBR) dye on unmodified biomass with linear regression coefficient value R^2 as 0.995 (slope:0.0082, intercept: 0.0551). A high R^2 value indicates that this kinetic model has a good correlation and the theoretical q_e value is consistent with the experimental *qe* value(Ahmad et al. 2014).

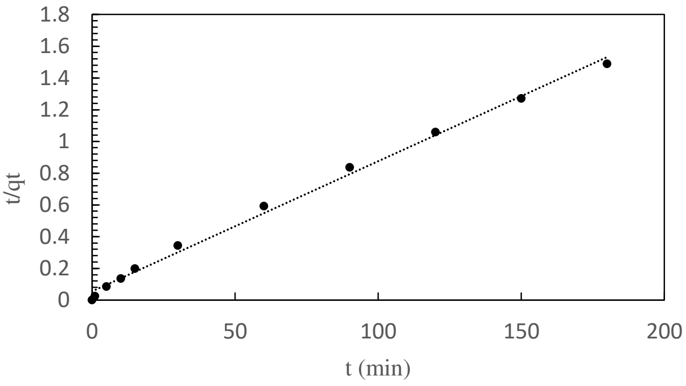

Fig 10. Pseudo-second-order kinetic model for RBBR dye biosorption

The theoretical amount of RBBR that can be adsorbed by unmodified biomass, q_e was calculated as 121.57 mg/g from the slope of the linear line. This value was found as 121 mg/g experimentally. The reaction rate constant for the pseudo-second-order adsorption process was calculated as 1.22×10^{-3} g/mg min. from an intercept.

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