Biosorption of Remazol Brilliant Blue R dye onto chemically modified and unmodified \textit{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 removal using chemically modified and unmodified \textit{Yarrowia lipolytica} biomass. 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 range. It has been found that the adsorption capacity is quite low at higher pH values. 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

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, electro-chemical 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
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. 1a) is an aerobic, dimorphic yeast used in various industrial applications such as the production of citric and isocitric acid, proteases, lipases, biosurfactants, and fruit aroma (Gonçalves et al. 2014). 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. Chemical modification of biomass is a useful technique to enhance biosorption capacity of dyes (Sun et al. 2011). Acid alkali treatment, washing with alcohol, formaldehyde fixation, and CTAB treatment can be given as examples of chemical modification methods used in previous studies (Bai and Abraham 2002; Jianlong 2002; Huang et al. 2016). Therefore, in this study, chemically modified and unmodified yeast biomass was investigated in terms of RBBR biosorption capabilities.

**Materials and methods**

**Chemicals, microorganism and statistical analysis**

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. Statistical analyses were performed with R software.

**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. The fungal biomass was autoclaved at 110 °C for 25 min and left to dry for 2 days at 40 °C. Dried biomass samples were pulverized into a powder and sieved through a screen (0.15 mm).

**Chemical modification methods**

Chemical modification method may use for increasing negative charge of cell surface, hardening cell wall, opening available binding sites and increasing total binding area (Luo 2006; Huang et al. 2016). In this study, potassium permanganate was chosen as the oxidizing agent and CTAB was chosen as the total binding surface enhancer.

**CTAB modification method**

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 24 h incubation period, biomass was separated by filtration, the filtrate was washed with 1 L distilled water. After the
washing process, modified biomass was left to dry 48 h at 40 °C and sieved through a 0.15 mm screen.

**Postassium permanganate modification method**

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 (48 h, 40 °C). After that sieved through a 0.15 mm screen.

**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.

**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 RBRR adsorption capacity (*q*) and removal rate % (*r*) as follows:

\[
q = \frac{(C_i - C_f)}{m(g)} \times V(L),
\]

\[
r = \frac{(C_i - C_f)}{C_i} \times 100,
\]

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).

**Isotherms**

In this study, two widespread used adsorption models, i.e., Freundlich and Langmuir isotherms, were employed to describe RBRR adsorption. Isotherm plots were drawn via R studio with a PUPAIM adsorption package (Saroyda et al. 2020). Linearized Langmuir and Freundlich models are expressed with following equations (Febrianto et al. 2009).

\[
\text{Langmuir} : 1/Q_e = (1/K_L Q_{\text{max}})1/C_e + 1/Q_{\text{max}},
\]

\[
\text{Freundlich} : \log Q_e = \log K_F + \frac{1}{n} \log C_e,
\]

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).

\[
R_L = 1/(1 + K_L C).
\]

**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).

\[
\ln (q_e - q_t) = \ln(qe) - K_1 t.
\]

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

\[
t/q_t = 1/(K_2 q_e^2) + t/q_e.
\]

**Results and discussion**

**Characterization**

Brunauer–Emmett–Teller (BET) surface areas were found to be 0.3630 m²/g for unmodified biomass, 3.9473 m²/g for CTAB modified and 3.5686 for potassium permanganate.
modified biomass. A previous study showed that modification of fungal biomass with CTAB, increases bet surface area 1.5–2.03 m²/g which means CTAB modification caused 26% increase in BET surface area (Huang et al. 2016). In this study, although the CTAB and potassium permanganate modification caused an increase in the surface area, the biosorption capacity did not increase. The fungal cell wall is composed of chitin, proteins, lipids melanin, and polysaccharides with several functional groups (such as, amino, carboxyl, thiol and phosphate groups) capable of adsorbing the dye molecules (Arica and Bayramoğlu 2007). Therefore, if there is no increase in the functional groups to which the dye molecules will bind, an increase in the biosorption capacity may not be observed.

FTIR spectra of unmodified (A), CTAB modified (B) and KMNO₄(C) modified biomass are shown in Fig. 1b. 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, 1375 cm⁻¹ (B), and 1378 cm⁻¹ may represent –CH₃ 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 and Webster 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.

**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 to 7 using unmodified, CTAB and potassium permanganate modified yeast biomass. As depicted in Fig. 2, 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-SO₃⁻ + 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, according to Student’s t test results at pH 3 there were no significant differences found among the modified and unmodified biomass groups (CTAB-Unmodified p value: 0.28, Permanganate-Unmodified p value: 1). Therefore, further studies will be continued with unmodified biomass.
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. 3). 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).

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. 4). 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).

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).
In this study, the biosorption capacity of unmodified biomass increases with the increasing temperature that corresponds to an endothermic process (Online resource 1). 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).

When the biosorption of RBBR against time is considered, it is seen that almost 50% of the dye is absorbed within the first 15 min (Fig. 5). 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.

### 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). The plot of experimental $1/Q_e$ vs $1/C_e$ fits well linear Langmuir adsorption model ($R^2 = 0.991$ and adjusted $R^2 = 0.989$, $K_L = 0.00027$, Online Resource 2).

The theoretically calculated $Q_{\text{max}}$ 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 $0 < R_L < 1$ 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).

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 are better for the Langmuir adsorption model than the Freundlich adsorption model ($R^2 = 0.804$, Adjusted $R^2 = 0.752$, Online Resource 3).

Adsorption of RBBR dye with activated carbon prepared from pinang frond better fit Freundlich adsorption model than Langmuir adsorption model (Saratale and Chang 2011). 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 ($R^2 = 0.804$).

### Kinetics

Kinetic adsorption data of Reactive Brilliant Blue R on biomass were considered using two kinetic models:

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| Initial dye concentration ($C_i$), (mg/L) | $R_L$  |
|------------------------------------------|--------|
| 25                                       | 0.993  |
| 50                                       | 0.987  |
| 100                                      | 0.974  |
| 125                                      | 0.967  |
| 150                                      | 0.961  |
| 175                                      | 0.955  |

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Fig. 5 Effect of contact time on biosorption of RBBR dye (Initial dye conc.:150 mg/L, pH:2, V: 50 mL, biomass dosage: 50 mg, T:25 °C, agitation speed:150 rpm)
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 ($R^2 = 0.891–0.9478$) indicates that the pseudo-first-order kinetic model does not fit the experimental value well. 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, Online Resource 4).

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 $q_e$ value (Ahmad et al. 2014).

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 $1.22 \times 10^{-3}$ g/mg min. from an intercept.

**Conclusion**

In this study, modified and unmodified *Y. lipolytica* biomass was used for the removal of RBBR dye. In terms of dye removal capacity, there are no difference observed chemically modified and unmodified biomass. Batch experiments showed that the initial pH of the solution is the most significant parameter of dye biosorption and the highest RBBR removal occurred at between pH 2 and 3 values. Almost half the amount of dye is removed from the solution within the first 15 min with unmodified biomass. The biosorption of RBBR dye with unmodified biomass follows the Langmuir isotherm model. The kinetics of RBBR dye biosorption onto unmodified biomass fits better to pseudo-second-order model. In conclusion, the results of this research show that unmodified biomass is a good candidate for RBBR dye removal.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s00203-021-02743-3.

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