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Research Article

Evaluation of Food Wastes in *Chlorella vulgaris* Cultivation for Remazol Brilliant Blue R Biosorption

Safiye Büşra Nazlı[1], Nazlıhan Tekin[1], Sevgi Ertuğrul Karatay[1], Gönül Dönmez[1]

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ABSTRACT

The current study demonstrates the biosorption efficiency of *Chlorella vulgaris* for the removal of Remazol Brilliant Blue R (RBBR), which is often used in the textile industry. For this, optimization of microalgal growth was investigated under photoautotrophic conditions including only BG-11 medium and photoheterotrophic conditions containing 0.5 g/L of pumpkin waste, apple pomace, or glucose. Some critical parameters for RBBR biosorption onto dry C. vulgaris biomass, such as pH (2-10), initial concentration of RBBR (100-800 mg/L), biosorbent concentration (1-3 g/L), and biosorption time (0-120 min) were optimized. As a result of the study, the best growth of microalgae was determined as 0.502 g/L under photoheterotrophic cultivation condition, including 0.5 g/L of pumpkin waste sugar. The highest dye removal was calculated as 99.49% in the presence of 3 g/L microalgal biosorbent and 103.38 mg/L RBBR concentration at pH 4. These results indicate that *C. vulgaris* has a promising biosorbent for waste management and dye removal.

Keywords: *Chlorella vulgaris*, agro-industrial waste, dye removal, biosorption, photoheterotrophic growth

ORCID IDs of the author:
S.B.N. 0009-0000-5149-6067;
N.T. 0000-0003-1922-594X;
S.E.K. 0000-0001-9544-0276;
G.D. 000-0001-7972-5570

[1]Ankara University, Science Faculty, Biology Department, Beşevler, Ankara, Turkiye

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Correspondence:
Sevgi Ertuğrul Karatay
E-mail:
sertugrul@ankara.edu.tr

INTRODUCTION

The growing textile industry and the use of synthetic dyes are the primary causes of environmental pollution. Moreover, water pollution caused by dyeing processes in the textile industry is approximately 17-20% (Premaratne et al., 2021). Synthetic dyes, defined as highly polluting, have toxic (cytotoxic, genotoxic, and mutagenic) effects (Verma, 2021). Up to 50% of dyes are used in different areas, such as skin and clothing, do not bind to the cloth's fibers, and are mixed into the aquatic environment as pollutants (Benkhaya, M'rabet & El Harfi, 2020). Therefore, the dyes used in the textile industry threaten the living ecosystem due to them mixing with natural water. Low amounts of dye in water can even significantly affect the photosynthetic activity of plants and toxicity for animals (Verma, 2021). Azo dyes constitute the biggest class (up to 60%) of textile industry dyes. Of these dyes, 15-50% of them are mixed with effluent because they can not remain fixed into the product (Al-Tohamy et al., 2022). For example, Remazol Brilliant Blue R (RBBR), an anionic-azo dye utilized in polymeric material production and the textile industry, is quite dangerous for aquatic organisms due to its low biological disjunction and high toxicity (Aracagök, 2022).

For many years, chemical (electrochemical etc.), physical (coagulation etc.), and biological (enzyme or microorganisms) methods have been utilized to remove these dangerous dyes from natural life (Shabir et al., 2022). Among the methods used, biosorption from biological methods is a cheap, effective, and successful mechanism. Biosorption is a passive process, without the need for energy, in which xenobiotic chemicals from contaminated sources are removed by microbial biomass such as bacteria,

yeast, and algae (Gadd, 2009). Furthermore, the microbial biomass used in biosorption can be active (live) or inactive (dead) character. However, the use of dead microbial biomass is more effective than live biomass because of its features, such as reusability, high surface-to-volume ratio, storage convenience, metabolic independence, economic efficiency, and environmental friendliness (Goud et al., 2020). The biosorption mechanism occurs as a result of the interaction of azo dyes and different functional groups (such as carboxyl, hydroxyl, and amino) on the microbial cell surface. Among microorganisms used, microalgae can live adaptively in an environment contaminated with textile dyes since they are aquatic organisms. Phosphorus and nitrogen compounds in dyes can be used as nutrient sources for microalgae growth (Zohoorian et al., 2020). It is also indicated in the literature that it is a good biosorbent with its high binding affinity and high surface-to-volume ratio for dyes because of its functional groups (carboxyl, sulfhydryl, etc.) on the cell wall surface (Chu & Phang, 2019).

Furthermore, biosorption with microalgal biomass has a low carbon footprint profile due to its photosynthetic properties (Mustafa et al., 2021). Thus, it can be said that using microalgae in industrial dye removal is quite advantageous. *Chlorella vulgaris* is among the microorganisms frequently used in the literature because it has a high affinity for removing heavy metals (Joo, Lee & Choi, 2021) and dyes (Aksu & Tezer, 2005). Even though they typically grow photoautotrophically, microalgae can be cultivated in heterotrophic, mixotrophic, or photoheterotrophic conditions containing a carbon source (Saratale et al., 2022). In addition, in the literature, it is indicated that microalgae growth and yield significantly were supported in photoheterotrophic conditions using organic carbon and a light source. For microalgal growth, industrial and agricultural wastes with rich sugar content are often utilized in photoheterotrophic conditions as a carbon source (Isleten-Hosoglu et al., 2013). Apple pomace (AP) and pumpkin waste (PW) are important by-products of the food industry and are abundant in carbohydrates, protein, lipids, minerals, vitamins, and polyphenols. While pumpkin production is about 23 million tons worldwide, 93,144,358.17 tons of apples were generated in 2021 worldwide (FAOSTAT, 2021; Kidoń & Uwineza, 2022). Therefore, AP and PW are promising carbon sources for microalgae production.

Moreover, the contents and surface structures of microbial cells may be associated with the growth conditions (Joo et al., 2021). In addition, it is emphasized that *C. vulgaris* cells change the efficiency of their contents (macro (protein, carbohydrates, lipids etc.) and micro (phosphorus etc.) compounds) under different growth conditions (Joo et al., 2021). In this context, as a result of the growth of *C. vulgaris* under photoheterotrophic conditions, it can be expected to obtain high biomass and have a more effective cell surface for biosorption.

This work aimed to evaluate the biosorption of anionic RBBR dye onto dead *C. vulgaris* biomass produced in photoheterotrophic conditions in the presence of AP or PW. For this aim, carbon source and its concentrations were optimized for *C. vulgaris* growth. Using microalgal biosorbent cultivated at optimized photoheterotrophic conditions, important parameters such as pH, initial dye concentration, biosorbent concentration, and time were optimized for RBBR biosorption. Thus, the present study aims to suggest an effective, eco-friendly, and cost-efficient biosorbent cultivated with AP or PW sugar in photoheterotrophic conditions for anionic dye biosorption to the literature for the first time.

MATERIAL AND METHODS

Microorganism and its cultivation conditions

C. vulgaris microalgae was supplied from the Ankara University culture collection. Microalgae stock culture was cultivated at 30°C with 2400 lx light in sterile BG-11 medium that contain of 1.5 g $NaNO_3$, 6 mg ferric ammonium citrate, 40 mg $K_2HPO_4.3H_2O$, 6 mg citric acid H_2O, 1 mg $Na_2EDTA{\cdot}2H_2O$, 75 mg $MgSO_4{\cdot}7H_2O$, 2.86 mg H_3BO_3, 20 mg Na_2CO_3, 1.81 mg $MnCl_2{\cdot}4H_2O$, 0.22 mg $ZnSO_4{\cdot}7H_2O$, 0.39 mg $Na_2MoO_4{\cdot}2H_2O$, 0.049 mg $Co(NO_3)_2{\cdot}6H_2O$, 0.08 mg $CuSO_4{\cdot}5H_2O$, and 36 mg $CaCl_2{\cdot}2H_2O$ per liter (Rippka, 1988; Park et al., 2014). The experiments were carried out in photoautotrophic and photoheterotrophic conditions for 10 days. In photoautotrophic conditions, *C. vulgaris* was cultivated in the standard BG-11 medium under constant illumination. For photoheterotrophic condition, microalgae cultivation was carried out in BG-11 medium adding 0.5 g/L glucose, 0.5 g/L AP sugar, or 0.5 g/L PW sugar with constant illumination in the first part of the study. Furthermore, in further experiments, photoheterotrophic cultivation was carried out at different sugar concentrations of PW, the waste from which the highest microalgal biomass was obtained.

Pretreatment of agro-industrial wastes

PW and AP as carbon sources for photoheterotrophic conditions were provided by a local company in Sakarya and Aroma Bursa Fruit Juices and Food Industry, Turkey, respectively. Agro-industrial wastes were dried at 80°C in an oven, ground in a laboratory-type mill (Miprolab, Turkey), and stored at room temperature.

Before experiments, dried and ground wastes were pretreated with 1% H_2SO_4 by dilute acid pretreatment method. The solution was autoclaved for 15 min at 121°C to obtain monomeric sugars (Germec & Turhan, 2018). Hydrolyzates were filtered with Whatman No. 1 filter paper, and the solutions were stored at +4°C.

The effect of initial PW sugar concentration on microalgal growth

Different concentrations of PW sugar (0.25-1 g/L) were investigated for the growth of *C. vulgaris* under photoheterotrophic condition. For this, 10% (v/v) of microalgae culture was added into 250 mL flasks including 100 mL of BG-11 media containing desired PW sugar concentrations and cultivated at 2400 lx and 30°C for 10 days.

Preparation of microalgal biosorbent and stock dye solutions for biosorption

To obtain biosorbent, microalgae culture photoheterotrophically cultivated in the presence of PW sugar was harvested at 5000 rpm for 10 min by centrifugation and dried overnight at 70 °C using an oven. For stock biosorbent solution, 10-30 g of dried microalgal biomass was homogenized in 1L of distilled water with a homogenizer (IKA T18 digital Ultra Turrax) for 45 seconds at 13400 rpm.

To prepare the RBBR stock solution, 20g of dye was dissolved in 1L of distilled water and the solution was diluted to arrive desired RBBR (100-800 mg/L) concentration in experiments.

Biosorption studies

The experiments for biosorption were actualized in 150 mL flasks including 50 mL of RBBR dye solution. The flasks containing desired concentrations of dye and biosorbent were shaken at 100 rpm for 120 min. The samples taken at definite minutes were centrifuged for 4 min at 5000 rpm. After centrifugation, the supernatants were spectrophotometrically measured to determine the dye removal efficiency.

The effect of pH on RBBR biosorption

The pH effect was analyzed in the range of 2 to 10 in the dye solution containing about 100 mg/L of RBBR with 1 g/L microalgal biosorbent. The pH of dye solutions was brought to the determined value with 0.1 M NaOH or 0.1 M H_2SO_4 before mixing the microalgal biosorbent.

The effect of initial RBBR concentration on biosorption

To determine the effect of increasing dye concentrations, biosorption experiments were performed approximately from 100 to 800 mg/L (from 103.38 mg/L to 818.30 mg/L) RBBR at optimum pH value. The flasks containing dye solutions were shaken at 100 rpm for 120 min.

The effect of biosorbent concentration and time on RBBR biosorption

1, 2 and 3 g/L of biosorbent were tested for all dye concentrations at optimum pH value. In addition, during all the biosorption experiments, the samples were taken at 0, 5, 15, 30, and 120 minutes and spectrophotometrically analyzed.

Analytical methods

Reducing sugar concentration in agro-industrial wastes was analyzed according to Dinitrosalicylic acid Method (Miller, 1959) and spectrophotometrically measured at 540 nm using a Shimadzu UV-1201 model UV-VIS spectrophotometer.

Microalgal growth in different conditions was spectrophotometrically analyzed at 600 nm using a Shimadzu UV-1201 model UV-VIS spectrophotometer. Absorbance measurements for the concentration of RBBR in the dye solutions were spectrophotometrically carried out at 590 nm.

The biosorption percentage (%) and removal capacity (q_m) of RBBR were calculated using the equations below (Eq. 1 and Eq. 2) (Gül, 2022):

$$Biosorption(\%) = \frac{Co-Cf}{Co} x100 \quad \text{(Eq.1)}$$

$$q_m(mg/g) = \frac{Co-Cf}{X_m} \quad \text{(Eq.2)}$$

Where C_o shows the initial RBBR concentration in solution (mg/L), C_f definites the final concentration of RBBR at any time (mg/L), and X_m shows the microalgal biosorbent concentration (g/L).

RESULT AND DISCUSSION

Microalgal growth in different cultivation conditions

Macro and microelements such as carbon, protein, mineral, and vitamins are important for microalgae production because they affect microbial metabolic activities. In this sense, agro-industrial wastes are unique sources containing substances required by microalgae. Therefore, the current study determined the effect of different cultivation conditions for the growth of microalgae with BG-11 media adding 0.5 g/L of AP, PW, or glucose. Figure 1 shows the growth of *C. vulgaris* in photoautotrophic (BG-11) and photoheterotrophic (agro-industrial wastes or glucose) condition. As shown in Figure 1, the highest microalgal biomass was 0.502 g/L in medium including 0.5 g/L sugar of PW after 10-days. According to this, the supplementation of PW sugar increased microalgal growth 2.3 times compared with photoautotrophic cultivation. The BG-11 medium containing PW sugar caused higher biomass production than the BG-11 medium containing synthetic glucose. This increase may be due to the fact that the pumpkin waste has compounds that support microbial growth, such as minerals, vitamins, protein and lipid, in addition to its sugar content (Valdez-Arjona & Ramírez-Mella, 2019). While proteins are important sources of nitrogen in cell growth (Wang et al., 2016), vitamins and minerals can participate as coenzymes or cofactors for enzymes involved in microalgal metabolism pathways such as carbon and lipid metabolism (Golub and Voyevoda, 2013). Thus, despite the same sugar concentration, microalgal growth was significantly increased thanks to the rich nutritional content of the pumpkin waste.

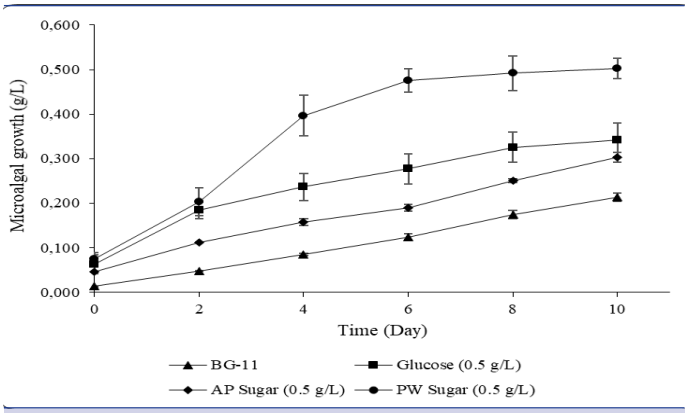

Figure 1. The effects of photoautotrophic and photoheterotrophic conditions on *C. vulgaris* growth (BG-11 media; 2400 lx; 30 °C; pH 7; 10 days).

Moreover, AP sugar supported microalgae production but was less effective than glucose. This situation may be due to the sugar and composition of apple pomace. Furthermore, 0.25 - 1 g/L of PW sugar was investigated in BG-11 Medium to determine the optimum PW sugar concentration and the results were demonstrated in Figure 2. According to the obtained results, when sugar concentration increased from 0.25 to 0.5 g/L, biomass production significantly enhanced and reached from 0.357 g/L to 0.502 g/L. Moreover, 1 g/L PW sugar caused a slight decrease in mi-

croalgal growth. This decline can be explained by the fact that high organic matter content causes a decrease in biomass productivity (Manzoor et al., 2020).

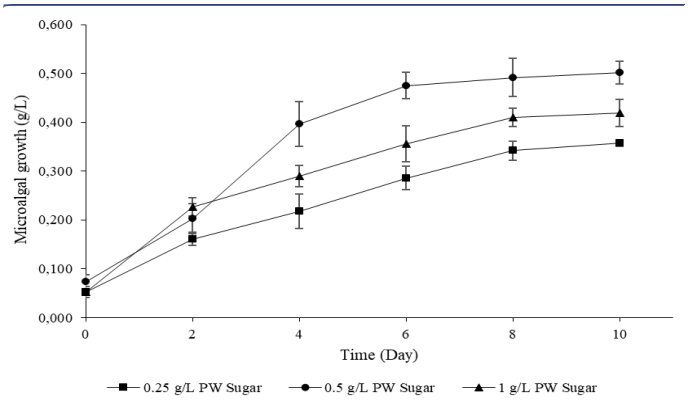

Figure 2. The effects of different PW sugar concentrations on *C. vulgaris* growth (BG-11 medium; 2400 lx; 30 °C; pH 7; 10 days).

In previous studies, the addition of various carbon sources has been shown to increase microalgal biomass production. For instance, Kassim et al. (2022) determined that the highest biomass of *Tetraselmis suecica* and *Halochlorella rubescens* was 0.669 ± 0.01 g/L and 0.653 ± 0.009 g/L, respectively, when molasses were used in photoheterotrophic conditions. Mohammad Mirzaie et al. (2016) observed that under the mixotrophic conditions using cane molasses and corn steep liquor for carbon and nitrogen sources, the dry weight of *C. vulgaris* was 4 and 2.5 times higher than the heterotrophic and autotrophic conditions, respectively. Moreover, adding a carbon source improves microalgae growth and its valuable content, such as carbohydrates, lipids, and protein, for fields such as biofuel production and food supplementation. For example, supplementation of carbon sources in growth of different *Chlorella* species has been stated to increase lipid accumulation for biodiesel production compared to the photoautotrophic condition (Sharma et al., 2016).

Thus, according to the results obtained, for use as a biosorbent in biosorption experiments, *C. vulgaris* biomass was produced under the photoheterotrophic cultivation conditions containing 0.5 g/L PW sugar.

The effect of pH on RBBR biosorption

The pH of the solution is a critical parameter in the biosorption activity of the biosorbent, depending on whether the dyes are anionic or cationic. Therefore, in the current study, the effect of pH on RBBR biosorption was tested in the range of 2-10 in the presence of 1 g/L microalgal biosorbent and about 100 mg/L RBBR. Figure 3 shows that the pH change significantly affected the biosorption of RBBR. For this dye, which has an anionic character, the increase at pH value caused a decrease on biosorption onto *C. vulgaris* biomass, and the highest biosorption percentage was obtained at pH 4 as 93.06% at the end of 120 min (Figure 3). In addition, there was not a significant difference between pH 2 and pH 4. Higher biosorption activity at low pH values for

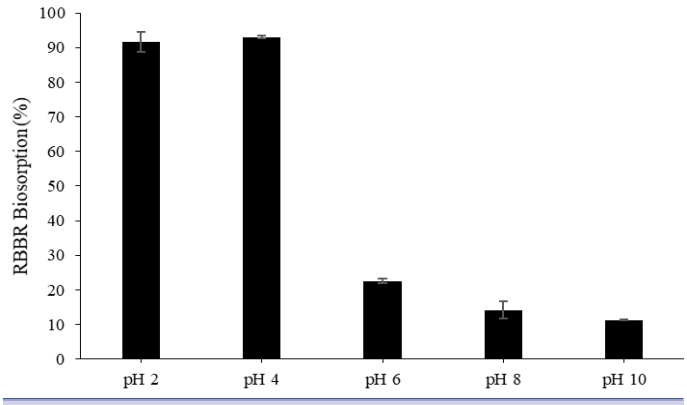

Figure 3. The effect of pH on RBBR biosorption (1 g/L biosorbent, 100 mg/L RBBR, 30 °C; 100 rpm, 120 min).

an anionic dye is due to the ionized dye molecules producing electrostatic charges. When the pH of the solution is low, the solution positively charges and the biosorbent surface also becomes protonated, and the adsorption of negative-charged dye increases (Salleh et al., 2011). In addition, the decrease in biosorption at high pH can be explained by decreasing attraction force between negative-charged cell surfaces and anionic dye molecules with the increase of negative charges in the environment (Yu et al., 2018).

Furthermore, in the studies where *C. vulgaris* was used as a biosorbent, Aksu & Tezer (2005) determined the highest biosorption capacities for Remazol Red RR, Remazol Black B and Remazol Golden Yellow at pH 2 while Kumar, Ahluwalia & Charaya (2019) obtained the maximum biosorption value at pH 5 in dye solution containing 5 ppm Orange G (an anionic dye) and 50 mg biosorbent. Similar effects of pH were shown in other studies using microalgal biosorbent. For example, Khataee, Vafaei & Jannatkhah (2013) used *Spirogyra* sp. biomass as a biosorbent for the removal of acid orange 7. In the study, the highest percentage of dye removal at pH values of 2, 4, 6, 8 and 10 was determined as 42% at pH 4, and a significant decrease in dye removal was observed after pH 4. Gunasundari et al. (2020) investigated the adsorption of Naphthol green-B using *Spirulina platensis* biomass. pH 3 was determined as the best solution pH for biosorption. The dye removal was noticeably decreased when the pH increased from 3 to 7. In another study, the removal of anionic Methyl orange dye using *Chlorella* biomass decreased from 96.3% to 18.7% when pH increased from 2.5 to 11, and the decrease was evident after pH 4 (El Amri, Elkacmi & Boudouch, 2023).

Moreover, in the current study, the biosorption percentage for RBBR sharply decreased when the pH value was increased from 4 to 10. This trend of RBBR biosorption was demonstrated in the study of Ergene et al. (2009) performed with *Scenedesmus quadricauda* biomass, and a significant decrease was determined after pH 4.

The effect of initial RBBR concentration on biosorption

In the present study, the effect of initial dye concentration was investigated approximately from 100 to 800 mg/L (103.38 mg/L -

818.30 mg/L) RBBR concentrations at pH 4 in the presence of 1 g/L biosorbent, and the results were demonstrated in Figure 4. It is clearly seen in Figure 4 that increasing RBBR concentration from 100 to 800 mg/L decreased the biosorption from 93.06% to 16.11% at the end of 120 min. RBBR biosorption efficiency was better for low initial dye concentrations due to the availability of active binding sites on the adsorbent (Aracagök, 2022). Similarly, Hernández-Zamora et al. (2015) observed that biosorption of the anionic azo dye Congo red onto inactive *C. vulgaris* biomass decreased when the initial concentration of Congo red increased from 5 mg/L to 25 mg/L. The authors stated that this is due to the fact that low concentrations of dye molecules in solution interact more easily with the binding sites of biosorbent.

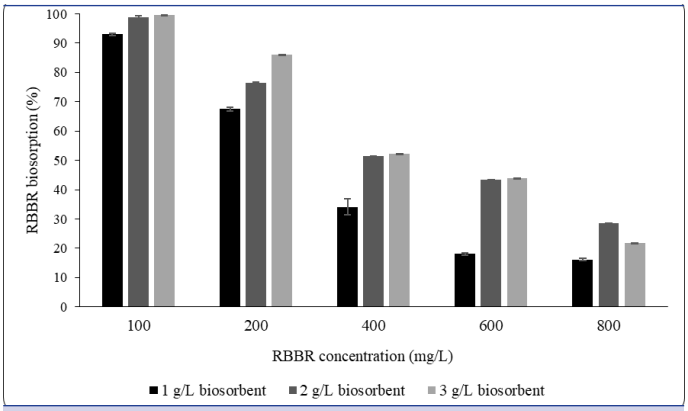

Figure 4. The highest biosorption values obtained with different biosorbent concentrations in increasing initial dye concentrations (pH 4; 30 °C; 100 rpm; biosorption time: the 30th min for 100 and 200 mg/L RBBR, the 120th min for 400, 600 and 800 mg/L RBBR in the presence of 3 g/L biosorbent; the 15th min for 100 mg/L RBBR, 30th for 400 and 800 mg/L RBBR, the 120th min for 200 and 600 mg/L RBBR in the presence of 2 g/L biosorbent; the 0th min for 400 mg/L RBBR, the 30th min 200 mg/L RBBR, the 120th min for 100, 600 and 800 mg/L RBBR in the presence of 1 g/L biosorbent).

In the current study, 600 and 800 mg/L RBBR removals were concluded with similar biosorption values at the end of the process. This situation can be explained by the fact that the amount of fixed dye molecules decreases the attractiveness of biosorbent surface's functional groups (Lai, 2021).

According to results obtained from the current study and literature, it has been seen that effective results can be obtained using *C. vulgaris* as a biosorbent for the biosorption of azo dyes.

The effect of initial biosorbent concentration and time on biosorption

Time and biosorbent concentration parameters for the removal of RBBR were found to be correlated with each other in the current study. The effect of biosorbent concentration (1, 2, and 3 g/L) on the RBBR biosorption is also shown in Figure 4. RBBR removal accelerated when biosorbent concentration in dye solution was risen from 1 to 3 g/L. According to this, the highest RBBR biosorption was 98.91% at 15th min in 100 mg/L dye solution in the presence of 2 g/L biosorbent. Furthermore, for 3 g/L biosorbent concentration, the highest dye removal was calculated as 99.49% at the 30th min while it was similarly examined as 99.18% at the 15th min, and no significant change was observed between these values. Correspondingly with the current study, Radwan et al. (2020) showed that the biosorption rate of the 10 mg/L reactive yellow 145 dye was accelerated when the concentration of *C. vulgaris* biosorbent modified with citric acid increased from 0.1 to 0.5 g/L. For example, while equilibrium was reached at 60 min with 0.5 g/L modified- *C. vulgaris*, the dye uptake process continued for 0.1 g/L modified- *C. vulgaris*. The authors also stated that this increase is due to the increase in active binding sites on surface with increasing biosorbent concentration.

In addition, for 200 mg/L initial RBBR concentration, an increase from 1 to 3 g/L in biosorbent concentration reduced biosorption time up to the 15th minute and the biosorption percentage ranged from 67.45% to 85.88%. However, at dye concentrations after 200 mg/L, removal percentages were slightly decreased at the end of 30 min when biosorbent concentration increased from 2 to 3 g/L. This decline can be clarified by the crowding of the biosorbent and by releasing some dye molecules from the biosorbent surface (Mohd Khori et al., 2018).

Behl et al. (2019) examined the range of 0.25-1.5 g/L of *C. pyrenoidosa* biomass in Direct Red 31 removal. As a result of the study, no increase in dye removal efficiency was observed at concentrations after 1 g/L biosorbent. Furthermore, Revathi et al. (2017) demonstrated that moderate *C. vulgaris* concentration is more effective than higher cell concentrations.

According to the results of the present study, a significant increase in RBBR biosorption was observed at biosorbent concentrations above 1 g/L. At low RBBR concentrations (about 100 and 200 mg/L), the most effective biosorbent concentration was detected as 3 g/L *C. vulgaris* biomass. In addition, although there is no significant difference between 2 and 3 g/L biosorbent concentrations for approximately 400 and 600 mg/L RBBR concentrations, the best biosorption of 800 mg/L RBBR was observed in the presence of 2 g/L biosorbent. The decrease for 800 mg/L RBBR biosorption percentage in the solution containing 3 g/L biosorbent concentration may be due to the crowding of the biosorbent particles, which declined the number of active binding sites on the surface for adsorption, and the adsorption sites overlapped. This causes a decrease in the dye biosorption percentage (Mohd Khori et al., 2018).

Biosorption capacity of microalgal biosorbent on biosorption

Table 1 shows q_m values at the 15th min on RBBR biosorption in the presence of increasing biosorbent concentrations and increasing RBBR dye concentrations. As seen in Table 1, maximum q_m values at the 15th min were obtained in 200 mg/L RBBR for 1 g/L microalgal biosorbent and in 600 mg/L RBBR for 2 and 3 g/L microalgal biosorbent. When the q_m values for the RBBR concentrations of about 100, 200, and 400 mg/L were evaluated, a de-

Table 1. q_m values at 15th min on RBBR biosorption in the presence of increasing biosorbent concentrations and increasing RBBR dye concentrations (30 °C; 100 rpm; pH 4)

100		Initial RBBR dye concentration (mg/L)				
		100	200	400	600	800
Biosorbent concentration (g/L)	1	90.14±2.72	135.92±0.11	132.92±0.01	81.34±4.37	79.21±5.67
	2	52.42±0.27	68.23±0.01	100.28±0.43	112.47±0.08	102.77±0.01
	3	34.18±0.04	55.97±0.04	65.31±0.04	78.72±1.61	56.31±0.15

crease in the q_m was observed as the concentration of biosorbent increased. The highest q_m values for 100, 200 and 400 mg/L dye concentrations were observed at 1 g/L biosorbent concentration. Furthermore, at 600 and 800 mg/L initial RBBR dye concentrations, the highest q_m value was observed at 2 g/L biosorbent concentration. The lowest q_m value in all dye concentrations was at 3 g/L biosorbent concentration.

Similarly, studies in the literature show that the biosorption capacities of microalgal biosorbents for the removal of dyes decreased by increasing biosorbent concentration. da Rosa et al. (2018) investigated the *C. pyrenoidosa* biosorbent amount effect on the biosorption of rhodamine B at pH 4.5 in the presence of 100 mg/L dye. The biosorption capacities decreased from about 20 mg/g to 3.6 mg/g due to increasing biosorbent concentration from 0.1 to 2.0 g. Seth et al. (2022) determined that the increasing biosorbent concentrations (1-5 g/L) caused the decrease on sorption (mg/g) of 100 mg/L anionic dye Indigo Carmine. The authors also indicated that a decrease in sorption with the increase in biomass concentration is associated with a decrease in the availability of dye molecules per unit of biomass. In addition, the aggregation of biomass particles as another factor can decrease sorption (mg/g) due to the slowing of the intraparticle diffusion of dye molecules. Another study also showed that biosorption capacity decreased from 34.89 mg/g to 9.61 mg/g with increasing concentrations (1-5 g/L) of wet-torrefied *Chlorella* biochar on Congo Red biosorption (Yu et al. 2021). Thus, the results from the current study are consistent with the mentioned studies.

CONCLUSION

In this study, the maximum *C. vulgaris* biomass for RBBR biosorption was obtained as 0.502 g/L under photoheterotrophic cultivation conditions containing 0.5 g/L PW sugar. After optimization of microalgal growth, the highest RBBR biosorption percentage was determined as 99.49% at pH 4 in the presence of 3 g/L biosorbent and 103.38 mg/L initial RBBR concentration. Thus, the current study shows that PW for microalgae production and *C. vulgaris* for RBBR removal are potent, eco-friendly, and cost-effectively materials.

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