Biosorption, Isotherm and Kinetic Properties of Common Textile Dye by *Phormidium animale*

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ABSTRACT

In this study, the potential textile dye biosorption capacity of a low-cost biosorbent (*P. animale*) was tested as functions of pH, biosorbent type, initial dye concentration, temperature, contact time and biosorbent dosage at batch scale level. The optimal conditions are 2, dried biosorbent, 93.16 mg/L, 45 °C, 1440 minutes and 4 g/L for pH, biosorbent type, initial dye concentration, temperature, contact time and adsorbent dosage, respectively. Dried *P. animale* removed 99.66 % of Remazol Black B (RBB). The isotherm and kinetic models were analyzed for biosorption mechanism and characteristic. According to the results, Langmuir isotherm and pseudo second order kinetic models were compatible with the experimental data obtained for RBB biosorption on algal biosorbent. Also FTIR and elemental analysis were done and resulted that the functional groups on the surface of algae had significant role in biosorption process. The results of this study supported that *P. animale* is an effective, inexpensive and eco-friendly biosorbent for treatment of textile dye wastewater.

Keywords: Biosorption; *Phormidium animale*; Remazol Black B; Langmuir isotherm; Freundlich isotherm

1. Introduction

Textile industry wastewater is still an important environmental problem today due to the high amounts of toxic and carcinogenic dyeing contents. Reactive dyes are extensively used in dyeing industry due to their advantages such as having simple application techniques, brightness color effect and low energy consumption. The chromophore groups of reactive dyes are azo groups and these chromophore groups are combined with different reactive groups as chlorotriazine, vinyl sulfone or trichloropyrimidine (Gul and Donmez, 2014). Azo dyes are very problematic for health and environment because their biotransformation products such as dye precursors or aromatic amines are mutagen and carcinogen for living organisms (Alves de Lima *et al*., 2007; Daneshvar *et al*., 2007). Remazol Black B is a vinyl sulfone type reactive dye and it has azo groups in its molecular structure (Figure1). The chemical property of RBB is given in Table 1.

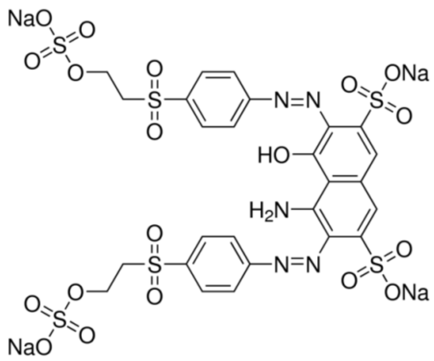

Figure 1. The molecular structure of Remazol Black B (RBB)

Table 1. The chemical properties of Remazol Black B (RBB)

Property	Remazol Black B
Color	Black- Dark Blue
Structure	Powder
Wave Length	593-600 nm
Chromotophore Group	Azo
Water Solubility	High
CI NO	306452
Molecular Weight	991.82
Molecular Formula	$C_{26}H_{21}N_5Na_4O_{19}S_6$

Reactive dyes are highly water soluble (Table 1) and easily disperse to rivers and, then diffuse to surface and ground water. These dyes have resistance to biodegradation and in some situations that biodegradation products have more toxic effects than these dyes. Dyeing wastewater needs to be treated before charging in to the receiving environment. The commonly methods for removal of dyes from textile effluents are listed as precipitation, ozonation, biosorption, membrane separation and electrochemical technology (Gonawala and Mehta, 2014). Biosorption is recommended as an economic, alternative and effective treatment method for textile effluents (Priya and Selvan, 2017). Recent studies are focused on finding suitable biosorbents for efficient biosorption process (Priya and Selvan, 2017; Lacin *et al*., 2015). Most of the recent studies have been interested in the usage of algal biomass as a compatible biosorbent (Omar *et al*., 2018). The aim of this study is to determine the potential usage of *P. animale*, which was isolated from Turkey, as an effective biosorbent. Two types of biosorbents were prepared and tested for removal of Remazol Black B. One of them was dried and the other one was an ash form. *P. animale* is prokaryotic blue green algae and its biomass can be easily and economically produced (Lage *et al.*, 2018) and the results of this study supported that the algal biomass can be used as efficient biosorbent for treatment of textile wastewater.

2. Materials and methods

2.1. Dye solution preparation

Remazol Black B (RBB) dye was obtained from textile factory in Bilecik. Stock dye solution was added in to distilled water as 1000 mg/L and diluted to the desired concentrations in the experiments.

2.2. Microalgal biomass preparation

Phormidium animale (a prokaryotic blue-green algae, also called as cyanobacterium) was isolated from Çanakkale, Turkey (unpublished study). The microalgal biomass was prepared in Gazi University Polatlı Faculty of Arts and Sciences Department of Biology, Research Laboratory. The microalgae were incubated in 100 mL of BG 11 culture media in 250 mL Erlenmayer flasks at 25 °C ± 2 at 48 μmol/m^{2}s (2400 lx) (Rippka, 1988). The pH of the culture media was adjusted with concentrated (1M) and dilutes (0.01M) sulfuric acid / sodium hydroxide solutions. And then, high level of biomass production was conducted in 5 liters of plastic sterile containers containing BG11 medium. The containers were sterilized with dilute sodium hypochlorite and/or ethanol solutions. Aquarium motors, a working capacity of 8W with 2-4 outputs, were used to supply continuous air in the containers. Microalgal biomass was harvested from the media by centrifugation after the end of logarithmic growth phase. Subsequently the biomass was dried at 70 ° C for overnight in aluminum beakers and homogenized biomass was sent to Bilecik Seyh Edebali University to use as biosorbent in biosorption experiments. Two types of biosorbents were used in experiments. One of them was dried as explained before and sieved to use as dried biosorbent. The other one was pyrolised at 500° C for 30 minutes and the sieved ash was used as ash biosorbent.

2.3. Biosorption studies

The biosorption experiments were carried out in 250 ml of Erlenmeyer flasks containing 100 mL distilled water with desired amounts of RBB dye. The effects of pH, contact time, dye concentration, biosorbent dosage and temperature were examined at different pHs (2, 4, 6, 8 and 10), times (0 to 1440 minutes), dye concentrations (65.76-114.13 mg/L), biosorbent dosages (1, 2 and 4 g/L) and temperatures (25, 35 and 45° C), respectively.

2.4. Analitical Methods

RBB dye concentration in the supernatant was determined spectrophotometrically (Labomed INC. 22 model spectrophotometer). In order to find maximum wave length of RBB dye, the dye solution was scanned between 550 to 650 nm. The concentration of RBB was determined by measuring the absorbance at 600 nm (Figure 2), which was the wave length of observing the maximum absorption peak for dye.

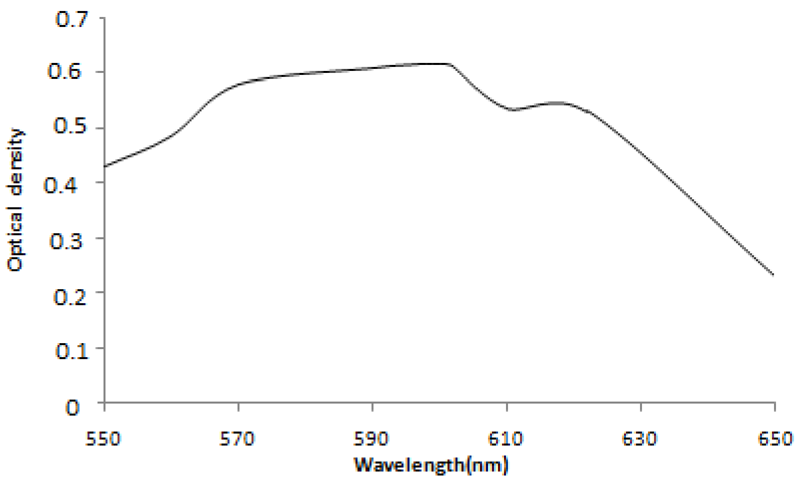

Figure 2. The UV spectrum of RBB

The samples (2 mL) were taken and centrifuged (Hettich EBTA12 model centrifuge) at 4000 rpm for 3 minutes. The supernatant was used for dye analysis.

The percentage of dye biosorption rate was calculated from Equations (1) and (2);

The percentage of dye biosorption rate (B %) = $(C_o – C_f) /C_o \times 100$ Eq. (1)

The uptake of dye by unit mass of biosorbent at any time (q_m: mg/g) was determined from

$q_m = C_o - C_f / X_m$ Eq. (2)

C_o: the initial dye concentration (mg/L)

C_f : final dye concentration at any time (mg/L)

X_m : the sorbent concentration (g/L)

2.5. Biosorption isotherms and kinetics

The data of most effective biosorbent (dried *P. animale*) was calculated for determining the most compatible biosorption isotherm and kinetic in this study. The experimental data were applied in isotherms models to determine the most extensively used biosorption isotherms called Langmuir and Freundlich. The biosorption percentages of dye were calculated from Equations (3) and (4) for these isotherms models, respectively.

Langmuir Isotherm Equation: $C_e/q_e = (1/q_m)C_e + 1/K_L q_m$ Eq. (3)

Freundlich Isotherm Equation: $\ln(q_e) = \ln(K_F) + 1/n \ln(C_e)$ Eq. (4)

q_m: the maximum capacity of adsorption (mg/g)

K_L: Langmuir isotherm constant (L/mg)

K_F: Freundlich isotherm constant (L/mg)

In order to examine the suitable biosorption kinetic for RBB biosorption on algal biosorbent, pseudo-first-order (Equation 5) and pseudo-second order (Equation 6) kinetic models were calculated.

Pseudo first-order kinetic model equation: $\log (q_e - q_t) = -k_1/2.303t + \log q_e$ Eq. (5)

Pseudo second-order kinetic model equation: $t/q_t = 1/k_2 q_e^2 + 1/q_e.t$ Eq. (6)

q_e: The theoretical (cal)) and

k_1: rate constant of pseudo first-order kinetic model

k_2: rate constant of pseudo second -order kinetic model

2.6. FTIR and elemental analysis

To have more information about RBB biosorption process on dried *P. animale*, the more detailed analysis as FTIR and elemental analysis, before and after biosorption were done.

3. Results and Discussion

The effects of pH, biosorbent type, initial dye concentration, temperature and biosorbent dosage were investigated at batch scale level.

3.1. The effect of pH and biosorbent type

To determine the most effective biosorbent type the RBB biosorption capacities of dried and ash *P. animale* biosorbents were compared. Dried and ash algal biosorbents removed maximum 97.06% and 9.77% of RBB dye, respectively. Dried biosorbent performed 10 times more successful dye biosorption rate than ash one. Dried biosorbent was selected for further experimental series.
The effect of pH was examined at pH values of 2, 4, 6, 8 and 10 with 1 g/L biosorbent dosage at 25°C. As seen on Table 2, the optimal pH for dried *P. animale* was found as 2. The sharp reductions were observed in the percentage of dye biosorption rate by dried *P. animale* from pH values 2 to 10 as 97.06 to 2.83 %, respectively (Table 2). Similarly, another cyanobacterium (*Nostoc linckia* HA 46) Reactive dye biosorption capacity was decreased from pH 2 to 6 (Mona *et al*., 2011). The azo chromophore group of reactive dyes charges negatively and the surface of the algal biosorbent charge as positively in acidic solutions, so electrostatical interactions occurred between dye molecules and algal biosorbent surface (Ozer *et al*., 2005). It was clear that the surface of *P. animale* was charged positive in acidic condition and, then negatively charged dye molecules adsorbed onto the algal surface.

Table 2. The effect of pH and biosorbent type on RBB biosorption (Co: Initial dye concentration; B%: the percentage of dye biosorption rate; Contact time: 1440 min., Biosorbent dosage: 1g/L; Temperature: 25 ˚C)

Biosorbent	Type	pH	C_o (mg/L)	B%
P. animale	Dried	2	92.39	97.06
P. animale	Dried	4	99.35	0.33
P. animale	Dried	6	100.00	0.87
P. animale	Dried	8	100.43	0.11
P. animale	Dried	10	99.89	2.83

3.2. The effect of dye concentration

The effect of dye concentration was investigated at pH 2 and 25 ˚C with 1 g/L biosorbent dosage while the different initial dye concentrations were tested as 65.76, 92.39, 102.83 and 114.13 mg/L. Water consumption and wastewater generation during the dyeing and finishing of textiles can reach 150–350 L per kg of product (Ghaly *et al.*, 2015). Bilinska *et al.* (2016) stated that the final dye concentration of the dye liquor exhausted from textile material was 730 mg/L and the discharged of the real wastewater from industry contained 125 mg/L dye. The dye biosorption studies, published in the literature, were investigated the effect of dye concentration with different range of dye concentrations between 20 and 800 mg/L (Aksu and Tezer, 2006), 100 and 500 mg/L (Mona *et al.*, 2011) and 25 and 2500 mg/L (Maurya *et al.*, 2014). In this study the effect of dye concentration between 65-114 mg/L was examined in order to reach the closest dye concentration of the real dye containing wastewater which was mentioned by Bilinska *et al.* (2016). Aravindhan et al., (2007) showed that initial dye concentrations provide significant power to cope with the mass transfer resistance of dye molecules between aqueous and solid phases. The augmentation of dye concentrations resulted in decreasement of the percentage of dye biosorption rate (Table 3). The similar situation was reported by Marungrueng and Pavasant (2007) for the biosorption of basic yellow dye by *Caulerpa scalpelliformis* and, also stated by Mona *et al.* (2007) for the removal of Reactive Red 198 by *Nostoc linckia* HA 46.

Table 3. The effect of dye concentration on the percentage of dye biosorption rate (Co: Initial dye concentration; Ce: Final dye concentration at equilibrium time; B%: the percentage of dye biosorption rate; Contact time: 1440 min., Biosorbent dosage: 1g/L; Temperature: 25 °C; pH: 2)

Biosorbent	**Type**	**C_o (mg/L)**	**C_e (mg/L)**	**B%**
P. animale	Dried	65.76	0.98	98.51
P. animale	Dried	92.39	2.72	97.06
P. animale	Dried	102.83	62.28	39.43
P. animale	Dried	114.13	78.04	31.62

3.3. The effect of biosorbent dosage

The biosorbent dosage is another significant parameter which influences biosorption properties of biosorbent determining the sorption capacity of the biosorbent. The different biosorbent dosages (1, 2 and 4 g/L) were tested at pH 2 and 25 $^{\circ}$C with 100 mg/L initial dye concentration. The increment of adsorbent dosage increased adsorption rates (Kousha *et al*., 2012). Similarly, in this study maximum percentage of dye biosorption rate occurred with 4 g/L adsorbent dosage as 98.29% (Figure 3). In addition to this, Maurya *et al*. (2014) showed that the percentage of methylene blue dye biosorption rate by algal biomass was increased with the augmentation of biosorbent dosage. Recently, Omar *et al*. (2018) reported that maximum malachite green dye removal rates were increased with the incensement of the amount of 3 algal biomasses called *U. reticulata*, *S. crassifolium* and *G. corticata.* Incensement of the amount of algal biomass caused to raise up the percentage of dye biosorption rate. The incensement of the biosorption rate was explained with the increase of available sorption sites on the biosorbent surface (Saeed *et al*. 2010). Therefore, the continious experiments were carried out with 4 g/L biosorbent dosage. The results of biosorbent dosage experiment were supported by the results of previous dye biosorption studies reported in the literature (Saeed *et al.*, 2010; Kousha *et al.*, 2012; Maurya *et al.*, 2014; Omar *et al.*, 2018).

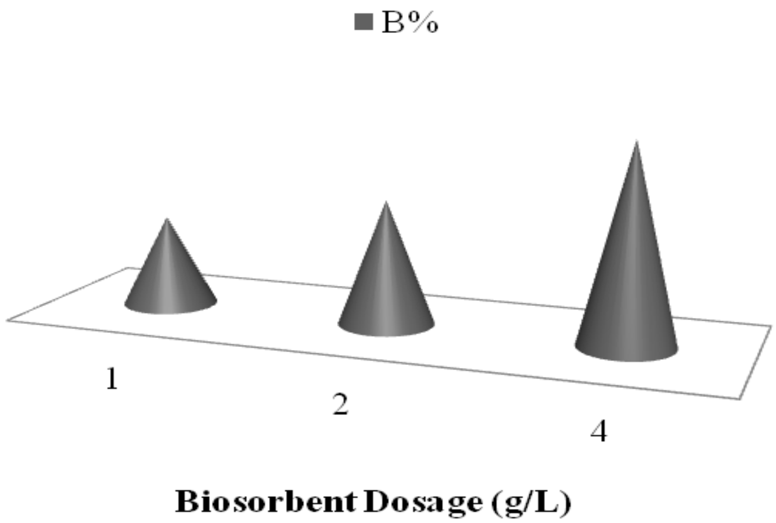

Figure 3. The biosorbent dosage (g/L) of *P. animale* (Contact time: 1440 min., pH 2; Temperature: 25 ◦C; pH:2)

3.4. The effect of temperature and contact time

Temperature is also important parameter for biosorption process that increases the solubility of the adsorbate molecules and causes the augmentation of adsorbate molecules diffusion through the outher sites of the adsorbents (Waranusantigul *et al.*, 2003). The effects of temperature and contact time on dried biosorbent decolorization were investigated in the same experimental series and given in Figure 4. At 25 ˚C the dried biosorbent removed 91.45% of dye after 360 minutes but further times the percentage of dye removal rates was decreased and, then raised up to 98.29% after 1440 minutes of incubation. The maximum percentages of dye biosorption rates were found as 99.29% and 99.65% at 35 and 45˚C, respectively (Figure 4). The similar of this study was reported by Sarioglu and Atay (2006) that the methylene blue dye biosorption on to the biosolid sorbent was increased with the increasing of temperature from 25 to 45 ˚C. At all tested temperatures the percentages of dye biosorption rates were found more than 95% and this situation showed that in

RBB biosorption on dried algal biomass the role of temperature was not significant. However, the maximum percentage of dye biosorption rate was observed at 45 $^{\circ}$C. In another study, Mona *et al.* (2011) reported that increasing temperature positively affected the number of active sites of another algal biosorbent for sorption of dye molecules. The increase in the percentages of the dye biosorption rate was indicated that the biosorption process was endothermic (Sarioglu and Atay, 2006). The optimal temperature was selected as 45 $^{\circ}$C for RBB biosorption by dried *P. animale*.

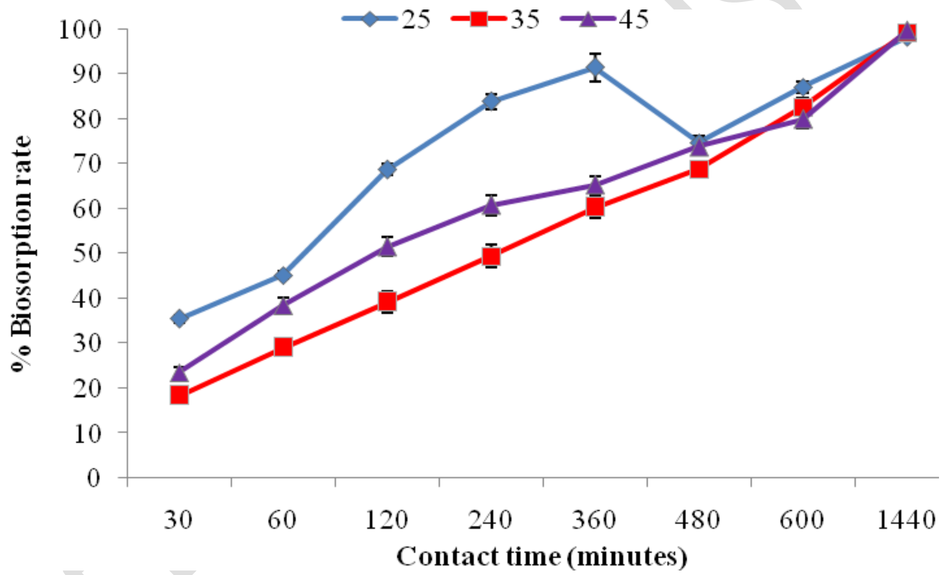

Figure 4. The effect of temperature (25, 35 and 45 $^{\circ}$C) and contact time on the percentage of biosorption rate (pH:2)

The contact time in interaction between adsorbate and adsorbent affects total biosorption process (Maurya *et al.*, 2014). It was reported that biosorption experiments were done for 24 h (1440 minutes) to examine the effect of time on the process (Aksu and Tezer, 2005). Sarioglu and Atay (2006) stated that the sorption of methylene blue dye by biosolid was increased with time.

Similarly, increasing contact time up to optimal time resulted in augmentaion of the biosorption rate, then the active sites on the surface of the algal biosorbent was saturated and kept constant after optimum time (Ibrahim, 2011; Ibrahim *et al.*, 2016). The optimum time was varied according to the biosorbent type (Bilal *et al.*, 2018). For instance, Aksu and Tezer (2005) stated that the optimal time for reactive dye biosorption by *Chlorella vulgaris* was 24 h (1440 minutes). Recently, Maurya *et al.* (2014) showed that the algal biomass originated from *Microspora* sp. performed maximum dye biosorption capacity at optimal time as 24 h (1440 minutes). The similar results were obtained in this study. The reactive dye called RBB uptake capacity of dried *P.animale* is given in Figure 4 at different contact times. Maximum dye uptake capacity was observed as 23.21 mg/g at 1440 minutes. The optimal contact time was determined as 1440 minutes.

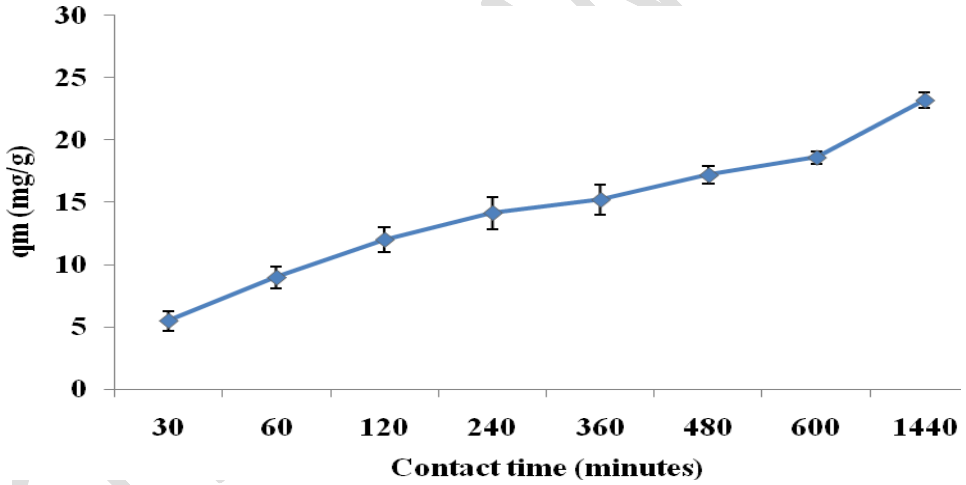

Figure 5. The effect of contact time on biosorption uptake capacity (q_m: mg/, pH:2; Biosorbent dosage: 4 g/L)

3.5. Biosorption isotherms and kinetics

The isotherms and kinetics models for RBB biosorption on the dried *P. animale* biosorbent were applied at optimal conditions. As seen in Table 4, the Langmuir and Freundlich isotherms correlations were calculated as 0.999 and 0.936 for the biosorption, respectively. The biosorption was compatible with Langmuir isotherm model because the correlation value was the highest one. The q_m value indicating the biosorption capacity from the Langmuir constants as 41.667 and the K_L value indicating the biosorption energy, were found as 2.4 L/mg (Table 4). The azo dye biosorption on the surface of a microalgae called *Spirulina platensis* was suitable with Langmuir isotherm model (Dotto *et al*., 2013). The Langmuir isotherm model indicates the homogeneous surface of the biosorbent covering with a single layer.

Table 4. Langmuir and Freundlich constants for RBB biosorption on dried *P. animale*

	Langmuir			Freundlich		
	q_{max} (mg/g)	K_L (l/mg)	R^2	K_F	1/n	R^2
Dried *P. animale*	41.667	2.4	0.999	22.08724	0.128	0.936

The pseudo first order model correlation was calculated as 0.973 which was a high value but on the other hand the difference between experimental qe_{exp} value (23.21 mg/g) calculated qe_{cal}. (13.67 mg/g) was also high. The correlation of the pseudo second order model was high as 0.986 and the experimental and calculated *qe* values were 23.21 and 25 mg/g. The RBB biosorption on the dried *P. animale* was fitted with pseudo second order kinetic model (Table 5). Similarly, the biosorption of RBB on dried *Chlorella vulgaris* was fitted with pseudo second order mode (Aksu and Tezer, 2005). The suitable with pseudo second order kinetic model indicated the occurrence of chemical activation between dried *P. animale* biosorbent and dye molecules.

Table 5 Kinetic parameters for the biosorption of RBB onto dried *P. animale*

	Pseudo first order model				Pseudo second order model			
	qe_{cal}	qe_{exp}	$k_1 \times 10^{-3}$	R^2	qe_{cal}	qe_{exp}	$k_2 \times 10^{-3}$	R^2
Dried *P. animale*	13.67	23.21	4.606	0.973	25	23.21	0.248	0.986

3.6. FTIR and elemental analysis

For having further information about RBB biosorption process, the FTIR and elemental analysis were done. The results of FTIR analysis show that the position of adsorption peaks at 1450 1/cm and 1438 1/cm of the microalgae changed after dye biosorption (Figure 6 a and b), which confirmed the involvement of C-H bonds revealed with aliphatic CH_3 groups in dye removal. Also the peak at 619 cm^{-1} appeared after biosorption (Figure 6 a and b) represented the presence of aromatic rings.

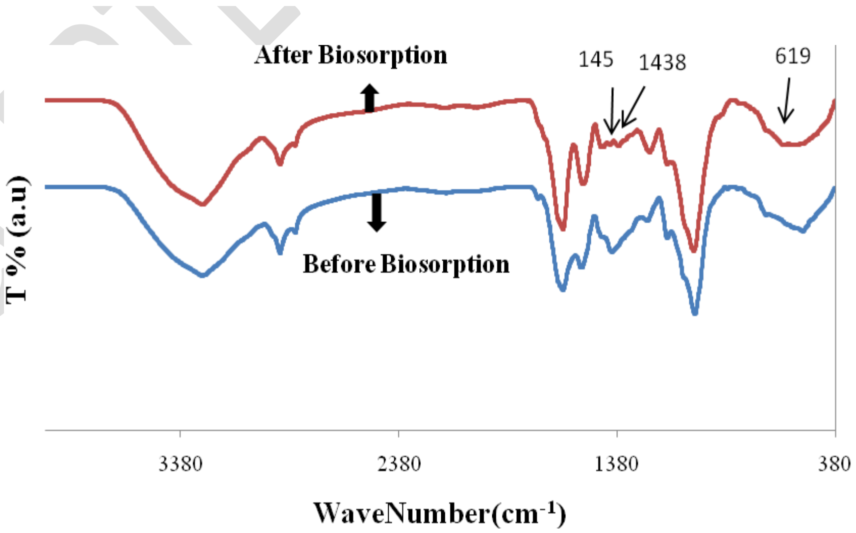

Figure 6. FTIR spectrum of dried *P. animale* before (b) and after (a) RBB dye biosorption

Elemental analysis results for RBB dye and the *P. animale* biomasses before and after biosorption are given in Figure 7. After biosorption of the RBB dye by *Phormidium animale*, in the algal biomass the amounts of Carbon, Hydrogen and Nitrogen were increased while the amount of Oxygen was decreased. The results indicated that oxygen was used during biosorption process.

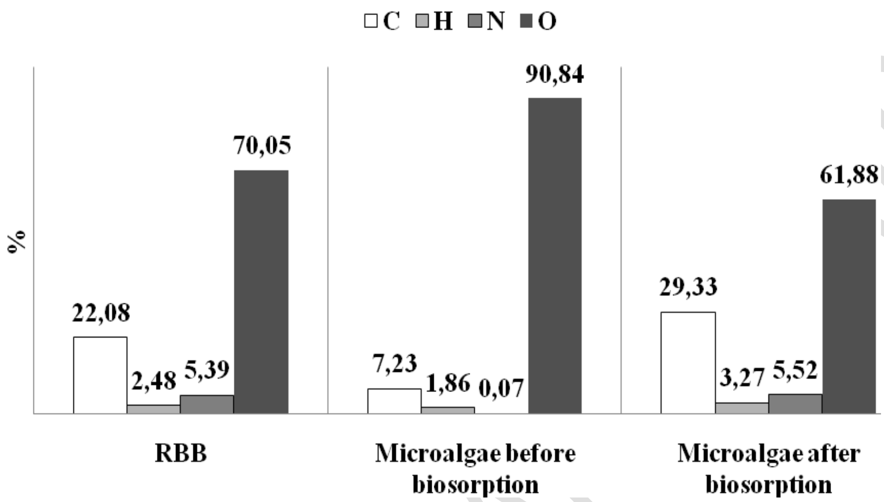

Figure 7. The data of elemental analysis (%) of RBB dye and *P. animale* before and after biosorption

The dye biosorption properties comparison of other algal biosorbents and *P. animale* is given in Table 6. As seen on the Table 6, optimal pH for biosorption was 2 for all anionic dyes and 8 for cationic dye. This is because of the opposite electrical charge of the algal surface and dye molecules. In low pH values the positive charged functional groups on the surface of algal biosorbents interact with negatively charged dye molecules. In alkaline conditions the surface of algae becomes negative charged and the electrostatic attractions occurred with cationic dye molecules. Optimal temperature values were changed as 25, 35 and 45 °C for all algae (Table 6). This study concluded that tested temperature values (25, 35 and 45 °C) had no significant effect on dye biosorption. While comparing other published studies results about algal biosorption, the most successful biosorbent was *P. animale*, which was used in this study (Table 6). According to the recent studies published in literature, algal biosorbents were efficiently and effectively used in variety of biotechnological and industrial applications including treatment of industrial wastewater contained potentialy toxic pollutans such as dyes (Bilal *et al.*, 2017; Centella *et al.*, 2017; Bulgariu and Bulgariu, 2017; Blue *et al.*, 2018). According to the results of this study the algal biosorbent of *P. animale* was a successful biosorbent for removal of reactive textile dyes from aquous solutions. This results indicate that *P. animale* may effectively utilized for the treatment of dye contaminated industrial wastewater.

Table 6. Comparison the dye biosorption percentages and optimal conditions by different algal biosorbents in literature (C_o: initial dye concentration; T: Temperature; B.D: biosorbent dosage; B%: Biosorption percentages)

Algal biomass	Dye	Dye Group	pH	C_o (mg/L)	T (°C)	B.D (g/L)	B%	Reference
Chlorella vulgaris	Remazol Black B	Anionic	2	18.60	35	1	85.20	(Aksu and Tezer, 2006)
Chlorella vulgaris	Remazol Black B	Anionic	2	86.3	35	1	73.00	(Aksu and Tezer, 2006)
Chlorella vulgaris	Remazol Red RR	Anionic	2	75.1	25	1	73.60	(Aksu and Tezer, 2006)
Chlorella vulgaris	Remazol Golden Yellow	Anionic	2	80.6	25	1	43.50	(Aksu and Tezer, 2006)
Nostoc linckia	Remazol Red 198	Anionic	2	100	35	1	94.00	(Mona *et al.*, 2011)
Sargassum crassifolium	Malachite Green	Cationic	8	5	25	2	95.60	(Omar *et al.*, 2018)
P. animale	Remazol Black B	Anionic	2	92.39	25	1	97.06	in this study
P. animale	Remazol Black B	Anionic	2	93.16	45	4	99.66	in this study

Conclusions

The effect of parameters such as pH, biosorbent type, initial dye concentration, temperature, contact time and biosorbent dosage was investigated at batch scale level experiments. The optimal parameters for RBB biosorption by *P. animale* were found as acidic pH conditions (pH 2), 93.16 mg/L initial dye concentration, 45 °C for temperature, 1440 minutes for contact time and 4 g/L adsorbent dosage. The biosorption isotherms and kinetic parameters were also calculated and the biosorption process was compatible with Langmuir isotherm model and pseudo secon order kinetic model. The results of FTIR and elemental analysis showed that the functional groups on the surface of algae had important role in biosorption of dye. Maximum percentage of the dye removal rate was found the highest as 99.66% comparing the results of other studies about dye biosorption by algae. It is concluded that *P. animale* is an effective, inexpensive and eco-friendly candidate for the usage in treatment of textile dye effluents.

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Figure Legends

Figure 1. The molecular structure of Remazol Black B (RBB)

Figure 2. The UV spectrum of RBB

Figure 3. The biosorbent dosage (g/L) of *P. animale* (Contact time: 1440 min., pH 2; Temperature: 25 ◦C; pH:2)

Figure 4. The effect of temperature (25, 35 and 45 ° C) and contact time on the percentage of biosorption (pH:2)

Figure 5. The effect of contact time on biosorption uptake capacity (q_m: mg/g, pH:2; Biosorbent dosage: 4 g/L)

Figure 6. FTIR spectrum of dried *P. animale* before (b) and after (a) RBB dye biosorption

Figure 7. The data of elemental analysis (%) of RBB dye and *P. animale* before and after biosorption