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Biosorption of Methylene Blue from Aqueous Solutions by Iron Oxide-Coated *Cystoseira barbata*

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Abstract: In this study, *Cystoseira barbata* was coated with iron oxide (Fe_3O_4) to obtain a magnetic biomaterial and used as a sorbent material for the removal of methylene blue from aqueous solution. This biosorbent was characterized by Scanning Electron Microscopy (SEM) and Fourier Transform Infrared spectroscopy (FTIR). Methylene blue adsorption capacity of this material was investigated as function of pH, contact time, initial methylene blue concentration and temperature. The equilibrium data was analyzed with Langmuir and Freundlich isotherms. The results showed that the maximum adsorption capacities were achieved in 300 min at pH 2 and reached to 5.74 and 1.08 mg/g at 25 ºC and 45 ºC respectively.

Keywords: Methylene blue, biosorption, *C. barbata,* iron oxide-coating.
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INTRODUCTION

Industrial and environmental pollutants mostly cause water pollution [1] and one of the most important pollutants is coloring agents. The synthetic dyes are in general toxic and can cause serious environmental problems [2, 3]. Dyes are mainly used in textile, plastics, tanneries, pharmaceuticals, leather, paint, and electroplating industries [4]. For example, methylene blue (MB), a common type of colorants, are usually used to dye cotton, wool and other materials [1, 5]. Several methods such as biosorption, chromatography, membrane separation, and ion exchange have been tested for the removal of dyes since they have destructive effect on aquatic life and human body [6-8]. The use of magnetic separation in biosorption process has increased in the last decades [9, 10]. This procedure might be problematic on biomass separation. Magnetic sorbents, following the adsorption procedure, simplify the separation of sample solutions from sorbents and no centrifugation is needed. Therefore, magnetic separation method, which is simple, fast, energy efficient and low cost, was used in the study [11-12].

Many kinds of adsorbents such as fruit peels, potato plant wastes and dried algae have been tested for the removal of the pollutants [13, 14]. In addition, tremendous developments have occurred in nanoparticle technology in the past decade. Magnetic nanoparticles, which generally consist of magnetic elements such as iron, nickel, and cobalt [15], have potential applications in some fields such as biotechnology/biomedicine and environmental remediation [12]. Magnetite (Fe_3O_4) is a common compound used for the synthesis of magnetic nanoparticles [16], especially for that of algae [10]. Algae have been shown to be proper biosorbents because of the functional groups such as amino, carboxyl, hydroxyl, and sulfate on the cell wall [17]. Brown algae are the renewable biomass used in many parts of the world [3]. *Cystoseira barbata*, a brown alga, has air vesicles and the biomass can be used in different areas such as food, medical, and pharmacological applications [18].

The aim of this work is to inquire the potential of iron-coated *C. barbata* for the removal of MB from aqueous solution. The effects of pH, contact time, concentration of MB solution and temperature were investigated on the biosorption of the alga. The data were fit to Langmuir and Freundlich isotherm models. SEM and FTIR analyses were utilized to compare the surfaces of raw and iron-oxide coated *C. barbata*.

MATERIALS AND METHODS

Biomass: Brown alga *Cystoseira barbata* (Stackhouse) C. Agardh was collected from the Dardanos Campus of Çanakkale Onsekiz Mart University. The biomass was rinsed to remove some impurities and dried in an oven at 60 °C until constant weight was reached. Dried biomass was ground and sieved.

Iron Oxide-Coated Biomass

The method proposed by Pokhrel and Viraraghavan (2008) was used for the preparation of magnetic biosorbent. A solution of 80 mL of 2 M $Fe(NO_3)_3.9H_2O$ was prepared and 1 mL of 10 M NaOH was added into this solution and mixed. The autoclaved *C. barbata* (20 g) and the mixture was poured into the porcelain pot, homogenized and kept at 80ºC for 3 h. The oven temperature was then increased to 110 ºC for 24 h. The prepared biosorbent powder was sieved to same particle size. The magnetic *C. barbata* was labeled as "Mag-*C. barbata*" in this study.

Reagents and Equipment

All chemicals used were of analytical grade (Merck). All the solutions were prepared with distilled water. For biosorption experiments, stock methylene blue (MB) solution (1000 mg/L) was used and different concentrations of MB (5, 10, 20, 50, 100, 150, 200, 250, 300, 350, 400, 450 and 500 mg/L) were prepared from stock solution using distilled water. The MB concentration in the solution was measured with a spectrophotometer (Rayleigh Vis-7220 G). The pH of aqueous solutions was adjusted using 0.1 M HCl or 0.1 M NaOH. The samples were filtered through Millipore Millex-HV hydrophilic PVDF 0.45 µm syringe filter. A Wise Bath WSB-30 model shaker was used for the adsorption experiments.

Biosorption Experiments

Batch adsorption technique was used to assess the adsorption of MB from the aqueous solution. Mag-*C. barbata* (100 mg) was put into a 50 mL falcon tube and treated with 10 mL of a MB solution. All the biosorption experiments were carried out using 10 mL aqueous MB solutions. The test solutions were shaken at 250 rpm at room temperature for 60 min, centrifuged at 3000 rpm and the supernatants were filtered through the syringe filter. The adsorbed amount of MB was calculated following the spectrophotometric measurement of supernatant at 665 nm.

Determination of Optimum pH

Five pH values (2, 3, 5, 7 and 9) were tested in the trials. Accordingly, 100 mg dried Mag-*C. barbata* was put into the Falcon tubes filled with 10 mg/L MB solutions at different pH values. The tubes were shaken at room temperature for 60 min at 250 rpm. After adsorption step, sorbents easily came together by means of a magnet kept from outside of the tube and supernatant is simply taken out by a syringe. The absorbance

value of the supernatant was measured with the spectrophotometer and the amount of adsorbed methylene blue was calculated.

The percentage of MB removal (*R*) from the aqueous solution was calculated as follows:

$$\% R = \frac{(C_o - C_s)}{C_o} \text{ x } 100 \quad \text{(Eq. 1)}$$

Where C_o is the initial MB concentration (mg/L) and C_s is the adsorbed MB concentration (mg/L).

Determination of Optimum Contact Time: Mag-*C. barbata* (100 mg) was added into 10 mL of MB (10 mg/L) solution and the pH was adjusted to 2. Falcon tubes were shaken at room temperature for different time intervals (10, 25, 45, 60, 80, 100, 150, 200, 300 and 400 min). Samples were centrifuged, filtered, and the absorbance of the supernatant was measured with the spectrophotometer. The amount of MB uptake, qt (mg/g), at each interval was calculated using the following equation:

$$q_t = \frac{(C_o - C_e)}{M} \text{ x } V \quad \text{(Eq. 2)}$$

Where C_o is the initial MB concentration (mg/L), C_e is the concentration of MB solution at a given time (mg/L), *V* is the volume of metal solution (L) and *M* is the mass of biosorbent (g) (dry weight).

Adsorption Isotherms: A 10 mL MB test solutions (pH 2) were prepared at different concentrations (5, 10, 20, 50, 75, 100, 150, 200 and 300 mg/L) and added into the Falcon tubes each containing 100 mg Mag-*C. barbata*. The test solution was shaken for 200 min at 25ºC and 45ºC. The amount of MB concentration was calculated using Eq. (2). In Eq. (2), q_t is substituted with q_e.

Characterization of Biomass: FTIR spectra were obtained using Perkin-Elmer FTIR Spectrometer (Spectrum BX-II). The biomass was dried at 60°C to the constant weight; 1 mg of *C. barbata* was then pelleted with 100 mg KBr. FTIR analysis was studied in the range 400-4000 cm^{-1} for the characterization of biomass. Morphological features of algal biosorbent particles before and after the adsorption of MB were obtained using the Scanning Electron Microscope (Jeol JSM 7100F) at accelerating voltages of 10 kV attached to an X-ray energy dispersive spectrometer (EDX). Before the scanning process, all samples were dried and coated with gold to enhance electron conductivity. In this study, SEM micrographs were taken at different magnifications.

RESULTS AND DISCUSSION

Determination of Optimum pH

Algal cell surfaces have several functional groups such as amino, carboxyl, and phosphate groups. The biosorption mechanism depends on these functional groups on the surface of the cell wall and the solution pH is important in the process [19]. The effect of pH was studied in the pH range of 2-9 at room temperature. The effect of the pH values on biosorption were shown in Figure 1. It was found that the removal percentage decreased by the increase in pH from 2 to 5, remained stable at pH 5 and 7, and then started to increase again until pH 9. However, the pH was in general not so important on the biosorption of MB by Mag-*C. barbata*. The maximal removal was 40.61% at pH 2. The surface of the algae is negatively charged and the strength of the negative charge is higher on acidic zone. Under normal conditions, the strength of the negative charge of adsorbent decreases in lower pH values, which also results in a reduction on the adsorption potential of positively charged ions. When the non-magnetic algae are treated with iron, an electrostatic interaction takes place between positive (Fe^{2+}) and negative (algal surface) charges. In the case of algae magnetized with iron, however, Van der Waals interaction takes place. In our opinion, the type of the interaction was the main reason as to why the adsorption capacity of the magnetic algae was independent from the pH changes.

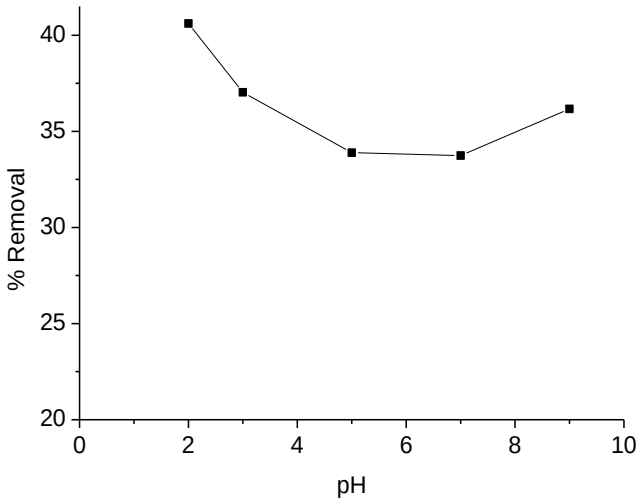

Figure 1. Effect of pH on the biosorption of MB.

Determination of Optimum Contact Time

The effect of contact time for MB biosorption onto Mag-*C.barbata* was studied at two different temperatures (Figure 2). The results showed that the q_t value increased in parallel to the rise in temperature. The adsorption reached to equilibrium within 300 min

at both temperatures. The biosorption capacity became stable at 0.57 and 0.71 mg/g for the temperatures 25 ºC and 45 ºC, respectively.

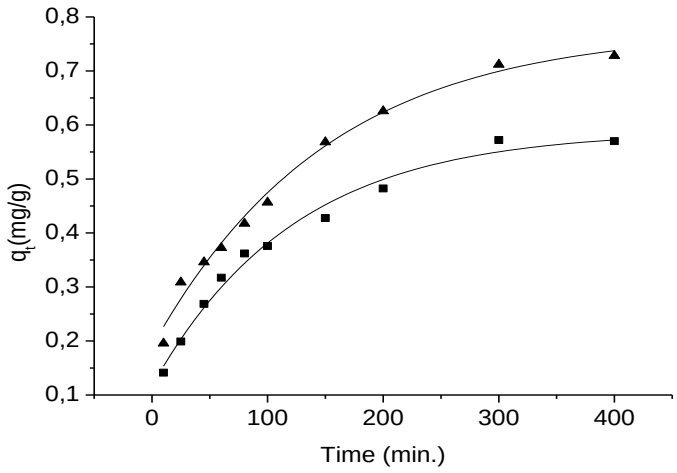

Figure 2. Effect of contact time on the biosorption of MB at 25 ºC (■) and 45 ºC (▲).

Adsorption Isotherms

Several isothermal models are used to determine the relationship between q_e and C_e. In the study, the equilibrium data were analyzed with Langmuir and Freundlich isotherms at different temperatures. The monolayer of the adsorbate on the adsorbent surface was predicted with the Langmuir model while the multi-layer adsorption isotherm, the Freundlich model, was applied to the heterogeneous surfaces. The Langmuir was shown below [20]:

$$\frac{C_e}{q_e} = \frac{1}{q_m a_L} + \frac{C_e}{q_m} \qquad \text{(Eq. 3)}$$

Where q_e is the amount of adsorbent (mg/g), C_e is the equilibrium concentration of the MB solution (mg/L), q_m is the maximum adsorption capacity and a_L is the Langmuir constant related to the energy of adsorption. A linear form of the Freundlich equation was shown below [21]:

$$log\ q_e = log\ K_f + 1/\ n\ log\ C_e \qquad \text{(Eq. 4)}$$

Where K_f (mg/g) is related to adsorption capacity and n is an empirical parameter that varies with degree of heterogeneity. Fig. 3 shows the adsorption isotherm used to characterize the interaction between MB and algal biomass. According to the results, MB amount adsorbed by *C. barbata* increased in higher solution concentrations. The maximum adsorption capacities of *C. barbata* were 5.74 and 1.08 mg/g at 25 ºC and 45 ºC, respectively. In this study, Freundlich isotherm model showed a better fit than the

Langmuir isotherm model at 25 ºC (R_F^2 = 0.989), while Langmuir isotherm model showed a better fit than the Freundlich isotherm model at 45 ºC (R_L^2 = 0.999) (Table 1).

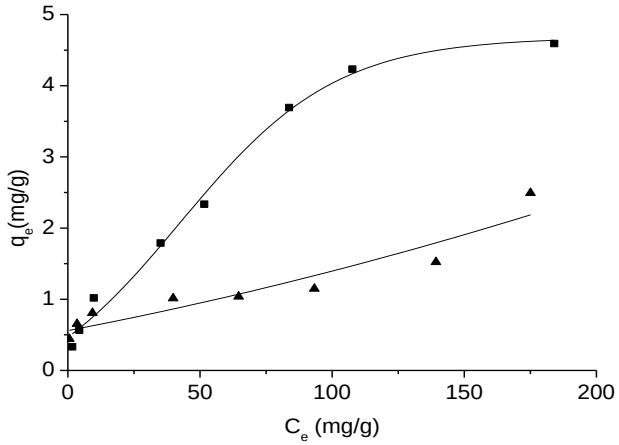

Figure 3. Sorption isothermal curves for biosorption of MB at 25ºC (■) and 45ºC (▲).

Table 1. A comparison of Langmuir and Freundlich isotherm models for MB by Mag-*C.barbata* at different temperature.

	Langmuir isotherm model			Freundlich isotherm model		
Temperature (ºC)	q_m (mg/g)	a_L	R_L^2	n_f	K_f (mg/g)	R_F^2
25	5.74	0.02	0.981	0.26	1.79	0.989
45	1.08	0.08	0.999	0.55	6.30	0.922

Characterization of Biomass

FTIR: FTIR spectroscopy is largely used to characterize the mechanism of binding on algal surfaces with the help of the hydroxyl, carboxylic acid, amine, amino, sulfonyl, and phosphate functional groups found in the structure of algae [8, 22, 23]. The FTIR analysis was carried out to identify the functional groups on unloaded *C. barbata* and Mag-*C. barbata* (Fig. 4). The functional groups of MB adsorbed Mag-*C. barbata* were also examined. It has been observed that Fe_3O_4 was connected to carboxyl groups of biomass since the peak at 2926 cm^{-1} (Figure 4a) was not observed after Fe_3O_4 loading (Fig. 4b). The peaks at 2325 cm^{-1} and 2058 cm^{-1} also disappeared when MB was loaded onto Mag-*C. barbata* (Fig. 4c). In general, the peak areas were changed by both Fe_3O_4 and MB loading. The functional groups of biomass were also given in Table 2. Similar results were also reported for the biosorption of different colorant agency [9, 15, 24-26].

SEM: SEM is used as a useful tool to examine the surface structure of the biosorbent. In this study, the surface microstructures of *C. barbata* samples were analyzed by the SEM technique while the existence of iron was analyzed by the EDX technique. Figure 5 shows SEM images of *C. barbata* (a), Mag-*C. barbata* (b) and MB loaded Mag-*C. barbata* (c). Structure of *C. barbata* indicated the presence of rough flat surfaces. After coating with

iron oxide, the surface of magnetic algae began to shine and the layered structure looked like a sponge (Figure 5b). After MB adsorption, MB possibly attached onto this porous surface (Figure 5c) and the surface of magnetized algae appeared roughed and more porous in comparison to the raw sample. Furthermore, the iron was detected as a signal in the EDX spectrum (Figure 5d).

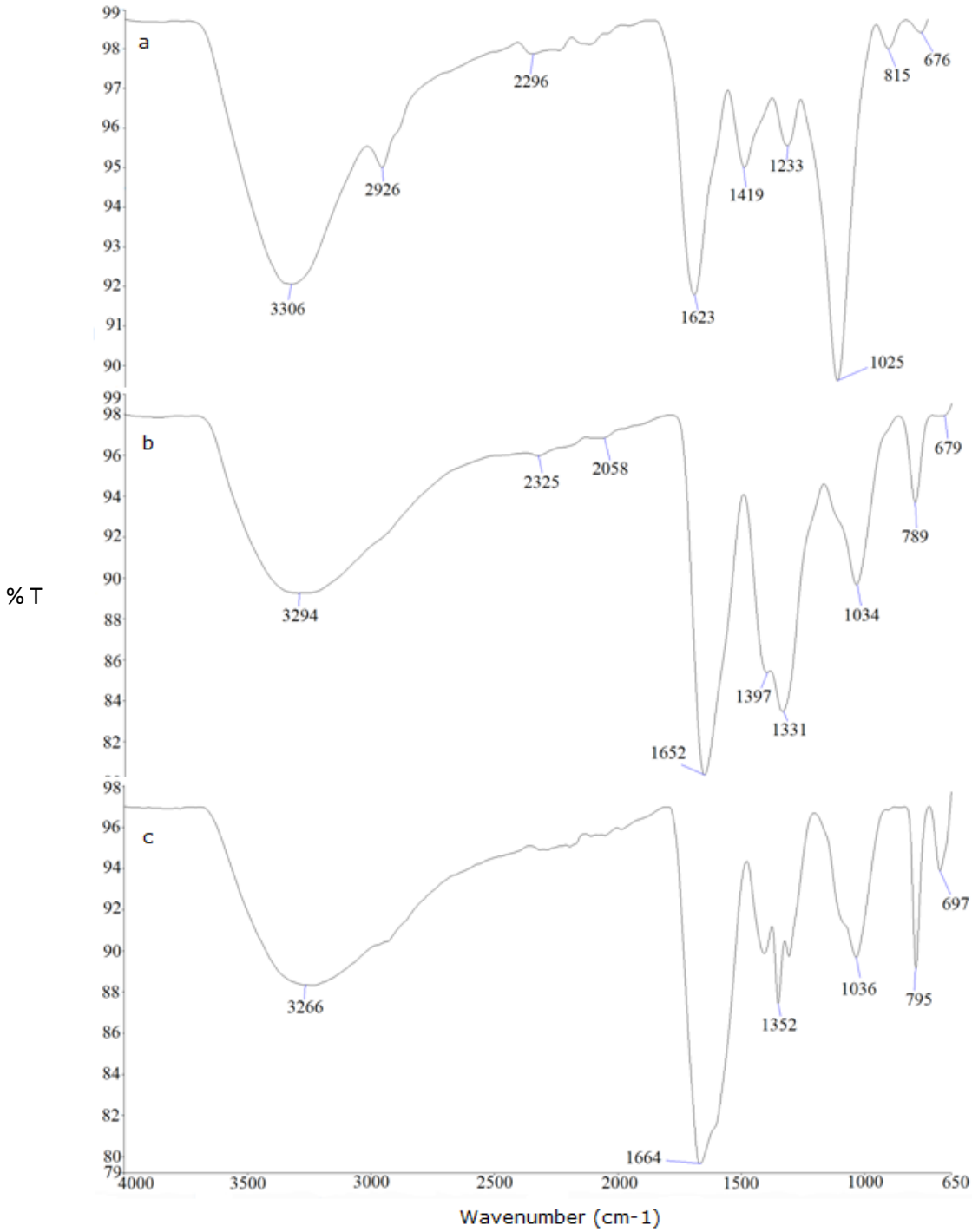

Fig.4. FTIR spectra of *C. barbata* unloaded (a), Mag-*C. barbata* unloaded (b) and Mag-*C. barbata* loaded with MB (c).

Table 2. Functional groups observed in FTIR of *C. barbata*

Functional group	Wavenumber (cm^{-1}) Unloaded	Mag- *C. barbata*	Mag- *C. barbata* loaded with MB
-OH and – NH stretching	3306	3294	3266
-CH stretching	2926	-	-
- OH bond	2296	2325	-
C≡C bond	-	2058	-
C = O groups in amide	1623	1652	1664
C-O stretching	1419	1397	1352
C-O carboxyl	1233	1331	-
S=O stretching	1025	1034	1036
S-O stretching	815	789	795

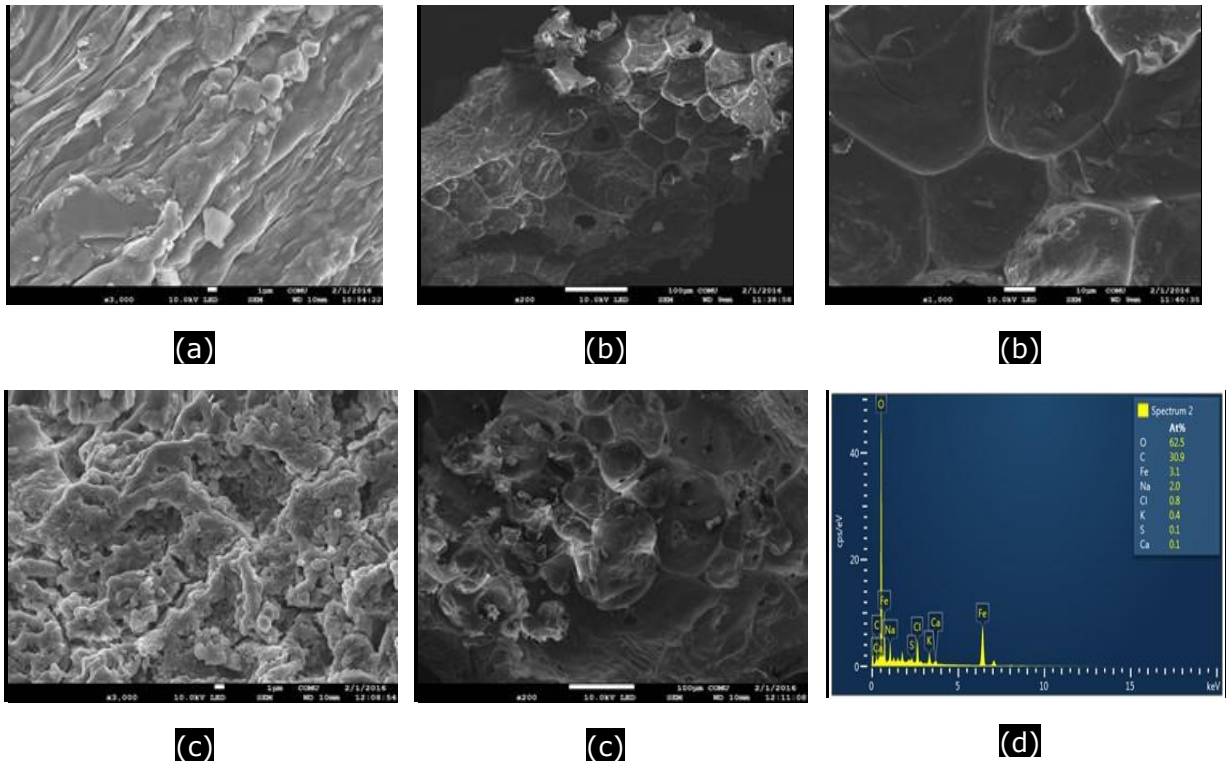

Fig.5. SEM images of *C. barbata*, unloaded (a), Mag-*C. barbata* (b), Mag-*C. barbata* loaded with MB (c), EDX images of Mag-*C. barbata* (d).

CONCLUSION

The removal of MB from aqueous solution was investigated with iron oxide-coated *C. barbata*. Effect of pH, contact time, initial MB concentrations and temperature were studied. The results showed that the pH value was not so effective on adsorption of MB. The biosorption reaction reached equilibrium within the first 300 min. Active sites of *C. barbata* surface decreased by the rising temperature. The maximum adsorption capacities (q_m) were 5.74 and 1.08 mg/g at 25 ºC and 45 ºC, respectively. Freundlich

isothermal model showed a better fit than the Langmuir isothermal model at 25 ºC, while Langmuir isothermal model showed a better fit at 45 ºC. The FTIR analyses and SEM images before and after biosorption of MB onto Mag-*C. barbata* showed the binding of several functional groups during biosorption. According to the results, Mag-*C. barbata* can be used as an alternative low-cost material for the removal of MB from aqueous solution.

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Türkçe Öz ve Anahtar Kelimeler

Demir Oksitle Kaplanmış *Cystoseira barbata* tarafından Sulu Çözeltilerden Metilen Mavisinin Biyosorpsiyonu

Yeliz Özüdoğru, Melek Merdivan, Tolga Göksan

Öz: Bu çalışmada, *Cystoseira barbata* demir oksitle (Fe_3O_4) kaplanmış ve manyetik bir biyomalzeme elde edilmiştir, bu malzeme sulu çözeltiden metilen mavisini gidermek için sorbent olarak kullanılmıştır. Bu biyosorbent Taramalı Elektron Mikroskopisi (SEM) ve Fourier Dönüşüm Kızılötesi Spektroskopisi (FTIR) ile karakterize edilmiştir. Bu malzemenin metilen mavisi adsorpsiyon kapasitesi pH, temas süresi, ilk metilen mavisi derişimi ve sıcaklığa bağlı olarak incelenmiştir. Denge verisi Langmuir ve Freundlich izotermleri ile analiz edilmiştir. Sonuçlara göre, maksimum adsorpsiyon kapasiteleri 300 dakikada ve pH 2'de elde edilmiş olup 25 °C ve 45 °C için sırası ile 5,74 ve 1,08 mg/g olarak elde edilmiştir.

Anahtar kelimeler: Metilen mavisi, biyosorpsiyon, *C. barbata,* demir oksit kaplaması.
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