

Research Article

Cationic and Anionic Dye Removal of Modified Ulva lactuca L. and Antioxidant Activity

Tugba SENTURK*¹, Mustafa OSKAY²

¹Section of Hydrobiology, Department of Biology, Faculty of Engineering and Natural Sciences, Manisa Celal Bayar University, Manisa, Türkiye

²Section of Basic and Industrial Microbiology, Department of Biology, Faculty of Engineering and Natural Sciences, Manisa Celal Bayar University, Manisa, Türkiye

¹https://orcid.org/0000-0002-9882-0079, ²https://orcid.org/0000-0001-8693-5621

*Corresponding author e-mail: tugba.sen@cbu.edu.tr

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Keywords

Antioxidant activity, Dye removal, Modified biomass, Pollution, *Ulva lactuca* Abstract: This study aimed to investigate the removal of fuchsin and nigrosin synthetic dyes using modified Ulva lactuca L. The study used the adsorption process under laboratory conditions to determine the removal effect of initial dye concentration (25, 50, 100 mg L⁻¹) at different exposure times (60, 90, 120 min), constant temperature (26 ± 1 °C), biomass dosage (1 g dw) and pH (7–8) values on the dried biosorbent chemically treated with magnesium chloride (MgCl₂) and potassium hydroxide (KOH). The research also provides information on changes in some biochemical properties of the biosorbent by exposure to MgCI₂ and KOH. The adsorption of the dyes on U. lactuca was modeled with the Langmuir and Freundlich isotherms. The study results determined maximum dye removal for fuchsin (70.81%) and nigrosin (61.29%) dyes at 120 min exposure time and 50 mg L^{-1} dye concentration onto non-modified U. lactuca biomass. The mean dye removal for fuchsin (60.08%, 99.12%) and nigrosin (56.23%, 54.27%) was obtained on U. lactuca biomass treated with MgCI2 and KOH, respectively. The sample prepared at 60 min contact time and 50 mg L⁻¹ dye concentration had the highest adsorption efficiency for fuchsin on U. lactuca biomass treated with KOH (99.40%). These results demonstrated that the KOH exposure onto Ulva is an efficient, non-polluting, and economical process for eliminating fuchsin from aqueous solutions.

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1. Introduction

As a result of the direct discharge, with or without pre-treatment, of wastewater generated in different industrial departments like paper, leather, textile, plastic, and medicine to the environment, it has become inevitable for dye-laden wastewater to affect the environment and organisms negatively (Li et al., 2022). Today, water pollution, which occurs primarily as a result of the inability of textile industries to fully treat wastewater containing dyes, negatively affects the global economy and environmental pollution in many countries (Almroth et al., 2021; Olisah et al., 2021; Ali et al., 2022). Approximately 7×10^7 tons of synthetic dyes are produced worldwide each year. Textile industries use ten thousand tons of dyes such as azo, direct, reactive, mordant, acid, basic, disperse, and sulfur dyes (Chandanshive et al., 2020). Textile dyes, however, do not adhere well to fabric and are released into

lakes, rivers, streams, and ponds without first being treated. They are also released with wastewater, constituting a significant ecotoxicological hazard that can harm living things (Parmar et al., 2022).

Contaminated colored wastewater is formed due to the combination of aromatic, water-soluble, and dispersible dyes used extensively in the textile industry. This dirty paint discharge into the environment causes an aesthetic problem and serious environmental problems due to its durability in nature and non-biodegradable properties (Stefanakis et al., 2014). In addition to threatening human health due to their carcinogenic and mutagenic properties, dyes significantly affect the distribution of plants, plankton, and neuston organisms in the aquatic ecosystem by blocking the penetration of sunlight into the water (Abbas et al., 2021; Alprol et al., 2021; Ashour et al., 2021; Sardar et al., 2021). These artificial synthetic dyes, which have very high pollutant levels even at low concentrations and negatively affect the aquatic food chain, are generally light-stable, oxidizing agents and are resistant to aerobic digestion, making it challenging to treat colored wastewater and difficult to degrade (Tahir et al., 2008; Oladipo et al., 2013; Stefanakis et al., 2014).

Dyes used in the textile industry are generally classified as cationic, anionic, and nonionic dyes (Demirbas, 2009). cationic dyes (Crystal violet, Methylene blue, Fuchsin, etc.) carrying a positive charge in their molecules are toxic basic dyes widely used in wool, nylon, and silk dyeing. They are water-soluble (Eren and Afsin, 2008). Anionic dyes (Indigo carmine, Methyl orange, reactive Brilliant red, Nigrosin, etc.) bound to a negative ion are acidic and reactive toxic dyes used in dyeing cotton, silk, and wool, and are well soluble in water (Qin et al., 2009; Eren et al., 2010). Some methods have been developed to purify wastewater from the harmful effects of these toxic dyes. These physical and chemical methods include flotation, flocculation, coagulation, ion exchange, irradiation, ozonation, precipitation, oxidation, and reduction. The low removal rate, large secondary sludge, and high cost of these methods make them unfavorable since the dyes' aromatic structures are resistant to heat, light, and oxidizing agents (Akceylan et al., 2009; Gupta et al., 2009; Chequer et al., 2013; Brahmbhatt and Jasrai, 2016). Conversely, in the bioremediation technique, where microorganisms such as fungi, bacteria, or micro-macro algae are used in-situ or ex-situ, pollutants can be converted into non-toxic products while being cleaned from the environment or an aqueous medium. In addition, this technique is environmentally friendly, economical, has a high absorption capacity, and is widely available in physico-chemical treatment technology (Abatenh et al., 2017; Bhuyar et al., 2020).

The biomaterial pores must be opened to be used as an efficient bioadsorbent in removing toxic materials. Studies have determined that cone-shaped cavities are formed with physical activation and bottle-shaped cavities with chemical activation. Alkaline agents that chemically activate the pores of the biomaterial are some acids and salts such as KOH, K₂CO₃, Na₂CO₃, and MgCl₂, and alkaline earth agents are H₃PO₄, H₂SO₄, AlCl₃, and ZnCl₂ (van Oss, 1990; Nahil and Williams, 2012; Nazem et al., 2020).

According to studies, the biomass of many microorganisms such as macroalgae, seaweeds, microalgae, fungi, and yeasts have been used as biological, sustainable, and low-cost adsorption materials for the removal of toxic dyes in the aqueous environment (El-Sheekh et al., 2009; Blaga et al., 2021; Kapoor et al., 2021; Mishra et al., 2021). Among all microorganisms, non-living or living algal cells are the most promising, sustainable, and low-cost biomaterials for adsorption due to the cell wall containing various functional groups such as phosphate, carboxyl, amino, carbonyl, and hydroxyl groups (Alprol et al., 2021). Algae can take up pollutants in the aquatic environment bioaccumulate and biotransform organic matter and immobilize inorganic elements, making them less toxic (Saleh, 2015). It has been stated that dye removal using especially non-living algae biomass as a result of physicochemical interactions such as electrostatic interaction, ion exchange, complexation, and microprecipitation is more advantageous due to reasons such as not needing maintenance and nutrition, being independent of metabolism and being able to be stored and reused (Aksu, 2005).

Ulva Linnaeus is a cosmopolitan genus that can grow very quickly, has a short life cycle and high pollution tolerance, and is tolerant to high pollutant concentrations, eutrophication, and wide changes in other abiotic factors (Koeman, 1985; Steneck and Dethier, 1994; Amaral et al., 2018; Eismann et al., 2020). *Ulva lactuca* (Sea lettuce), a type of green macroalgae that can be obtained in large quantities in shallow water near low water bodies, is a biomaterial that is suitable for the extraction of color and metal ions from water, is highly preferred in the removal of toxic heavy metals, and is used in biological treatment methods (Salima et al., 2013; Ibrahim et al., 2016; Heidarpour et al., 2019; Mourad et al., 2019; Soliman et al., 2019). However, most previous studies used *U. lactuca* without activation (El Sikaily et al., 2006; Tahir et al., 2008; Pratiwi et al., 2019; El Nemr et al., 2021). The

study aimed to improve the biosorption surface by activating the surface of the seaweed *U. lactuca* biomass as a low-cost adsorbent. The removal of fuchsin (cationic) and nigrosin (anionic), widely used in different industrial fields such as textile, pharmaceutical, and chemical, was analyzed on modified (with MgCl₂ and KOH chemicals, 2:1 ratio) and non-modified dried *U. lactuca* L. biomass collected from the Aegean Sea. The adsorption capacity of *U. lactuca* towards fuchsin and nigrosin was studied using different concentrations of dye solution and different contact times for the adsorption performance. The experiment under laboratory conditions kept pH, algal biomass dose, and temperature constant. Additionally, the research also provides information on changes in some biochemical properties of the biosorbent, including total phenolic, flavonoid ingredient, and antioxidant activity in the modified and non-modified green macroalgae *U. lactuca*. The adsorption of the dyes on *U. lactuca* was supported by Langmuir and Freundlich isotherm models. The results obtained from this study, which was conducted on water contaminated by the used synthetic dyes, would be significant in addressing the increasing demand for bioabsorbents for cleaning toxic dyes from aqueous solutions.

2. Material and Methods

2.1. The pretreatment of the organism and modified biomass

The seawater and organism employed in this study were gathered between March 9–10, 2024, from the coastal zone of İzmir Bay, İnciraltı ($38^{\circ}24'39''N$, $27^{\circ}02'10''E$). After sampling, the *U. lactuca* sample was transferred to the laboratory (4 °C). The thallus was washed with double distilled water to eliminate salts and other particles on the surface. The cleaned thallus was dried at room temperature and then in an oven (Memmert UNB 100) at 60 °C for 24 h until the mass stabilized and measured the dry weight.

The dried biomass, which was ground into a fine powder with the grinder, was sieved to obtain particle sizes below $350 \,\mu\text{m}$. The sieved powdered biomass was stored in glass containers for future use.

2.2. Dyes and batch adsorption experiment

The adsorbates used in this study were basic fuchsin (Synthetic cationic dye, Merck 42510, with the molecular formula $C_{19}H_{17}N_3 \cdot HCl$, 323.82 g mol⁻¹) and nigrosin disodium (Synthetic anionic dye, Merck 115924, with the molecular formula $C_{22}H_{14}N_6Na_2O_9S_2$, 202.21 g mol⁻¹).

The chemical activation is carried out by impregnating small particle-sized biomass into an aqueous solution containing an activating agent such as $MgCI_2$ or KOH at a ratio of 2:1 (biomass: chemical). This chemical method can also be modified to related literature according to the purpose of the study (Tseng, 2006; Cazetta et al., 2011; Sultan et al., 2020; Wahlström et al., 2020).

An adsorption experiment was conducted in batch conditions with 250 mL glass beakers containing 100 mL of dyes in an aqueous solution. Using the prepared 1000 mg L⁻¹ stock solution, dilutions were prepared in three different concentrations. Makeswari et al. (2016) determined in their dye removal study that the absorption rate decreased with the increase in the initial dye concentration. The concentration ranges were adjusted taking this study into account. 1 g of dry algal powder was added to each beaker containing 100 mL of dye solution and agitated continuously using an orbital shaker. Beakers were shaken on an incubator at 100 rpm and 26 °C. The effect of suitable time interval was tested at 60, 90, and 120 min, then centrifuged for 10 min at 2000 rpm. pH was kept constant between 7 and 8. All dye experiments were carried out at room temperature. Fuchsin and nigrosin concentrations were assayed colorimetrically using a spectrophotometer (Varian Cary 50). Spectrophotometric wavelengths of 544 nm and 570 nm were used for fuchsin and nigrosin, respectively. The initial pH was calibrated with concentrated 0.1 M HCl or 0.1 M NaOH.

The absorption capacity and its effect are given in the Equations 1 and 2, respectively.

$$qe (mg/g) = \frac{(CO - Ce) * V}{m}$$
(1)

Dye removal efficiency (%) =
$$\frac{(CO - Ce) * 100}{CO}$$
 (2)

Co and Ce (mg L^{-1}): Initial and final concentrations of dyes in the solution, V (L): The volume of the solution.

m (g): The mass of the adsorbent. The percentage of dye removal can also be displayed by the dye removal efficiency (Denniz and Saygideger, 2011).

All experiments in the study were performed with 3 repetitions, and the results were expressed as an average value.

2.3. Total phenolic compounds (TPC), total flavonoids content (TFC), and antioxidant activity

TPC was determined with Folin-Ciocalteu and gallic acid (1 mg mL⁻¹) as a reagent and standard, respectively, according to the described method by Saeed et al. (2012). Briefly, 50 μ L of the crude extract (5 mg) in 150 μ L of the methanol at different concentrations was taken and followed by the addition of 250 μ L of Folin-Ciocalteu diluted in a 1:1 ratio was added and left for approximately 1 min. Then, 1 mL of Na₂CO₃ (10%) was added, and the total reaction volume was completed to 5 mL with ddH₂O, with the remaining 95 min in darkness for the reaction. After the reaction period was completed, the color change in the reaction solution was determined at 760 nm absorbance and the results were expressed as mg equivalents per gram of gallic acid equivalents (mg GAE g⁻¹) by comparing it with the standard absorbance subjected to the reaction under the same conditions.

The TFC was determined by the colorimetric method with minor amendments, using ddH_2O solutions of 10% AlCl₃ and 1 M sodium acetate as a reagent. Briefly, 100 µL of extract was taken and followed by the addition of 1700 µL of methanol, 100 µL of AlCl₃, and 100 µL of sodium acetate. After, it was incubated at room temperature for 30 min for the reaction, then, the absorbance measurements were completed at 415 nm with quercetin (1 mg mL⁻¹) as a standard prepared in different concentrations under the same reaction conditions. TFC of the extract was expressed as quercetin equivalents per gram of sample (mg QE g⁻¹) (Zhishen et al., 1999).

Antioxidant activity was assayed using the standard method with a different concentration of 1,1-diphenyl-2-picrylhydrazyl (DPPH) (0.002%, 2 mg 100 mL⁻¹ in methanol). Briefly, different methanol extracts (0.1-1 mg mL⁻¹) of the *U. lactuca* (5 mg stock solution) were added to 150 μ L methanol; then, 2 mL of DPPH was added to each tube and kept for 40 min at a room temperature in the darkness and the absorbance was measured at 517 nm. The various ascorbic acid concentrations from the stock solution (1 mg mL⁻¹) were tested as a standard at the same experiment conditions for comparing the results. Thereafter, DPPH radical scavenging activity was determined at 517 nm, and the antioxidant activity was calculated using the following equation:

DPPH free Radical scavenging activity:

$$RSA\% = \frac{\text{ControlA517} - (\text{SampleA517} - \text{BlankA517})}{\text{ControlA517}}X100$$
(3)

ControlA517: The absorbance of the control (DPHH-without sample), SampleA517: The absorbance of the test sample (the sample and DPPH), BlankA517: The absorbance of the sample blank (Sample without the DPPH) (Huang et al., 2005; Prakash et al., 2007).

2.4. Adsorption isotherms

The adsorption isotherm and the Langmuir and Freundlich isotherm models supported the results obtained. These adsorption isotherms are two popular isotherm models frequently preferred in studies that provide information about the adsorption capacity among the adsorbate and the adsorbent. The Langmuir isotherm is a model that predicts the monolayer absorption (homogeneous points) of a solute from a liquid solution without the adsorbate movement on the surface plane (Langmuir, 1916). In other words, this isotherm was used to estimate the highest layered adsorption capacity. The linear form of the Langmuir isotherm is represented in the Equation 1 (Langmuir, 1917).

$$qe = \frac{qmkLCe}{1 + kLCe} \tag{4}$$

qe (mg g⁻¹): The equilibrium amount of biosorbent, qm (mg g⁻¹): The maximum capacity for sorption at equilibrium, kL (L mg⁻¹): Langmuir constant represents a coefficient related to the affinity. Ce (mg L⁻¹): Dye concentration at balance.

 R^2 , the regression correlation coefficient value, is used to determine whether the isotherm model is suitable. R^2 value close to 1 indicates that the isotherm model is suitable. The kL constant factor is the separation factor of the Langmuir isotherm, which indicates the suitability of the absorption process. If the kL value is between 0 and 1, it is suitable; if it is greater than 1, it is not appropriate (kL = 1 linear; kL = 0 irreversible). Ce/qe and Ce graphs were created using experimental data to determine the regression coefficient and Langmuir isotherm model parameters.

A model known as the Freundlich adsorption isotherm assumes that the dye molecule is adsorbed on the solid surface in several layers or heterogeneous spots. When all of the surface's points are filled with dye molecules, the system reaches equilibrium, forming an isotherm (Freundlich, 1906). The following equation provides the Freundlich adsorption isotherm in its linear form.

$$qe = kFCe^n \tag{5}$$

kF (L mg⁻¹): Freundlich constant related to the adsorption capacity of adsorbent,

n: The intensity of biosorption (the adsorbate's affinity for the adsorbent),

Favorable sorption is indicated by a value of 1/n that falls between 0 and 1 (El Qada et al., 2006).

3. Results and Discussion

3.1. Effect of initial dye concentrations on U. lactuca dye uptake

To determine the effect of dye concentration on *U. lactuca*, pH, temperature, and time were kept constant at 7–8, 26 °C, and 120 min., respectively. The removal of fuchsin by both modified and non-modified *U. lactuca* increased in correlation with the increase in dye concentration after exposure to three different dye concentrations. However, high nigrosine removal was found at 50 mg L⁻¹ dye concentration (Figure 1).

For non-modified U. lactuca biomass, maximum fuchsin removal was determined as 420.5±1.072 mg g⁻¹ dw at 100 mg L⁻¹ dye exposure. In comparison, it was defined as 104.14±0.193 mg g⁻¹ dw at 50 mg L⁻¹ dye concentration for nigrosin. A decrease in dye removal was observed in U. lactuca biomass modified with MgCI₂. Dye removal decreased from 270.12 \pm 2.570 mg g⁻¹ dw to 234.01 \pm 1.855 mg g⁻¹ dw for fuchsin and from 85.69 ± 0.977 mg g⁻¹ dw to 75.12 ± 0.814 mg g⁻¹ dw for nigrosin on average compared to non-modified biomass. On the other hand, an increase (46.29%) in fuchsin removal was determined in KOH-modified U. lactuca biomass (average value 395.06±3.455 mg g⁻¹ dw). In contrast, a decrease in nigrosin removal (average value 66.41±0.947 mg g⁻¹ dw) was observed compared to unmodified biomass. Similar to the results obtained in the study, Salima et al. (2013) obtained maximum uptake of 400 mg g⁻¹ and 526 mg g⁻¹ for the removal of malachite green and safranin by phosphoric acidmodified Ulva lactuca and Systoceira stricta, respectively. To obtain an effective biosorbent for dye removal, the pores of the biomass must be formed into a porous structure on the biomass surface by activating agents such as MgCI₂, KOH, H₂SO₄, and NaOH. These agents' primary purposes are to eliminate water from the material structures and lower the temperature needed for carbonization. In this way, a bottle-shaped porous structure is obtained on the biomass surface (Bansal et al., 1988; Nahil and Williams, 2012). As a result of the porous structure formed on the biomass surface due to the KOH treatment, the removal of fuchsin, in particular, is higher than that of the nonmodified biomass. This situation has been confirmed by the information given in other studies (Bansal et al., 1988; Nahil and Williams, 2012).



Figure 1. The effect of different concentrations of dye treatments on non-modified (a) and modified (b-c) *U. lactuca* biomass dye removal (mg g⁻¹).

3.2. Effect of different contact times on dye removal efficiency

To determine the effect of contact times on *U. lactuca*, pH and temperature were kept constant at 7–8 and 26 °C, respectively. In non-modified *U. lactuca* biomass, fuchsin and nigrosin uptake was exceptionally high at 50 mg L⁻¹ dye concentration and 90-120 min dye exposure. Average fuchsin removal was determined as 33.68%, 68.20%, and 60.92% at three different dye concentrations (25, 50, and 100 mg L⁻¹), respectively. Similarly, the average nigrosin removal was determined to be 43.91, 61.17, and 41.43% in non-modified biomass, respectively (Figure 2).



Figure 2. The effect of different contact times on fuchsin (a) and nigrosin (b) removal efficiency (%) of non-modified *U. lactuca*.

The fuchsin removal of *U. lactuca* modified with MgCl₂ increased parallel to the increasing time and dye concentration. The average fuchsin removal percentage was determined as 33.78%, 43.22%, and 60.08%, respectively. In nigrosin removal, the highest removal percentage was determined to be 56.23% at a 50 mg L⁻¹ dye concentration ratio. Dye removal of *U. lactuca* modified with KOH

was determined to be 99.12% and 98.46%, especially in fuchsin removal at 50 and 100 mg L⁻¹ dye concentration exposure, respectively. In nigrosin removal, these values were determined as 35.12%, 54.27%, and 26.13%, respectively (Figure 3). The findings indicate that, for most treatments at low and high concentrations, but not all of them, the ideal contact duration was 120 min, which equates to a sufficient elimination of the dyes. Additionally, it was found that increasing the contact period from 60 to 120 min had no discernible effect on the rates of dye removal for either low or high concentrations of the modified *U. lactuca* adsorbent. However, El-Skaily et al. (2006) studied the removal of methylene blue at different concentrations by *U. lactuca* biomass at various contact times, and they found that dye removal stabilized and reached saturation after a contact time of 45 min, after which it remained more or less constant up to 120 min. In another study (Safarik et al., 2024), it was determined that *Ulva rigida* modified with iron oxide had maximum capacity for malachite green (202 mg g⁻¹) and safranin (227 mg g⁻¹) removal among fifteen macroalgae species collected from Saros Bay, Türkiye.



Figure 3. The effect of different contact times on dye removal efficiency (%) of modified *U. lactuca* with MgCI₂ (a₁: fuchsin, a₂: nigrosin removal) and with KOH (b₁: fuchsin, b₂: nigrosin removal).

It can be seen that the removal percentage of fuchsin increased from 68.20% to 99.12% on average for *Ulva* biomass modified with KOH at low dye concentration (50 mg L^{-1}) compared to unmodified biomass. This could be because the adsorbent surface has unoccupied functional groups. Active sites may become occupied if the dye concentration rises further. As a result, the algal cell surface becomes saturated, preventing further adsorption. This is confirmed by previous studies (M'sakni and Alsufyani, 2021). In addition, the fact that the absorption capacity of fuchsin, a cationic dye, increased more than that of unmodified biomass as a result of KOH application can be explained by the increase in the binding sites formed in the surface active regions of the adsorbent as a result of modification.

3.3. Effects of MgCI₂ and KOH on total phenolic, flavonoid content, and antioxidant activity of modified *U. lactuca*

The present study determined the TPC, TFC, and DPPH radical scavenging activity (RSA%) of the methanol extract from *U. lactuca* (Table 1.). The TPC values were 7.8, 4.6, and 30.40 mg GAE g⁻¹ for the non-modified, modified with MgCI₂, and modified with KOH of the *U. lactuca*, respectively. Interestingly, the TPC value of *U. lactuca* modified with KOH was the highest and may have shown a synergistic effect in the reactions. On the other hand, the TPC amount of *U. lactuca* that was not treated

at all was determined to be consistent with previous studies. Tong et al. (2020) determined the TPC value of the distilled water extract of *U. lactuca* as 7.72. mg GAE g⁻¹. In the same study, the RSA value of *U. lactuca* was approximately 85%, which is considerably lower than our values (98.13%). However, although the TPC value of *U. lactuca* treated with KOH was high (30.40 mg GAE g⁻¹), the RSA value decreased considerably (38.70%). The reason for this is not understood; perhaps DPPH, which was used as a reagent to determine antioxidant activity, may have reacted negatively with KOH. As a result, there was no positive correlation between the TPC value and RSA value of *U. lactuca* extract treated with KOH. In a recent study, the amount of TPC (2.4 mg g⁻¹) was lower than the value obtained in this study. The same survey clearly emphasizes that the amount of TPC can be affected by the extraction process, the organic solvent used, and the extraction time (Pappou et al., 2022). It was also observed that the highest RSA value was obtained in 0.11 mg mL⁻¹ of *U. lactuca* methanol extract, which is much lower than in earlier research (Farasat et al., 2014; Tong et al., 2020; Ouahabi et al., 2024). The organic solvent used in extraction significantly affects the determination of primary and secondary metabolites and pigments of macroalgae. In a study, the ratio of Chlorophyll *a*, carotenoids, and total phenolic compounds was higher in ethyl acetate extracts obtained from *U. lactuca* (Hidayati et al., 2020).

Table 1. The effect of MgCI₂ and KOH treatments on TPC, TFC, and antioxidant activities of U. lactuca.

	TPC* (mg GAE g ⁻¹)	TFC (mg QE g ⁻¹)	RSA (%)
Non-modified U. lactuca	7.8±0.015	0.74 ± 0.00025	98.13±2.95
Modified <i>U. lactuca</i> with MgCI ₂	4.6 ± 0.050	1.47 ± 0.00050	93.90±0.42
Modified U. lactuca with KOH	30.40±2.14	0.82 ± 0.00062	38.70 ± 0.05

*TPC, TFC, and RSA refer to Total Phenolic Contents, Total Flavonoid Contents, and DPPH Free Radical Scavenging Activity, respectively.

Besides, total flavonoid amounts were determined between 0.74 and 1.47 (mg QE g⁻¹), and the highest TFC value was determined in *U. lactuca* treated with MgCI₂. Interestingly, these amounts are much lower than the results obtained in previous studies from the literature (Prasedya et al., 2019; Benítez García et al., 2020; Ouahabi et al., 2024). Many studies have been conducted on the health benefits of *U. lactuca*; these benefits are generally based on the amounts of proteins, fatty acids, phenolics, flavonoids, polysaccharides, minerals, and other bioactive components. In addition, the antimicrobial, antioxidant, and anti-inflammatory properties of *U. lactuca* increase due to these compounds, especially flavonoids (Shobier and El Ashry, 2021; Madhusudan and Baskaran, 2023; Putra et al., 2024). As a result, it is thought that the differences in TPC, TFC values, and antioxidant activities in our research may be due to differences in extraction method, organic solvent, time, habitat, and period in which *U. lactuca* grows.

3.4. Adsorption isotherms

Langmuir and Freundlich's isotherms were used to indicate the efficiency of the dye activation process on *Ulva lactuca*. The Langmuir Model is a model that predicts the monolayer absorption (homogeneous points) of a solute from a liquid solution without the adsorbate movement on the surface plane (Langmuir, 1916). The Freundlich isotherm model describes the binding occurring in a heterogeneous adsorption site (multiple layers). Ce/qe and Ce graphs were created to determine the regression coefficient and Langmuir isotherm model parameters using experimental data. In qe and Ce graphs were created to determine the Freundlich isotherm model parameters. Figure 4 shows the Langmuir and Freundlich isotherm models for dye adsorption on unmodified *U. lactuca* biomass.



Figure 4. Langmuir (a_1/a_2) and Freundlich (b_1/b_2) Isotherms for the fuchsin and nigrosin (100 mg L⁻¹) dye onto non-modified *U. lactuca*.

Because the Langmuir isotherm model had the highest regression coefficient ($R^2 = 0.9857$ and 0.9828, respectively), the results demonstrated that it was the best model for the adsorption of fuchsin and nigrosins onto non-modified *U. lactuca*. According to the Langmuir model, which was used to fit the adsorption data, the monolayer adsorption capacity for the removal of fuchsin and nigrosin was 420.50 and 104.14 mg g⁻¹, respectively. However, as it has the highest regression coefficient ($R^2 = 0.9832$ and 0.9035 for modified biomass with MgCI₂; 0.9462 and 0.9492 for modified biomass with KOH), it has been demonstrated that the Freundlich isotherm model is the most suitable model for the removal of dyes onto modified *U. lactuca* biomass (Figure 5 and 6; Table 2).



Figure 5. Langmuir (a₁/a₂) and Freundlich (b₁/b₂) Isotherms for the fuchsin and nigrosin (100 mg L⁻¹) dye onto modified *U. lactuca* with MgCI₂.



Figure 6. Langmuir (a_1/a_2) and Freundlich (b_1/b_2) Isotherms for the fuchsin and nigrosin (100 mg L⁻¹ cons.) dye onto non-modified *U. lactuca* with KOH.

Table 2. Isotherms for the adsorption of fuchsin and nigrosin (100 mg L⁻¹ cons.) dye onto modified and non-modified *U. lactuca*

tuca	Adsorption Isotherm Constants	Fuchsin	Nigrosin
	Freundlich		
Ľ.	1/n (L g ⁻¹)	0.759	1.001
p	$k_{\rm F}$ (L g ⁻¹)	0.65	0.55
ifi	\mathbb{R}^2	0.9397	0.6705
po	<u>Langmuir</u>		
E.	$q_{\rm m} ({\rm mg}~{\rm g}^{-1})$	420.50	104.14
On	k _L (L mg ⁻¹)	0.52	0.16
Z	\mathbb{R}^2	0.9857	0.9828
ICA	Freundlich		
ict i	1/n (L g ⁻¹)	0.9460	0.6639
la	$k_F (L g^{-1})$	0.5839	0.4043
[²	\mathbb{R}^2	0.9832	0.9035
J g	<u>Langmuir</u>		
M ²	$q_m (mg g^{-1})$	414.12	107.93
th d	k _L (L mg ⁻¹)	4.1963	0.018
M IN	\mathbf{R}^2	0.0378	0.5759
ICa	Freundlich		
icti	$1/n (L g^{-1})$	0.1285	0.6545
la	$k_F (L g^{-1})$	0.1791	0.4199
U.	\mathbb{R}^2	0.9462	0.9492
eq	<u>Langmuir</u>		
KC	$q_m (mg g^{-1})$	674.71	104.69
(†) (†)	$k_L (L mg^{-1})$	0.003	0.020
N II	\mathbb{R}^2	0.3388	0.5313

As shown in Table 2, the maximum adsorption capacity of fuchsin was determined to be higher than the adsorption of nigrosin on unmodified *U. lactuca*. Similarly, when previous studies were examined, it was stated that cationic dyes were absorbed faster than anionic dyes (Gong et al., 2005; Namane et al., 2005; M'sakni and Alsufyani, 2021). The negatively charged carboxyl group in the cell wall molecular structure of *U. lactuca* is one of the main functional groups in cationic dye adsorption (Gong et al., 2005). For this reason, it was predicted that the cationic dye would be absorbed more than the anionic dye by the adsorbent. In addition, dye removal studies conducted on *Ulva lactuca* determined that biosorption showed a monolayer, i.e., homogeneous absorption, and was by the Langmuir isotherm model (M'sakni and Alsufyani, 2021). Removal of cationic organic dye from aqueous solution by chemical and pyrolysis-activated *U. lactuca* biomass by the other studies conducted is consistent with the results of this study. However, it was determined that the surface biosorption of the adsorbent modified with MgCl₂ and KOH was heterogeneous, and the adsorption was multilayered for the two dyes determined, i.e., it was more suitable for the Freundlich model. As indicated in Table 2, the 1/n value between 0 and 1 and the R² value between 0.9 and 1 support the result.

Conclusion

This study investigated the removal of fuchsin and nigrosin synthetic dyes by using nonmodified and modified *U. lactuca* with MgCl₂ and KOH. According to the results, the sample prepared at 60 min contact time and 50 mg L⁻¹ dye concentration had the highest adsorption efficiency for fuchsin on *U. lactuca* biomass treated with KOH (99.40%). In addition, the TPC value of *U. lactuca* modified with KOH was the highest and may have shown a synergistic effect in the reactions. The RSA value decreased significantly. No positive correlation was found between the TPC and the RSA values of the *U. lactuca* extract treated with KOH. As a result, it is thought that the differences in TPC, TFC values, and antioxidant activities in our study may be due to differences in extraction method, organic solvent, time, habitat, and the period in which *U. lactuca* was grown. The most suitable adsorption isotherm for *U. lactuca* biomass treated with MgCl₂ and KOH was determined as the Freundlich isotherm model. These results demonstrated that applying KOH on *U. lactuca* is an effective, non-polluting, and economical process, especially for removing fuchsin cationic dye from an aqueous solution.

Ethical Statement

Ethical approval is not required for this study because it does not include any studies on human or animal subjects.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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Author Contributions

This study is performed by both authors. Both authors contributed 50%.

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