ORIGINAL ARTICLE



Environmental performance of two marine algae *Ulva fasciata* and *Pterocladia capillacea* in the biological treatment of four reactive dyes from aqueous solutions based on fresh and dried biomass

Nagwa I. El-Agawany¹ · Suzan A. R. Abdel-Razik¹ · Mohamed S. Abdel-Kareem¹ · Mona I. A. Kaamoush²

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Abstract

The runoff of textile dye effluents into public water bodies is a major environmental and health problem. Color removal, in particular, has recently become of substantial scientific interest, as revealed by the multitude of related research reports. The present study focused on measuring the efficiency of two marine algae, *Ulva fasciata* and *Pterocladia capillacea*, in bio-removal of four synthetic dyes (RY2, RR195, RB19, and RB5) based on the fresh and dried biomass of the tested algal species. According to the highest removal efficiencies of the dyes, two algal species were chosen from among nine different algae. Bio-removal efficiency was examined under the effects of salinity and contact time. The results of this experiment revealed that algae achieved high bio-removal efficiency of the examined dyes in both fresh and saline water, but the removal percentages were higher in saline water compared to fresh conditions. The highest removal percentage recorded after 8 h by fresh *U. fasciata* in saline water reached 82.75 and 83.23% for RY2 and RR195 dyes, respectively. Contact time has the highest impact on dye removal in both algal species. The highest removal values were obtained in the case of dried *U. fasciata*, which achieved impressive removal percentages that reached 100% for RB195 and RB5 and fresh *P. capillacea* which had high removal percentages of 91.11, 94.85 and 97.13% for RR195, RB19, and RB5 dyes, respectively, after 8 h. Our results revealed that the used algal species were highly significant in the biosorption of most used dyes.

Keywords Biological treatment · Dyes · Salinity · Contact time · Ulva fasciata · Pterocladia capillacea

Introduction

Over the last few decades, the utilization of dyes has increased drastically due to the rapid industrialization of dye-based industries and the increase in demand for textiles and clothes. However, due to rapid industrialization, the dyes remained the same synthetic ones, which are very difficult to degrade in the environment. New initiatives for environmental restoration have emerged as a result of the realization that environmental contamination poses a global threat to human health for both ecological and economic reasons (Elumalai and Saravanan 2016). Many physicochemical decolorization processes have been approved over the last two decades, but only a few have been used by the textile industry. Their lack of deployment has mostly been attributed to their high cost, low efficiency, and ineffectiveness with a wide range of dyes. The ability of microorganisms to decolorize dyes has gained a lot of interest (Ghazal et al. 2018). The discharge of effluent from dyeing industries into adjacent bodies of water contaminates the water, has negative effects on aquatic life, and disturbs the environment. Given that textile companies use a lot of water for the dyeing and finishing processes, their effluent can be considered the most polluting of all industrial sectors. Due to the rising demand for textile products, textile effluents play a significant part in the environment's severe pollution. (Sahoo et al. 2022)

The textile industry is one of the most important industries in all countries throughout the world, and it requires a great amount of water during textile manufacture via numerous processes such as desiring, bleaching, mercerizing,

Mona I. A. Kaamoush mona.ibrahim.abdelnaby2021@gmail.com; monakaamoush@aast.edu

Botany and Microbiology Department, Faculty of Science, Alexandria University, Alexandria, Egypt

² Environmental Protection and Crises Management Department, Simulator Complex, Arab Academy for Science, Technology and Maritime Transport (AAST), 23 Ali Ben Abi Talib St. Abu Qir, P. O. Box: 1029, Alexandria, Egypt

dying, printing, and finishing and produces a large number of dyes in wastewater effluent (Imtiazuddin et al. 2012). Environmental experts are concentrating on efforts to degrade these pollutants' presence in water in order to minimize, if not eliminate, their harmful impacts on the ecosystem. This is due to the continual accumulation and widespread availability of these pollutants in our surroundings (Hadibarata et al. 2019; Hu et al. 2020). Different microorganisms can be employed to decompose a variety of colors since they have a variety of pathways and mechanisms to achieve this (Cao et al. 2019; Ebrahimi et al. 2019). Connections among colors and biosorbent rely upon the idea of color and explicit the feature surface of biomass, and natural conditions (pH, dye concentration, salinity and contact time). Unlike most organic compounds, dyes possess color because they absorb light in the visible spectrum (400-700 nm), have at least one chromophore (color-bearing group), have a conjugated system, i.e., a structure with alternating double and single bonds, and exhibit resonance of electrons, which is a stabilizing force in organic compounds (Abd El-Razik 2010). Dyes can be classified according to how they are used in the dyeing process into acid dyes, direct dyes, vat dyes, disperse dyes, sulfur dyes, azo dyes, and reactive dyes (Aksu 2005). Dyeing is the process of adding color to the fibers, which normally requires large volumes of water not only in the dye bath, but also during the rinsing step. Depending on the dyeing process, many chemicals like metals, salts, surfactants, sulfide, and formaldehyde may be added to improve dye adsorption onto the fibers. To obtain the target color, normally a mixture of red, yellow and blue dyes is applied in the dye baths (Aksu 2001).

Adsorption has been observed to be an effective process for color removal from dye wastewater. Many studies have been undertaken to investigate the use of low-cost adsorbents such as peat, bentonite, steel plant slag, fly ash, maize cob, wood, and silica for color removal. However, these lowcost adsorbents have generally low adsorption capacities. So in recent years, a number of studies have focused on some microorganisms that are able to biodegrade or bioaccumulate the dyes in wastewaters (Gupta et al. 2003). Algal communities have been utilized in tests of toxicity for environmental monitoring of heavy metal pollution and can be used in determining general water quality and growth-limiting nutrients. Likewise, they can be used in the removal of metals from contaminated wastewater (Kaamoush et al. 2022b).

Textile dye wastewater remediation is based not only on color removal (decolorization), but also on the degradation and mineralization of the dye molecules. As non-living biomasses, macroalgae can be utilized to remove different textile dyes. The most effective mechanisms for algal utilization to decolorization of azo dyes were production of algal biomass by assimilation, the production of carbon dioxide and H2O while converting color to an uncolored molecule. Mechanisms of algal decolorization can involve enzymatic degradation, adsorption, or both (Abd El-Razik 2010).

Additionally, dried algae can be reused once 99% of the dye has been removed from them by a desorption process with 0.1 NaOH, and they are easily removed from wastewater following dye adsorption by filtration (Singh and Kaur 2013). The state and kind of coloring of the algae greatly influence their capacity for the micropollutant removal event. Algae can be divided into groups that are living and non-living depending on their status, while they can also be divided into green, red, and brown categories depending on their pigment (Syafiuddin and Boopathy 2021).

Algae have been found to be potential biosorbents of different environmental pollutants because of their availability in both fresh and salt water. The biosorption capacity of algae may be attributed to their relatively high surface area and high binding affinity (El Agawany et al. 2021; Kaamoush and El-Agwany 2021). This high binding affinity is due to their cell wall structure, which contains functional groups like amino, carboxyl, sulfate, phosphate, and imidazoles that are connected with polysaccharides, alginic acid, and proteins to bind different contaminants. Dye biosorption is a surface process in which an anionic dye is chemically attached to the algal cell wall's active groups (amino, sulfate, and carboxyl) (Donmez and Aksu 2002; Gupta et al. 2003) or may be due to electrostatic attraction and complexation taking place during algal biosorption (Satiroglu et al. 2002). Adsorption is the accumulation of dye at the surface of algae that occurs in two steps (an initial quick phase that occurs within minutes to hours followed by a slower process that might take several hours to a day) (Tien (2002)). Adsorption mechanisms are regulated by a variety of parameters, such as the surface charge density of the interacting species, time, structure, electrostatic interactions, hydrogen bonding, salinity, and pH (Sarojini et al. 2022).

Due to the large amount of dyes present, the wastewater released by the dyeing industry is the main contributor to water pollution. Because of the high levels of dyes in water bodies, photosynthesis and aquatic biodiversity are hampered by the blocking of sunlight and decreased oxygenation capacity of incoming water. Environmentally friendly biological treatment techniques are becoming more and more popular in the present day. It is possible to effectively use many algae species, including *Ulva fasciata* and *Pterocladia capillacea*, to remove a variety of dyes' colors from industrial effluent and save the environment.

Aim of the work

The main aim of this research is to estimate performance of two marine algal species (*Ulva fasciata* and *Pterocladia capillacea*) for treatment of four synthetic dyes (Reactive Yellow 2, Reactive Red 195, Reactive Blue 19, and Reactive Black 5) using fresh and dry algal biomass based on impact of salinity and contact time.

Materials and methods

Biological material

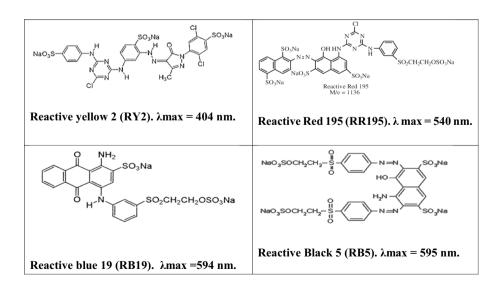
Algal species were harvested from Alexandria's Abu-Qir area along the Mediterranean Sea shore (Supplemental data), washed numerous times by sea water to remove sand and vegetation, and then carried to the laboratory in plastic sheets filled with sea water. The harvested species were air-dried at room temperature for 24 h before being dried in an oven at 60 °C for 24 h, ground to a fine powder, and saved in a dry place until used. The dye-biosorption survey consists of the following species: Corallina officinalis, Jania rubens, Laurencia papillosa, Pterocladia capillacea, Sargassum hornschuchii, Colpomenia sinuosa, Ulva linza, Ulva intestinalis, and Ulva fasciata. Both Ulva fasciata and Pterocladia capil*lacea* were chosen for the controlled laboratory experiments, because of their significant efficacy in the removal of dye in the survey experiment. Every experiment for this study, which was done at room temperature, used algal biomass as the biosorbent. In a 250-ml pre-sterilized Erlenmeyer flask, the appropriate weight of algal biomass (fresh or dried) was blended with 100 ml of the appropriate dye solution. Several algal biomasses were investigated to see how they affected the decolorization process. Both Ulva fasciata and Pterocladia capillacea were fed 1, 2, 3, 4, and 5 g of fresh biomass and 0.5, 1, 1.5, 2, and 2.5 g of dried biomass.

Chemical composition of the four studied dyes

Four commercial grade reactive dyes were employed in this study: Reactive Yellow 2, Reactive Red 195, Reactive Blue 19, and Reactive Black 5. They were donated by Misr El Beida Dyers S.A.E., Kafr El Dawar, El Beheira, Egypt. Each dye's maximum wavelength was determined by measuring its absorption using a dual-beam UV–visible spectrophotometer (PerkinElmer Lambda 4B). 100 mg of pure powdered dye were dissolved in 100 ml of filtered sea water to generate a stock dye solution. The dye concentrations that followed were created by diluting the stock solution. Diluting the dye stock solution with sea water yielded the dye concentrations. Using a single-beam visual spectrophotometer (Unico 7200), the absorbance at the characteristic dye wavelength was measured before and after treatment and compared to the control (sea water).

Algal survey for decolorization

The nine algae species were employed to demonstrate their removal capacity for the prespecified four reactive dyes. In each flask, 2 g of algal fresh biomass or 0.5 g of algal dried biomass was mixed with a single dye (each with a different beginning concentration) and shaken on a regular basis. These values are as follows: RB5 = 10 mg/l, RB19 = 20 mg/l, RY2 = 30 mg/l, and RR195 = 10 mg/l. At certain time intervals (2, 4, 6, 8, and 24 h), a sample (5 ml) was collected from each flask. This sample was centrifuged at $503 \times g$ for 20 min, the algal biomass was removed, and the maximum absorbance of the dye was determined in the supernatant at the maximum concentration of the corresponding dye.



Biosorption experiments and Assay for decolorization

All of the dyes tested in this investigation were commercial grade, Reactive Yellow 2 (RY2), Reactive Red 195 (RR195), Reactive Blue 19 (RB19), and Reactive Black 5(RB5). To make the stock dye solution, dissolve 100 mg of pure powdered dye in 100 ml of filtered sea water. Dilution of this stock dye solution yielded subsequent dye concentrations. Biosorption experiments were conducted at room temperature $(25-30 \,^{\circ}C)$ with algal biomass as the biosorbent. All biosorption studies were carried out in 250-ml Erlenmeyer flasks containing 100 ml of filtered sea water. The influence of algal biomass, dye concentration, and pH on the biosorption process was studied using a variety of tests.

The dye concentration in solution was determined at its characteristic wavelengths RB5 λ max = 595, RY2 λ max = 404 nm, RR195 λ max = 540 nm, and RB19 λ max = 594 nm both before and after removal (PerkinElmer Lambda 4B). As a control, dye-enhanced sea water was employed. A single color was applied to the medium in each experiment. The decolorizing efficiency was represented as a percentage and was measured by measuring the reduction in absorbance at each dye's absorption maxima (max). All biosorption studies were done in triplicate, and the mean results were utilized to analyze the data.

Effect of salinity and contact time

This experiment was conducted to compare dye removal performance in fresh and normal saline conditions. Distilled and sea water (38%) were used. The pH of the solution was adjusted to 8, the algal biomass was 2 g (fresh) and 0.5 g (dry), and the dye concentration for each color was constant.

To evaluate the effect of contact time on biosorption capacity at optimum conditions, all variables were kept constant (algal biomass, 5 g fresh or 2.5 g dried); beginning dye concentration (each dye individually); pH 2 and salinity, 38%, while absorbance was evaluated at different times (sea water supplemented with the dye was used as control, times 2, 4, 6, and 8 h).

Calculations

In all prior studies, the decolorization capacity was estimated as a percentage (dye removal %) by measuring the concentration of dye before (at time 0) and after (at a specific time, t) the biosorption experiment. The dye clearance % was obtained using the equation given:

Dye removal $\% = \frac{C_0 - C_t}{C_0}$

where C_0 is the initial dye concentration at zero time and C_t is the dye concentration at time *t*.

All biosorption studies were carried out in triplicate, and the mean results were chosen for data analysis. All findings were reported as mean \pm SD (standard deviation).

Results and discussion

A decolorization survey experiment was carried out to assess the potential of the researched algae species to serve as biosorbents for four reactive dyes and to determine the best algal species with the greatest dye removal rates. The dye-biosorption survey indicated varying dye removal percentages depending on the type of dye and algae species. In general, the proportion of dye eliminated rose over time and reached a maximum after 24 h for all studied species. Microalgae degrade dye rapidly in the presence of cyanobacteria in dye effluent and have the potential to clean wastewater released to local water sources from the textile industry and other enterprises that employ colors (Ghazal et al. 2018). The ability of different synthetic dyes to be bioremoved by different algae species is dependent on several parameters, such as pH, dye concentration, contact duration, and salinity (Abd El-Razik 2010). To provide ecologically acceptable solutions, living and non-living algae have recently been recognized and widely exploited as potential agents in the bioremediation of dyes from industrial wastewater. Microalgae can be used as a low-cost and secure agent in wastewater bioremediation to protect water bodies from harmful contaminants (Kaamoush 2019; Ratnasari et al. 2022).

Algal biomass

The dye-biosorption survey revealed that dye removal percentages varied depending on dye type and algae species. For all tested species, the dye removal % increased with time and reached its maximum after 24 h. Table 1 and Fig. 1 illustrate that RB19 was removed efficiently by all studied species whether green, red, or brown. The maximum removal value (95.56%) was recorded by the fresh red alga *Jania rubens*, and the minimum removal value (63.90%) was recorded by the brown alga *Laurencia papillosa*.

Fresh *Ulva fasciata, Jania rubens,* and *Sargassum horns-chuchii* achieved high removal ability for RB5 with 92.94%, 91.64% and 71.19%, respectively, as shown in Table 2 and Fig. 2. The minimum removal value was recorded by *Laurencia papillosa* (36.99%). It is clear from Table 3 and Fig. 3 that the maximum (69.01%) and the minimum (4.0%) removal percentages of RY2 were recorded by the red algae *Corallina officinalis* and *Laurencia papillosa*, respectively.

As shown in Table 4 and Fig. 4, the maximum removal values of RR195 were recorded by *Ulva intestinalis*

Table 1 Removal percentage of Reactive Blue 19 (RB19) by the nine studied fresh algal species

| Algae | | Green algae | e | | Red algae | | | | Brown algae | |
|-------|------|-------------|---------------|-------------------|--------------------------------|------------------------|--------------------------|--------------|---------------------------|------------------------------|
| | | Ulva linza | Ulva fasciata | Ulva intestinalis | Pterocladia capillacea 0 | Laurencia papillosa | Corallina officinalis | Jania rubens | Sargassum hornschuchii | Colpome- nia sinuosa 0 |
| Time | 0 h |)h 0 | 0 0 | | | 0 | 0 | 0 | 0 | |
| | 2 h | 63.89 | 63.89 | 60.56 | 57.22 | 42.78 | 60.56 | 56.67 | 53.33 | 47.22 |
| | 4 h | 65.00 | 67.78 | 62.78 | 75.56 | 60.56 | 83.33 | 82.78 | 70.00 | 66.67 |
| | 6 h | 67.78 | 75.56 | 72.78 | 75.56 | 61.67 | 88.33 | 93.89 | 76.67 | 73.33 |
| | 8 h | 70.56 | 85.56 | 88.89 | 78.89 | 63.89 | 88.33 | 95.56 | 76.67 | 73.33 |
| | 24 h | 80.56 | 85.56 | 89.00 | 78.90 | 63.90 | 88.33 | 95.56 | 76.67 | 77.78 |

Fig. 1 Removal percentage of Reactive Blue 19 by the nine studied fresh algal species

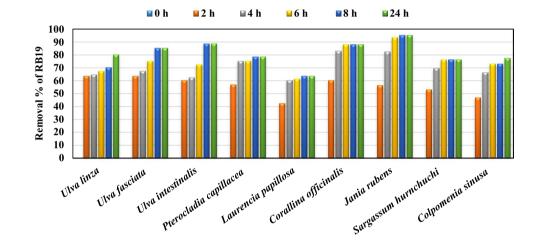


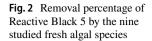
Table 2 Removal percentage of Reactive Black 5 (RB5) by the nine studied fresh algal species

| Algae | | Green algae | | | Red algae | | | | Brown algae | |
|-------|------|-------------|---------------|-------------------|---------------------------|------------------------|--------------------------|--------------|---------------------------|------------------------------|
| | | Ulva linza | Ulva fasciata | Ulva intestinalis | Pterocladia capillacea | Laurencia papillosa | Corallina officinalis | Jania rubens | Sargassum hornschuchii | Colpome- nia sinuosa 0 |
| Time | 0 h | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | 2 h | 18.96 | 39.96 | 37.55 | 37.17 | 18.77 | 32.34 | 45.91 | 24.16 | 13.57 |
| | 4 h | 20.82 | 50.93 | 40.33 | 39.78 | 18.96 | 43.31 | 57.62 | 28.81 | 17.10 |
| | 6 h | 21.56 | 60.22 | 40.89 | 40.00 | 22.49 | 50.93 | 74.35 | 31.41 | 19.52 |
| | 8 h | 23.98 | 65.80 | 44.42 | 47.40 | 30.00 | 52.23 | 80.00 | 32.16 | 30.00 |
| | 24 h | 68.03 | 92.94 | 81.78 | 83.64 | 36.99 | 90.52 | 91.64 | 71.19 | 52.42 |

(46.36%) within green species, *Jania rubens* (85.11%) within red species, and *Sargassum hornschuchii* (34.86%) within brown species. However, the minimum removal percentages were reached by *Ulva linza* (9.64%), *Laurencia papillosa* (11.17%), and *Colpomenia sinuosa* (25.55%) for green, red, and brown species, respectively. The green algae species *Ulva fasciata* generally has the best capacity to remove practically all types of dyes. *Pterocladia capillacea* and *Jania rubens*, two red algae species, demonstrated

excellent dye removal. Alaguprathana and Poonkothai (2015) demonstrated that the green alga Spirogyra gracilis was effective in reducing all of the physicochemical components of the effluent, including EC, BOD, COD, and color.

For dried biomass, maximum and minimum removal percentages of RB19 by green algal species were achieved by *Ulva fasciata* (75.2%) and *Ulva intestinalis* (67.1%), respectively, as represented in Table 5 and Fig. 5. The maximum values within red and brown algae were by *Pterocladia*



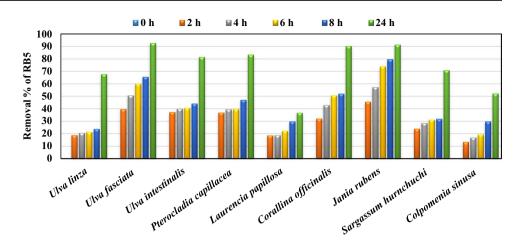
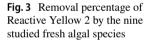
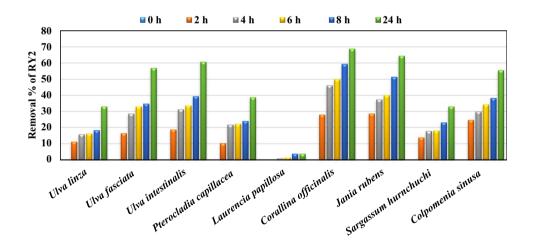


 Table 3
 Removal percentage of Reactive Yellow 2 (RY2) by the nine studied fresh algal species

| Algae | | Green algae | e | | Red algae | | | | Brown algae | |
|-------|------|-------------|---------------|-------------------|---------------------------|------------------------|--------------------------|--------------|---------------------------|-------------------------|
| | | Ulva linza | Ulva fasciata | Ulva intestinalis | Pterocladia capillacea | Laurencia papillosa | Corallina officinalis | Jania rubens | Sargassum hornschuchii | Colpome- nia sinuosa |
| Time | 0 h | 0 | 0 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 2 h | 11.43 | 16.70 | 18.90 | 10.55 | 0.44 | 28.13 | 28.79 | 14.07 | 24.84 |
| | 4 h | 16.04 | 28.79 | 31.43 | 21.98 | 1.10 | 46.37 | 37.58 | 18.02 | 30.11 |
| | 6 h | 16.48 | 33.19 | 33.85 | 22.42 | 1.54 | 50.11 | 40.22 | 18.24 | 34.51 |
| | 8 h | 18.46 | 34.95 | 39.56 | 24.18 | 3.96 | 59.56 | 51.65 | 23.30 | 38.46 |
| | 24 h | 33.19 | 57.14 | 60.88 | 38.90 | 4.00 | 69.01 | 64.62 | 33.19 | 55.82 |





capillacea (56.33%) and *Colpomenia sinuosa* (44.00%), respectively, while the minimum ones were by *Corallina officinalis* (47.00%) and *Sargassum hornschuchii* (34.40%), respectively. As shown in Table 6 and Fig. 6, *Ulva fasciata* removed the RB5 with maximum value 70.91%. However, *Corallina officinalis* recorded the minimum removal percentage which was 17.64%. Table 7 and Fig. 7 illustrated that the maximum removal percentages of RY2 was 65.14, 41.43, and 29.00% by *Ulva fasciata*, *Pterocladia capillacea*,

and *Colpomenia sinuosa*, respectively. The minimum values was reached by *Ulva intestinalis* (48.57%) and *Laurencia papillosa* (21.71%), and *Sargassum hornschuchii* recorded the lowest removal percent within all algal species (19.23%). All algal species decreased in removal at 24 h except *Ulva intestinalis* and *Jania rubens*. The removal percent of RR195 was increased gradually with time till the end of experiment at 24 h. As shown in Table 8 and Fig. 8, the maximum removal percentage was recorded by *Ulva fasciata*

Table 4 Removal percentage of Reactive Red 195 (RR195) by the nine studied fresh algal species

| Algae | | Green algae | e | | Red algae | | | | Brown algae | |
|-------|------|-------------|---------------|-------------------|--------------------------------|------------------------|--------------------------|--------------|---------------------------|------------------------------|
| | | Ulva linza | Ulva fasciata | Ulva intestinalis | Pterocladia capillacea 0 | Laurencia papillosa | Corallina officinalis | Jania rubens | Sargassum hornschuchii | Colpome- nia sinuosa 0 |
| Time | 0 h | | 0 | 0 | | 0 | 0 | 0 | 0 | |
| | 2 h | 0.34 | 8.80 | 10.83 | 23.18 | 5.08 | 25.89 | 23.18 | 18.95 | 16.75 |
| | 4 h | 1.52 | 16.75 | 17.77 | 27.92 | 6.26 | 25.89 | 35.03 | 18.95 | 17.00 |
| | 6 h | 2.37 | 19.63 | 21.66 | 30.12 | 7.28 | 31.30 | 48.90 | 19.97 | 17.43 |
| | 8 h | 4.00 | 22.84 | 25.89 | 37.56 | 8.46 | 35.53 | 56.35 | 24.20 | 21.15 |
| | 24 h | 9.64 | 42.13 | 46.36 | 54.15 | 11.17 | 52.62 | 85.11 | 34.86 | 25.55 |

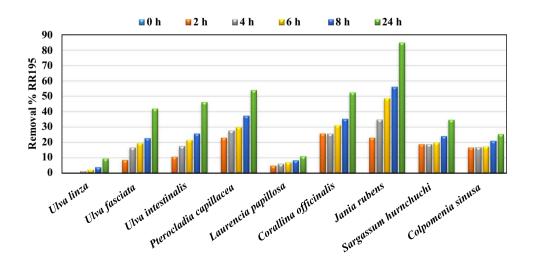
(75.99%). A very low record was reached by *Colpomenia sinuosa* which was just 6.19%. Our results are going with harmony with those obtained by Ghazal et al. (2018) who tested five microalgae strains (*Chlorella vulgaris, Anabaena variabilis, Nostoc ellipsosporum, Nostoc linckia,* and *Anabaena flos-aquae*) for their capability to remove synthetic dyes from waste water effluent and noticed *that Nostoc ellipsosporum* had the highest percentage dye removal of 100%, followed by *C. vulgaris* with 96.16% and *A. variabilis* with

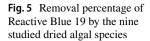
88.71%. Various *Chlorella spp.* demonstrated remarkable bio-removal efficiency for a variety of environmental contaminants including heavy metals, antifouling compounds, and textile dyes (Kaamoush et al. 2022a). In comparison with wet algal biomass, dry algal biomass of *Chlorella pyrenoidosa* was shown to be a more effective biosorbent of methylene blue with a larger surface area and a high binding affinity for MB dye (Pathak et al. 2015).

Table 5 Removal percentage of Reactive Blue 19 (RB19) by the nine studied dried algal species

| Algae | | Green algae | e | | Red algae | | | | Brown algae | |
|-------|------|-------------|---------------|-------------------|---------------------------|------------------------|--------------------------|--------------|---------------------------|-------------------------|
| | | Ulva linza | Ulva fasciata | Ulva intestinalis | Pterocladia capillacea | Laurencia papillosa | Corallina officinalis | Jania rubens | Sargassum hornschuchii | Colpome- nia sinuosa |
| Time | 0 h | 0 h 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 2 h | 42.33 | 44.67 | 40.67 | 31.33 | 26.00 | 28.67 | 28.00 | 20.00 | 28.33 |
| | 4 h | 53.00 | 62.67 | 53.00 | 42.67 | 35.00 | 34.67 | 36.00 | 26.33 | 33.67 |
| | 6 h | 59.00 | 70.33 | 60.33 | 49.67 | 41.33 | 38.00 | 41.33 | 31.00 | 38.00 |
| | 8 h | 63.33 | 75.00 | 67.00 | 56.00 | 47.00 | 42.33 | 46.00 | 34.33 | 42.00 |
| | 24 h | 68.00 | 75.20 | 67.10 | 56.33 | 47.20 | 47.00 | 50.00 | 34.40 | 44.00 |

Fig. 4 Removal percentage of Reactive Red 195 by the nine studied fresh algal species





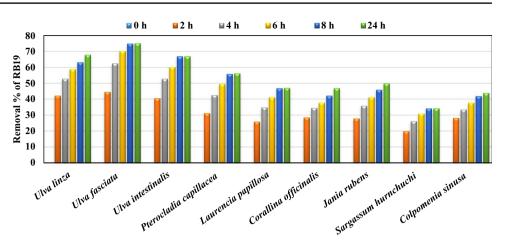
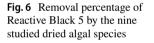
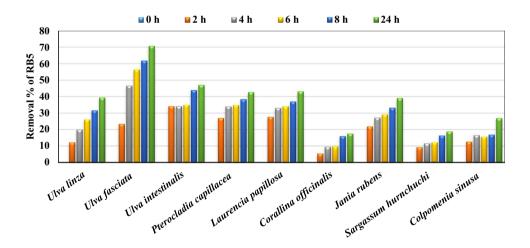


Table 6 Removal percentage of Reactive Black 5 (RB5) by the nine studied dried algal species

| Algae | | Green algae | | | Red algae | | | | Brown algae | |
|-------|------|-------------|---------------|-------------------|---------------------------|------------------------|--------------------------|--------------|---------------------------|------------------------------|
| | | Ulva linza | Ulva fasciata | Ulva intestinalis | Pterocladia capillacea | Laurencia papillosa | Corallina officinalis | Jania rubens | Sargassum hornschuchii | Colpome- nia sinuosa 0 |
| Time | 0 h | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | 2 h | 12.28 | 23.55 | 34.35 | 27.15 | 27.89 | 5.54 | 21.98 | 9.42 | 12.74 |
| | 4 h | 20.04 | 46.81 | 34.35 | 34.16 | 33.33 | 9.70 | 27.42 | 11.73 | 16.71 |
| | 6 h | 26.22 | 56.69 | 35.18 | 35.09 | 34.35 | 10.06 | 29.36 | 12.37 | 15.79 |
| | 8 h | 31.76 | 62.05 | 44.04 | 38.50 | 37.21 | 16.07 | 33.43 | 16.44 | 16.99 |
| | 24 h | 39.52 | 70.91 | 47.18 | 44.84 | 43.31 | 17.64 | 39.24 | 18.93 | 27.05 |





According to our results in previous tables and figures, it was clear that both green and red algal species especially *Ulva fasciata*, *Pterocladia capillacea*, and *Jania rubens* have an effective and high removal ability for all types of dyes. But, the brown algal species even fresh or dried with all types of dyes exhibited very poor removal. For this reason, *Ulva fasciata* and *Pterocladia capillacea* were selected for performing the conditional decolorization experiments, and the brown algal species were excluded. Four dyes were chosen for the further experiments (Reactive Yellow 2, Reactive Red 195, Reactive Blue 19, and Reactive Black 5) due to their abundance in the industrial effluent of textile factories, and their good affinity with almost all algal species. Khaled et al. (2005) studied the ability of *Ulva lactuca*, as a feasible biomaterial for the

Table 7 Removal percentage of Reactive Yellow 2 (RY2) by the nine studied dried algal species

| Algae | | Green algae | | | Red algae | | | | Brown algae | |
|-------|------|-------------|---------------|-------------------|--------------------------------|------------------------|--------------------------|--------------|---------------------------|------------------------------|
| | | Ulva linza | Ulva fasciata | Ulva intestinalis | Pterocladia capillacea 0 | Laurencia papillosa | Corallina officinalis | Jania rubens | Sargassum hornschuchii | Colpome- nia sinuosa 0 |
| Time | 0 h | h 0 | 0 0 | 0 | | 0 | 0 | 0 | 0 | |
| | 2 h | 34.57 | 38.57 | 28.29 | 27.43 | 14.00 | 26.57 | 26.86 | 13.00 | 23.11 |
| | 4 h | 41.43 | 43.43 | 33.43 | 28.86 | 14.29 | 23.43 | 27.14 | 13.90 | 23.99 |
| | 6 h | 50.00 | 59.43 | 43.43 | 35.43 | 16.29 | 28.57 | 31.43 | 15.00 | 26.00 |
| | 8 h | 53.43 | 65.14 | 48.57 | 41.43 | 21.71 | 30.86 | 34.57 | 19.23 | 29.00 |
| | 24 h | 53.43 | 47.14 | 52.29 | 32.00 | 0.0 | 26.00 | 38.00 | _ | 18.32 |

Fig. 7 Removal percentage of Reactive Yellow 2 by the nine studied dried algal species

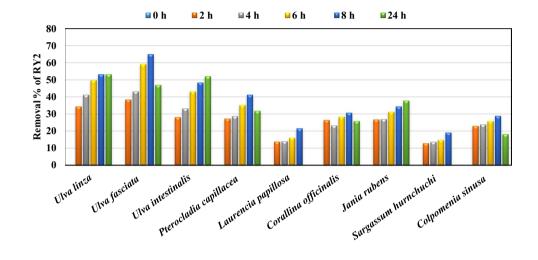


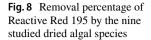
Table 8 Removal percentage of Reactive Red 195 (RR195) by the nine studied dried algal species

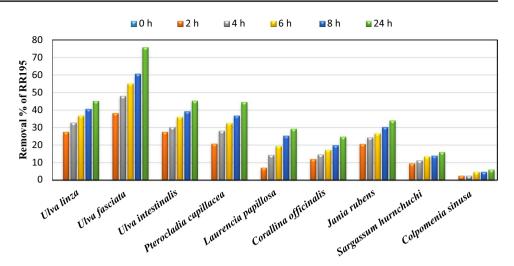
| Algae | | Green algae | e | | Red algae | | Brown algae | | | |
|-------|------|-------------|---------------|-------------------|---------------------------|------------------------|--------------------------|--------------|---------------------------|------------------------------|
| | | Ulva linza | Ulva fasciata | Ulva intestinalis | Pterocladia capillacea | Laurencia papillosa | Corallina officinalis | Jania rubens | Sargassum hornschuchii | Colpome- nia sinuosa 0 |
| Time | 0 h | h 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | 2 h | 27.69 | 38.44 | 27.69 | 20.91 | 7.22 | 12.22 | 20.77 | 9.72 | 2.65 |
| | 4 h | 32.99 | 48.31 | 30.49 | 28.28 | 14.58 | 14.87 | 24.45 | 11.49 | 2.65 |
| | 6 h | 36.97 | 55.38 | 36.23 | 32.70 | 19.59 | 17.38 | 26.80 | 13.70 | 4.86 |
| | 8 h | 40.80 | 60.82 | 39.47 | 36.97 | 25.48 | 20.03 | 30.49 | 14.14 | 4.86 |
| | 24 h | 45.36 | 75.99 | 45.51 | 44.77 | 29.46 | 24.89 | 34.17 | 16.20 | 6.19 |

biological treatment of synthetic basic blue 9 effluents, and they proved, from the batch experiments, the ability of the green alga to remove the blue color, and this ability was dependent on the dye concentration, pH, and algal biomass. Also, Abd El-Razik (2010) proved that potential decolorization ability of some marine algal species to remove textile reactive dyes depends on algal biomass, initial dye concentration, and pH.

Effect of salinity

Textile wastewater is categorized based on its color, salinity, temperature, pH, BOD, COD, total phosphorus, TDS, nitrogen, and heavy metals, all of which have an impact on diverse microalgal species (Khan et al. 2022). Decolorization by sorption is controlled by a number of physicochemical factors, such as interaction between the dye and the sorbent, the surface area, and the biomass, contact period, and





the salinity. Decolorization by sorption happens through two mechanisms: sorption and ion exchange (Dajic et al. 2019). The main aim of this experiment is to compare the ability of both algal species (Ulva fasciata and Pterocladia capillacea) for decolorization in fresh and saline water. After several experiments, the optimum salinity obtained was 38%, freshwater was used as distilled water for 2 g fresh algae and 0.5 g dry, pH of the solution was adjusted to 8, and dye absorbance was evaluated at different times (2, 4, 6, and 8 h). Many authors have observed different effects of salinity on acidic and basic dye removal (Gong et al. 2007; Won and Yun 2008; Esmaeli et al. 2013). As was obvious from the results that both algal species U. fasciata and P. capillacea have good ability to remove the four reactive dyes not only in saline water, but also in freshwater, although with a higher affinity in saline water than in freshwater. At the same time, U. fasciata exhibited better dye removal than P. capillacea, especially in freshwater.

As shown in Table 9 and Fig. 9, fresh *U. fasciata* exhibited higher dye removal percentages for the four studied dyes in saline water than in freshwater, where the maximum removal percentages of RY2, RR195, RB19 and RB5 after 8 h in saline water were 82.75, 83.23, 60.06, and 78.73%, respectively, and in case of freshwater, maximum removal percentage was 58.15, 13.64, 24.40, and 17.77%, respectively. In case of fresh *P. capillacea*, removal percentages of RY2, RR195, RB19, and RB5 after 8 h in saline water

were 51.50, 51.98, 44.20, and 67.08%, respectively, while in freshwater, they were 18.95, 7.56, 13.79, and 12.29%, respectively (Table 9 and Fig. 9). The above results are expected, since physiological performance is perfect in normal salinity conditions. *Ulva sp.* is also known to exhibit physiological plasticity in response to salinity and may be induced to activate the osmoregulatory systems in order to effectively carry out its physiological operations (Esmaeli et al. 2013).

Table 10 and Fig. 10 show that the fresh red algae P. capillacea followed the same pattern as U. fasciata. It exhibited higher removal percentages for the four studied dyes in saline water than in freshwater. As is clear, the removal was rapid at the beginning of the experiment, exponential phase; after that, the biosorption process starts to proceed at a slower rate (transition phase), and then the removal becomes roughly constant (saturation phase). In addition, when the electrostatic forces between the adsorbent surface and dye ions are attractive, an increase in ionic strength will decrease the adsorption capacity. This may be due to the ionic competition between Na⁺ and the cationic dye. This result is consistent those of several authors as Alberghina et al. (2000), Yupeng et al. (2005), Gong et al. (2007) and Salima et al. (2013); they indicated that the high adsorption capacity of Safranin O under salt addition can also be attributed to the aggregation of dye molecules induced by the action of salt ions, increasing the extent of dye adsorptions.

| Table 9 Removal percentages | |
|-------------------------------------|---|
| of the four studied dyes by fresh | h |
| Ulva fasciata under saline and | |
| fresh conditions | |

| | Ren | noval % | of dyes | in saline v | water (389 | %) & fres | h water (| distilled) | |
|------------|---------|---------|-----------|-------------|------------|-------------|-----------|------------|-------|
| D | ye | RY | /2 | RR195 | | RB19 | | RB5 | |
| Туре о | f water | saline | fresh | saline | fresh | saline | fresh | saline | fresh |
| | 2 h | 65.09 | 26.84 | 77.64 | 11.36 | 53.85 | 14.00 | 59.30 | 6.60 |
| T : | 4 h | 77.21 | 40.26 | 82.61 | 12.50 | 60.36 | 24.00 | 70.96 | 9.14 |
| Time | 6 h | 82.14 | 51.76 | 83.23 | 17.05 | 60.65 | 24.00 | 78.12 | 10.66 |
| | 8 h | 82.75 | 58.15 | 83.23 | 13.64 | 60.06 | 24.40 | 78.73 | 17.77 |

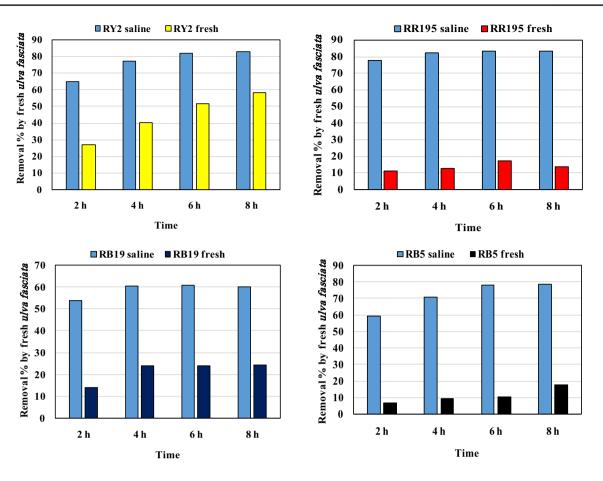


Fig. 9 Removal percentages of the four studied dyes by fresh Ulva fasciata under saline and fresh conditions

| Table 10 | Removal percentages |
|------------|----------------------------|
| of the fou | r studied dyes by fresh |
| Pteroclad | <i>ia capillacea</i> under |
| saline and | l fresh conditions |
| | |

| | Ren | noval % o | of dyes in | saline wa | ter (38% |) & fresh | water (di | istilled) | |
|---------|---------|-----------|------------|-----------|----------|-------------|-----------|-----------|-------|
| Dye | | RY2 | | RR195 | | RB19 | | RB5 | |
| Type of | f water | saline | fresh | saline | fresh | saline | fresh | saline | fresh |
| | 2 h | 18.94 | 12.42 | 24.86 | 1.74 | 25.45 | 10.34 | 31.35 | 8.38 |
| Time | 4 h | 34.22 | 14.38 | 38.42 | 5.23 | 35.71 | 15.09 | 54.23 | 11.17 |
| Time | 6 h | 48.17 | 19.61 | 46.33 | 7.56 | 42.41 | 15.52 | 64.58 | 12.29 |
| | 8 h | 51.50 | 18.95 | 51.98 | 7.56 | 44.20 | 13.79 | 67.08 | 12.29 |

The dried algal biomass exhibited better removal in saline water than in freshwater, as is the case with the fresh algal biomass. It is remarkable in Table 11 and Fig. 11 that the removal percentages of the four dyes by dried *U. fasciata* are higher in saline water than in freshwater, but the differences between them are not drastic. The maximum (58.45%) recorded for the RB19 dye in saline medium, while the maximum removal percent in fresh medium was recorded at 50.50% for the RB5 dye. Dried *P. capillacea* exhibited a lower dye removal rate than *U. fasciata* in saline and freshwater except in RB5 (Table 12 and Fig. 12). The dye's first rapid absorption shows that the sorption process is ionic in nature. Reactive dye molecules that are anionic bond to the

many positively charged organic functional groups on the surface of the biomass (Gulnaz et al. 2004).

Different salinity effects on the elimination of acidic and basic dyes have been seen in earlier research. Esmaeli et al. (2013) studied the effects of different concentrations of NaCl on AB1 dye biosorption and found that in the absence of NaCl, the dye decolorization was 62.14%. And biosorption was increased from 65.14 to 72.24% by adding 0.1–40 g/L NaCl to the dye solution. Also, Al-Tohamy et al. (2022) established that salt content, co-substrate, and electron donor all have an effect on the dye decolorization process, which must be tuned in order to produce a high decolorization response. Won and Yun (2008) suggested that NaCl is

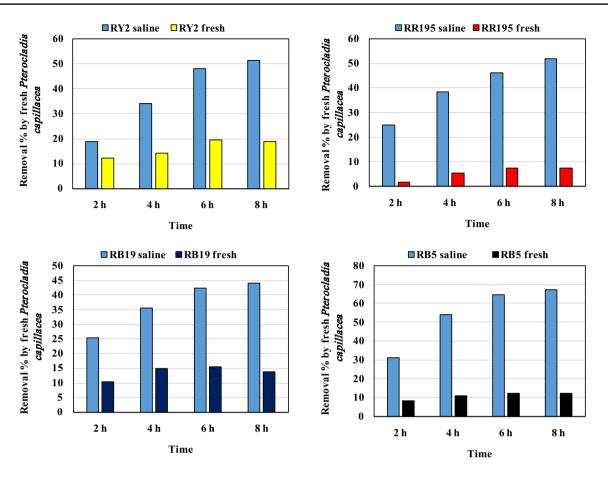


Fig. 10 Removal percentages of the four studied dyes by fresh Pterocladia capillacea under saline and fresh conditions

| | Re | emoval % | of dyes in | n saline w | ater (38% | 6) & fresh | water (d | istilled) | |
|---------|-------|----------|------------|------------|-----------|-------------|----------|-----------|-------|
| Dye | | RY2 | | RR195 | | RB19 | | RB5 | |
| Type of | water | saline | fresh | saline | fresh | saline | fresh | saline | fresh |
| Time | 2 h | 13.84 | 19.47 | 25.73 | 23.30 | 43.48 | 11.65 | 21.59 | 46.80 |
| | 4 h | 31.59 | 30.70 | 37.43 | 29.66 | 56.52 | 17.90 | 35.68 | 48.80 |
| | 6 h | 32.90 | 41.27 | 39.77 | 32.00 | 57.97 | 24.15 | 37.89 | 50.20 |
| | 8 h | 41.51 | 42.93 | 29.24 | 34.90 | 58.45 | 25.45 | 38.00 | 50.50 |

the most common salt used to increase bath dye degradation; as a result, a considerable amount of NaCl was present in several wastewaters, along with dyes that might alter their biosorption.

Effect of contact time

 Table 11
 Removal percentages

 of the four studied dyes by dried
 Ulva fasciata under saline and

fresh conditions

Contact time is the most critical variable impacting biosorption efficiency (Pratiwi et al. 2019). Each and every abiotic characteristic will be highly variable and change quickly across time (El-Sheekh et al. 2021). The dye absorbance was evaluated at different contact times (2, 4, 6, and 8 h), algal biomass, 5 g fresh or 2.5 g dried, and pH of the solution was adjusted to 2. The primary objective of this experiment is to illustrate the power of the optimum conditions on the ability of algae to remove the four dyes; the results show that using these optimum conditions resulted in a brilliant effect on the decolorization process. Table 13 and Fig. 13 show the removal percentages of the four studied dyes by fresh *Ulva fasciata* under the optimum conditions; the fresh alga exhibited remarkable high removal percentages of 69.47, 98.88, 99.56, and 97.06% for RY2, RR195, RB19, and RB5 respectively, after 8 h. As obvious in Table 14 and Fig. 14, the dried *U. fasciata* expressed impressive removal percentages which reached to 100% (RB19 and RB5) after 8 h. The removal of RR195 was also very high (99.05%), while the

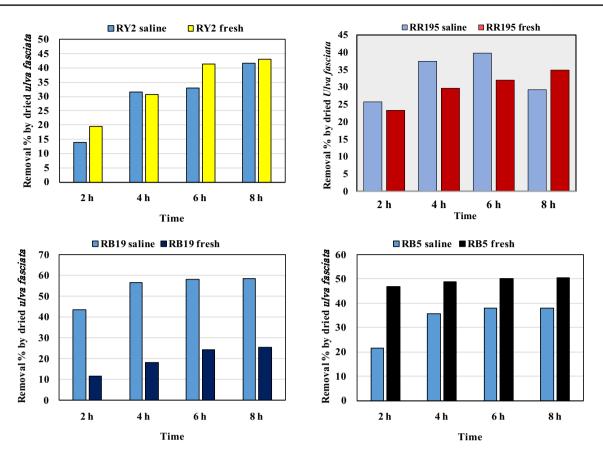


Fig. 11 Removal percentages of the four studied dyes by dried Ulva fasciata under saline and fresh conditions

| Removal % of dyes in saline water (38%) & fresh water (distilled) | | | | | | | | | |
|---|-------|--------|----------|--------|-------|--------|-------|--------|-------|
| Dye | | RY | 2 | RR195 | | RB19 | | RB5 | |
| Type of v | vater | saline | fresh | saline | fresh | saline | fresh | saline | fresh |
| Time | 2 h | 8.88 | 5.20 | 23.39 | 5.88 | 35.75 | 10.78 | 25.55 | 5.26 |
| | 4 h | 17.75 | 11.93 | 35.09 | 11.23 | 43.00 | 19.70 | 37.44 | 7.66 |
| | 6 h | 18.54 | 20.49 | 39.18 | 11.76 | 45.89 | 21.93 | 40.09 | 9.09 |
| | 8 h | 23.76 | 20.49 | 40.00 | 11.76 | 46.00 | 22.30 | 42.29 | 15.31 |

Table 12Removal percentagesof the four studied dyes by driedPterocladia capillacea undersaline and fresh conditions

minimum removal value recorded (36.57%) for the fourth dye (RY2) was comparatively low. Our results agree with those obtained by Omar et al. (2018) which indicated that the adsorption efficacy of *Ulva lactuca* improves with contact time up to 110 min, after which it becomes less stable, and that the bulk of adsorption onto adsorbents was obtained after the first 110 min. Also, Pratiwi et al. (2019) proved that the ability of the marine algae *Ulva lactuca* to remove the dye color (Methylene blue) was dependent on contact time, algae biomass, dye concentration, and pH. The optimum adsorption was found at around pH 8; contact time 110 min; adsorbent dose 1.25 g/L; initial concentration 25 mg/L; and maximum percentage dye removal value of 91.92%. Rajeshkannan et al. (2010) demonstrated that the optimal parameters for maximal removal of Acid Blue 9 from a 100 mg/l aqueous solution using the brown marine algae *Turbinaria conoides* were as follows: temperature (33 °C), adsorbent dosage (3 g/l), contact duration (225 min), and maximum adsorption capacity (38.46 mg/g).

The removal percentages of the four studied dyes by fresh *Pterocladia capillacea* are represented in Table 15 and Fig. 15. After 8 h, the removal percentage of RY2 dye is 30.61%, while the removal percentages of the other dyes (RR195, RB19, and RB5) are 91.11, 94.85, and 97.13%, respectively. *P. capillacea* as dried biomass showed high removal for the four studied dyes (Table 16 and Fig. 16)

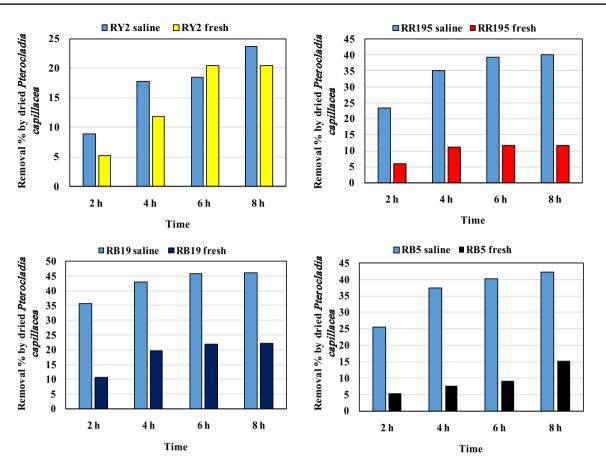
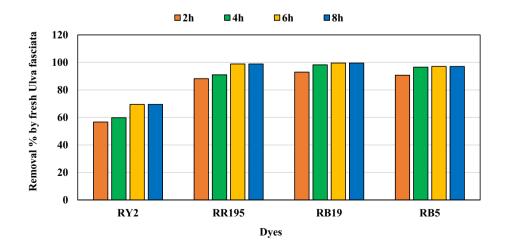


Fig. 12 Removal percentages of the four studied dyes by dried Pterocladia capillacea under saline and fresh conditions

Table 13Effect of contact time
(at optimized conditions) on
the removal percentages of the
four studied dyes by fresh Ulva
fasciata

| Removal % of dyes by fresh Ulva fasciata | | | | | | | | |
|--|----|-------|--------------|-------------|-------|--|--|--|
| Dye | | RY2 | RR195 | RB19 | RB5 | | | |
| Time | 2h | 56.70 | 88.20 | 92.95 | 90.69 | | | |
| | 4h | 59.81 | 91.01 | 98.24 | 96.57 | | | |
| | 6h | 69.47 | 98.88 | 99.56 | 97.06 | | | |
| | 8h | 69.47 | 98.88 | 99.56 | 97.06 | | | |



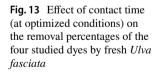


Table 14Effect of contact time(at optimized conditions) onthe removal percentages of thefour studied dyes by dried Ulvafasciata

| | Removal % of dyes by dried Ulva fasciata | | | | | | | |
|------------------------|--|-------|--------|-------|--------|--|--|--|
| Dye RY2 RR195 RB19 RB5 | | | | | | | | |
| | 2 h | 27.31 | 97.18 | 96.69 | 96.50 | | | |
| T • | 4 h | 35.27 | 98.31 | 99.06 | 98.69 | | | |
| Time | 6 h | 36.57 | 100.00 | 99.05 | 99.57 | | | |
| | 8 h | 36.57 | 100.00 | 99.05 | 100.00 | | | |

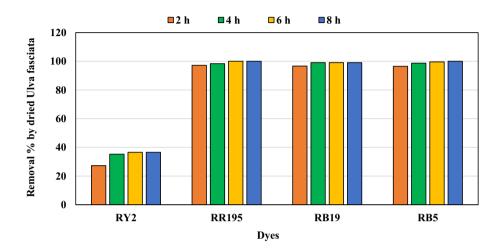
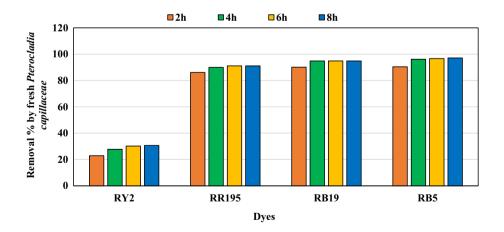


Fig. 14 Effect of contact time (at optimized conditions) on the removal percentages of the four studied dyes by dried *Ulva fasciata*

Table 15Effect of contacttime (at optimized conditions)on the removal percentages ofthe four studied dyes by freshPterocladia capillacea

Fig. 15 Effect of contact time (at optimized conditions) on the removal percentages of the four studied dyes by fresh *Pterocladia*

| Removal % of dyes by fresh Pterocladia capillaceae | | | | | | | | |
|--|----|-------|-------|-------|-------|--|--|--|
| Dye | | RY2 | RR195 | RB19 | RB5 | | | |
| | 2h | 22.86 | 86.11 | 90.13 | 90.43 | | | |
| Time | 4h | 27.76 | 90.00 | 94.85 | 96.17 | | | |
| | 6h | 30.20 | 91.11 | 94.85 | 96.65 | | | |
| | 8h | 30.61 | 91.11 | 94.85 | 97.13 | | | |



but it could not achieve the same fascinating results as *U. fasciata*. The maximum removal percentages were 32.54, 88.27, 92.27, and 91.88% for RY2, RR195, RB19, and RB5, respectively, after 8 h. These changes in removal rate

could be due to a lack of early adsorbent sites and a strong solute concentration gradient (El Nemr et al. 2006). Ayele et al. (2021) proved that the dye absorption rate of various sorbent species to different dyes is fast at the start of the

Table 16Effect of contacttime (at optimized conditions)on the removal percentages ofthe four studied dyes by driedPterocladia capillacea

| Removal % of dyes by dried Pterocladia capillacea | | | | | | | | |
|---|----|-------|-------|-------|-------|--|--|--|
| Dye RY2 RR195 RB19 RB5 | | | | | | | | |
| | 2h | 27.78 | 74.86 | 80.00 | 74.11 | | | |
| | 4h | 28.97 | 84.36 | 91.82 | 87.82 | | | |
| Time | 6h | 32.54 | 88.27 | 92.27 | 90.36 | | | |
| | 8h | 32.54 | 88.27 | 92.27 | 91.88 | | | |

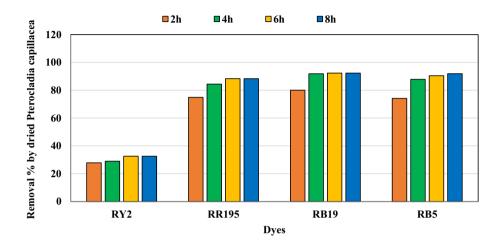


Fig. 16 Effect of contact time (at optimized conditions) on the removal percentages of the four studied dyes by dried *Pterocladia capillacea*

contact period; but, as the contact time approaches equilibrium, the uptake rate slows or stops. The higher biosorption during the early contact period might be attributable to dye driving forces through the surface (El Sikaily et al. 2006). El-Agawany and Kaamoush (2022) proved that environmental contaminants' toxicity toward *Dunaliella tertiolecta* is primarily determined by their concentration and the length of the culture period.

Conclusion

The objective of the study is to evaluate the bio-removal of four synthetic dyes (RY2, RR195, RB19, and RB5) from fresh and dried biomass using two marine algae, Ulva fasciata and Pterocladia capillacea, which were selected from nine algal species based on their removal efficiency for the studied dyes. Our findings showed that the various ratios of the selected algae species were significantly relevant to the biosorption of the most commonly used dyes. In order to examine the effects of salt on the two studied algae throughout the decolorization process, saline and freshwater were employed, and the removal rates of the four dyes were higher in saline than in fresh circumstances. According to our findings, both algae species, U. fasciata and P. capillacea, are capable of effectively removing reactive dyes from both freshwater and saltwater, however with a higher affinity in saline conditions. The removal efficiency of the four studied dyes (RY2, RR195, RB19, and RB5) after 8 h by fresh U. fasciata in saline water reached 82.75, 83.23, 60.06, and 78.73%, respectively, and in fresh P. capillacea, they reached 51.50, 51.98, 44.20, and 67.08%, respectively. In freshwater, fresh U. fasciata recorded 58.15, 13.64, 24.40, and 17.77%, respectively, and fresh P. capillacea recorded 18.95, 7.56, 13.79, and 12.29%, respectively. For dried U. fasciata, the maximum removal percentages recorded were 58.45% for RB19 in saline medium and 50.50% for RB5 in fresh medium. Dried P. capillacea exhibited a lower removal rate than U. fasciata of all dyes in saline and freshwater except in RB5. The most critical factor influencing biosorption efficiency was contact time. Fresh U. fasciata under the optimum conditions exhibited remarkable high removal percentages of 69.47, 98.88, 99.56, and 97.06% for RY2, RR195, RB19, and RB5, respectively, after 8 h, while dried U. fasciata achieved impressive removal percentages that reached 100% for RB195 and RB5 after 8 h. Fresh P. capillacea has high removal percentages of 91.11, 94.85, and 97.13% for RR195, RB19, and RB5 dyes, respectively, after 8 h, while RY2 dye has the lowest removal percentage (30.61%). Dried P. capil*lacea* recorded maximum removal percentages of 88.27, 92.27, and 91.88% for RR195, RB19, and RB5, respectively, and a minimum removal rate of 32.54% for RY2, after 8 h. Our results revealed that the used algal species were highly significant in the biosorption of most used dyes. We suggest extending our research into the use of diverse algae species for removing different pollutants from wastewater in future studies.

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Authors' contributions N.I.A., S.A.R.A., M.S.A., and M.I.A.K. contributed equally to the manuscript preparation. N.I.A., S.A.R.A., M.S.A., and M.I.A.K. designed the experiment and performed the laboratory analyses. N.I.A., S.A.R.A., M.S.A., and M.I.A.K. carried out the statistical analyses and tabulated the study results. N.I.A., S.A.R.A., M.S.A., and M.I.A.K. wrote the first draft and revised the final version of the paper. N.I.A., S.A.R.A., M.S.A., and M.I.A.K. read and agreed on the submitted paper.

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Availability of data and materials All data generated or analyzed during this study are included and available in this article.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Not applied.

Consent to participate I voluntarily agree to participate in this research study. I understand that even if I agree to participate now, I can withdraw at any time or refuse to answer any question without any consequences of any kind.

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