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Dyes Bio-Sorption by Two Marine Algae and Their Applications on Industrial Effluents from Borg El-Arab region, Egypt

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BATCH biosorption experiments were carried out for the removal of four dyes; Drimarene yellow CL4R (Y), Drimarene blue K2RL (B), Congo Red (CR) and Malachite Green (MG) from their aqueous solutions using two algal biomasses namely *Gelidium latifolium* (Grev.) Bornet et Thuret (red alga) and *Ulva lactuca* Linnaeus (green alga). The optimum conditions were applied in the treatment of eleven coloured industrial effluents from Borg El-Arab region, Egypt. Some modifications of the algal surface due to the bio-sorption process were noticed using SEM such as protuberance, wrinkle, shrinkage, stickiness, rupture and roughness. The bio-sorption process led to several band shifts that were detected using FTIR revealing the participation of C-N-S, alcoholic C-O, carboxylic C-O, carboxylic C=O, NH and OH groups in dyes bio-sorption. This bio-sorption was nonspontaneous, endothermic and chemical in nature. Freundlich isotherm model fitted slightly better than the Langmuir model indicating multilayer coverage (heterogeneous sorption). Utilization of C_2H_5OH for Y and B; NH_4OH and HNO_3 for CR and MG, respectively was showed the most desorption efficiency for 4 reuse cycles of sorption-desorption. However, desorption and resorption efficiencies decrease with increasing the reusability cycle number. *Ulva lactuca* has shown much better dyes bio-sorption efficiency particularly with solid/liquid ratio: 1/1000 g/mL, initial dye concentration: 400 mg/L, temperature: 40° C, contact time: 1 hour, pH: 1, 2, 8 and 7 for Y, B, CR and MG, respectively. The applied decolourization of coloured samples reached 97.46 %.

Keywords : Industrial wastewater treatment, Bio-sorption, Macro-algal biomass, Dyes, Borg El-Arab city

Bio-sorption is the removal of pollutants from an aqueous solution by the passive binding to living or nonliving biological biomass. In contrast, the term bioaccumulation describes an active process (Davis *et al.*, 2003). Bio-sorption is possible by both living and non-living biomasses; however, bioaccumulation is mediated only by living biomass. Further, bioaccumulation is a growth dependent

process and it is difficult to define a variety of effluents in contrast to bio-sorption which is growth independent (Gupta *et al.*, 2000). The use of inactivated biomass is advantageous as the process is free from nutrient supply and moreover there are no toxicity constraints in the organism employed (Özer *et al.*, 2005).The major advantages of bio-sorption over conventional treatment methods are low cost, high efficiency, eco-friendship, regeneration of bio-sorbents and possibility of pollutant recovery (Ahalya *et al.*, 2003).

The Color is the first contaminant to be recognized in wastewaters and has to be removed before discharging into water bodies or on land. The presence of very small amounts of dyes in water (less than 1 ppm for some dyes) is highly visible and affects the aesthetic merit, water transparency and gas solubility in lakes, rivers and other water bodies (Gajare and Menghani, 2012). Dye wastewater is very difficult to treat, because of its synthetic origin and complex aromatic structure.

The currently used anionic and cationic synthetic dyes (as Y, B, CR and MG) are highly water soluble azo or anthraquinone-based reactive dyes that must be removed from wastewater completely (Leechart *et al.*, 2009). Most of these dyes are toxic and potentially carcinogenic in nature and hence their removal from the industrial effluents is a major environmental problem (Ali *et al.*, 2014). Moreover dyes inhibit development of aquatic animals and plants by blocking out sunlight penetration and being concentrated throughout the food chain (Özer *et al.*, 2005). Due to the chemical stability and low biodegradability of these dyes, conventional biological wastewater treatment systems are inefficient in treating colored wastewater. Algae are low-cost and locally available adsorbing materials with high removal and bio-sorption capacity to colors from industrial wastewater. Bio-sorption in algae has mainly been attributed to the contents and properties of cell wall polysaccharides, where the electrostatic attraction and complexation can play a role (Davis *et al.*, 3003).

Scanning Electron Microscopy (SEM) is widely used to study the morphological features and surface characteristics of the adsorbent material. It also reveals the surface texture and porosity of the adsorbent. SEM micrograph demonstrated the highest affinity for the dye by bio-sorbent (Mahmoud, 2014). Fourier Transform Infrared (FTIR) spectroscopy involves collecting absorption information in the form of spectra. These spectra specify the absorption signals and the corresponding functional groups on the pure biomass surface to be compared with the referenced data of IR absorption (Fakhry, 2013). The objectives of this paper were: (1) to detect the optimum factors and methods to remove some dyes from artificial aqueous solutions using highly efficient Egyptian algal biomasses via batch biosorption. (2) to apply these optimum conditions in the treatment of actual polluted industrial wastewater collected from Borg El-Arab region.

Materials and Methods

Marine algal (Seaweed) biomasses were collected from Abou-Keer and Almontazah shores, Alexandria (Egyptian Mediterranean Sea) during April 2013. The algal taxa were identified and classified according to Aleem (1993) and Zinova (1967). The collected algae immediately washed with the surrounding water to remove extraneous matters, sand particles, epiphytes and water squeezed out. *In vitro,* algal biomasses were severally washed with distilled water prior to air drying.

Both of Drimarene yellow CL4R and Drimarene blue K2RL were a gift from Clariant textile industries company, UK. Both of Congo Red and Malachite Green were purchased from Bioworld chemicals, USA.

Bio-sorption batch experiments

The dried algal biomasses were conducted in pretreatment experiment by shaking in 0.1 N solutions of acids (H_2SO_4 and H_3PO_4) and alkalis (NaOH and NH_4OH) by S/L ratio of 1:50 g/mL for 1 hour then filtered, washed and dried. Afterwards the dried alga was immersed in dye aqueous solution (Solid/liquid ratio 1:500 g/mL) for 24 hr at room temperature. The initial dye concentration was 200 mg/L. Solutions' pH are 4.8, 5.4, 7.0 and 4.9 for Y, B, CR and MG, respectively.

All the batch experiments were carried out in triplicate. Unless otherwise stated, 0.025 g of algal biomass was put in contact with 25 mL of dye aqueous solution at room temperature for 1 hour. The solution was then filtered and the remained dye concentration was analyzed using UV/Visible Spectrophotometer, Unicam UV 300, Thermo Spectronic, USA. Each dye was determined at the appropriate wavelength of the highest absorbance (λ_{max}) that was 420, 619, 497 and 617 nm for Y, B, CR and MG, respectively.

pH of the dye solutions was adjusted using pH meter by addition of dilute solutions of HCl and NaOH. No further adjustment of pH was made during the experiments.

Calculations and data evaluation

a- The amount of biosorbed dye to algal biomass was expressed according to Vitor and Corso (2008) and Kumar *et al.* (2005) where:

$$q_1 = [(C_i - C_f)\ V]/M \quad \& \quad q_2 = [(C_i - C_f)/C_i]\ X100$$

(q_1) is the amount of sorbed dye onto the unit amount of the biomass (mg/g), (C_i) is the initial concentration of dye in aqueous solution (mg/L), (C_f) is the final (remained) concentration of dye in aqueous solution (mg/L), (V) is the volume of

dye aqueous solution (L) and (M) is the biomass weight (g) & (q_2) is the amount of removed dye from the aqueous solution (%).

b- In biosorption isotherm modeling, the linearized Langmuir and Freundlich adsorption isotherms were applied according to Salima *et al.* (2013) and Farah and El-Gendy (2013) where: $C_e/q_e = 1/(Q_e K_L) + (1/Q_e) C_e$

(Ce) is the dye concentration in aqueous solution at equilibrium (mg/L), (q_e) is the experimental amount of adsorbed dye at equilibrium (mg/g), (Q_e) is the calculated amount of adsorbed dye at equilibrium (mg/g) and (K_L) is the Langmuir constant indicating the adsorption affinity of the binding sites (L/mg). Via plotting (C_e/q_e) versus (C_e), (Q_e), and (K_L) can be determined from the slope and intercept of the obtained straight line respectively.

Whereas the linearized logarithmic Freundlich equation assumes as:

$$\text{Log } q_e = \text{Log } K_F + (1/n) \text{ Log } C_e$$

(K_F) is the Freundlich constant indicating adsorption capacity, (n) is the Freundlich constant indicating adsorption intensity. By plotting (Log q_e) versus (Log C_e), (n) and (K_F) can be determined from the slope and intercept of the obtained straight line respectively.

c- The thermodynamic parameters such as changes in standard free energy (ΔG), enthalpy (ΔH) and entropy (ΔS) are determined by using the following equations according to Farah and El-Gendy (2013) and Saibaba and King (2013), where:

$K_c = C_a/C_e$ & $\ln K_c = (-\Delta H/R).(1/T) + \Delta S/R$ and $\Delta G = \Delta H - \Delta ST$

(K_c) is the equilibrium constant, (C_a) is the adsorbed dye "initial concentration-final concentration" (mg/L), (C_e) is the remained "final concentration" (mg/L), (ΔH) is the change in enthalpy "heat content" (j/mol), (R) is the gas constant (8.314 j/mol.K), (T) is the temperature (K), (ΔS) is the change in entropy "randomness" (j/mol.K) and (ΔG) is the change in Gibbs' free energy of dye removal(j/mol). The ΔH and ΔS values can be obtained from the slope and intercept, respectively of the Van't Hoff plots of ln K_c versus 1/T. Meanwhile ΔG values are calculated based on ΔH and ΔS values.

d- The amount of desorbed dye from algal biomass (de-loaded) using C_2H_5OH, NH_4OH and HNO_3 as eluents was expressed according to Bulgariu and Bulgariu (2014) and Kanwal *et al.* (2013), where Desorption %= ($q_{desorbed}/q_{sorbed}$) X 100

e- The amount of resorbed dye to algal biomass (re-loaded) was expressed according to Aboulsoud (2008), where Resorption %= ($q_{resorbed}/q_{sorbed}$) X100

Examination of algal surface by Scanning Electron Microscopy (SEM)

The dried algal samples (raw and dyes loaded biomasses) were coated by gold sputter coater (SPI-Module). The coated samples were examined by Scanning Electron Microscope, JSM-5500 LV, JEOL, Japan. The accelerating voltage used was 18 KV.

Determination of functional groups onto the algal biomass by Fourier Transform Infrared (FTIR)

Fine powdered dried algal samples (raw and dyes loaded biomasses) were pressed into KBr pellets prior to analysis by FTIR Spectrophotometer, 8201 DC, Shimadzu, Japan (wavenumber range from 400-4000 cm^{-1}).

Application of algal biomasses in the treatment of polluted industrial effluents in Borg El-Arab region

Eleven color wastewater samples were collected from four industrial zones of Borg El-Arab city during March to December 2010. The samples were collected from the factories effluents of four different activities representing textile dyeing, metal, steel, concrete, soap and food, in addition to two samples of a combined wastewater of collection of factories. The green algal biomass *Ulva lactuca* was used in decolourization of wastewater samples. The concluded optimum conditions from the batch experiments (solid/liquid ratio 1/1000 g/mL for 1 hr at 40°C) were conducted and no pH adjustment was done. Colored samples were scanned using UV/Visible Spectrophotometer, Unicam UV 300, Thermo Spectronic, USA within the range from 400-1000 nm to detect the peak of the appropriate wavelength of measurement.The removal percentage of colour intensity was calculated using the following equation: Removal (%) = $(C_i - C_f / C_i)$ X 100; where C_i is the initial color intensity before treatment, and C_f is the final color intensity after treatment.

Statistical analysis

All determinations were made in triplicate for all assays. Data were subjected to an analysis of variance (ANOVA) with statistical significance at $P<0.05$ being tested using the Duncan's Test. Mean having the same alphabetical letters in the same column are not significant at P (significance probability value) =0.05 level.

Results and Discussion

Pretreatment experiment

As can be noticed from Figure 1, the studies raw algal biomass is the most efficient form for the biosorption of the four investigated dyes. The two algal biomasses follow the same behavior. However algal cell wall modification with acid and alkali pretreatment can greatly alter the binding of sorbate and may remove lipids and proteins that mask reactive sites (Gupta *et al.*, 2000).

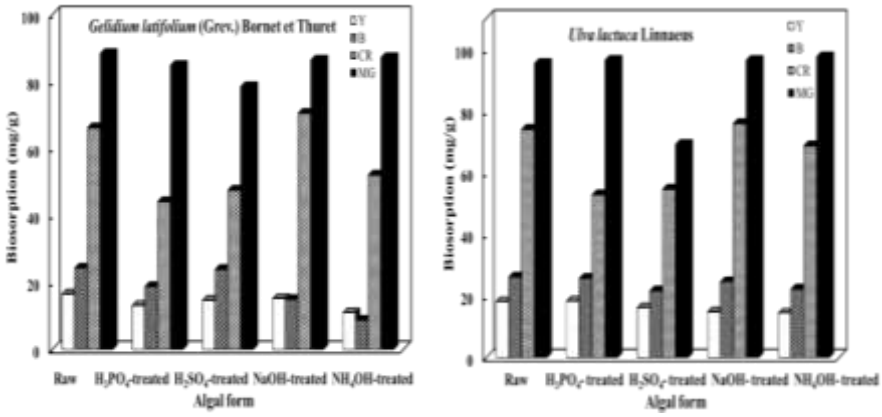

Fig. 1. Effect of acidic and basic pretreatment of algal biomasses on dyes biosorption efficiency (Experimental conditions: contact time: 24 hours, temperature: 25°C, initial dye concentration: 200 mg/L, S/L ratio: 1/500 g/mL, solutions' pH: 4.8, 5.4, 7.0 and 4.9 for Y, B, CR and MG, respectively).

Effect of solid/liquid ratio on dyes bio-sorption efficiency

Data in Table 1 indicated that, bio-sorption efficiency (%) is leveled off at S/L ratio of 1/1000 g/mL. The two studied algal biomasses follow the same behavior. Moreover, for the four investigated dyes, *Ulva lactuca* recorded the highest bio-sorption efficiency values. (Khaled *et al.*, 2005) reported that, the increase in dye bio-sorption (%) with the S/L ratio increasing is due to increase in availability of dye binding sites resulting from an increase in adsorbent dosage.

Effect of pH on dyes bio-sorption efficiency

Previously, several researchers had proven that bio-sorption processes using algae are highly pH dependent and the most important parameters to be considered (Kumar *et al.*, 2005). pH affects not only the bio-sorption capacity, but also the colour of the dye solution and the solubility of some dyes (Özer *et al.*, 2005). Results of pH range for each dye (Table 2) showed to vary according to its type and the stability of its color. The pH studying ranges are 1-11, 2-11, 8-11 and 3-9 for Y, B, CR and MG, respectively. Lower or higher pH values than these ranges cause a change in dye color and thus be unsuitable to be measured at the same wavelength (λ_{max}). Optimum pH values (Table 2) using the studied two algal biomasses gave the most significantly efficient bio-sorption (1, 2, 7 and 8) for Y, B, CR and MG are respectively. For the four investigated dyes, *Ulva lactuca* showed the highest bio-sorption efficiency values. This can be explained on the basis of zero point of discharge for biomass. For algal species, the isoelectric point would be at a pH of 3. Above this pH the surface of algae may acquire a negative charge leading to increased dye cation uptake due to the electrostatic force of attraction (Kumar *et al.*, 2005). MG is a cationic dye, so it attracted to the negatively charged algal biomass at high pH values. Both of Y and B

are anionic reactive dyes, therefore attracted due to the electrostatic forces with the positively charged algal biomass at low pH values.

TABLE 1. Effect of solid/liquid ratio on dyes bio-sorption efficiency (Experimental conditions: contact time: 24 hours, temperature: 25°C, initial dye concentration: 200 mg/L, solutions' pH: 4.8, 5.4, 7.0 and 4.9 for Y, B, CR and MG, respectively).

S/L ratio (g/mL)	Biosorption efficiency															
	Y		B		CR		MG		Y		B		CR		MG	
	mg/g	%	mg/g	%	mg/g	%	mg/g	%	mg/g	%	mg/g	%	mg/g	%	mg/g	%
	Gelidium latifolium								*Ulva lactuca*							
1/100	9.14	45.83	11.95	59.86	17.66	88.79	18.60	93.09	10.75	53.93	8.99	45.04	18.22	91.6	18.85	94.35
1/500	16.65	16.89	24.36	24.84	66.29	66.5	88.47	88.56	18.23	18.29	26.19	26.24	74.11	74.52	95.45	95.55
1/1000	31.1	15.6	46.22	23.16	118.89	59.77	153.74	76.95	34.53	17.32	48.44	24.27	136.6	68.69	158.2	79.18

TABLE 2. Interaction effect between algal type and aqueous solution pH on dyes bio-sorption efficiency (Experimental conditions: contact time: 24 hours, temperature: 25°C, initial dye concentration: 200 mg/L, S/L ratio: 1/1000 g/mL).

Algal type	pH	Biosorption (mg/g)							
		Y		B		CR		MG	
Gelidium latifolium	**1**	100.81	b						
	2	62.96	c	62.96	b			132.94	g
	3	32.56	ghi	22.09	gh			133.38	g
	4	33.60	fghi	39.04	cd			168.22	d
	5	32.06	ghijk	31.72	def			177.26	b
	6	28.06	jklm	38.56	cde			177.78	ab
	7	25.50	m	33.20	def	105.99	f	178.43	ab
	8	26.80	lm	24.00	fgh	160.98	b	179.08	ab
	9	32.30	ghij	26.66	fgh	109.99	e		
	10	35.12	fgh	24.83	fgh	97.68	g		
	11	41.25	e	27.61	fgh	90.01	h		
Ulva lactuca	**1**	122.25	a						
	2	55.03	d	77.85	a			131.88	g
	3	37.23	f	28.16	fgh			150.01	f
	4	30.86	hijkl	19.36	h			163.10	e
	5	33.40	fghi	32.66	def			170.31	c
	6	27.76	klm	42.10	c			178.18	ab
	7	36.46	fg	30.08	efg	122.40	d	179.72	a
	8	29.93	ijkl	32.76	def	142.0	c	179.68	a
	9	34.66	fgh	27.10	fgh	178.73	a		
	10	42.86	e	29.46	fg	177.56	a		
	11	31.76	hijk	26.63	fgh	106.22	f		

Statistical data in Table 3 indicated that the bio-sorption efficiency reaches a plateau at 200 mg/L to give the highest removal values at 400 mg/L (optimum initial dye concentration) where equilibrium is attained. The two studied algal biomasses follow the same behavior. Moreover, for the four investigated dyes, *Ulva lactuca* recorded the highest bio-sorption efficiency values. At equilibrium, no further increase in bio-sorption is noticed as all bio-sorbents have a limited number of active sites, which become saturated at a certain concentration (Saha *et al*., 2012 and Mulugeta and Lelisa, 2014).

TABLE 3. Interaction effect between algal type and initial dye concentration on dyes bio-sorption efficiency (Experimental conditions: contact time: 24 hours, temperature: 25°C, S/L ratio: 1/1000 g/mL, solutions' pH: 1, 2, 8 and 7 for Y, B, CR and MG, respectively).

Algal Type	Dye concentration (mg/L)	Biosorption (mg/g)			
		Y	B	CR	MG
Gelidium latifolium	**0.5**	0.422 ^{m}	0.399 ^{n}	0.473 ^{n}	0.487 ^{j}
	1	0.899 ^{m}	0.880 ^{n}	0.899 ^{n}	0.910 ^{j}
	5	3.969 ^{l}	3.639 ^{m}	3.923 ^{m}	4.511 ^{i}
	10	6.090 ^{k}	7.750 ^{k}	8.357 ^{k}	7.987 ^{h}
	25	23.83 ^{h}	20.30 ^{j}	22.31 ^{j}	23.05 ^{g}
	50	39.72 ^{g}	36.64 ^{h}	43.48 ^{h}	45.05 ^{f}
	100	55.42 ^{f}	53.39 ^{f}	81.51 ^{f}	91.46 ^{e}
	200	98.93 ^{d}	60.37 ^{d}	163.26 ^{d}	181.44 ^{c}
	400	101.50 ^{c}	61.76 ^{c}	166.70 ^{c}	185.76 ^{a}
Ulva lactuca	**0.5**	0.444 ^{m}	0.435 ^{n}	0.487 ^{n}	0.487 ^{j}
	1	0.891 ^{m}	0.936 ^{n}	0.910 ^{n}	0.910 ^{j}
	5	3.940 ^{l}	4.477 ^{l}	4.511 ^{l}	4.511 ^{i}
	10	6.637 ^{j}	7.720 ^{k}	7.987 ^{k}	7.987 ^{h}
	25	21.55 ^{i}	22.27 ^{i}	23.05 ^{i}	23.05 ^{g}
	50	39.92 ^{g}	39.36 ^{g}	45.06 ^{g}	45.05 ^{f}
	100	59.15 ^{e}	58.36 ^{e}	87.31 ^{e}	94.04 ^{d}
	200	122.42 ^{b}	79.33 ^{b}	178.38 ^{b}	181.58 ^{c}
	400	125.19 ^{a}	83.08 ^{a}	181.32 ^{a}	184.83 ^{b}

Bio-sorption isotherm modeling

As can be observed, the calculated (Q_e) values (Table 4, Fig. 2 and 3) more or less match with the experimental ones ($q_{e\ (exp)}$) that are determined also in the level of high concentrations. These may reflect the applicability of bio-sorption in the treatment of water samples that contain low and high concentrations of dyes. On the other hand, K_L values indicate the adsorption affinity of the binding sites where the good sorption is indicated by low values of Langmuir parameter (Kumar *et al.,* 2005). Freundlich constant (n) values (Table 4) are greater than unity; this indicate the favourability of the studied algal biomass to biosorb dyes from water. Also the higher adsorption capacity (K_F) indicates the strong electrostatic attraction force (Kumar *et al.,* 2005); therefore the higher (K_F) calculated values are found to be proportional to the actual high bio-sorption capacities ($q_{e\ (exp)}$) and vice versa. It can be reported that Freundlich isotherm model fitted slightly better than the Langmuir model. *Ulva lactuca* recorded the highest (K_F) and ($q_{e\ (exp)}$) values except in bio sorption of MG dye. This finding assumes that the adsorbent (algal biomass) consists of a heterogeneous surface composed of different classes of adsorption sites and the sorption of the investigated dyes were a heterogeneous sorption or multilayer coverage (Aravindhan *et al.*, 2007).

Effect of temperature on dyes bio-sorption

The bio-sorption of dyes is investigated as a function of temperature (Table 5) ranging from 298 to 333 K (25° - 60° C). Working at higher temperature than this range leads to evolving of the internal pigments of algal biomasses therefore changes the colour of dye aqueous solution. Data in Table 5 showed that, dye bio-sorption slightly increases with rise in temperature from 298 to 333 K. This can be explained by the increased affinity of binding sites for dye molecules. Saha *et al.* (2012) reported that an increase in temperature also results in an increase in mobility of the dye molecules and a decrease in the retarding forces acting on the molecules. These may enhance of dye binding capacity of the bio-sorbent.

Although there is no significant difference between bio-sorption efficiency at 40° and 60°C but 40°C was chosen as the optimum temperature for dyes bio-sorption as it is as similar as possible to the Egyptian climatic conditions. These results agree to some extent with Fakhry (2013) findings about adsorption of fast yellow dye onto dried biomass of *Padina pavonica* where the optimum temperature was 35°C.

TABLE 4. Langmuir and Freundlich equilibrium parameters for dyes bio-sorption.

Dye type	Langmuir parameters			Freundlich parameters			$q_{e\,(exp)}$ (mg/g)	Langmuir parameters			Freundlich parameters			$q_{e\,(exp)}$ (mg/g)
	K_L (L/mg)	Q_e (mg/g)	R^2	K_F	n	R^2		K_L (L/mg)	Q_e (mg/g)	R^2	K_F	n	R^2	
	Gelidium latifolium							**Ulva lactuca**						
Y	0.08	101	0.997	6.52	1.1	0.998	101.5	0.062	122	0.991	6.58	1.08	0.997	125.19
B	0.106	64.1	0.991	5.2	1.18	0.994	61.76	0.084	85.5	0.997	5.85	1.12	0.996	83.08
CR	0.061	169.5	0.983	7.57	1.05	0.998	166.7	0.055	185.2	0.991	8.72	1.05	0.998	181.32
MG	0.052	181.8	0.994	9.89	1.06	0.999	185.76	0.054	188.7	0.989	9.13	1.05	0.998	184.83

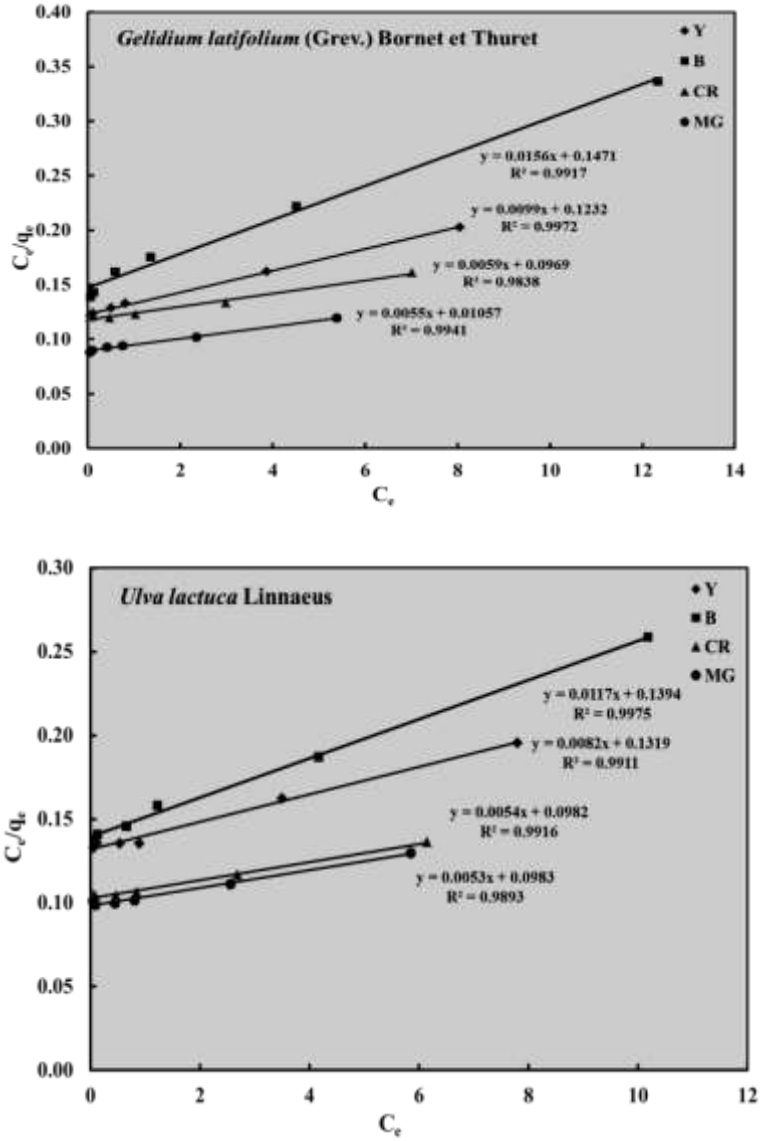

Fig. 2. Linearized Langmuir adsorption isotherms for dyes.

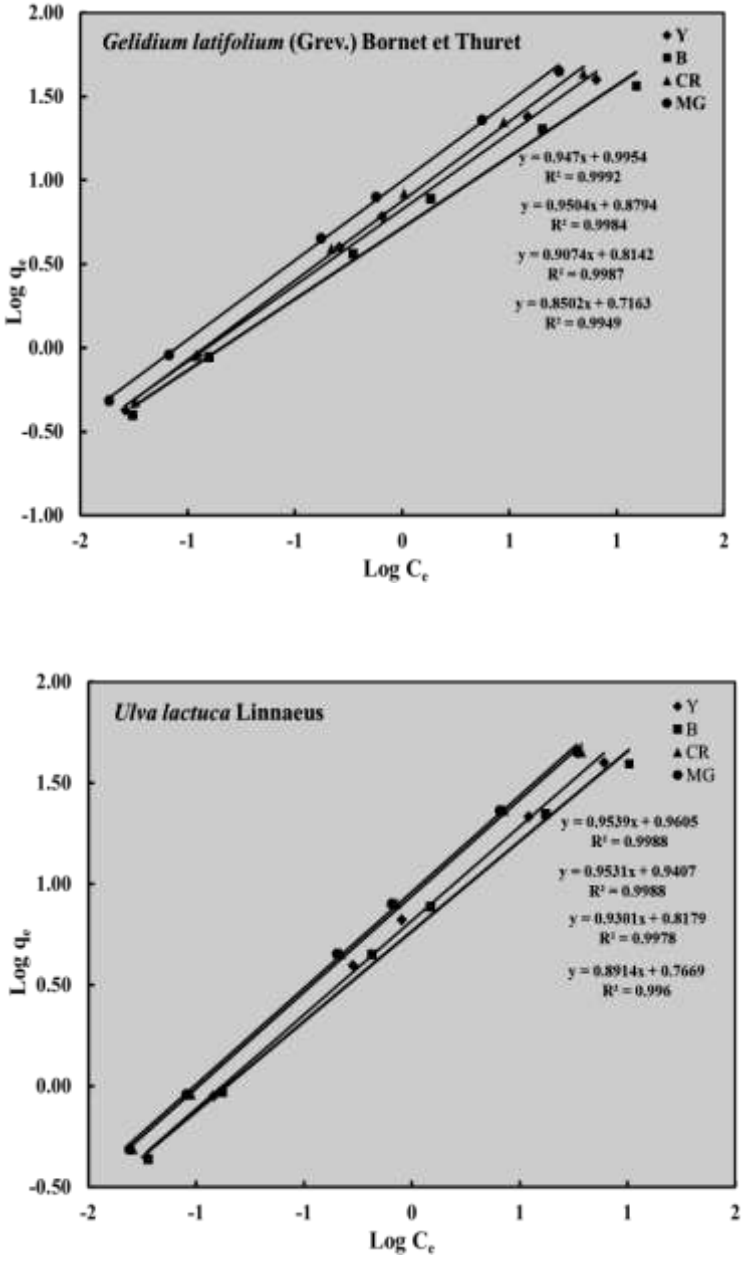

Fig. 3. Linearized Freundlich adsorption isotherms for dyes.

TABLE 5. Interaction effect between algal type and temperature on dyes bio-sorption efficiency (Experimental conditions: contact time: 24 hours, initial dye concentration: 400 mg/L, S/L ratio: 1/1000 g/mL, solutions' pH: 1, 2, 8 and 7 for Y, B, CR and MG, respectively).

Algal type	Temperature		Biosorption (mg/g)					
	C	K	Y	B		CR	MG	
Gelidium latifolium	**25**	**298**	100.74 [d]	60.80 [e]		167.53 [f]	183.37 [c]	
	40	**313**	102.63 [c]	62.77 [d]		168.53 [e]	184.13 [b]	
	60	**333**	103.62 [c]	63.73 [c]		169.73 [d]	184.63 [b]	
Ulva lactuca	**25**	**298**	124.30 [b]	83.67 [b]		180.47 [c]	184.70 [b]	
	40	**313**	125.20 [b]	84.63 [a]		181.10 [b]	185.37 [a]	
	60	**333**	126.43 [a]	85.00 [a]		182.20 [a]	185.87 [a]	

Thermodynamic studies

The data obtained from the batch bio-sorption experiments at different temperatures are used to evaluate the thermodynamics of the bio-sorption process. The current results in Table 6 and Figure 4 revealed that, Van't Hoff plots of ln K_c versus 1/T is considered satisfactory due to the high coefficient of correlation values (R^2). The positive values of (ΔG) indicate the nonspontaneous nature of adsorption (Aksu, 2002) for the four tested dyes using both algal biomasses. The positive values of (ΔH) show the endothermic nature of the bio-sorption (Aksu, 2002), which is an indication of the existence of a strong interaction between algal biomasses and the four dyes. The positive enthalpy of adsorption obtained indicates chemical adsorption. This suggests that the chemical bonds between the algal surface and the dye molecules are strong enough and the dye molecules cannot be easily desorbed by physical means such as simple shaking or heating (Farah and El-Gendy, 2013). The negative values of (ΔS) reflecting that the dyes molecules were orderly adsorbed on the surface of the algal biomasses (Saibaba and King, 2013). By summarizing up the results, the reaction between the studied algal biomass and different dyes is nonspontaneous, endothermic, chemical and orderly adsorption on the algal surface.

Effect of contact time on dyes bio-sorption

The interaction effect between the algal type and contact time (Table 7) showed that the optimum time is 1 hr. The dye bio-sorption profiles distinctly show three phases as described by Khaled *et al.* (2005). The rate of removal is less initially (phase 1) progressively increases and attains a rapid phase with progression of contact time (phase 2) and finally is leveled and attains saturation after a contact time of 1 hour and remains more or less constant thereafter up to 24 hr. In this *Ulva lactuca* also recorded the highest bio-sorption efficiency values. However, the highest bio-sorption efficiency reached 125.18, 84.63, 181.1 and 185.36 mg/g for Y, B, CR and MG, respectively using the green algal biomass *Ulva lactuca*. The lower bio-sorption efficiency reached 102.63, 62.76, 168.53 and 184.13 mg/g for Y , B , CR and MG , respectively using the red

algal biomass *Gelidium latifolium*. These data reflects the economical application of the algal biomass in the treatment of actual polluted water samples as well as the algal biomass itself is a low-cost tool (Aboulsoud, 2008).

TABLE 6. Thermodynamic parameters for dyes bio-sorption.

Dye	Temperature (K)	Thermodynamic parameters			R^2	Thermodynamic parameters			R^2
		ΔG (j/mol)	ΔS (j/mol.K)	ΔH (298-333) (j/mol)		ΔG (j/mol)	ΔS (j/mol.K	ΔH (298-333 (j/mol)	
		Gelidium latifolium				*Ulva lactuca*			
Y	**298**	4400.1	-11.78	888.76	0.9473	3075.4	-8.33	591.51	0.9986
	313	4466.7	-11.43			3143.0	-8.15		
	333	4602.5	-11.15			3228.3	-7.91		
B	**298**	6816.1	-18.50	1302.55	0.9889	4177.3	-12.45	466.84	0.9891
	313	6929.8	-17.97			4302.5	-12.25		
	333	7155.7	-17.57			4502.5	-12.11		
CR	**298**	1827.4	-4.330	537.05	1	1826.4	-3.77	701.31	0.9894
	313	1838.3	-4.157			1830.9	-3.608		
	333	1852.8	-3.95			1827.2	-3.381		
MG	**298**	1415.9	-2.91	546.25	0.9707	1396.1	-2.82	553.28	0.9824
	313	1411.7	-2.765			1392.9	-2.682		
	333	1417.9	-2.61			1397.1	-2.534		

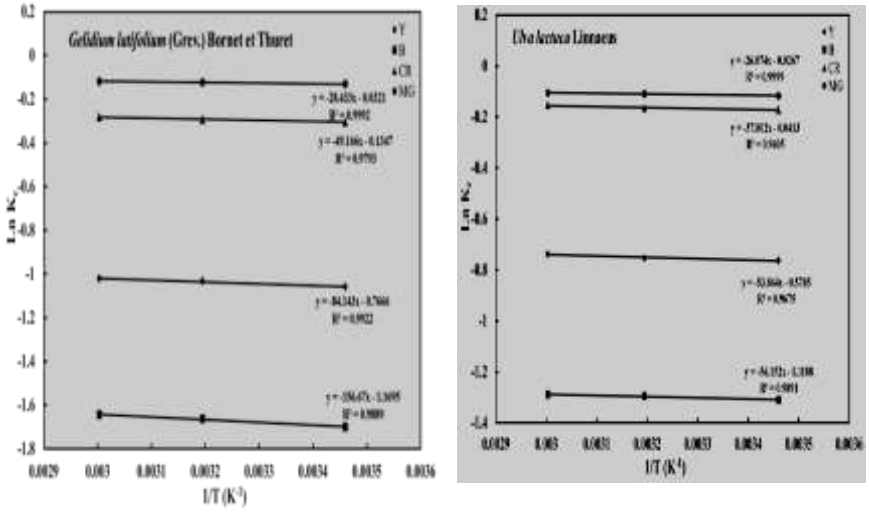

Fig.4. Van't Hoff Plot of ln K_c versus 1/T for dyes biosorption.

TABLE 7. Interaction effect between algal type and different time intervals on dyes bio-sorption efficiency (Experimental conditions: temperature: 40°C, initial dye concentration: 400 mg/L, S/L ratio: 1/1000 g/mL, solutions' pH: 1, 2, 8 and 7 for Y, B, CR and MG, respectively):

Algal type	Time	Biosorption (mg/g)			
		Y	B	CR	MG
Gelidium latifolium	**5 min**	10.30	[l] 9.36	[k] 19.26	[k] 20.29 [k]
	10 min	25.40	[j] 16.78	[i] 48.33	[j] 58.14 [i]
	15 min	47.89	[h] 31.15	[g] 82.46	[h] 92.73 [g]
	30 min	69.30	[g] 41.15	[f] 109.33	[f] 129.71 [e]
	45 min	89.95	[e] 51.91	[e] 142.01	[d] 155.99 [d]
	1 h	102.63	[c] 62.76	[c] 168.53	[b] 184.13 [b]
	2 h	102.33	[c] 62.73	[c] 168.36	[b] 184.46 [ab]
	4 h	101.96	[c] 62.63	[c] 168.36	[b] 184.50 [ab]
	8 h	102.43	[c] 62.73	[c] 168.46	[b] 184.60 [ab]
	24 h	102.13	[c] 62.50	[c] 168.50	[b] 184.63 [ab]
Ulva lactuca	**5 min**	13.10	[k] 11.95	[j] 19.56	[k] 21.53 [j]
	10 min	45.66	[i] 25.00	[h] 57.57	[i] 60.56 [h]
	15 min	70.57	[f] 42.10	[f] 91.44	[g] 100.49 [f]
	30 min	91.99	[d] 57.35	[d] 122.82	[e] 130.0 [e]
	45 min	115.85	[b] 72.82	[b] 158.94	[c] 160.83 [c]
	1 h	125.20	[a] 84.63	[a] 181.10	[a] 185.36 [a]
	2 h	125.23	[a] 84.66	[a] 181.10	[a] 185.16 [a]
	4 h	125.30	[a] 84.56	[a] 181.06	[a] 185.23 [a]
	8 h	125.23	[a] 84.70	[a] 180.96	[a] 185.26 [a]
	24 h	125.26	[a] 84.70	[a] 181.10	[a] 185.33 [a]

Reusability and regeneration of algal biomasses

Recovery of sorbent apart from loaded biomass without damaging its capacity is a very important aim for the success of the bio-sorption technology development. The optimal eluent must be effective, non-damaging to the biomass, non-polluting and cheap (Aboulsoud, 2008).

Figure 5 shows the desorption efficiency for 4 reuse cycles of sorption-desorption using C_2H_5OH for Y and B; NH_4OH and HNO_3 for CR and MG, respectively. After one reuse cycle, the desorption % in case of the biomass of *Gelidium latifolium* reaches 90.45, 90.23, 96.83 and 96.89 % for Y , B , CR and MG , respectively. In case of biomass of *Ulva lactuca*, the desorption % reaches 91.72, 89.66, 96.26 and 97.38 % for Y, B, CR and MG, respectively. Similar results were obtained by Kanwal *et al.* (2013) where ethanol recovered almost 90% of B dye from *Opuntia dillenii* seeds. Fig.6 shows the resorption efficiency of dyes under study for 4 reuse cycles of desorption-resorption. The calculated resorption efficiency referring to the maximum achieved bio-sorption efficiency of dyes. After one reuse cycle, the resorption % reaches 79.21, 78.17, 91.17 and 92.95 % for Y , B , CR and MG , respectively in case of the red algal biomass *Gelidium latifolium*. Meanwhile in case of the green algal biomass *Ulva lactuca*, resorption % reaches 79.73, 79.68, 89.65 and 93.39 % for Y, B, CR and MG, respectively. The algal biomasses can be reused up to 3 cycles with a reasonable efficiency in case of Y and B and up to 4 cycles in case of CR and MG. As can be seen both of desorption and resorption efficiencies decrease with increasing the reusability cycle number. These may attributed to the adverse effect of the eluents on the binding sites of the algal cell wall components. Additionally, accumulation of dyes inside the algal biomass in each cycle acts to decrease the further resorption efficiency in the next cycle. (Tüzün *et al.*, 2005).

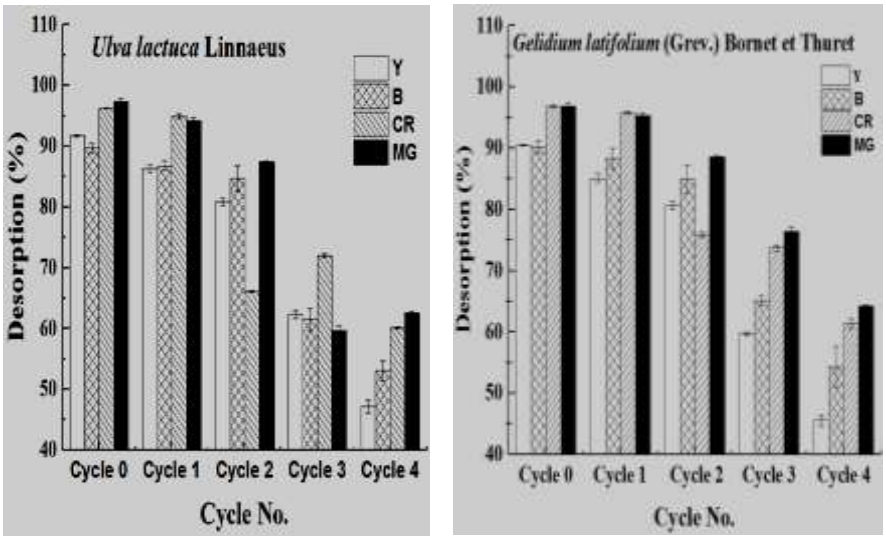

Fig.5. Effect of reuse cycles on desorption efficiency of dyes (Experimental conditions: S/L ratio: 1/500 g/mL, elution time: 4 hours, temperature: 25°C, eluent concentration: 0.01 M, eluent type: C_2H_5OH for Y and B; NH_4OH and HNO_3 for CR and MG, respectively).

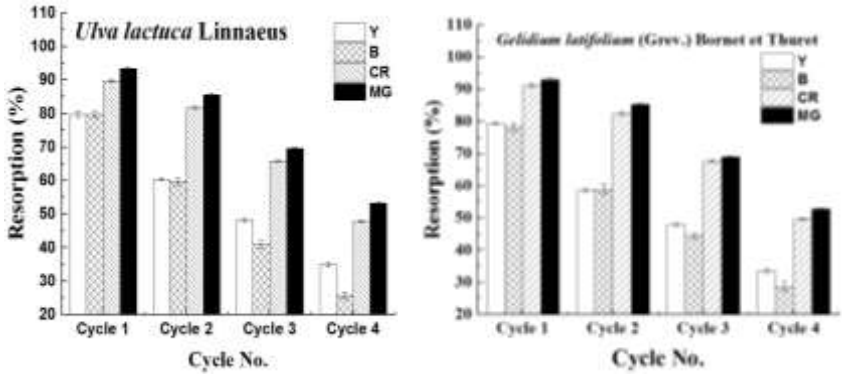

Fig.6. Effect of reuse cycles on resorption efficiency of dyes (Experimental conditions: contact time: 1 hour, temperature: 40°C, initial dye concentration: 400 mg/L, S/L ratio: 1/1000 g/mL, solutions' pH: 1, 2, 8 and 7 for Y, B, CR and MG, respectively).

Examination of algal biomass surface using Scanning Electron Microscope (SEM)

By comparing with the surfaces (Fig. 7), of the raw biomasses between both algae using Scanning Electron Microscope (SEM) before dyes bio-sorption showed that *Ulva lactuca* poses a papillary surface structure supplying a large exposed surface area for bio-sorption, meanwhile the surface of *Gelidium latifolium* appears smooth and non-porous. This finding may help in interpretation of the order of the bio-sorption efficiency where the green alga *Ulva lactuca* > the red *Gelidium latifolium*.

The effect of dye loading on green alga *Ulva lactuca* is however quite interesting. After being in contact with four types of dyes, in case of loading with Y and CR, the alga appears as thick cell wall and surface protuberance can be observed, meanwhile in case of B the algal surface appears wrinkly. In case of MG, the matrix layers of the cell wall are seen to shrink and stick.

On the other hand, loading of dyes causes some structural changes of the red algal biomass *Gelidium latifolium*. The red alga loaded with Y seems as quite rough; meanwhile loading of B is uniformly bound on the cell wall surface that becomes ruptured. CR loaded algal biomass has a plump shape with cloud-capped layer on the surface. Lastly, MG loaded algal biomass become wrinkled and rough.

These may attributed to the strong cross-linking due to the chemisorption (concluded previously from thermodynamic studies and reusability of algal biomasses in Table 6 and Fig.8) between the dyes' particles and the active groups in the cell wall matrix. The mechanism of biosorption varies according to the dye type. These results are confirmed by Tan *et al.* (2012).

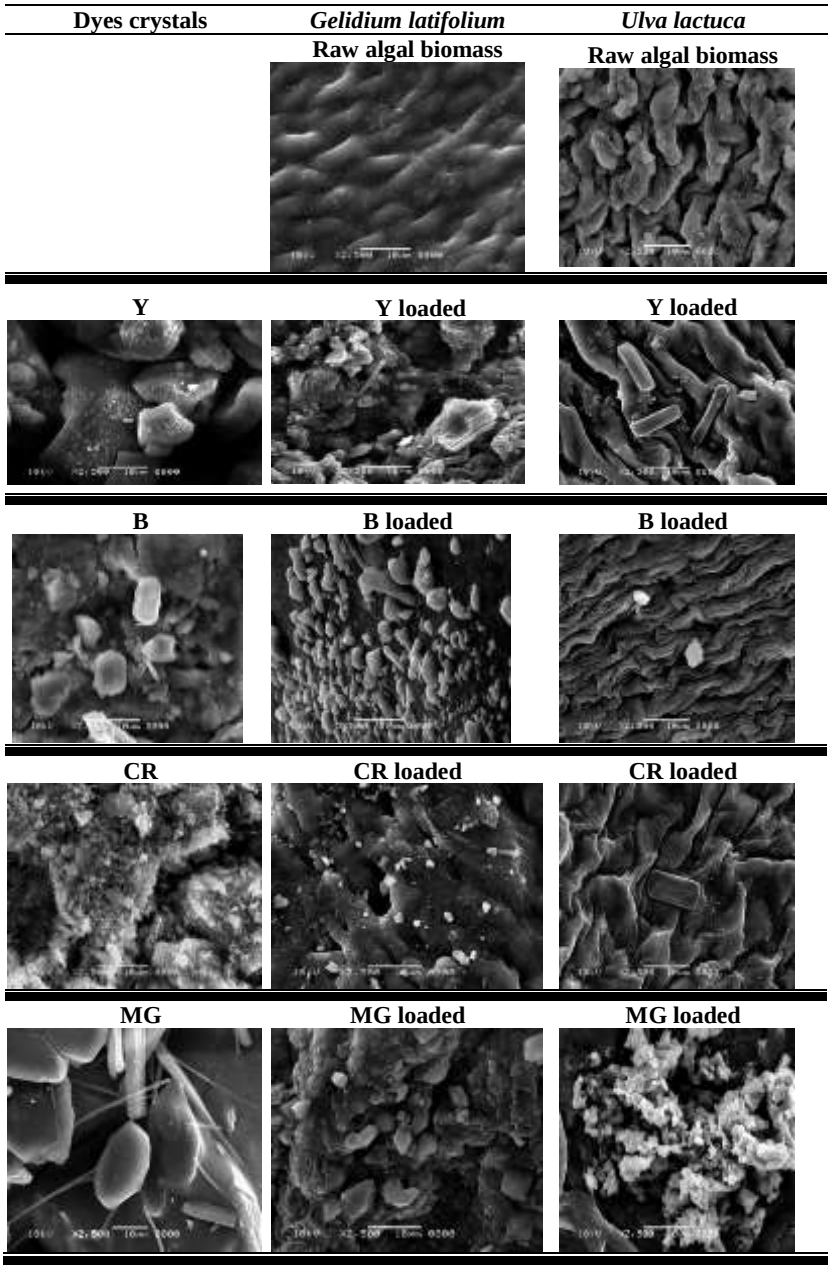

Fig.7. The SEM micrographs of dyes crystals and algal biomasses before and after dyes biosorption at X 2500 magnification.

Fourier Transform Infrared (FTIR) analysis of algal biomasses

The results of FTIR spectra (Range 400 - 4000 cm^{-1}) in Table 8 and Fig.8 showed several band shifts can be summarized as the two algal biomasses contributing functional groups in dyes bio-sorption process are C-N-S, alcoholic C-O, carboxylic C-O, carboxylic C=O, NH and OH while SO_3 is not contributing for the four dyes under investigation. This assumes that the bio-sorbents consist of a heterogeneous surface composed of different classes of bio-sorption sites. This finding is supported by SEM observations where the green alga *Ulva lactuca* poses a papillary surface structure supplying a large exposed surface area for biosorption; meanwhile the surface of the red alga *Gelidium latifolium* appears smooth and non-porous. These results agree with Bekci *et al.* (2009) that reported stretching of O-H, C-H, C=O and C-O groups in the biosorption of MG onto the marine alga *Caulerpa racemosa* var. *cylindracea.* Also, Jayaraj *et al.* (2011) reported participation of OH, and NH groups in the removal of MG using the seaweed *Enteromorpha* sp. biomass. On the other hand, Fakhry (2013) found that carboxyl and hydroxyl groups are mainly responsible for fast yellow dye sorption on *Padina pavonica*

The two algal biomasses affinity to dyes bio-sorption follow the same sequence of MG > CR > Y > B . MG is a cationic dye; therefore it is highly attracted to the negatively charged algal biomass giving the highest bio-sorption efficiency. CR is an anionic di-azo dye and the presence of these two N=N bonds gives it more staining ability than Y (anionic dye also) that constitutes one N=N group. Lastly, B that is also an anionic dye but is azo free dye and therefore shows the lowest staining efficiency.

TABLE 8. FTIR frequencies for algal biomasses before and after dyes bio-sorption.

Algal form	Wavenumber (cm^{-1})													
	C-N-S	C-O alcoholic	S=O	Carboxylic group C-O	Carboxylic group C=O	NH	OH	C-N-S	C-O alcoholic	S=O	Carboxylic group C-O	Carboxylic group C=O	NH	OH
	Gelidium latifolium							*Ulva lactuca*						
Raw	474	1114	1284	1411	1635	3417	3645	470	1114	1280	1404	1631	3414	3749
Y loaded	624	1095	1284	1392	1643	3441	3741	667	1095	1280	1411	1639	3433	3834
B loaded	682	1099	1284	1408	1639	3437	3749	644	1068	1280	1411	1635	3437	3846
CR loaded	624	1087	1284	1392	1639	3433	3749	690	1083	1280	1400	1637	3444	3846
MG loaded	678	1083	1284	1388	1639	3437	3745	675	1103	1280	1381	1635	3441	3761

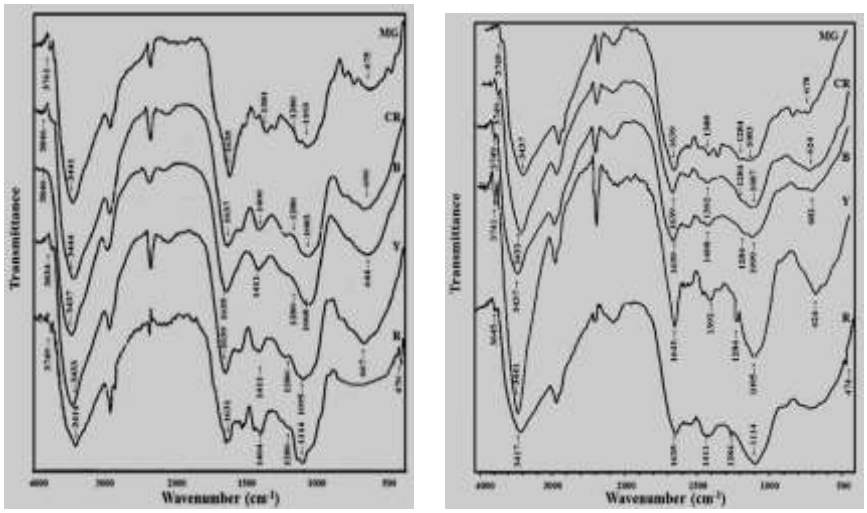

Fig.8. FTIR spectra of (A) *Gelidium latifolium* and (B) *Ulva lactuca* algal biomasses before and after dyes bio-sorption.

Application of bio-sorption optimum conditions in the treatment of the actual polluted wastewater samples of Borg El-Arab region:

The batch experiments results show that the green algal biomass *Ulva lactuca* is an effective and economical bio-sorbent material for the removal and recovery of dyes, thus it is applied in decolourization of eleven industrial wastewater samples expressed as absorbance. Data of Table 9 showed the absorbance of coloured samples before and after the bio-sorption process as well as the removal %. It can be observed that textile dyeing sector is the most abundant source of coloured wastewater where the majority belongs to this sector. Dye wastewater from textile and dyestuff industries is one of the most difficult industrial wastewaters to treat. The synthetic origin and complex aromatic structures of dyes make them stable and difficult to be biodegraded (Srinivasan and Viraraghavan, 2010).

The lowest absorbance value reached 0.075 was observed in sample No. 3 which is belonging to textile dyeing sector, while the highest value reached 0.944 was found in sample No. 8 that is belonging to metals, steel and concrete sector. After the biosorption using algae, the absorbance of all samples was greatly lowered by percentage ranged from 82.93 (sample No. 6) to 97.46 % (sample No. 10).

TABLE 9. Absorbance of coloured wastewater samples before and after the bio-sorption treatment process using the green algal biomass *Ulva lactuca*.

Activity sector	Textile Dyeing	Textile Dyeing	Textile Dyeing	Textile Dyeing	Textile Dyeing	Collective industrial wastewater	Collective industrial wastewater	Metals, steel &Concrete	Soap	Textile Dyeing	Food
Sample No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Before	0.078	0.108	0.075	0.089	0.184	0.082	0.097	0.944	0.085	0.209	0.122
After	0.002	0.011	0.008	0.007	0.018	0.014	0.0072	0.14	0.0034	0.0053	0.0082
Removal (%)	97.44	89.81	89.33	92.13	90.22	82.93	92.58	85.17	96.00	97.46	93.28

Conclusions

Two Egyptian algal biomasses, namely *Gelidium latifolium* (Grev.) Bornet et Thuret (a red alga) and *Ulva lactuca* Linnaeus (a green alga) were used in their raw dried forms to remove four dyes from their aqueous solutions by batch procedure. The optimum bio-sorption conditions were Solid/Liquid ratio; 1/1000 g/mL, initial dye concentration; 400 mg/L, temperature; 40° C, contact time; 1 hour, pH; 1, 2, 8 and 7 for bio-sorption of Drimarene yellow CL4R, Drimarene blue K2RL, Congo Red and Malachite Green, respectively. The highest bio-sorption efficiency reached 125.18, 84.63, 181.1 and 185.36 mg/g for Drimarene yellow CL4R, Drimarene blue K2RL, Congo Red and Malachite Green, respectively using the green algal biomass *Ulva lactuca*. Algal biomasses were applied under the optimum conditions in the decolourization of wastewater samples that were achieved up to 97.46 %.

Thus, algal biomasses showed promising results in the field of water treatment and removal of water coloring. Besides that the studied algal biomasses are considered as natural, low-cost and eco-friendly tool, so that they can help to a great extent in improving quality of water resources and to keep a clean environment via elimination of industrial pollution and its subsequent hazards on all biota especially human being.

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الإدمصاص الحيوي للصبغات باستخدام نوعين من الطحالب البحرية ثم تطبيق ذلك على الصرف الصناعي لمنطقة برج العرب بجمهورية مصر العربية.

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تهدف هذه الدراسة إلى استخدام الإدمصاص الحيوي لإزالة أربعة أنواع من الصبغات من محاليلها المائية بطريقة النقع للكتل الحيوية لنوعين من الطحالب البحرية وهما: *Gelidium latifolium* (Grev.) Bornet et Thuret من الطحالب الحمراء و *Ulva lactuca* Linnaeus من الطحالب الخضراء. ثم تطبيق الظروف المثلى في معالجة أحدى عشر عينة صرف صناعي سائلة مجمعة من منطقة برج العرب بجمهورية مصر العربية. و قد أظهرت النتائج أن فحص سطح الكتل الطحلبية باستخدام الميكروسكوب الإلكتروني الماسح قبل وبعد عملية الإدمصاص الحيوي للصبغات بأن لكل من الطحلبين قيد الدراسة في صورته الخام شكل سطحي مميز. فى حين أن إدمصاص الصبغات قد أظهر العديد من التغيرات المظهرية الواضحة مثل التجعد والأنكماش والتقطيع والخشونة فى الشكل السطحى للطحلبين. كما أوضحت النتائج أيضا أن طبيعة الإدمصاص كانت كيميائي وماص للحرارة وغير تلقائي.و يتم هذا الإدمصاص على سطح الكتل الطحلبية في تغطية متعددة الطبقات. أما نتائج التحليل الطيفي باستخدام الأشعة تحت الحمراء أظهرت أن المجموعات المشاركة في عملية إدمصاص الصبغاتعلى سطح الطحلبين قيد الدراسة هى الثيوسيانات والكحوليات والكربوكسيلات والأمينات والهيدروكسيلات.

كما دلت الدراسة أن كلا من C_2H_5OH خاصا لل Drimarene yellow (Drimarene blue K2RL and CL4R) لل NH_4OH , HNO_3، and (Malachite Gree Congo Red MG) على الترتيب و خلال أربعة دورات كان لهم أعلى كفاءة لإزالة الصبغات (التى تم إدمصاصها وتحميلها) من على سطح الكتل الحيوية للطحلبين قيد الدراسة. حيث أن كفاءة الإدمصاص تقل مع زيادة دورات إعادة إستخدام الكتلة الطحلبية.

كما أسفرت النتائج على أن أعلي نسبة إدمصاص للصبغات قد تم تحقيقها باستخدام طحلب *Ulva lactuca* تحت الظروف المثلى لعملية الإدمصاص الحيوي وهي نسبة الطحلب/ المحلول: ١٠٠٠/١ جم/ملليلتر، درجة الحرارة: ٤٠° سيليزية، تركيز الصبغة: ٤٠٠ مجم/لتر، زمن التماس: ساعة واحدة، الأس الهيدروجيني: ١ و٢ و٨ و ٧ لكل من Drimarene yellow CL4R و Drimarene blue K2RL و Congo Red و Malachite Green على الترتيب. وبتطبيق الظروف المثلى على عينات الصرف الصناعي الملونة لمنطقة برج العرب مع إستخدام الطحلبين أدى إلى أزالة تلونها بنسبة وصلت إلى ٩٧،٤٦ ٪.