

UPTAKE OF DIFFERENT DYES BY TWO NEW STRAINS OF MICROALGAL DRY BIOMASS

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ABSTRACT

The control of wastewater pollution has become of increasing importance in recent years. The release of dyes into the environment constitutes only a small proportion of water pollution, but dyes are visible in small quantities due to their brilliance. In this study *Spirulina subsalsa* and *Scenedesmus ecornis* microalgae isolated from Chnarok and Taq-Taq Koya city and its ability to uptake different dyes. Batch studies were conducted at separate biosorbent doses ,dye concentration, pH, temperature and agitation speed. Optimum adsorption of dyes by *Spirulina subsalsa* and *Scenedesmus ecornis* showed in reactive yellow (89.3%, 90.4%) respectively at 50 mg l⁻¹, 30°C, pH 8, 150 rpm, dosage 1.2 g ml⁻¹.

Key words: dye uptake, biosorbent, optimum removal, biosorption, environment.

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تحديث الأصباغ المختلفة بواسطة سلالات جديدة ثنائية الكتلة الحيوية

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المستخلص

أصبحت السيطرة على تلوث المياه العادمة ذات أهمية متزايدة في السنوات الأخيرة. لا يشكل إطلاق الأصباغ في البيئة سوى نسبة صغيرة من تلوث المياه ، ولكن الأصباغ مرئية بكميات صغيرة بسبب تألقها. في هذه الدراسة تم عزل طحالب *Spirulina subsalsa* و *Scenedesmus ecornis* من جناروك و طق في مدينة كويا و قدرتها على امتصاص الأصباغ المختلفة. وأجريت دراسات دفعة في جرعات منفصلة ماصة، وتركيز الصبغة، ودرجة الحموضة ودرجة الحرارة وسرعة الانفعالات. أظهرت الامتزاز الأمثل للأصباغ بواسطة *Spirulina subsalsa* و *Scenedesmus ecornis* باللون reactive yellow (89.3% ، 90.4%) على الترتيب في 50 ملغم/ لتر⁻¹، 30 °C ، درجة الحموضة 8 ، 150 دورة في الدقيقة ، جرعة 1.2 غرام/ مل⁻¹.

الكلمات المفتاحية: امتصاص الصبغ، الماصة الحيوية، الإزالة المثلى، الامتصاص الحيوي، البيئة.

INTRODUCTION

Dyes are substance that adhere to substrate and colored the substrate, Dyes are toxic, mutagenic, and dangerous to aquatic living organisms (22). Dyes are used in different industries such as production of pulp and paper, leather tanning or textile dyeing and in manufacture of dyestuffs after application the more amount of effluents release to the environment that cause pollution and effects to water and terrestrial environments. Physicochemical method for the removal of dyes from wastewater such as coagulation and flocculation, advanced oxidation, activated carbon, ozonation and photocatalysis unable to predict the effects on the organisms in the ecosystem and sometime byproducts produce during treatment process (15). Various biomaterials such as bacteria, fungi and algae can reduce and remove industrial and agricultural pollutants in wastewater and can be utilized in purification of contaminated water by the substances such as dyes and metals that cannot be easily resolved due to their cost-effectiveness, rapid, reversible, and in several ways algae have used under different conditions to decrease environmental pollution their production of less sludge. Their eco-friendly nature has attracted interest as a valuable technology in the area of water treatment and depend on the possible interactions between pollutant and the cellular surface of the biomaterial (10,11,14). Microalgae are photosynthetic organism that are the primary biomass producers and have different functional group on the biomass surface like hydroxyl, carboxyl, sulfhydryl, amino, phosphoryl, and thiol and are requires minimal preparatory steps, naturally renewable that have been suggested as ideal biosorbent for wastewater treatment systems using biosorption process (9, 12, 26, 27). This study was aimed to evaluate ability of microalgae biomass (biosorbents) to reduce different dyes.

MATERIAL AND METHODS

Isolation and identification of microalgae: Water samples were collected from Hamamok- Koya city. Water obtained from 4ml depth were diluted and plated on Blue Green medium (BG11) The cultures were incubated at pH 7.8 and 28°C under

constant light 800Lx. Two weeks later, following the growth of colonies on the agar media, the colonies were removed with pasture micropipettes and gently blown into the liquid medium, then incubated at 28 °C, 800 Lx and pH 7.8. as described by (3,23).

Biosorption of Dyes

Preparation of biomass: Microalgae biomass initially dried from moisture on an aluminum tray, kept in the oven at 80°C for 24 hours, after cooling, the samples were subjected to sieve analysis.

Biosorption of dyes

Aqueous dye solutions with the required levels were prepared from 0.2g/10ml inventory alternatives. Batch adsorption studies were conducted in 250 ml conical flask containing 100 ml aqueous dye solution of the required concentration and known quantity of biosorbent. Initial pH was adjusted to the desired level with 1 N NaOH or 1 N HCl solutions. The dye solution was then separated from the adsorbent by centrifugation and the dye concentration was determined by spectrophotometer, the rate of biosorption recorded by spectrophotometer (507 nm for Direct red 254, 610 nm for Reactive Blue 214, 493, for Reactive black 5 and Reactive Yellow 410 nm. Batch studies were conducted at separate biosorbent doses ranging from 0.3 g ml⁻¹, 0.6 g ml⁻¹, 0.9 g ml⁻¹, 1.2 g ml⁻¹ to 0.5 g ml⁻¹, original dye concentrations ranging from 25, 50, 75, 100 and 125 mg l⁻¹ and pH ranging from 6.5 to 8.5 agitation speed from zero, 50, 100, 150 and 200rpm temperature from 20, 25, 30, 35 to 40 °C were studied. The uptake of dyes calculated using the following formula (5)

$$\text{Biosorption} = [(C_0 - C) / C_0] * 100$$

C₀ = Initial Dye concentration

C = Final Dye concentration

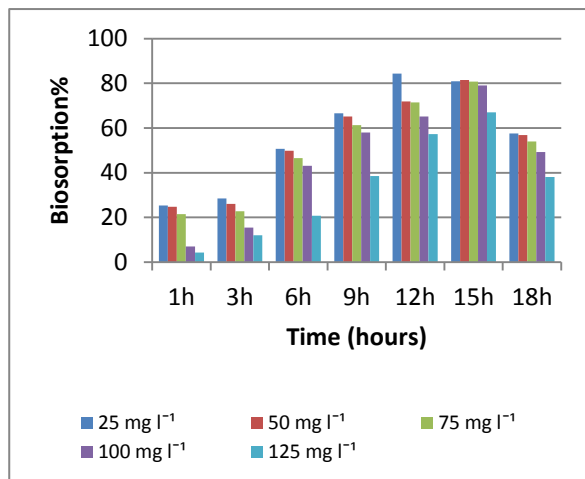
The characterization techniques were done by using Infrared spectroscopy (FTIR) (College of Education-Salahaddin University) were performed using KBr pellet technique in the frequency range of 4000 to 500 cm⁻¹ by pressing the powder of samples before and after biosorption process with KBr discs then subjected to spectral analysis as described by (7).

RESULT AND DISSCUSION

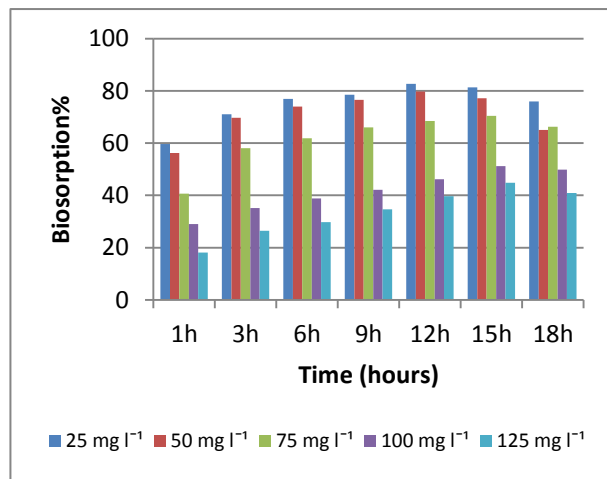
Biosorption of Dyes by *Spirulina subsalsa* and *Scenedesmus ecornis*:

Effect of dye concentrations on: biosorption by *Spirulina subsalsa* and *Scenedesmus ecornis* : Biosorption of direct red 254, reactive blue 214, reactive orange 16, reactive black 5 and reactive yellow at different initial concentration (25-125 mg l⁻¹) by *Scenedesmus ecornis* was studied as functional of contact time. As shows in Figure 1 (a-j) optimum adsorption of all dyes by *Spirulina subsalsa* and *Scenedesmus ecornis* showed at low concentration 25 mg l⁻¹ with different contact times which varied from 84.3%, 82.7%

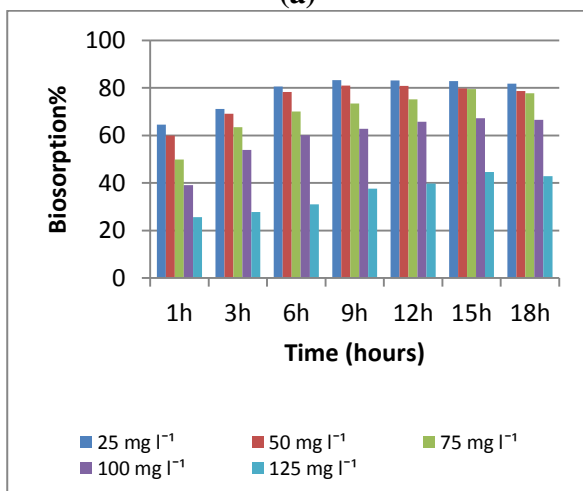
(Reactive yellow) at 12. hours, 83.3%, 82.5% (Reactive black 5) at 9. hours, 81.7% at 15. hours, 79.7% at 9. hours (Reactive Blue 214), 21.9%, in *Spirulina subsalsa* and *Scenedesmus ecornis* by increase initial dye concentration, the percentage of biosorption of all dyes decreased. A similar trend was reported by (16, 24, 28). (6) conducted that the higher removal of dyes observed at 25 ml by *Pseudomonas fluorescens*. The initial rapid uptake of the dye indicates that the sorption process could be ionic in nature where the acidic (anionic) dye molecules bind to the various positively charged organic functional groups present on the surface of the biomass (17).



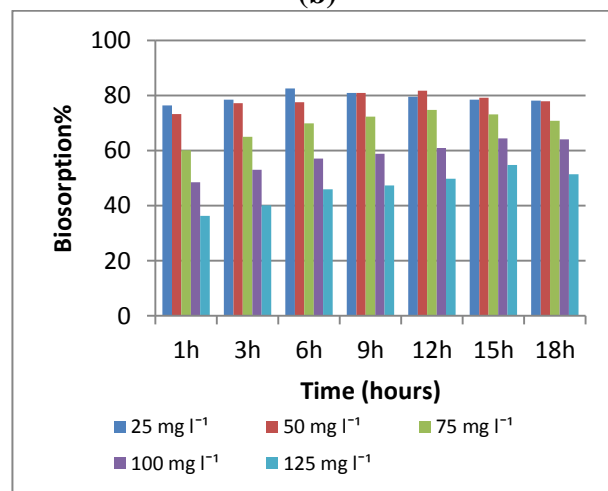
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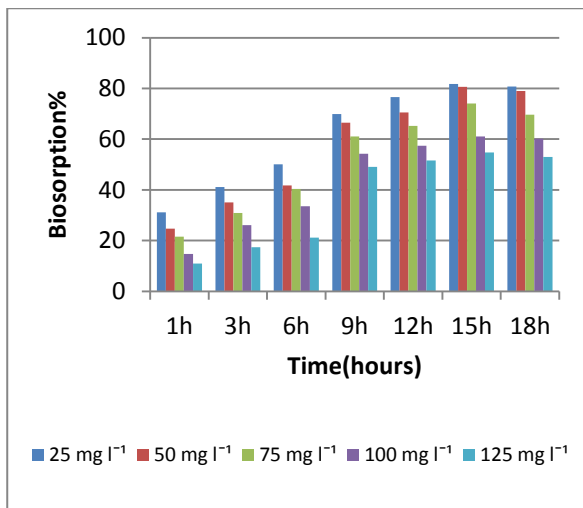
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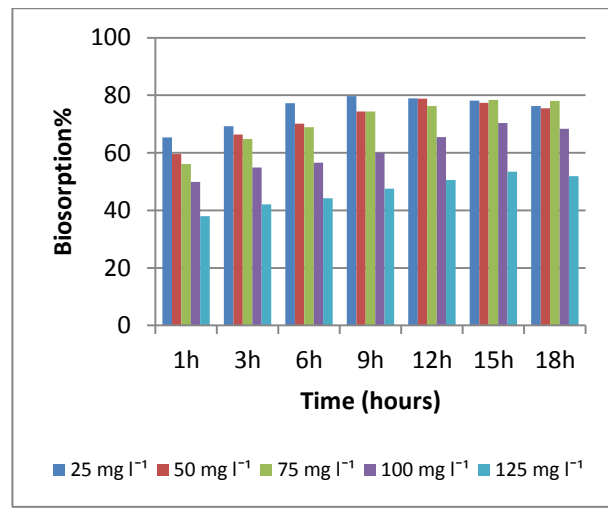
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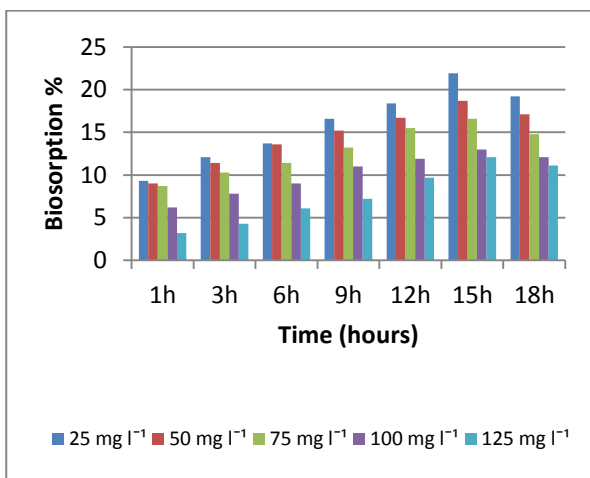
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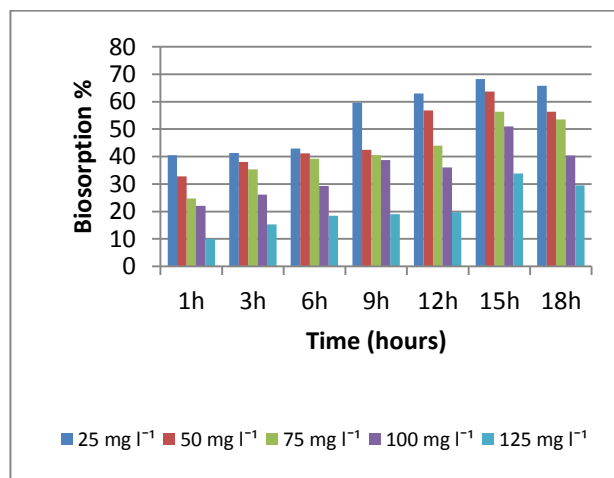
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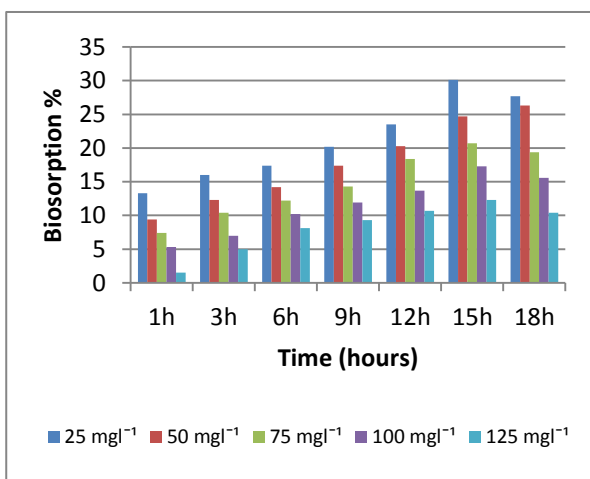
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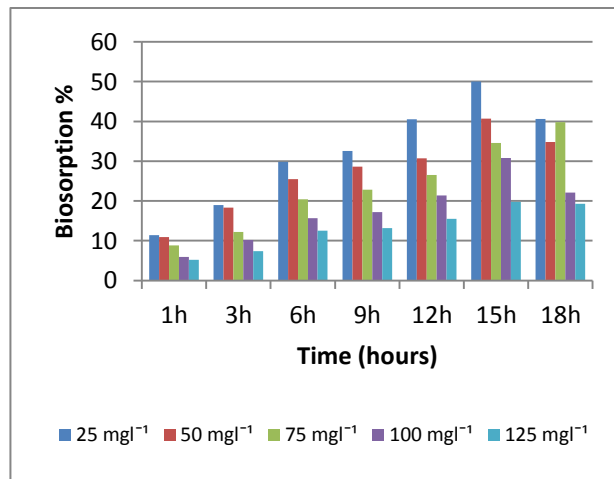
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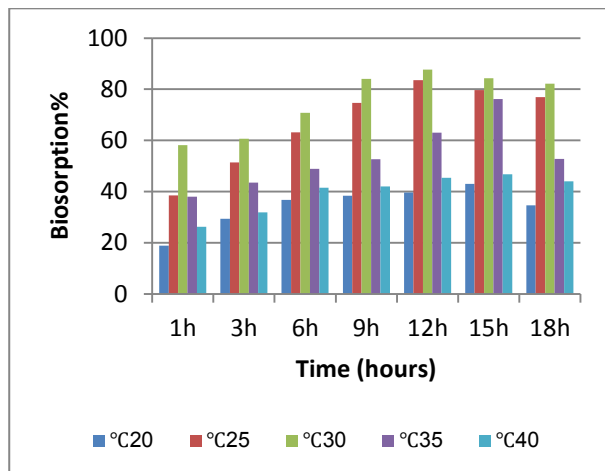


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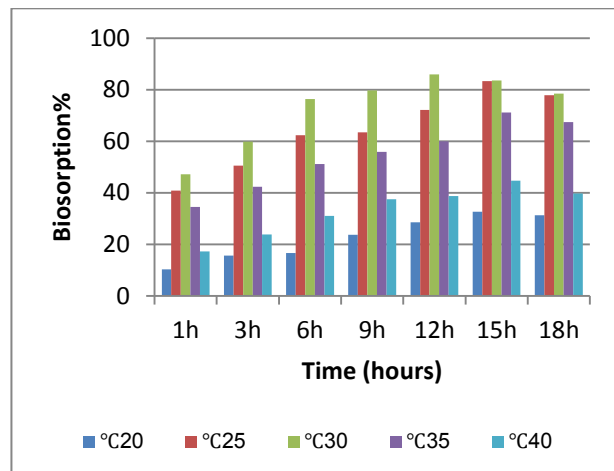
Fig. 1. Effect of [(a,b)Reactive yellow, (c,d)Reactive black 5, (e,f) Reactive Blue 214, (g,h) Reactive Orange 16, (i,j) Direct Red 254] concentration on biosorption process (pH 7, 35°C, 100rpm) by (a, c, e, g, i) *Spirulina subsalsa* and (b, d f, h, j) *Scenedesmus ecornis*

Effect of temperature on biosorption of dyes by *Spirulina subsalsa* and *Scenedesmus ecornis*: The effect of different temperature (20, 25, 30, 35, 40°C) on adsorption of different dyes by *Spirulina subsalsa* and *Scenedesmus ecornis* using 50 mg l⁻¹ initial dye concentration shows in Figure 2 (a-j). The results indicated maximum adsorption of reactive yellow (87.7%, 85.9%) at 12 hours, reactive black 5 (86.5% at 12. hours, 84.6 at 9. hours), reactive blue 214 (84.5% at 12. hours, 86.4% at 9. hours), within the temperature

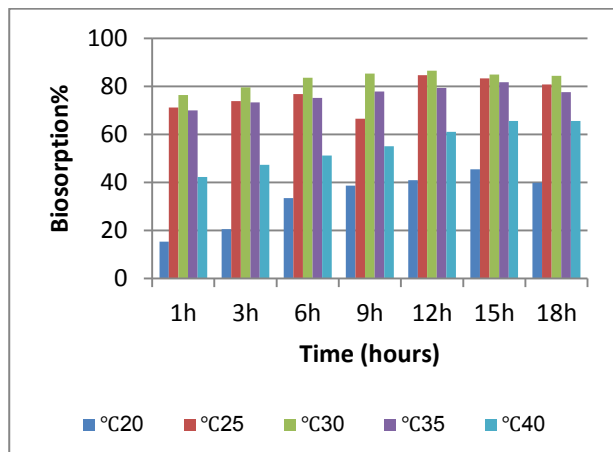
30°C. The percentage of dye uptake was increased from 20 to 30 °C and then showed decrease in sorption percentage with further increase in temperature. Similar observation was made by (18) where they observed an increase in biosorption of Remazol Brilliant Blue R by *Phanerochaete chrysosporium* with increase in temperature up to 30°C, (19) showed that the optimum dye removal for fungus and algae was at 30°C. (6) reported that the maximum removal of different dyes by *Pseudomonas fluorescens* was 30°C.



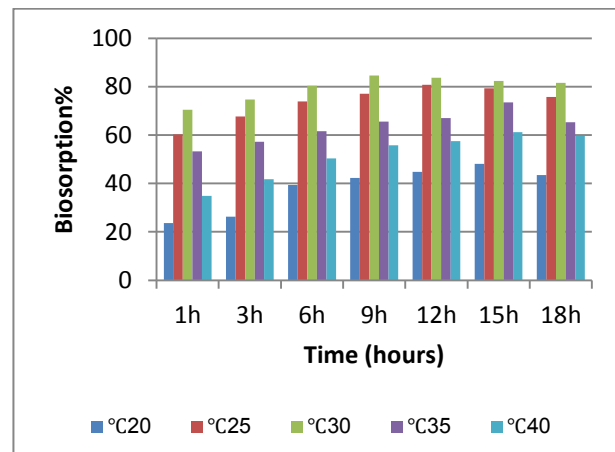
(a)



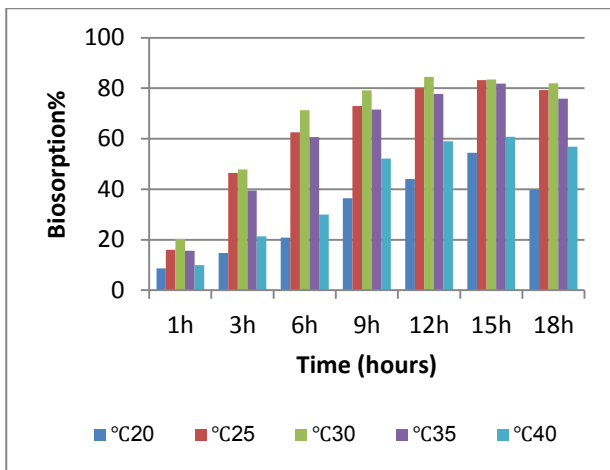
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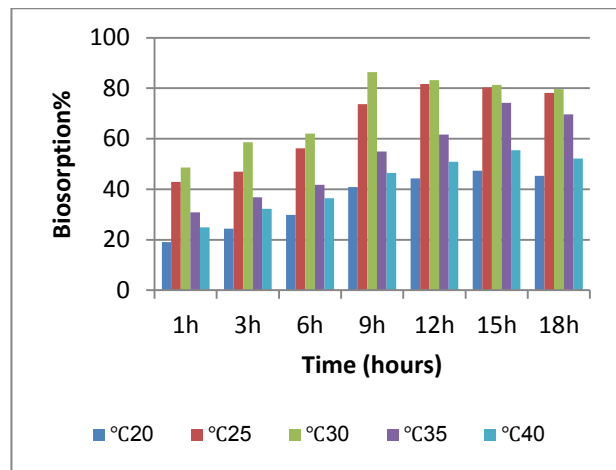
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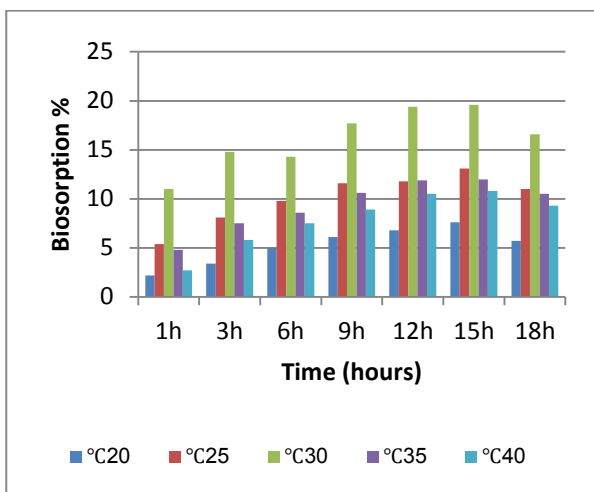
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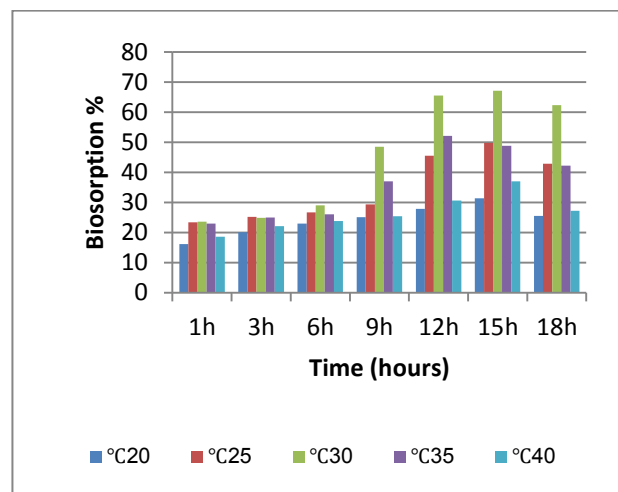
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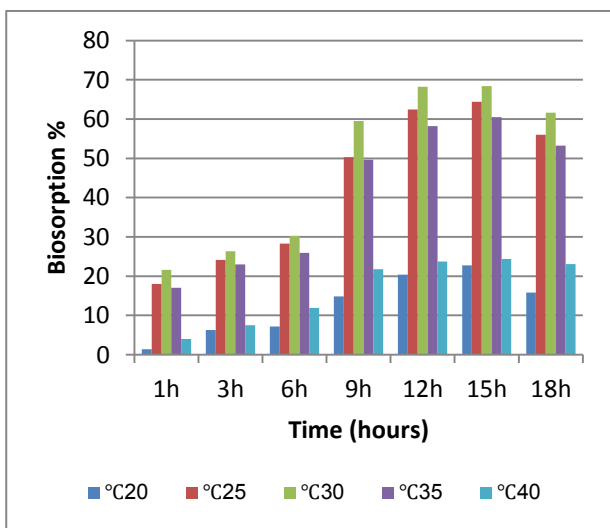
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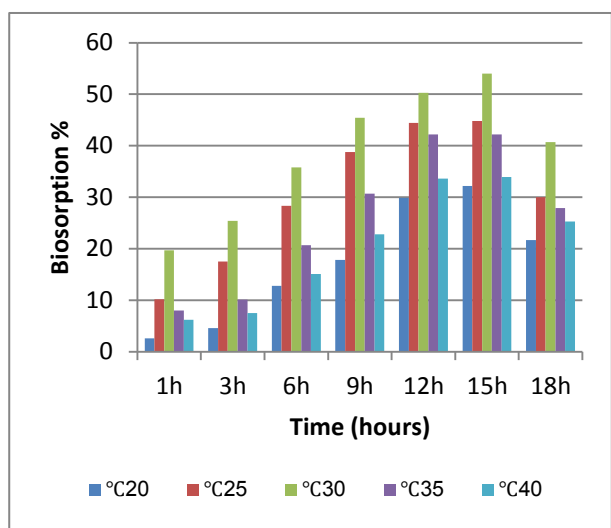
(g)



(h)



(i)



(j)

Fig. 2. Effect of temperature on [(a,b) Reactive yellow, (c,d) Reactive black 5, (e,f) reactive Blue, (g,h) Reactive Orange 16, (i,j) Direct Red 254] adsorption 50 mg/l, pH 7, 100 rpm (a, c, e, g, i) *Spirulina subsalsa* and (b, d, f, h, j) *Scenedesmus ecornis*

Effect of pH on biosorption of dyes by *Spirulina subsalsa* and *Scenedesmus ecornis*
potential hydrogen (pH) is one of the important parameter of adsorption as it depends on the surface chemistry of the adsorbent and the ion state of the solution (1,21). The effect of different pH values (6.5, 7, 7.5, 8,8.5) to biosorption capacity 50 mg l⁻¹ initial dye concentration and 30°C shows in Table (1-5) the effect of pH on adsorption of different dye molecules by *Spirulina subsalsa* and *Scenedesmus ecornis*. It was observed that different types of dye molecules adsorbed at different pH by algal biomass. Dyes such as Reactive yellow, reactive black 5, reactive

Blue 214 showed maximum adsorption at pH (8.5) by *Spirulina subsalsa* as shows in Table (1, 2, 3, 5) and reactive orange 16 adsorbed at pH (8) in Table (4), while optimum adsorption of reactive yellow, reactive black 5, by *Scenedesmus ecornis* was at pH (8) Table (1, 2, 4, 5) and reactive blue 214 at pH (8.5) Table (3). Biosorption of dye increased with increased pH. A similar trend was also observed for the adsorption of dye by Cyanobacteria (5.25). The algal cell wall contains several functional groups and the adsorption is depends on the protonation or deprotonation of these functional groups (21).

Table 1. Effect of pH on biosorption of dye (reactive yellow) by *Spirulina subsalsa* and *Scenedesmus ecornis* (50 mg l⁻¹, 30°C, 100rpm)

pH	<i>Spirulina subsalsa</i> (reactive yellow)				<i>Scenedesmus ecornis</i> (reactive yellow)			
	C ₀ (mg l ⁻¹)	Time (hours)	CC ₀ (mg l ⁻¹)	% Biosorption	C ₀ (mg l ⁻¹)	Time (hours)	CC ₀ (mg l ⁻¹)	% Biosorption
6.5	91.82	15h	35.6	38.8	91.82	15h	29.0	31.6
7	92.52	15h	48.3	52.3	92.52	15h	41.6	44.9
7.5	93.21	15h	64.5	69.2	93.21	15h	69.0	74.1
8	94.6	12h	82.4	87.1	94.6	12h	83.3	88.1
8.5	96.69	9h	86.4	89.4	96.69	12h	81.7	84.5

Table 2. Effect of pH on biosorption of dye (reactive black 5) by *Spirulina subsalsa* and *Scenedesmus ecornis* (50 mg l⁻¹, 30°C, 100rpm)

pH	<i>Spirulina subsalsa</i> (reactive black 5)				<i>Scenedesmus ecornis</i> (reactive black 5)			
	C ₀ (mg l ⁻¹)	Time (hours)	CC ₀ (mg l ⁻¹)	% Biosorption	C ₀ (mg l ⁻¹)	Time (hours)	CC ₀ (mg l ⁻¹)	% Biosorption
6.5	34.92	15h	14.4	41.1	34.92	15h	16.7	47.8
7	49.85	15h	33.7	67.5	49.85	15h	26.1	52.4
7.5	54.22	15h	43.0	79.2	54.22	15h	38.7	71.3
8	59.01	9h	50.3	85.2	59.01	9h	51.1	86.6
8.5	62.39	9h	54.6	87.5	62.39	12h	53.5	85.8

Table 3. Effect of pH on biosorption of dye (reactive blue 214) by *Spirulina subsalsa*. and *Scenedesmus ecornis* (50 mg l⁻¹, 30°C, 100rpm)

pH	<i>Spirulina subsalsa</i> (reactive blue 214)				<i>Scenedesmus ecornis</i> (reactive blue 214)			
	C ₀ (mg l ⁻¹)	Time (hours)	CC ₀ (mg l ⁻¹)	% Biosorption	C ₀ (mg l ⁻¹)	Time (hours)	CC ₀ (mg l ⁻¹)	% Biosorption
6.5	47.54	15h	17.9	37.6	47.54	15h	18.4	38.7
7	47.68	15h	23.8	50.0	47.68	15h	24.4	51.1
7.5	48.07	15h	26.0	54.0	48.07	15h	28.8	59.9
8	48.6	15h	39.8	81.9	48.6	15h	39.0	80.2
8.5	49.27	12h	41.1	83.5	49.27	12h	42.6	86.4

Table 4. Effect of pH on biosorption of dye (reactive orange 16) by *Spirulina subsalsa* and *Scenedesmus ecornis* (50 mg l⁻¹, 30°C, 100 rpm)

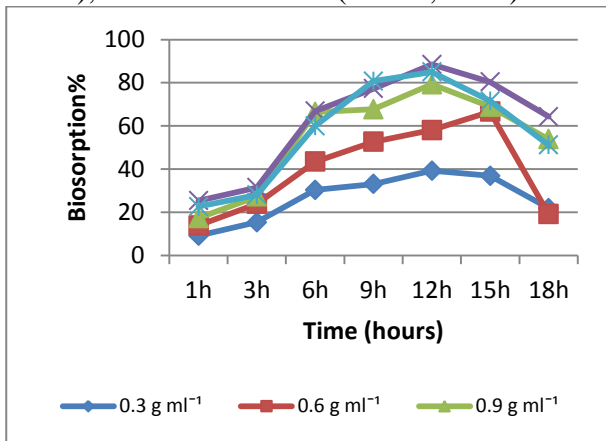
pH	<i>Spirulina subsalsa</i> (reactive orange 16)				<i>Scenedesmus ecornis</i> (reactive orange 16)			
	C ₀ (mg l ⁻¹)	Time (hours)	CC ₀ (mg l ⁻¹)	% Biosorption	C ₀ (mg l ⁻¹)	Time (hours)	CC ₀ (mg l ⁻¹)	% Biosorption
6.5	73.03	15h	9.3	12.8	73.03	15h	35.4	48.5
7	73.26	15h	8.4	11.4	73.26	15h	44.0	60.1
7.5	73.6	15h	9.1	12.4	73.6	15h	47.8	65.0
8	74.06	15h	12.9	17.4	74.06	15h	51.0	68.8
8.5	74.51	15h	12.7	17.0	74.51	15h	48.6	65.2

Table 5. Effect of pH on biosorption of dye (direct Red 254) by *Spirulina subsalsa* and *Scenedesmus ecornis* (50 mg l-1, 30°C, 100 rpm)

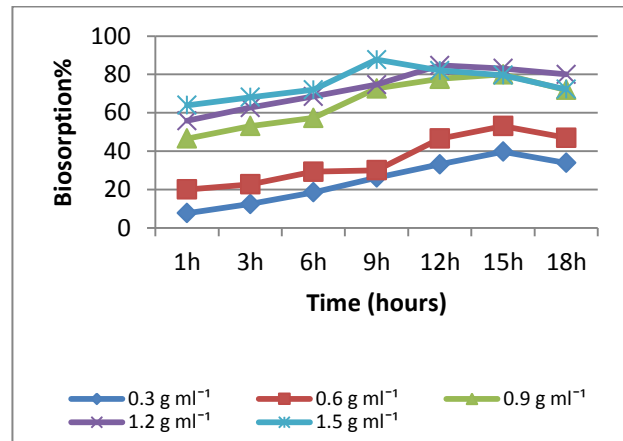
pH	<i>Spirulina subsalsa</i> (direct red 254)				<i>Scenedesmus ecornis</i> (direct red 254)			
	C0 (mg l-1)	Time (hours)	CC0 (mg l-1)	% Biosorption	C0 (mg l-1)	Time (hours)	CC0 (mg l-1)	% Biosorption
6.5	50.37	15h	20.8	41.3	50.37	15h	17.8	35.3
7	50.67	15h	22.5	44.5	50.67	15h	20.3	40.0
7.5	50.98	15h	24.3	47.6	50.98	15h	20.7	40.7
8	51.28	15h	26.5	51.6	51.28	15h	25.8	50.3
8.5	51.58	15h	30.0	58.2	51.58	15h	23.5	45.6

Effect of algal biomass on biosorption of Dyes by *Spirulina subsalsa* and *Scenedesmus ecornis*: The effect of different biomass (0.3, 0.6, 0.9, 1.2, 1.5 g ml⁻¹) on biosorption of different dyes by *Spirulina subsalsa* and *Scenedesmus ecornis* were studied. In Figure 3 (a-j) *Spirulina subsalsa* and *Scenedesmus ecornis* recorded high adsorption of reactive yellow (88.5% at 12. hours, 87.7% at 9. hours), reactive black 5 (88.1%, 87%) at 12.

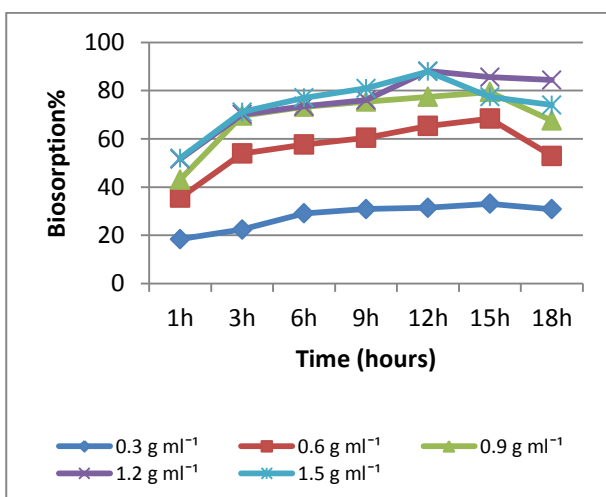
hours, reactive blue 214 with 1.5 g ml⁻¹ (83.3%, 86.8%) at 12. hours, optimum adsorption of reactive black 5 and reactive orange 16 showed with biomass 1.2 g ml⁻¹ of *Scenedesmus ecornis* and for reactive yellow, reactive blue 214, direct red 254 was with biomass 1.5 g ml⁻¹. It was observed that biosorption percentage increased with increase in biomass.



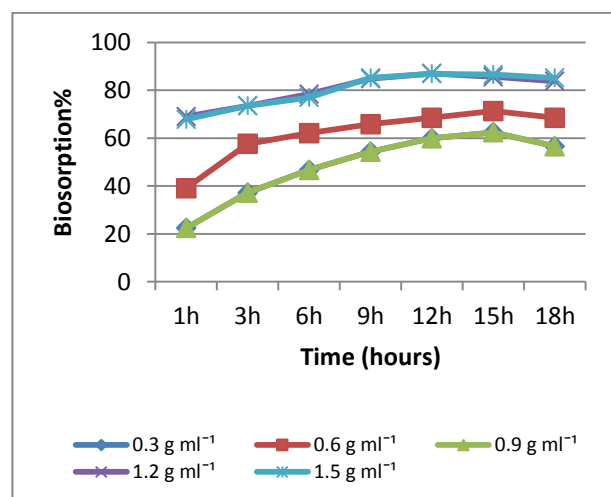
(a)



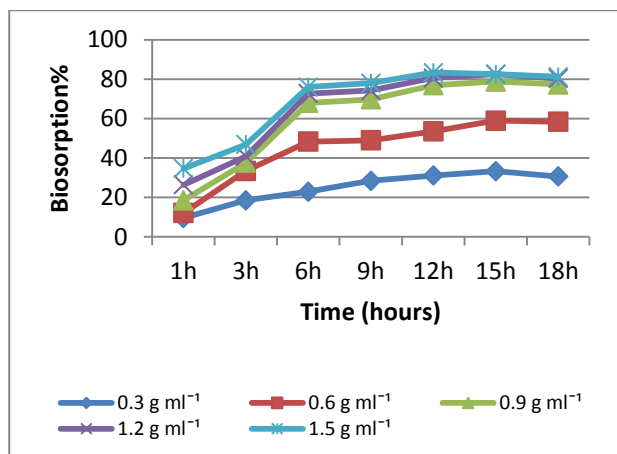
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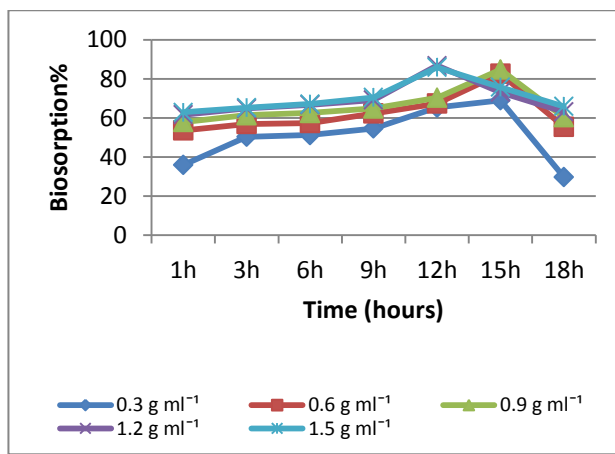
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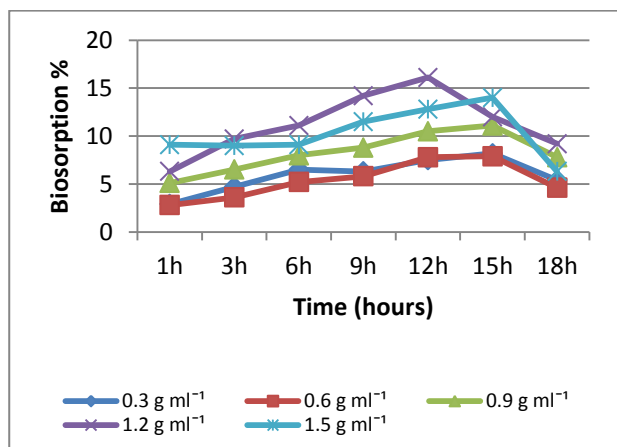
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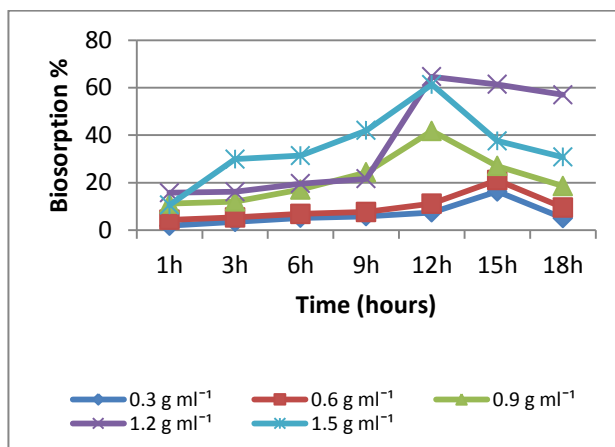
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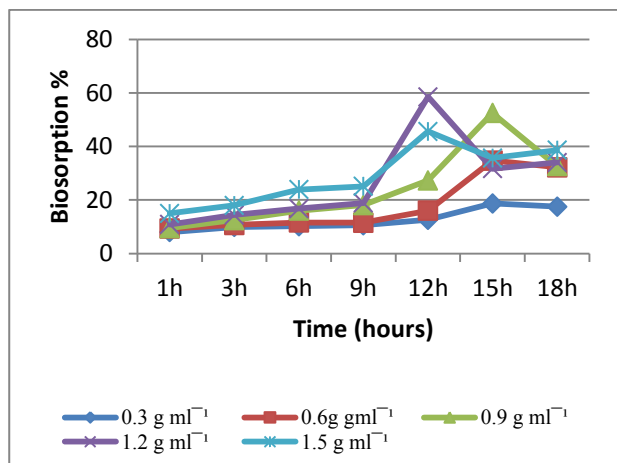
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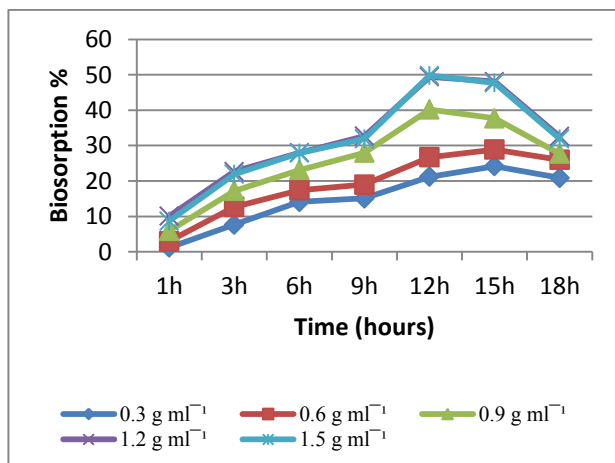
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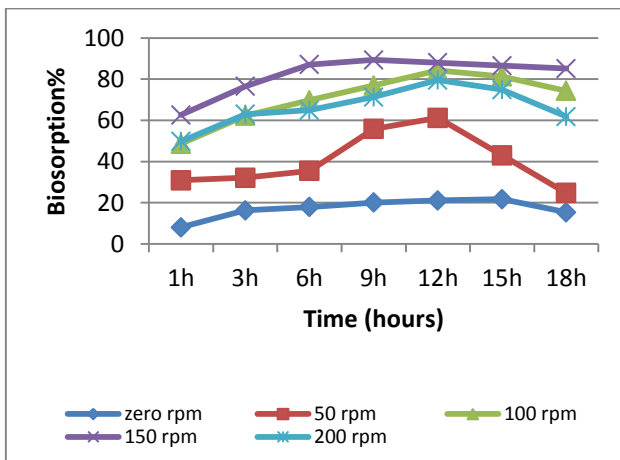
Fig. 3. Effect of biosorbent dosage on dye [(a,b) Reactive yellow, (c,d) Reactive black 5, (e,f) reactive Blue, (g,h) Reactive Orange 16, (i,j) Direct Red 254] (50 mg l⁻¹, 30°C, pH 8, 100rpm) by (a, c, e, g, i) *Spirulina subsalsa* and (b, d, f, h, j) *Scenedesmus ecornis*

Effect of agitation speeds on biosorption of dyes by *Spirulina subsalsa* and *Scenedesmus ecornis*: To determine effect of agitation speed on dye biosorption a series of experiments at different degrees of agitation

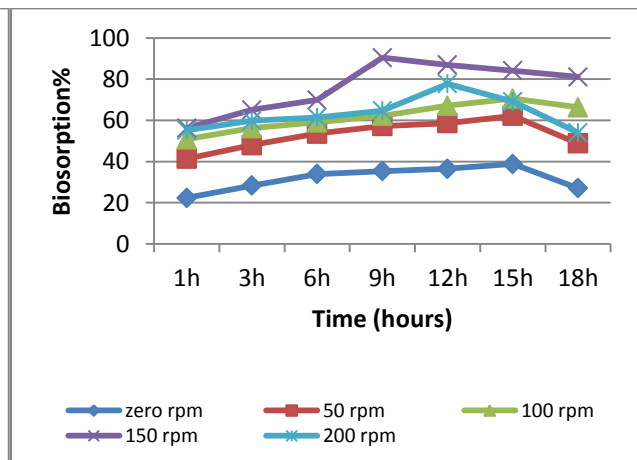
(zero rotation, 50, 100, 150 and 200 rpm) were undertaken and shown in Figure 4 (a-j) which indicates that the degree of agitation influences the sorption rate as the agitation speed increases from zero rotation to 200 rpm.

Both strains *Spirulina subsalsa* and *Scenedesmus ecornis* showed optimum uptake at 150 rpm. The uptake of Reactive yellow was (89.3%, 90.4%) at 9 hours, reactive black 5 (88.7%, 88.3%) at 12. hours, reactive Blue 214 (86.1% at 15 hours, 86.9% at 12. hours. By increasing of agitation to 200 rpm the sorption rates showed differ to a quite small extent, indicating that the film thickness has insignificant effect when the agitation rate is higher than 200 rpm and the weak binding dyes. Hence, an agitation rate of 150 rpm was

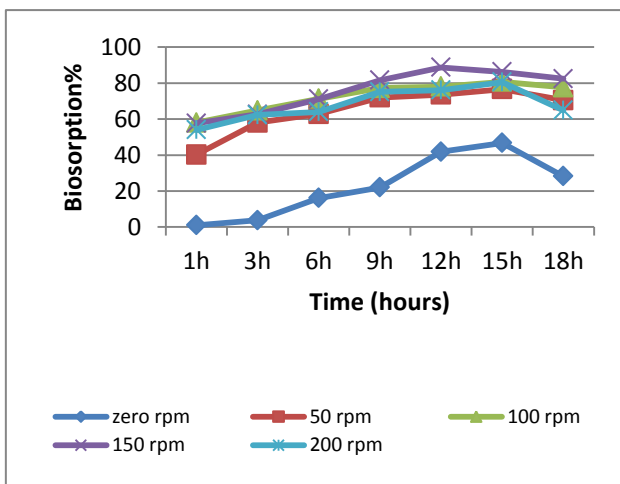
selected. This results same with (13) Indicated that the contact between biomass and dye solution is more effective at moderate agitation (150 rpm). An increase in agitation speed of up to 150 rpm could have facilitated proper contact between dye solution and biomass binding sites, thereby encouraging the effective transfer of sorbate ions to sorbent sites (8). The observed decrease in dye removal beyond 150 rpm could be attributed to an increase in turbulence associated with the desorption of adsorbates in the solution and therefore an increase in the residual concentration of dyes (2).



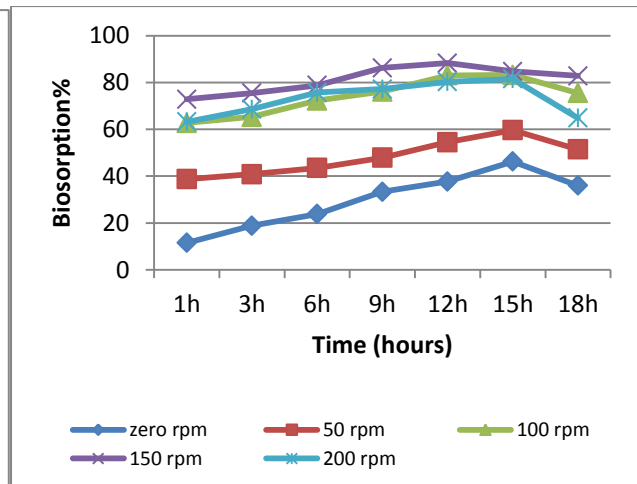
(a)



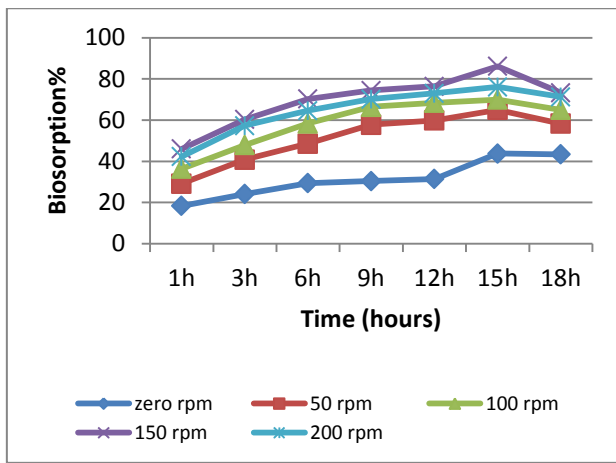
(b)



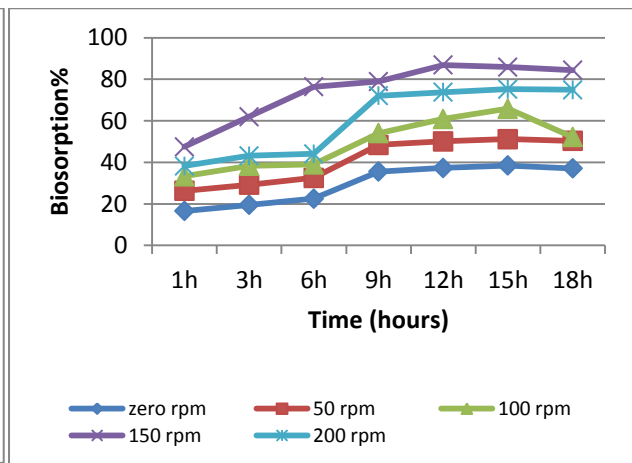
(c)



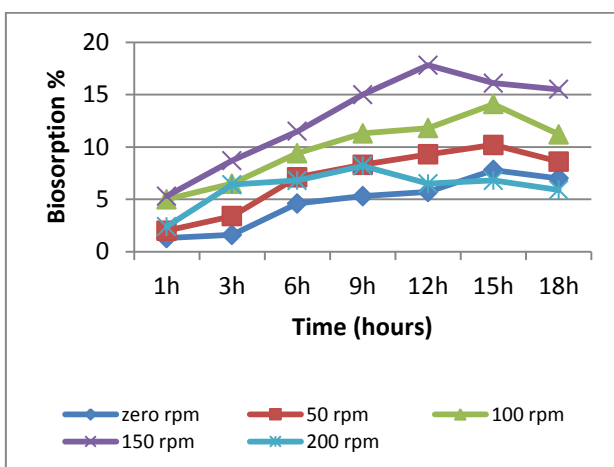
(d)



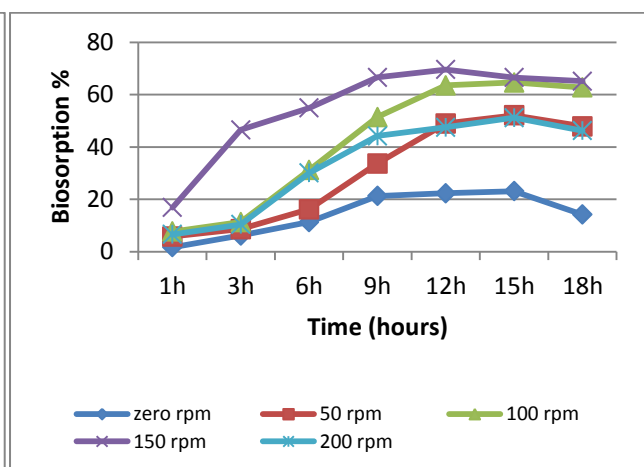
(e)



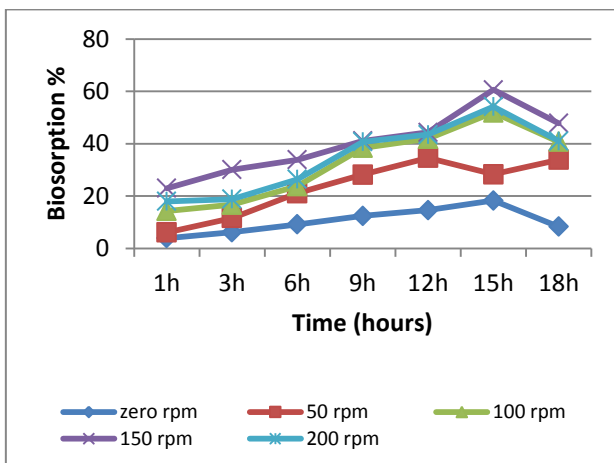
(f)



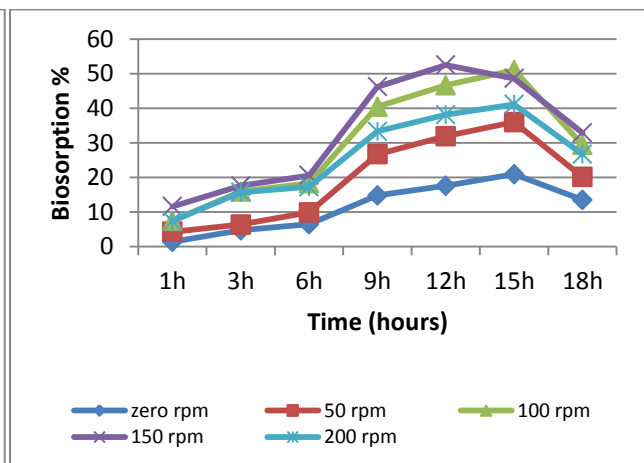
(g)



(h)



(i)



(j)

Fig. 4. Effect of agitation speed on biosorption of dye [(a,b) Reactive yellow, (c,d) Reactive black 5, (e,f) reactive Blue, (g,h) Reactive Orange 16, (i,j) Direct Red 254] (50 mg l^{-1} , 30°C , pH 8, dosage 1.2 g ml^{-1}) by (a, c, e, g, i) *Spirulina subsalsa* and (b, d, f, h, j) *Scenedesmus ecornis*



Fig. 5. Effect of agitation speed on biosorption of dye (reactive yellow, reactive orange 16, reactive black 5, direct red 254 and reactive blue 214) by *Spirulina subsalsa* compared with control without biomass



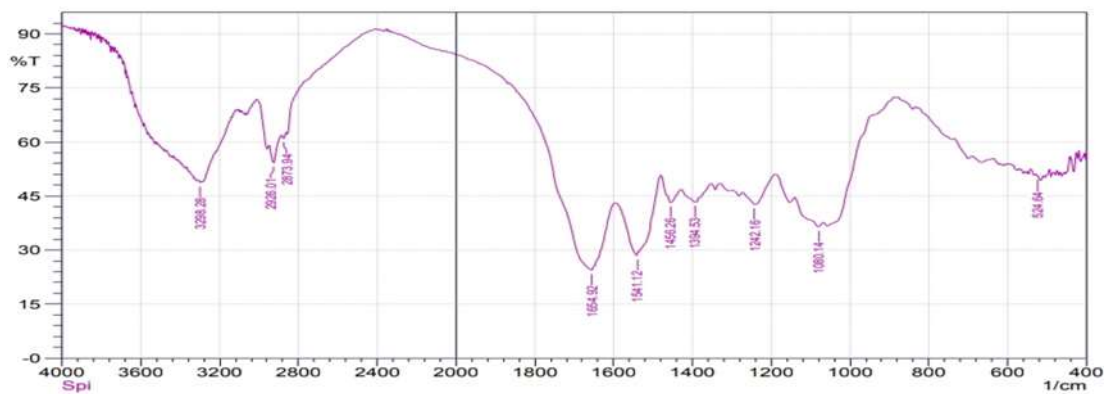
Fig. 6. Effect of agitation speed on biosorption of dye (reactive yellow, reactive orange 16, reactive black 5, direct red 254 and reactive blue 214) by *Scenedesmus ecornis*.

Fourier-transform infrared spectroscopy (FT-IR) characterization of microalgae cell wall: Fourier transformation infrared (FT-IR) spectroscopy was used in the study to identify and determine spectral features of *Spirulina subsalsa* and *Scenedesmus ecornis* surface characterization for Reactive yellow dye that showed optimum adsorption before and after dye biosorption. FTIR was carried out in order to investigate the functional groups of algal cell wall responsible for dye biosorption. The FT-IR spectral region was between (400-4000) cm^{-1} and (FT-IR) means Fourier transformation infrared spectrometer with high

resolution. FT-IR spectra gives 10 chemical adsorption bands with 10 different frequencies before adsorption, at 3298 cm^{-1} , 2926 cm^{-1} , 2873 cm^{-1} , 1654 cm^{-1} , 1541 cm^{-1} , 1456 cm^{-1} , 1394 cm^{-1} , 1242 cm^{-1} , 1080 cm^{-1} and 524 cm^{-1} . The FT-IR spectra of control cell (before dye adsorption) shows broad and prominent peaks at 3298 cm^{-1} which represents the stretching vibration of carbohydrates (O-H) group of the *Spirulina subsalsa* biomass (Figure 7). The peak at 2926 cm^{-1} assigned to (C-H) stretching of alkene. The peak between $1541\text{-}1456 \text{ cm}^{-1}$ which represents the symmetric and asymmetric stretching of (N-O) group. After

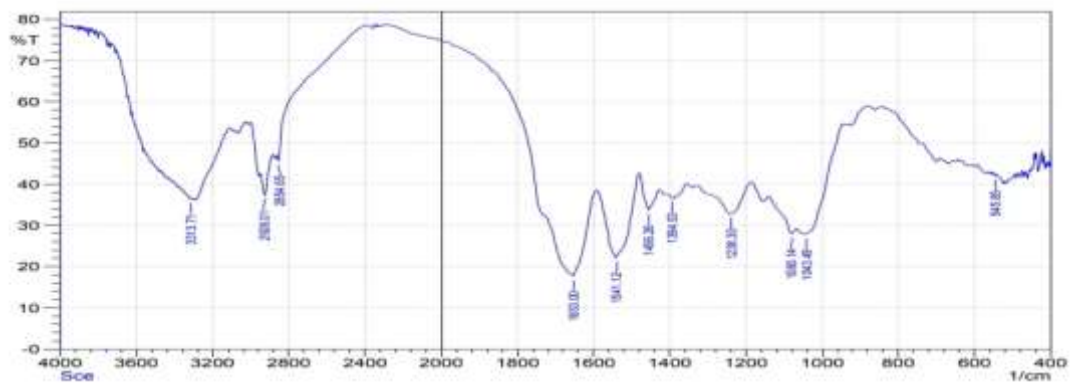
yellow dye the adsorption of pollution on the carboxylic functional group or hydroxyl group of protein of *Spirulina subsalsa* was increase science the band become broad due to H-band and shifting to the higher wave number or frequency. For *Scenedesmus ecornis* (FT-IR) gives 11 signal or chemical adsorption vibration for functional groups before adsorption by dye. Which are : 3313 cm^{-1} , 2926 cm^{-1} , 2854 cm^{-1} , 1653 cm^{-1} , 1541 cm^{-1} , 1456 cm^{-1} , 1394 cm^{-1} , 1238 cm^{-1} , 1080 cm^{-1} , 1043 cm^{-1} , 545 cm^{-1} (Figure 8). The peak between 2926-2854 cm^{-1} represents the

symmetric and asymmetric stretching for (C–H) CH_2 lipid and carbohydrate. The peak between 1541-1456 which represents the (N–H) bending protein amide. Where these chemical adsorption bands are shifted to the higher or lower frequency region depending on the how much adsorption occur after using Reactive yellow dye and rotating 150 rpm and pH 8 on the functional groups present in the *Scenedesmus ecornis*. (4,20) showed that the FT-IR data of five functional groups were probably involved in the adsorption of the dye.

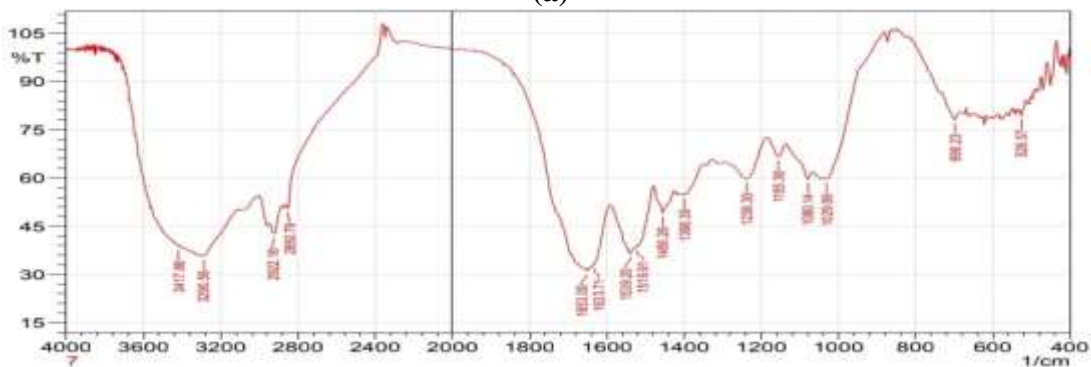


(b)

Fig. 7. FT-IR spectrum of *Spirulina subsalsa* (a) before and (b) after dye biosorption



(a)



(b)

Fig. 8. FT-IR spectrum of *Scenedesmus ecornis* (a) before and (b) after dye biosorption

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