

TETRACYCLINE ANTIBIOTIC REMOVAL FROM AQUEOUS SOLUTION USING CLADOPHORA AND SPIRULINA ALGAE BIOMASS

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ABSTRACT

Cladophora and Spirulina algae biomass have been used for the removal of Tetracycline (TC) antibiotic from aqueous solution. Different operation conditions were varied in batch process, such as initial antibiotic concentration, different biomass dosage and type, contact time, agitation speed, and initial pH. The result showed that the maximum removal efficiencies by using 1.25 g/100 ml Cladophora and 0.5 g/100 ml Spirulina algae biomass were 95% and 94% respectively. At the optimum experimental condition of temperature 25°C, initial TC concentration 50 mg/l, contact time 2.5hr, agitation speed 200 rpm and pH 6.5. The characterization of Cladophora and Spirulina biomass by Fourier transform infrared (FTIR) indicates that the presence of functional groups of different components such as the Hydroxyl group (-OH), amides(N-H stretch) were responsible of surface adsorption processes. The isothermal study has been applied using Freundlich, Temkin, and Langmuir models. The data best fitted with the Langmuir model. Finally, The pseudo-second-order kinetic model was best fitted the kinetic data with a high coefficient of determination ($R^2 > 0.97$ and 0.99) when used Cladophora and Spirulina algae biomass, respectively. The study showed that both Cladophora and Spirulina algae were promising and economical biomass that could be used for a large scale bioreactor.

Keywords: adsorption, pharmaceuticals, batch, kinetic, isotherm

عبد و محمدرضا

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إزالة المضاد الحيوي تتراسايكلين من محاليل مائية بواسطة كتلة الطحالب الحويية الكلاذفورا والسبارولينا

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باحث

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المستخلص

تم استخدام الطحالب الخضراء *Cladophora* والطحالب المزرقية *Spirulina* في هذه الدراسة كمادة مازة للمضاد الحيوي (Tetracycline TC) في النظام الدفعي تمت دراسة تأثير الرقم الهيدروجيني ، كمية المادة المازة ونوع الطحالب، سرعة الاهتزاز ، تركيز الملوث الأولي، زمن التلامس. أفضل كفاءة إزالة للتتراسايكلين كانت (95%، 94%) عند درجة حرارة 25 ، حامضية 6.5 = pH، زمن تلامس 2.5 ساعات، سرعة اهتزاز 200 rpm و تركيز الملوث الأولي 50 ملغم/لتر. تم تشخيص المواد المازة المستخدمة بأستخدام جهاز محلل (FTIR) طيف الاشعة تحت الحمراء وتم ايجاد المجاميع الفعالة- (OH) و amides (N-H stretch) . كانت البيانات متطابقة مع الموديلات الثلاثة المستخدمة Langmuir . Temkin و Freundlich . وتم اختيار افضل موديل رياضي يُمثل النتائج العملية فوجد ان موديل لانكمير (Langmuir) هي افضل الموديلات.. البيانات الحركية اتبعت موديل الحركية من الدرجة الثانية للمضاد الحيوي حيث معامل الارتباط (Correlation) $0.97 < \text{coefficient}$

الكلمات المفتاحية: امتزاز، المستحضرات الصيدلانية، النظام الدفعي ، الحركية، الايسوثرم.

INTRODUCTION

Pharmaceutical compounds are considered emerging environmental pollutants that have a potentially harmful effect on the environment and human health. The excessive use of antibiotics increased their presence in water sources, affecting the aquatic ecosystems and the quality of water(2). Antibiotic usage has been rapidly increased all over the world. Of particular concern are antibiotic residues in the environment, which can induce antibiotic-resistant genes (ARGs) from extended exposure at relatively low concentrations (9). In these conditions, the environment encourages bacteria to evolve ways to protect themselves, causing "Superbugs" is a term used to describe strains of bacteria that are resistant to the majority of antibiotics commonly used today. Worse, these bacteria, most of which are strains harmless to humans, can then share this resistance mechanism with disease-causing microbes(1). Traditional techniques for the removal of antibiotic from wastewater include evaporation, chemical precipitation, ion exchange, ozone treatment, photochemical oxidation, cation exchange membranes, ultrafiltration, nano-filtration, electro-chemical degradation, reverse osmosis, coagulation, membrane separation, and catalytic oxidation. These processes may be ineffective for large scale subtraction of antibiotic(22). The adsorption process is being widely used by various researchers for the removal of antibiotics from waste streams, offering significant advantages like the low cost, availability, profitability, ease of operation, and efficiency, in comparison with conventional methods, especially from

economic and environmental points of view (4). Algae biomass used for biosorption antibiotics from wastewater. In this work, the ability of algae biomass to remove tetracycline from the water will be studied under different operation conditions using a batch system. In (Al-taweel, 2019) study the use of a pure and mix algae culture in a free and immobilized form for removing of lead and copper ion from liquid solution. Batch experiments relevant that efficient pH value lies between (4 and 5) , the required equilibrium time was attained within 60 minute for all algae type and forms, the data result fitted with Langmuir and Freundlich isotherm model and conducted well with the Langmuir isotherm with R2 more than 0.99. By using chlorella (CA) , and mixes algae (MA) Pseudo 2nd order model of the kinetic study fit the obtained data well, that prefer chemisorption mechanism (8).

MATERIALS AND METHODS

Sorbate (Tetracycline) : Types of TC class Chlortetracycline, doxycycline, minocycline, oxytetracycline, and tetracycline belong to this group (15). Tetracycline (TC) used in this study **Fig. 1** shows the scan UV-V for the TC test at AlKhawarizmi University lab where max wavelength 360 nm. Table 1 summarizes TC characteristics. Wavelength scanning at different TC concentrations yielded the respective adsorption spectra, the determination of TC (15). Stock standard solutions were prepared by adding 1 g of the pure substance and dissolving it in distilled water to obtain TC concentration 1000 mg/L. The pH value of the solutions was controlled during the experiments by addition of the buffer solution dropwise.

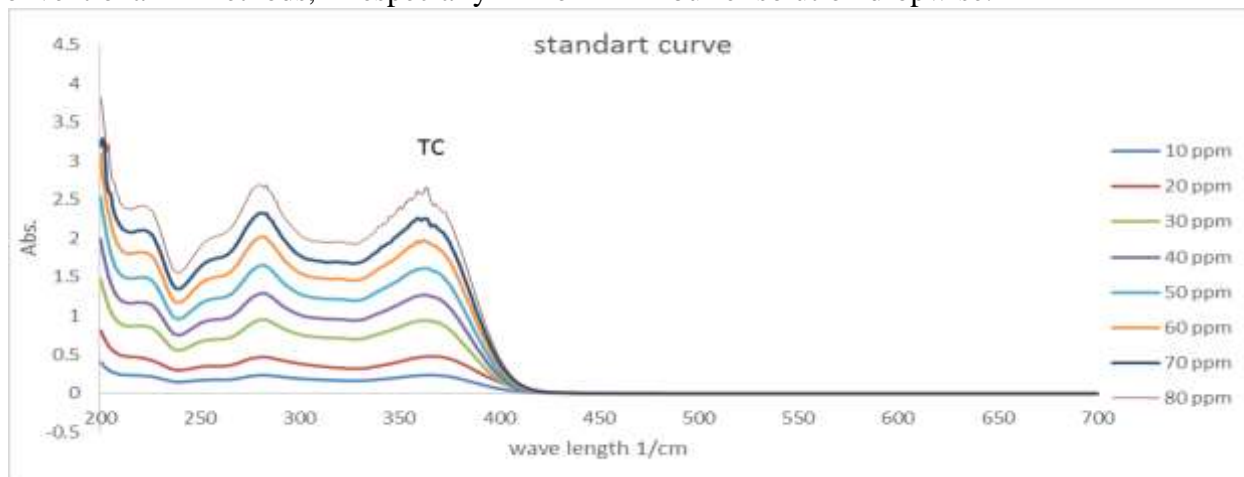
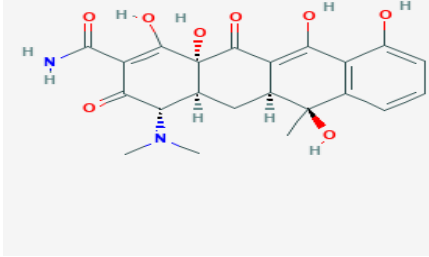


Fig. 1. Full scan UV-Vis spectrum of TC.

Table 1. Characteristics of tetracycline. (15)

Property	value
Chemical structure	
pKa with different pH value	$C_{22}H_{24}N_2O_8$ pKa1 =3.30 pKa2 = 7.70 pKa3 = 9.27
molecular weight	480.9 g/mol

Sorbent (algae biomass preparation)

Two kinds of algae biomass were used; the first one was local algae biomass [Cladophora]. It was used in this work as biosorbent for tetracycline removal from aqueous solution. A mass of wetted algae

collected from the artificial irrigation canal near the College of Engineering at the University of Baghdad in March of 2019. The water in this canal fed from the Tigris River. **Fig. (2)** shows the location of the collected algae.

**Fig. 2. The algae collection location (Cladophora)**

A random sample of the collected wet mix algae was analyzed for their species and content percentage of each type by using a microscope at the laboratories of the Biology Department, Science College, the University of Baghdad as given in Table 2.

Table 2. The genus, species and percentage weight for mixed algae

Algal species	Percentage %
<i>Cladophora</i>	85
<i>Microalgae</i>	10
<i>Impurities</i>	5

The results showed that two types were found in this sample, Cladophora algae were the highest percentage. After the collected step, algae were washed many times with tap water to get rid of impurities, dirt and other unwanted materials such as (non-vertebrate animals, small worms, crustaceans, bird feathers), then with distilled water twice to ensure clearness. The washed algae were left under the sun for three days to dry (20). The dried algal was cut off, ground and sieved to get grain size powder or <math><63 \mu\text{m}</math> for biosorption in batch experiments. **Fig. (3)** shows Cladophora algae biomass.



Fig. 3 A. Show the Cladophora algae drying on the sun , (B) Show the Cladophora algae drying on the sun after 3 days, (C) Powder size of Cladophora dried algae biomass

The second type used in this study was a pure Spirulina Algae biomass (supplied from amazon) was used in this work as biosorbent for tetracycline removal from aqueous solution. As powder less than 63 μm in particle size. **Fig. (4)** shows Spirulina algae biomass.

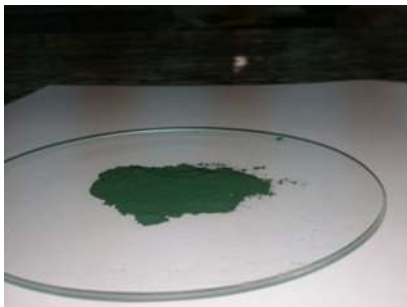


Fig. 4. Pure Spirulina algae powder

Instrumentation that used in research work

- 1- UV visible spectrophotometer: was used to measure the concentration of antibiotics in the solution (Model: Cary-100 conc., Varian, USA). Tetracycline concentration measured at wavelength 360 in this research.
- 2- Shaking Incubator (ISO 9001, Model: LSI-3016A, NO.B110416002, Korea).
- 3- pH meter: ISOLAB
- 4- Sensitive Balance with compartment (Model: Sartorius ENTRIS124-1S Lab Balance, Germany).
- 5- Cellulose acetate membrane Filter (0.2 μm)
- 6- Sieve (RETSCH sieves).
- 7- Grinder (Agate mortar, Retsch, Type BB1A, Masch.Nr.4323, Germany).

RESULTS AND DISCUSSION

Fourier Transform Infra-Red (FTIR)

Analysis: In the measurement of infrared spectroscopy of the samples, IR radiation passed through the sample. Some of that radiation absorbed via sample and some of the radiation transmitted. The spectrum result represented the transmission and molecular absorption capacity, making molecular fingerprint to the sample. That makes FTIR useful for several types of analysis (19). In order to identify functional groups (carbonyl, carboxylic, hydroxyl and others) involved in the biosorption process, the FTIR techniques were used. FTIR test can also provide an excellent information on the bands present nature present on the surface of the algae before and after the biosorption process, and has many advantages when used as an analytical technique: the test fast, nondestructive and requires only small quantities of the samples (21). Fourier transform infrared (FTIR) in the region of 4000-400 cm^{-1} resolution accomplished at AlKhawarizmi University. **Fig.5** shows the FTIR spectrum of algae biomass before and after sorption of TC. Some peaks after TC biosorption disappeared, shifting or decrease in its intensity. Among the active sites in **Fig.5 (a)** were Hydroxyl group (-OH), amides(N-H stretch) and amine have been suggested to be responsible for the adsorption of TC. While the active sites in **Fig.5 (b)** were Hydroxyl group (-OH), amides(N-H stretch), Carboxyl (C-H aldehyde stretch), Alkyl halides.

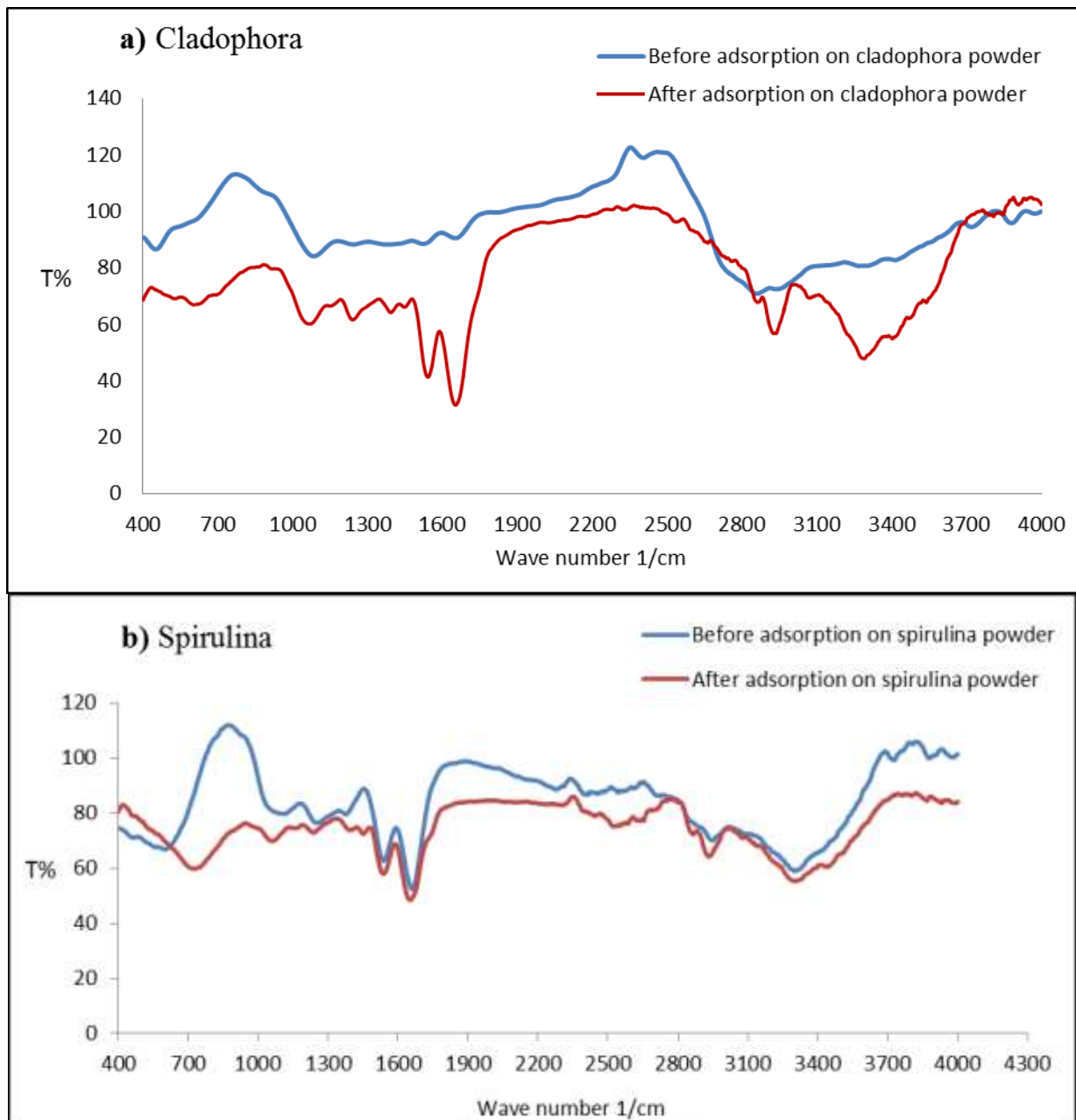


Fig. 5. Functional groups before and after algae biomass a) Cladophora b) Spirulina loaded with TC

Effect of parameters on batch process

Effect of Initial Concentration: Experiments were carried out to study the effect of various initial concentrations (10, 20, 30, 40, 50, 60, 70 and 80) mg/l of TC on removal efficiency by Cladophora and Spirulina algae biomass while keeping other parameters constant and their results were presented in **Fig. 6**. show a decrease in the uptake rate of tetracycline with rising initial TC concentration. Reduction in the efficiency explained by the saturation of

the available reactive adsorption sites on the sorbent surface while increasing initial TC concentration (7). All TC molecules present in solution have ability to interact with available sites at lower concentrations and the removal efficiency was high in comparison with higher concentrations. This can be attributed to large active (binding) sites available in the biosorbent dosage used in the set. Hence, the initial concentration of 50 mg/L was used for remaining batch experiment

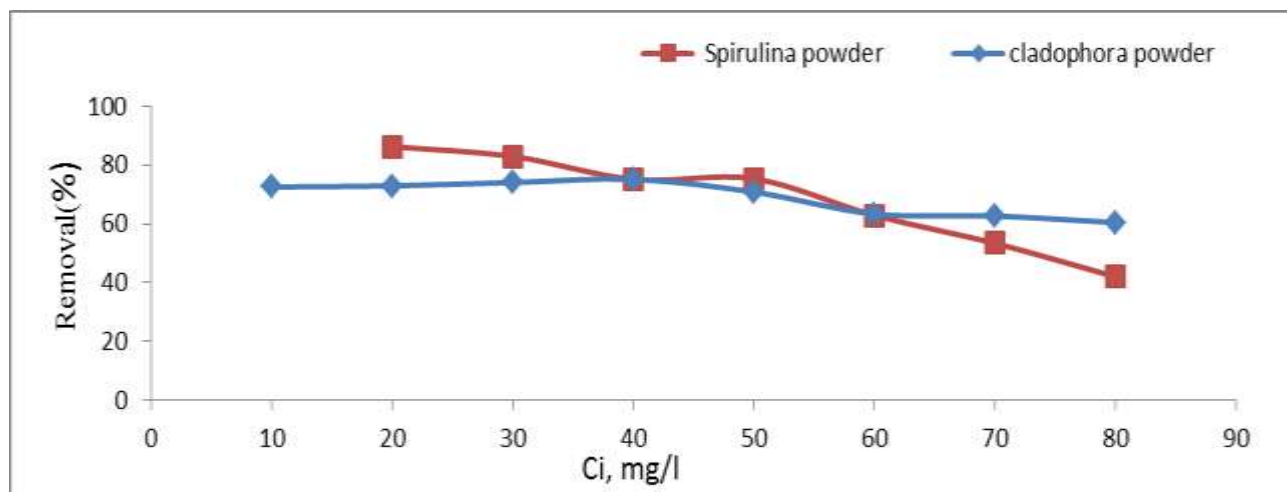


Fig. 6. Effect of TC initial concentration on the removal efficiency by cladophora and spirulina algae biomass (pH 6.5, $m_{(\text{cladophora})} = 0.5$ g/100 ml, $m_{(\text{spirulina})} = 0.2$ g/100 ml, 25°C, speed= 200rpm and time=3hr).

Effect of Algal Biomass Dosage

One of the important parameters that strongly affect the sorption capacity is the biosorbent dosage. This effect was studied by adding different dosages of biosorbent (0.05-1.25)gm. The batch tests were conducted with initial concentration of TC is 50 mg/L agitation speed of 200 rpm and contact time of 3 hr. at room temperature of 25°C. TC removal has been studied by a wide range of algal biomasses, as shown in Fig. 7, the maximum dosage of Cladophora and Spirulina used are

1.25 g/100 ml and 0.5 g/100 ml respectively, which removal efficiency 94% for two type. This is a logical behavior because the increasing of biosorbent dosage means a greater number of biosorption sites and, consequently, a higher removal of contaminant (18). It is clear there are not significant changes in the removal efficiency of TC in response to variation of biosorbent dosage from 0.5 to 1.2 g for spirulina algae biomass and this can be attributed to reaching the maximum sorption capacity.

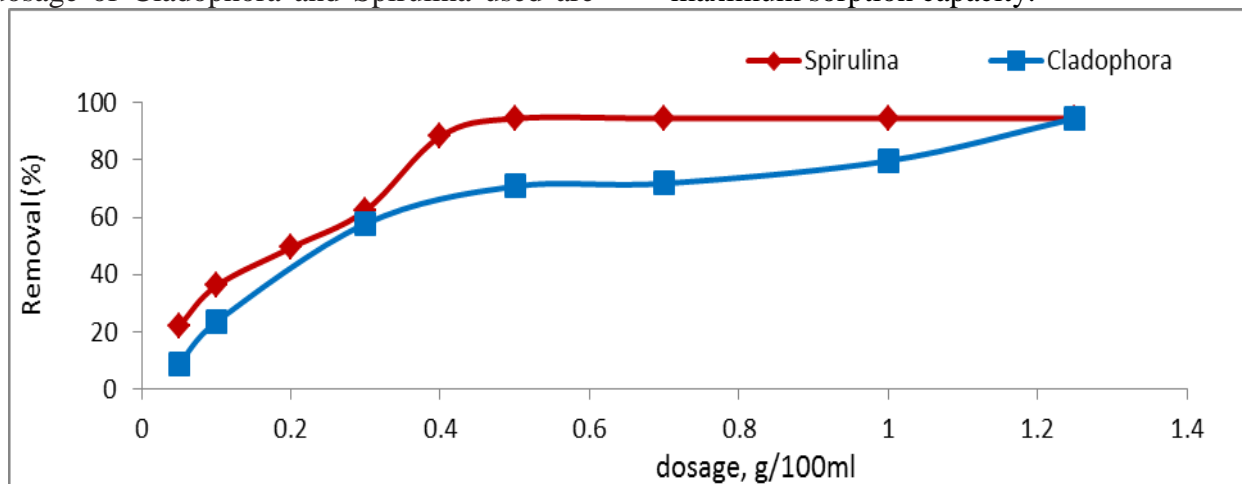


Fig. 7. Effect of sorbent dosage on the percentage removal of TC by Cladophora and Spirulina algae biomass (pH 6.5, 25°C, $C_{i,tet} = 50$ mg/L, speed= 200 rpm and time=3hr).

Effect of contact time

The effects of contact time onto removal efficiency of TC is shown in Fig. 8. It was observed that the removal efficiency increases as the contact time increases and it remains constant after reaching equilibrium. This is due to a larger surface area of the biomass at the beginning of the biosorption process. the

removal rate was gradually decreased due to decrease the vacant sites on the surface of the biosorbent and formation of repulsive forces (13). The maximum removal of TC was 95% percent after 150 minutes (2.5 h) of shaking time. Therefore, 2.5 h was taken as the equilibrium time for subsequent experiments.

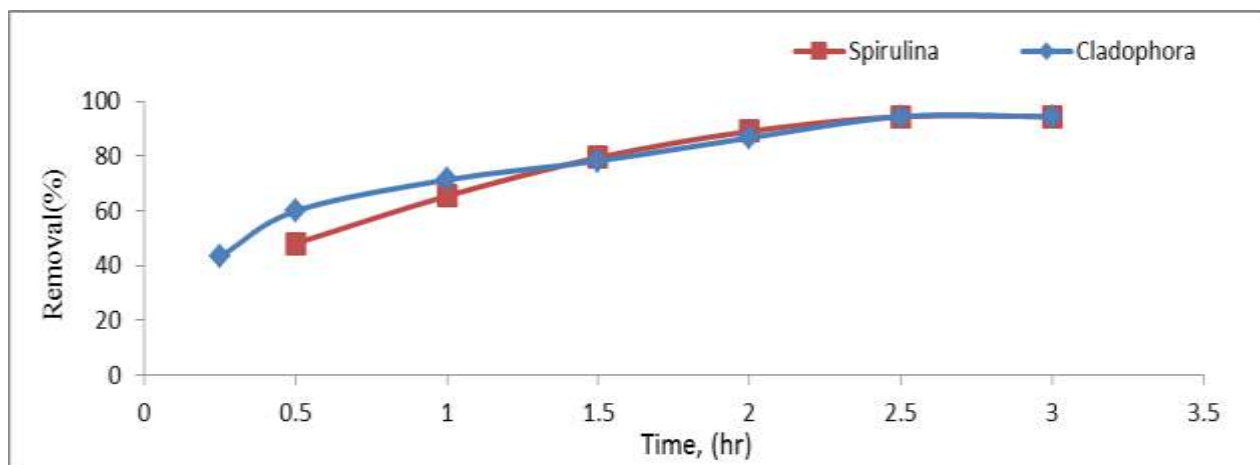


Fig. 8. Effect of time on the removal efficiency of TC by Cladophora and Spirulina algae biomass (pH 6.5, $m_{(\text{cladophora})} = 1.25 \text{ g/100ml}$, $m_{(\text{spirulina})} = 0.5 \text{ g/100ml}$, 25°C , agitation speed= 200 rpm $C_{o \text{ tet}} = 50 \text{ mg/L}$).

Effect of pH

The effect of TC solution pH has been studied in the range of pH (3-10), **Fig. (9)** shows clearly that TC removal increased significantly between pH 6 and 7 removal percentage reaching around 95%. Removal of TC was approximately low at pH equal to 3 and this is due to high concentration of hydrogen ions. These ions can be competed the TC molecules for binding with available sites on the algae biomass which have high affinity for H^+ ions. Therefore, the decreasing of TC removal can be caused by increasing the proton concentration in the aqueous phase. Consequently, the increase of the TC removal (from 41 to 94.5 %) and (from 22 to 94.5%) by cladophora and spirulina algae biomass

respectively as the pH increases (from 3 to 6.5) can be explained on the basis of a decrease in competition between TC and hydrogen species for the binding sites (23). It is clear that the maximum removal efficiency of TC was achieved at an initial pH value of 6.5. The pH_{pzc} (Point Zero Charge) of the cladophora and spirulina algae biomass was determined to be (7-7.8) respectively (10). Oppositely at $\text{pH} < \text{pH}_{\text{pzc}}$, ions of H^+ are transferred to the particle surface and combined with OH^- groups leading to a positive charge algae surface. Under these circumstances, the net surface charges of the algae biomass at $\text{pH} < 7$ were positive (2).

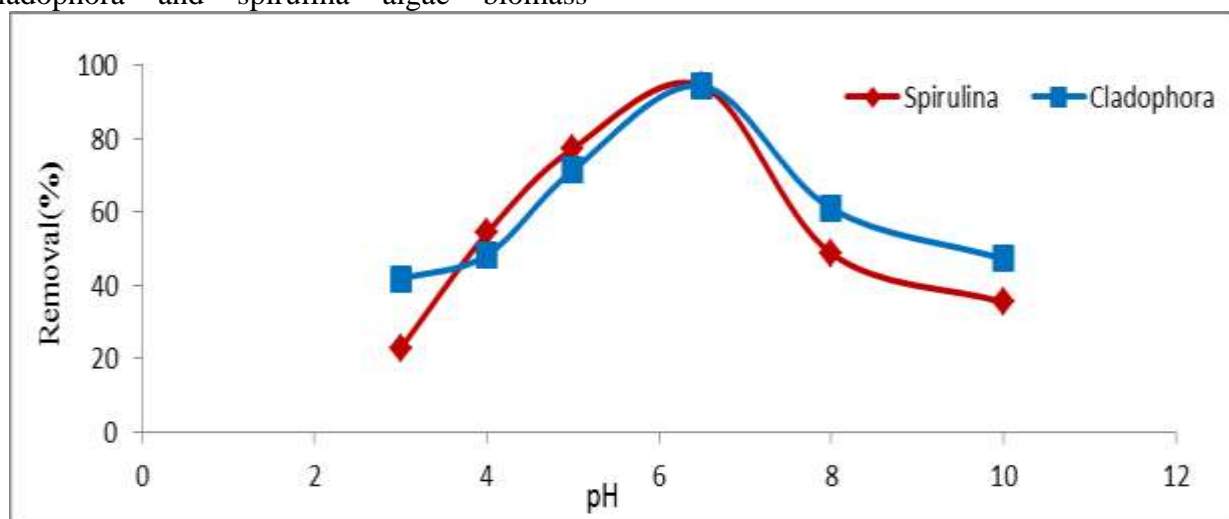


Fig. 9. Effect of pH on the removal efficiency of TC by Cladophora and Spirulina algae biomass (time=2.5hr, $m_{(\text{cladophora})} = 1.25 \text{ g/100ml}$, $m_{(\text{spirulina})} = 0.5 \text{ g/100ml}$, 25°C , agitation speed= 200 rpm $C_{o \text{ tet}} = 50 \text{ mg/L}$).

Effect of agitation speed

The effect of agitation speed onto removal efficiency is shown in **Fig. 10**. It is observed that, the removal efficiency increased as the agitation

speed increased. This is due to the fact that, at higher agitation speed the film thickness decreased and this eliminates the film resistance (14). Indeed, the high agitation speed is enhanced the diffusion

of contaminants through reactive medium and a suitable contact can be developed between binding sites and the contaminant (7). In addition, the results signified that a shaking speed with the values ranged from 100 to 300 rpm was adequate for ensuring maximum TC uptake

and no considerable change can be recognized after these values. Accordingly, the present study denoted that the required agitation speed for achieving the maximum removal efficiency is; 200 rpm for TC onto algae biomass.

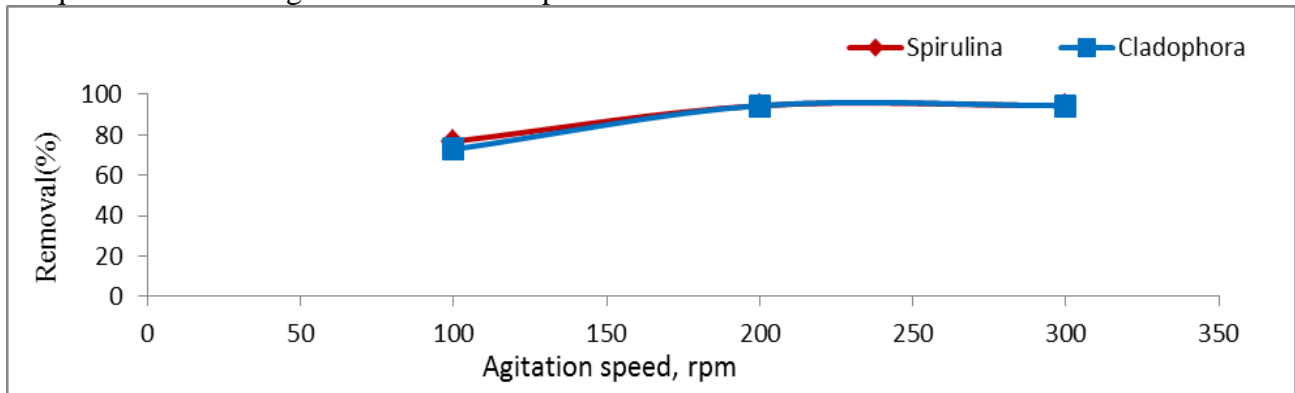


Fig. 10. Effect of agitation speed on the removal efficiency of TC by cladophora and Spirulina algae biomass (pH 6.5, $m_{(cladophora)} = 1.25 \text{ g/100ml}$, $m_{(spirulina)} = 0.5 \text{ g/100ml}$, 25°C , $C_{i \text{ tet.}} = 50 \text{ mg/L}$ and $\text{time} = 2.5 \text{ hr}$)

Isotherm Models

The importance of sorption isotherm comes from its representation of how antibiotic molecules distribute between the solution and biosorbents at equilibrium (12). Several isothermal models were used for this purpose, including Langmuir and Freundlich, which are considered the most common models (5). Selecting a suitable model depends on fitting the experimental data onto model equations, and the correlation coefficient helps by indicating the models that are suitable to fit the data.

1-Langmuir Isotherm

The Langmuir adsorption has been the most widely used adsorption isotherm for the adsorption of a solute from a liquid solution. Langmuir equation relates the coverage of molecules on a solid surface at a fixed temperature. The Langmuir model assumes a monolayer adsorption of sorbate onto a surface contained identical groups with homogeneous biosorption energy (10). It is simply represented by the linear equation:

$$q_e = q_{\text{max.}} * b * C_e / (1 + b * C_e) \dots \dots \dots (1)$$

where C_e (mg/L) is the TC concentration at equilibrium, q_e (mg/g) is the amount of TC adsorbed per gram adsorbent, $q_m =$ (mg/g) maximum capacity for sorption of TC from the solution, $b =$ (L/mg) constant depend on binding sites alliance

2-Freundlich Isotherm

an empirical expression in the Freundlich isotherm, and expressed as follows(13):

$$q_e = K_F C_e^{1/n} \dots \dots \dots (2)$$

Where $K_F =$ Freundlich's constant, a constant that is relative to the adsorption capacity (mg/g) $(\text{L/mg})^{1/n}$.

$1/n =$ constant indicates the intensity of the adsorption and gives indication on the favorability of the adsorption. Both n and K are constants, being indicative of the degree of non-

linearity between solution & concentration, and the extent of adsorption, respectively.

If $n = 1$, the partition between the two phases is independent of the concentration. $1/n > 1$ shows cooperative adsorption, but a $1/n < 1$ shows normal biosorption (20). The Freundlich constant n (adsorption intensity) in the range (1–10) suggested that the bonding affiliation of adsorbate and the biosorbent was strong (7)

3- Temkin isotherm model (Temkin, 1940):

Temkin isotherm takes into account the adsorbate-adsorbent interaction. By ignoring the extremely low and large value of concentrations, the model assumes that the fall in the heat of sorption (function of temperature) of all molecules in the layer is linearly rather than logarithmic with coverage (16). Adsorption is characterized by a uniform distribution of binding energies, up to some maximum binding energy (8). The Temkin isotherm equation is given by (11):

$$q_e = B_T \ln(K_T * C_e) \dots \dots \dots (3)$$

$$B_T = \frac{R * T}{b_T} \dots \dots \dots (4)$$

Where:

K_T is the equilibrium binding constant (L/g), B_T = Temkin isotherm constant, b_T Constant related to the heat of sorption (J/mole). R is the universal gas constant (8.314 J/mole K) and T is the absolute temperature (K).

All experimental data were analyzed and compared by using non-linear isotherm models, and the models parameters were evaluated by using Microsoft Excel SOLVER software. Furthermore, sum square error (SSM) and coefficient of determination (R^2)

were used to measure the goodness of fit. SSE is defined as (10):

$$SSR = \sum_{i=1}^n (q_{e, calc} - q_{e, exp})^2 \dots (5)$$

In the present study, *Cladophora* and *Spirulina* are characterized for its ability in TC removal, considering the parameters affecting the removal of TC from water such as pH, algal biomass dose, time, agitation speed, initial concentration. The adsorption of tetracycline on adsorbent is illustrated by the isothermal study. As show in **Fig. 11, 12 and Table 3.**

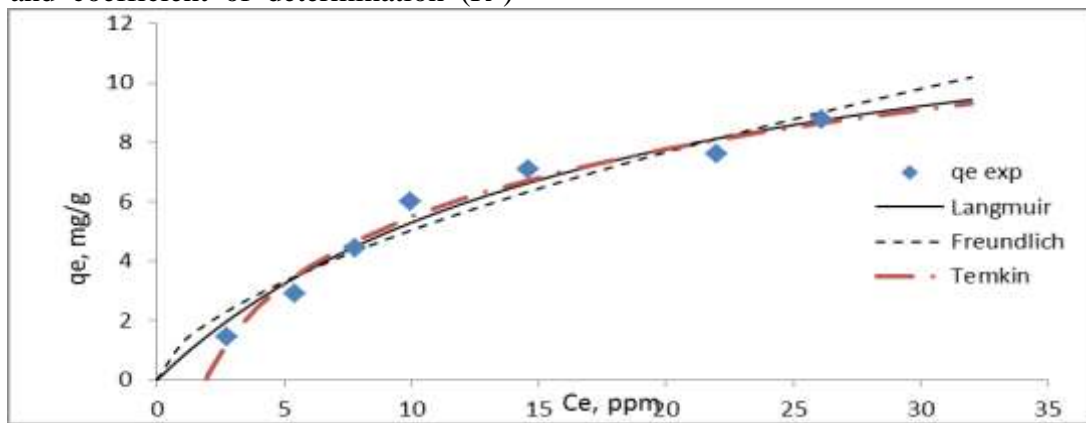


Fig. 11. Isotherm model for sorption of TC on algae biomass *Cladophora*

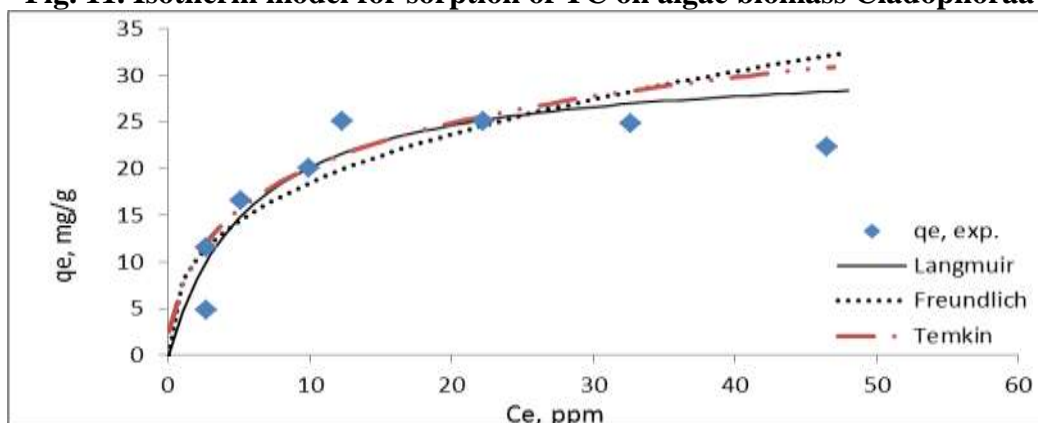


Fig. 12. Isotherm model for sorption of TC on algae biomass *Spirulina*

Table 3. Parameters of isotherms for sorption of TC

Model	Parameters	Isotherms equations constant	
		Cladophora powder	Spirulina powder
Langmuir Model	q_{max} (mg/g)	17.4	19.7
	b (L/mg)	1.22	0.350
	R^2	0.99	0.98
	SSE	26.3	10.4
Freundlich Model	k (mg/g)	8.6	7.1
	n	3.98	3.3
	R^2	0.98	0.95
	SSE	2.6	26.7
Temkin Model	K_T (mg/g)	86.4	3.9
	$B=RT/b$ (L/mg)	2.2	4.04
	R^2	0.97	0.97
	SSE	11.6	15.05

Sorption kinetics model

The kinetics of tetracycline adsorption onto algae biomass (*Cladophora*, *Spirulina*) were investigated using pseudo first order and pseudo second order model, using the experimental data at various initial concentrations. The calculated values obtained from the application of these models are tabulated in **Tables 4**. The values of R^2 (coefficient of determination) and q_e calculated from the second order kinetic model show a well fit with the experimental data compared to other mentioned models. The linear plot of each biosorbent did not pass through the origin, as a result, intraparticle diffusion was not the rate-limiting step (3). While, the second order kinetic model expected that the rate limiting step may be chemical sorption (23). The linear form of first-order kinetic model can be expressed by the following equation (24):

$$\ln(q_e - qt) = \ln(q_e) - k_1 t \dots\dots(6)$$

where qt and q_e (mg/g), respectively, are the adsorption capacity at any time (t) and at equilibrium. k_1 (1/min) is the pseudo-first-order rate constant.

From the kinetic model data in **Table 3** and **Fig. 13**, for the adsorption of TC, it can be

concluded that data are poorly fitted to the kinetic model for TC.

However, the second-order kinetic model **Fig. 14**, which expresses the presence of chemisorption process, is related to the difference between the equilibrium vacant adsorptive sites and the occupied sites (6, 17). The second-order kinetic model can be expressed by the following linear equation:

$$t/qt = 1/q_e^2 k_2 + t/q_e \dots\dots(7)$$

where qt and q_e (mg/g), respectively, are the adsorption capacity at any time (t) and at equilibrium. k_2 (g/mg min) is the pseudo-second-order rate constant.

By plotting $\ln(q_e - qt)$ versus t and t/qt versus t in the previous equations (Eqs. (6) and (7)), all the adsorption kinetic parameters can be determined from the slope and the intercept. Data shown in **Table 4** indicated that the adsorption of TC fit with the second-order kinetic model. Furthermore, differences between Q_e calculated and Q_e experiments are lower in the second-order kinetic model than the first-order kinetic model for TC. According to the fitness of the data to the second-order kinetic model, the adsorption of TC by *Cladophora* and *Spirulina* biomass may be chemisorption.

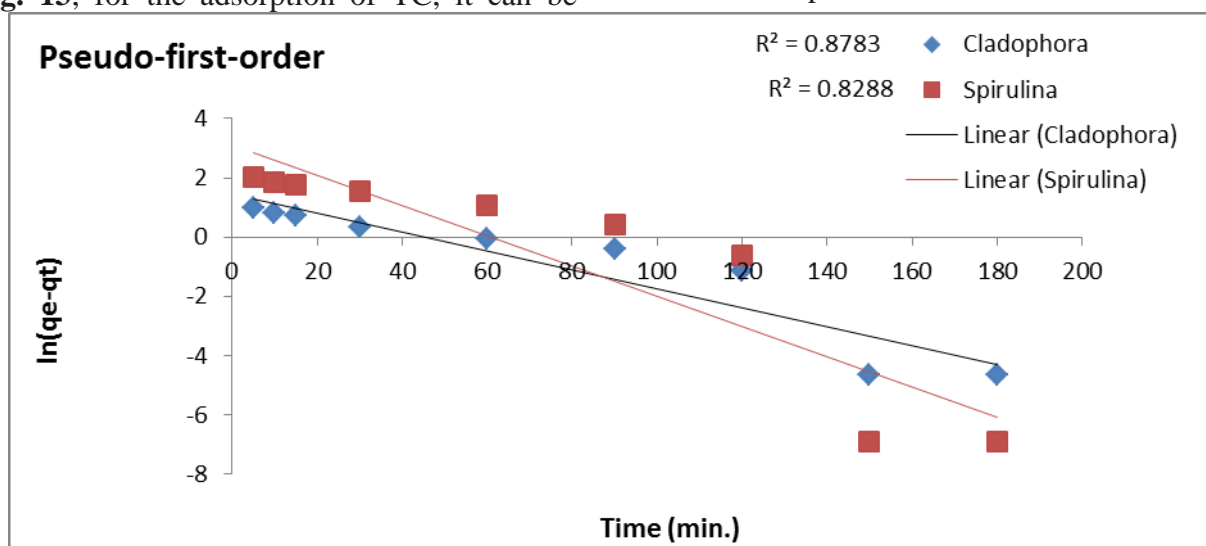


Fig. 13. Pseudo first order for sorption of TC using algae biomass (pH 6.5, T=25°C, agitation speed =200 rpm, and $m_{(cladophora)} = 1.25$ g, $m_{(spirulina)} = 0.5$ g, $C_0 = 50$ mg/L)

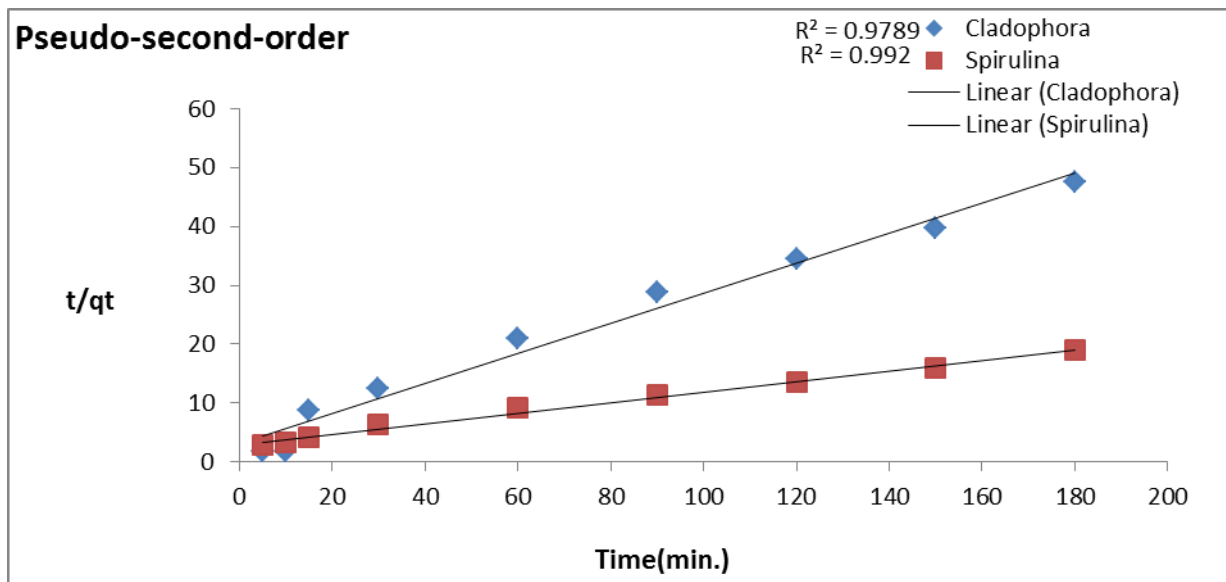


Fig. 14. Pseudo second order for sorption of of sorption of TC using algae biomass (pH 6.5, T=25°C, agitation speed =200 rpm, and $m_{(cladophora)} = 1.25$ g, $m_{(spirulina)} = 0.5$ g, $C_0 = 50$ mg/L
Table 4. Parameters of kinetic for sorption of TC

Kinetics models	Parameters	Cladophora powder	Spirulina powder
Experimental	qe(mg/g)	3.79	9.45
	qe	4.28	22.19
Pseudo first order	K_1 (1/min)	0.03	0.05
	R^2	0.88	0.83
	qe	3.79	11.14
Pseudo second order	K_2 (mg/g.min)	0.02	0.003
	R^2	0.97	0.99

CONCLUSION

The ability of Cladophora and Spirulina biomass to remove TC from water samples reached 94%. Moreover, TC removal reached equilibrium within 2.5 hour contact time for both type of algae. The optimum pH of solutions is 6.5, agitation speed 200 rpm and TC concentration 50 ppm. Nevertheless, algal biomass cladophora and spirulina dose of 1.25 g/100 ml and 0.5 g/100 ml respectively were shown to be the optimum. The data best fitted with the Langmuir model. According to the fitness of the data to the second-order kinetic model, the adsorption of TC by Cladophora and Spirulina biomass may be chemisorption.

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