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Bioadsorption of CI Reactive Blue 203 dye by duckweed *Lemna gibba*'s powder

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Abstract: Many contaminants, especially dyes used to manufacture paint, textiles, and other products, are released into the aquatic environment as a result of human population increase and industrialization. In this study, it was chosen to develop a plant biomaterial compatible with the demand of the world market in cost terms, and to focus on the reuse of powder duckweed *Lemna gibba* as a new effective material for the removal of dyes in particular, considering this plant's capacity for purification even in the waste state after assisting a lagoon plant achieve phytoremediation. After analyzing the effects of pH, contact time, adsorbate concentration, and adsorbent dosage on the dye (CI Reactive Blue 203)'s adsorption process on the plant powder *Lemna gibba*, it was determined that the adsorption kinetics follows the pseudo first order model and the adsorption isotherm is best described by the Langmuir model, and that the percentage of dye removal reaches 90% at pH 3 of the solution, and the adsorption capacity reaches its maximum of 96% at only 0.5g of the powder and 25 mL of the solution. As a result, this study recommends using powder *Lemna gibba* plant as an alternative for activated carbon in adsorption.

Keywords: Duckweeds, *Lemna gibba*, bioadsorbent, powder, adsorption, CI RB 203.

I. Introduction:

Morocco like all countries in the world, is going through a severe water crisis that could lead to water stress in the upcoming years. According to the World Resources Institute (WRI) (National Geographic). Water is a necessity for life, but the excessive production of pollution, especially industrial waste, that is dumped into water, is affecting its supply (Gupta *et al*., 2013). These effluents, especially synthetic dyes used in the textile and food industry (Yan *et al*., 2018), which are typically organic and have an aromatic chemical structure are harmful to human health and challenging to clean up (Sen *et al*., 2013, Yagub *et al*., 2014). These dyes affect aquatic life by limiting light penetration and destabilizing biological processes even at low concentrations (Rodríguez Couto 2009). Coagulation, flocculation, oxidation, ozonation, membrane filtration, reverse osmosis, and adsorption are some of the physical, chemical, and biological processes that have been used in this purpose over time (Körbahti *et al*., 2011, Raizada *et al*., 2019, Naseem *et al*., 2001, Nataraj *et al*., 2009).

Due to its simplicity and low cost, adsorption remains to be the most frequently used treatment method (Mohammed et *al*., 2018, Villegas *et al*., 2016, Elouardi *et al*., 2017). Activated carbon (and activation) continues to be the most widely used adsorbent for dye removal despite its high cost and excessive use (Aboua *et al*., 2018, Aloui *et al*., 2016, Trachi *et al*., 2014). This explains the numerous researches carried out throughout the centuries on activated carbon as an ideal adsorbent for the removal of dyes from the most basic methylene blue as an example to the most complex of dyes (Sakr *et al*., 2015, Kifuani *et al*., 2018, Khelifi *et al*., 2016, Miyah *et al*., 2015; Akartasse *et al.* 2022) However scientists are currently examining and researching new biomaterials that are environmentally friendly (Abida *et al.*, 2023, Kali *et al.* 2022; Turgut *et al.* 2021). in this context we have opted to research the dye's adsorption on a duckweed-based biomaterial.

A small, floating, vascular plant in the family Lemnaceae known as duckweed is well-known all over world for its ability to phytoremediation (Liu *et al*., 2021, Nguyen *et al*., 2021, Sarkheil *et al*., 2020). Given the purifying characteristics of this plant and its simplicity of accumulation (as a living plant) and adsorption (in powder form) of heavy metals and dyes (Halaimi *et al*., 2014, Imron *et al*., 2019, Reyes Ledezma *et al*., 2020, Singh *et al*., 2021, Chen *et al*., 2013, Chen *et al*., 2015), it is crucial to be interested in recycling this biomass in various applications. We were able to apply the adsorption of an azo dye (CI Reactive Blue203) on the powder's duckweed *Lemna gibba*.

2. Methodology:

2.1 Characterization of the dye:

We used the CI Reactive Blue 203 dye, also known by its marketing name Remazol Marine Blue, which belongs to the family of azo dyes. Its chemical formula is ($C_{28}H_{29}N_5O_{21}S_6.4Na$), and its maximum wavelength is λ_{max}=605nm. its chemical structure is illustrated (**Figure 1**) (Erden *et al.*, 2009).

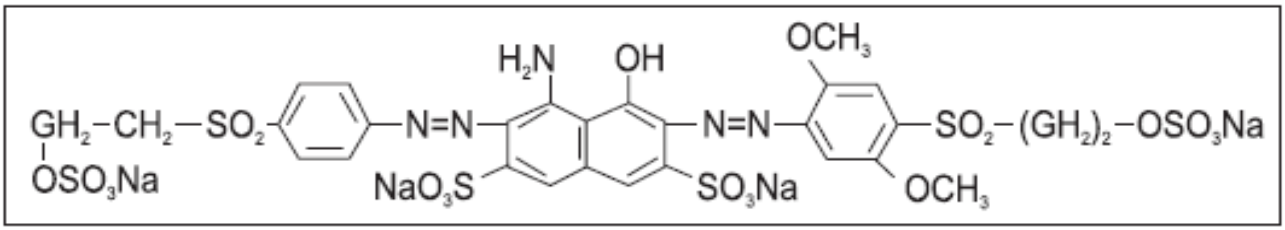


Figure 1: Chemical structure of CI Reactive Blue 203.

2.2 Preparation and characterization of the bioadsorbent:

- Powder preparation:

The bioadsorbent used in this study was prepared from *Lemna gibba* biomass collected after purification at the Bouregreg-Rabat pilot lagoon (33°56'33.6 "N 6°48'00.5 "W). After already being multiple times washed with tap water and then with distilled water, the plant was dried in the open air for a few days. To get rid of the chlorophyll, the dried plant was left to drip with distilled water for 24 hours, then we dried the product in an oven at 80°c for one hour, and finally we crushed and sieved it to obtain a fine powder.

- Granulometric analysis:

It is known that the finer the particle size, the greater the specific surface area and the better the adsorption, so we tried to fix the specific surface area by using a stainless steel sieve with cloth and sheet (DIN 4188) with a diameter of 250µm.

- Determination of moisture and ash of the bioadsorbent:

We chose to determine the moisture content by the quantitative method of gravimetry of volatilization, i.e. the measurement of the loss of mass after the release of water from the powder. For this purpose, 1g of powder was weighed and placed in the oven at 105°c for one day until the weight was stabilized, then placed in a desiccator and weighed after cooling. The percentage of moisture is given according to **(Eq.1)**:

$$\% H = \frac{(ma-mb)}{ma} \times 100 \quad \textbf{(Eq.1)}$$ (Kifuani *et al*., 2018)

ma: mass of the powder before steaming in g and mb: mass of the powder after steaming in g

The same method was used to calculate the total ash content, with the exception that the powder was calcined for 3 hours at 800°C in a muffle furnace. The difference in mass before and after calcination is then used to determine the total amount of ash. (Kifuani *et al*., 2018)

Table1: Characteristics of the Bioadsorbent *Lemna gibba*.

parameters	Values
Grain size (Granulometry)	250 µm.
Moisture (%)	10%
Ashes (%)	85%

- Characterization of the bioadsorbent by FTIR:

It was decided to analyze the biomaterial using Fourier transform infrared spectroscopy (FT/IR-4600 ATR PROONE) in order to understand the adsorption mechanism. By using Kbr disk method, the powder is compressed into a pellet and placed between two KBr windows, with the wave number set in the range of 4400 cm^{-1} to 400 cm^{-1}.

- Thermogravimetric analysis (TGA):

On the measuring rod, the powdered sample of duckweed was placed, after being accurately weighed (initial mass = 6.2 mg). The decomposition temperature of the duckweed sample was determined using an ATG instrument, from 30°C to 900°C at a heating rate of 10°C min^{-1} under air.

- Characterization of the bioadsorbent by SEM and BET:

The surface morphology of the dried *Lemna gibba* powder sample was observed on a reference scanning electron microscope (SEM): (Scanning electron microscope Zeiss with EVO 15 kV). The quantitative analysis of the elemental composition was studied by energy dispersive X-ray spectroscopy (EDX). BET was achieved using the reference instrument (Micromeritics Instrument Corp. Gemini VII Version 3.04).

2.3 Adsorption process:

All manipulations were done using the stock solution of the dye's initial concentration 50 mg/L prepared in a 1L flask, in an Erlenmeyer flask, 0.25g of powder and 25 mL of dye are mixed. The adsorbent/adsorbate mixture is then agitated at room temperature (25°C) for 3 hours at a speed of 350 rpm in an orbital shaker (Edmund Buhler GmbH), finally the solution is filtered by a water pump using

a 0.45 µm membrane filter. We varied the mass of the bioadsorbent from 0.0625 g to 0.5 g to analyze the mass effect. We changed the time from 30 minutes to 360 minutes to evaluate the kinetic effect. We adjusted the solution with H_2SO_4 (0.1 M) or NaOH (0.1 M) by adjusting the pH from 3 to 11 in order to assess the impact of pH. Finally, we used dilution to evaluate the impact of dye concentration, adjusting the dosage from 10 to 100 mg/L.

Table2: Summary table of the evaluated effects, their varied and fixed parameters

Effects studied	Fixed parameters	Varied parameters
Mass effect	Initial concentration:50 mg/L Volume:25 mL Time :3h	Mass of adsorbent in g: 0.0625 ;0.125 ;0.1875 ;0.25 ;0.375 ;0.5
Kinetic effect	Initial concentration :50 mg/L Volume : 25 mL Mass : 0.25 g	Time in min : 30 ;60 ;90 ;120 ;180 ;210 ;360
pH effect	Initial concentration: 50 mg/L Volume: 25 mL Mass : 0.25 g Time : 3h	pH:3 ;5 ;7 ;9 ;11
Concentration effect	Volume : 25 mL Mass : 0.25 g Time : 3h	Solution concentration in mg/L : 10 ;15 ;25 ;50 ;100

2.4 Analysis method:

After filtering, UV/Visible spectrophotometric analysis (SP-UV1100 DLAB) was used to determine the impact of the various effects on adsorption at a wavelength of 605 nm. Thus the adsorption capacity (Qads) in (mg/g) and the removal rate (R%) are calculated according to the following equations **(Eq. 2)** and **(Eq.3)** (Laabd *et al*., 2015):

$$Qads = \frac{(c0-ce)\times V}{m} \qquad \textbf{(Eq. 2)}$$

$$R\ (\%) = \frac{(c0-ce)\times 100}{c0} \qquad \textbf{(Eq.3)}$$

With c_0: initial concentration in (mg/L)

Ce: equilibrium concentration in (mg/L)
m: mass of the powder in (g)
V: volume of the dye (mL)

3. Results and discussion

3.1 Bioadsorbent characterization by FTIR

The following functional groups were able to be identified by analysis of the infrared spectrum in **Figure 2** as being most likely to be in responsible of the adsorption:

-The broad band identified between 3700 and 3300 cm^{-1} may be due to the overlap between hydroxyl (-OH) and amino (-NH) groups.
-The minor peak at roughly 2900 cm^{-1} might reflect the hydrocarbon bonds' stretching vibration (C-H).
- the 3 peaks between 1700 cm^{-1} and 1400 cm^{-1} can be attributed to the (-CO/NHR) group, i.e. (C=O), (N-H) and (C-N).
-The peak identified at almost 1000 cm^{-1} is attributed to the single bond (C-O).
-The presence of starch may be indicated by peaks between 700 and 400 cm^{-1}.
These detected peaks in the region from 1000 cm^{-1} to 400 cm^{-1} are attributed to carbohydrate compounds, the other peaks in the band are attributed to amide.
These results support the analysis of several studies (Nasser *et al*., 2021; Lim *et al*., 2014; Yu *et al*., 2011), showing that these chemical groups are a part of the plant's structure and are in charge of creating the adsorption sites.

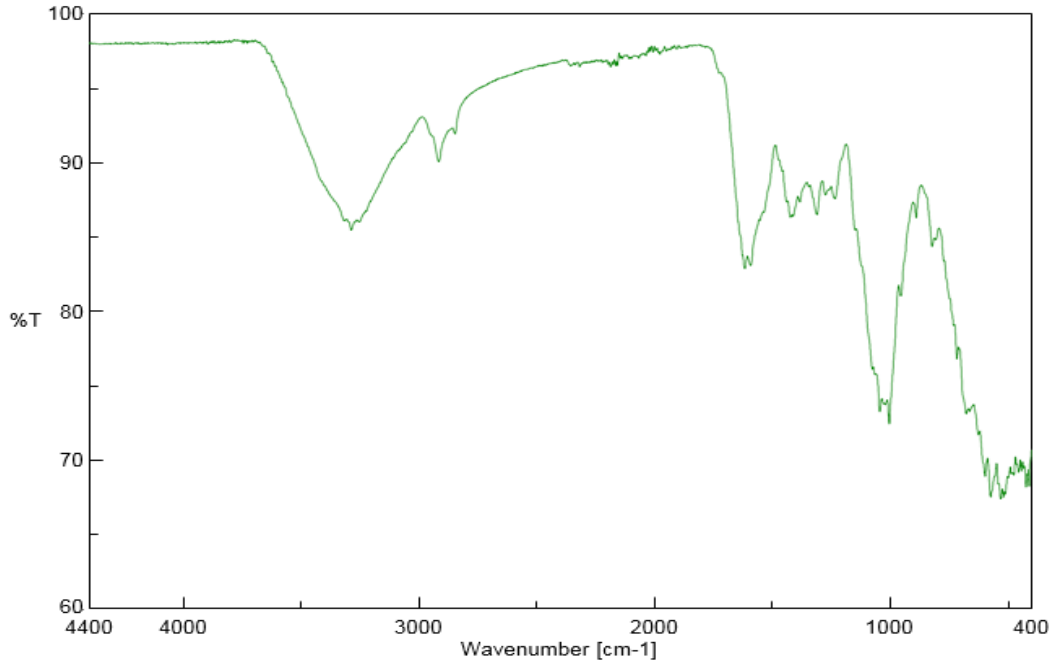


Figure 2: FT-IR band of *Lemna gibba*'s powder.

3.2 thermic degradation:

It is observed that at 250°C the sample has lost about 10% of its mass, this effect is due to the loss of water. The thermal stability, was around 206.6°C, but this value may not be accurate due to the gradual appearance of GTA resulting from impurities in the sample.
Between 250°C and 500°C, the sample has seen about 75% degradation, and this is the temperature at which pyrolysis of the duckweed proteins has been occurring.
The 20% of residue that remained above 750°C can be related to ash and solid carbon, which decompose at a higher temperature.

3.3 characterization of the bioadsorbent by SEM and BET:

- **Texture morphology:**

By using scanning electron microscopy (SEM) to examine powdered *Lemna gibba* duckweed, the surface of the powder can be viewed in great detail at a microscopic scale. SEM uses an electron beam to scan the surface of the sample and create a three-dimensional image of its topography.

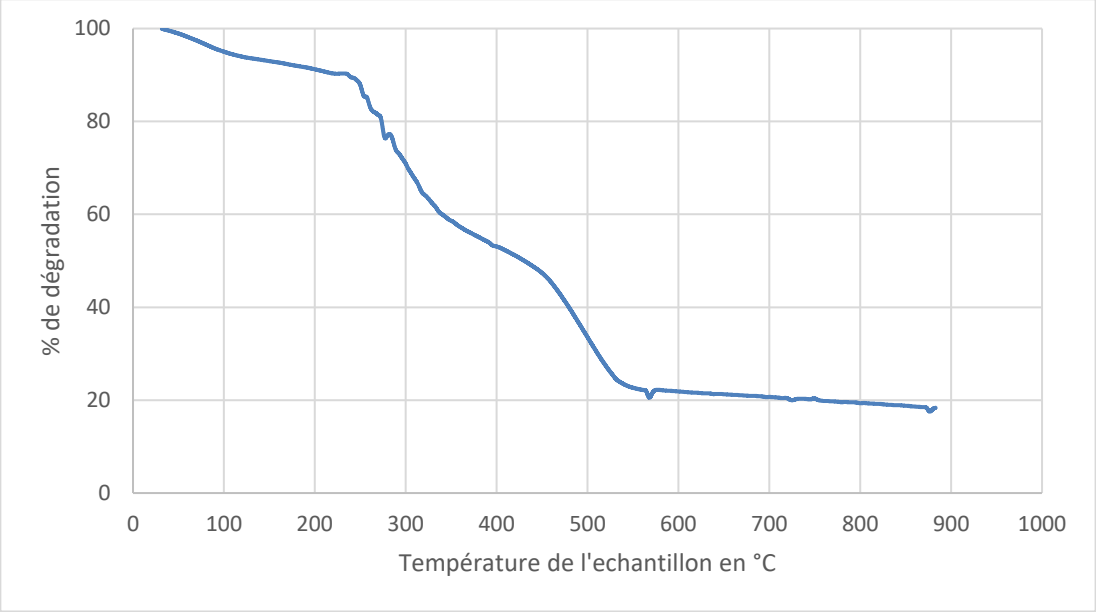


Figure 3: TGA curve of the *Lemna gibba*'s powder sample.

By examining *Lemna gibba* duckweed powder using this technique, one can gain insight into such characteristics as the size, shape, and structure of individual particles, as well as the organization and arrangement of the particles in the powder. This analysis could also reveal details about the surfaces and textures of the particles, as well as the interactions between the particles themselves or between the particles and their environment. EDs analysis **Figure 4** was used to determine the major constituents of the crude biosorbent prior to the adsorption of RB203 dye. As can be seen in **Figure 4**, the main constituents of the raw *Lemna gibba*'s powder sheets employed were only O, C, K, Mg, Na and Ca without reflecting the presence of any inorganic metals.

- **BET analysis:**

The BET method is used for the calculation of the specific surface. Starting from the basic assumptions of the BET theory, the BET equation is revealed as follows:

$$\frac{P/P_0}{Q\left(1-P/P_0\right)} = \frac{1}{Q_m C} + \frac{C-1}{Q_m C} \times P/P_0$$

With :Q = amount adsorbed at pressure P

Qm = amount of gas required to cover 1 g of adsorbent with a single layer of gas

c = BET constant defined as follows:

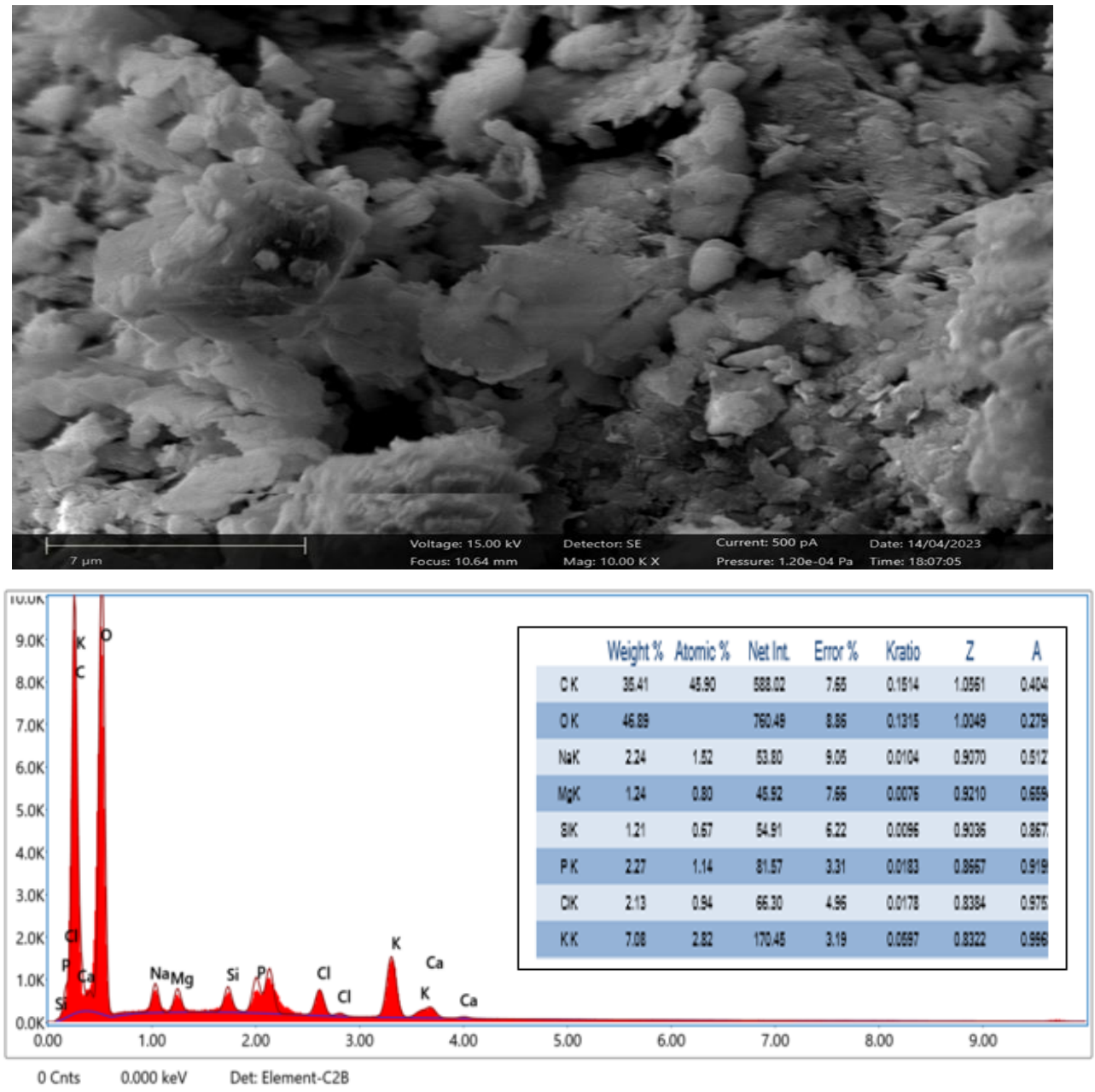
$$C = \exp[\,(\varepsilon_1 - \varepsilon_1)/RT]$$

With: ε_1 : Differential heat of adsorption of molecules on the surface of the solid L

ε_L : Latent heat of liquefaction of the vapour at the temperature considered, R: Constant of perfect gases and T: Absolute temperature (K).

The trace of $\frac{P/P_0}{Q(1-P/P_0)} = f(P/P_0)$ leads to an affine line , y = ax + b with slope a= $\frac{C-1}{Q_m C}$ and b=$\frac{1}{Q_m C}$

The values of Qm and c can be deduced: $Q_m = \frac{1}{(a+b)}$ et c = 1+ $\frac{a}{b}$



	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	A
C K	35.41	45.90	588.02	7.65	0.1514	1.0561	0.404
O K	46.89		760.48	8.86	0.1315	1.0049	0.279
NaK	2.24	1.52	53.80	9.05	0.0104	0.9070	0.512
MgK	1.24	0.80	45.52	7.66	0.0076	0.9210	0.659
SiK	1.21	0.67	54.91	6.22	0.0096	0.9036	0.857
P K	2.27	1.14	81.57	3.31	0.0183	0.8667	0.919
Cl K	2.13	0.94	66.30	4.96	0.0178	0.8384	0.975
K K	7.08	2.82	170.45	3.19	0.0597	0.8322	0.996

Figure 4: SEM and EDX micrograph of *Lemna gibba* 's powder.

The trace of the transform of the BET equation is shown in **Figure 5**. The resulting line with slopeα and y-intercept β leads to the determination of the constants Qm and c. The specific surface area is determined from the value of Qm by applying the following formula:

$$S_{BET} = Qm \,.\, N \,.\, \sigma$$

With: σ:Surf ace occupied by an adsorbate molecule and N: Avogadro number = $6.02.10^{23}$ mol^{-1}
In order to determine the specific surface area of a material, the use of nitrogen as an adsorbate remains the most commonly used with σ (N_2) = 0.162 nm^2. The BET equation remains valid for low relative pressures ($P/P_0 \leq 0.35$). Above this value, certain assumptions are no longer accepted. The specific surface of the sample *Lemna gibba*'s powder C gives a value of 0.2417 m^2/g.

Measuring the single point specific surface area of P/P0 = 0.301355860, which is 0.2138 m²/g, means that there is 0.2138 square metres of surface area available for gas adsorption per gram of adsorbent at this specific pressure. This may imply the presence of porous meso-structure of the material. A BET specific surface area of 0.2417 m²/g indicates that the total surface area available for gas adsorption is 0.2417 m² per gram of adsorbent. This value can be interpreted as the active surface of the adsorbent, which is in contact with the aqueous solution to achieve adsorption.

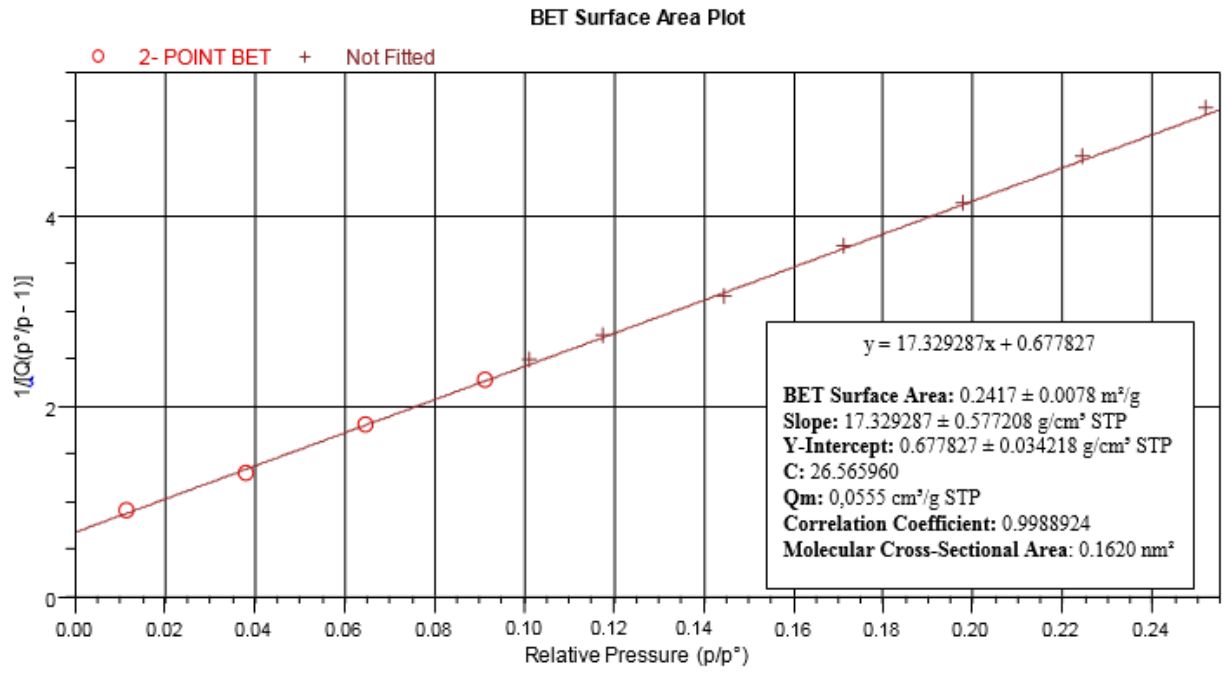


Figure 5: Straight line obtained from the transforms of the BET equation of *Lemna gibba*'s powder.

3.4 Effect of bioadsorbent dosage:

It is important to remember that the dosage of bioadsorbent is an essential factor in the adsorption process and depends on the available adsorbent sites. The effectiveness of the different doses of bioadsorbent was evaluated on the removal of CI Reactive Blue 203 dye at an initial concentration of 50 mg/L for 3 h as the optimum time. **Figure 6**'s results indicate that a 60% removal for a mass of 0.0625g of duckweed only, and it achieves 96% removal effectiveness for a mass of 0.5g of bioadsorbent, which explains the duckweed's inherent adsorption capacity even without modifying the sites (Qu *et al*., 2021).

3.5 Effect of the solution's pH:

Figure 7 illustrates that pH 3 relates to the best removal value of 90%, furthermore it has been found that lowering the pH from 3 to 11 slightly influences the amount of color removed, which drops to 80% at pH 11. This can be explained by the properties of the dye, which make adsorption more effective at acidic pH levels and slightly less effective at alkaline pH levels. This is mainly due to the presence of an excess of OH- ions from the hydrogen bond established with the water molecules in the solution competing with CI RB203 (Bagheri *et al*.,2018). These results, which reveal that a pH range of 3 to 9 is ideal for the absorption of IC RB203, are comparable with the adsorptive properties of duckweed in general and the genus Lemna in particular (Chen *et al*.,2015).

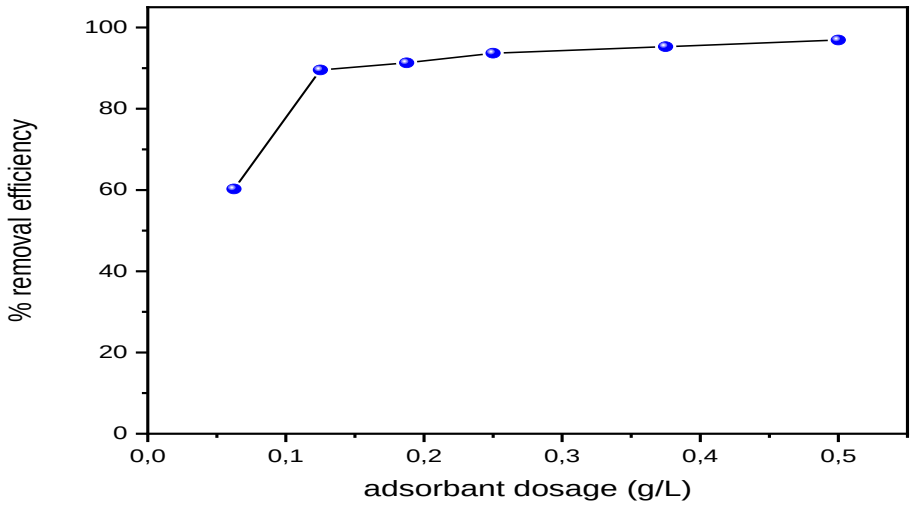


Figure 6: The effect of *Lemna gibba* bioadsorbent dosage on CI Reactive Blue 203 removal efficiency, Co = 50 mg /L, t=3h, V=25 mL, and T=25°C.

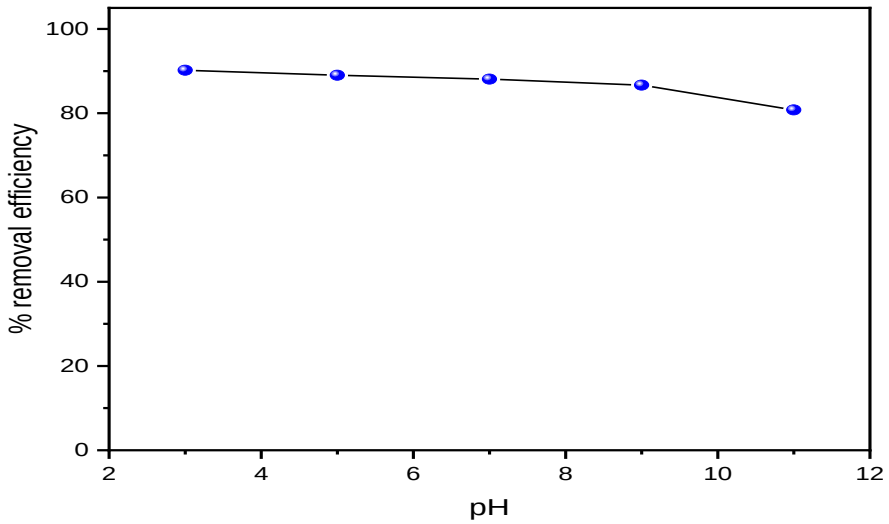


Figure 7: Effect of initial pH on the removal efficiency of CI RB203 from the bioadsorbent *Lemna gibba* (C0 = 50 mg/L, t = 3h, V = 25 ml, bioadsorbent mass = 0.25g, T = 25 ˚C).

3.6 Study of adsorption kinetics:

To determine the adsorption kinetics of the dye on the bioadsorbent the variation of the contact time was evaluated, the results shown in figure 5 are modelled from the pseudo first order **(Eq.4)** and pseudo second order **(Eq.5)** kinetic equations and the Elovich model **(Eq.6)** (Aboua *et al*., 2018) as follows:

$$\log(Qe - Qt) = \log(Qe - \frac{k1}{2{,}303}t) \quad \textbf{(Eq.4)}$$

Where: Qe and Qt the quantities of dye adsorbed at equilibrium and at time t respectively in (mg/g), k1 the pseudo-first order adsorption constant (in min^{-1}).

$$\frac{t}{Qt} = \frac{1}{h} + \frac{t}{Qe} \quad \textbf{(Eq.5)}$$

Qe and Qt are the quantities of dye adsorbed at equilibrium and at time t (mg/g), h is the initial adsorption rate which is defined by 2k2(Qe)2 (in mg/g/min) with k2 the adsorption rate constant (in g/mg/min).

$$Qt = \frac{1}{\beta}\ln(\alpha\beta t + 1) \qquad \textbf{(Eq.6)}$$

Where: Qt is the amount adsorbed at time t (mg/g) and α (mg/g/min) and β (g/mg) are the initial adsorption rate constant and desorption rate, respectively.

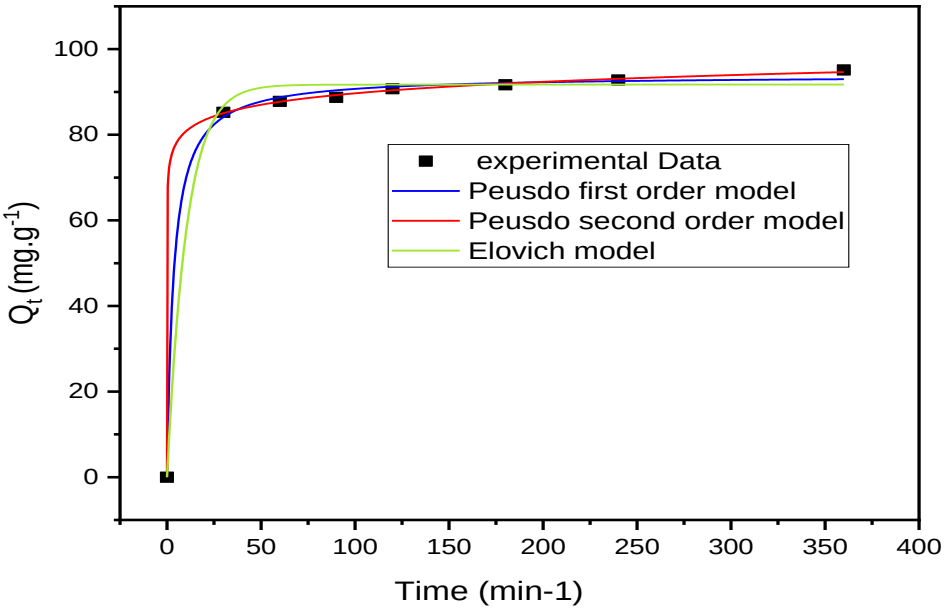


Figure 8: Non-linear fitting of kinetic models for the adsorption of the CI dye RB203 on the bioadsorbent *Lemna gibba* (Co = 50 mg/L, T = 25°C, V = 25 mL, mass of bioadsorbent = 0.25 g).

Figure 8's observations demonstrate that the dye quickly binds to the *Lemna gibba* plant during the first 30 minutes, which explains the plant's potential for adsorption, then, a plateau spread was seen at 180 min, indicating equilibrium (**Table 3**); this could be due to the dye molecules had occupied some adsorption sites; as a result, a decline in adsorption sites or the remaining color in the solution was seen about 360 min (Fayoud *et al.*, 2015).

From the parameters in **Table 3** and the non-linear fit of the kinetic model presented in **Figure 8** we can see that the adsorption process of the CI dye RB 203 is well described by the Elovich model (R^2 = 0.99987) and the pseudo-first-order model because (R^2 = 0.99984) as well as the value of Qe (exp) is very close to the Qe (cal) value, in contrast to the pseudo second-order kinetics which describes the bioadsorption of Co^{2+} on the same plant (Reyes Ledezma *et al.*, 2020).

3.7 Adsorption isotherm:

According to **Figure 9**, non-linear versions of the Langmuir, Freundlich, and Temkin isothermal models were used to confirm the results of dye fixation on the *Lemna gibba* plant. We were able to analyze the dye removal equilibrium data using these mathematical models by computing the maximum adsorption capacity and the adsorption parameters shown in **Table 4**.

Table 3: Parameters of the pseudo-first order, pseudo-second order and Elovich kinetic models.

Model	Parameters	value
Pseudo first order	Qe (exp) in (mg/g)	91.64
	Qe (cal) in (mg/g)	91.71
	K_1 in (min^{-1})	0.09671
	R^2	0.99984
Pseudo second order	Qe (exp) in (mg/g)	91.64
	Qe (cal) in (mg/g)	93.87
	K_2 in (g/mg/min)	0.00306
	R^2	0.99861
Elovich	α in (mg/g/min)	4.57
	β in (g/mg)	0.25
	R^2	0.99987

The Langmuir isotherm, which is represented by equation **(Eq.7)**, characterizes monolayer adsorption by assuming that the adsorption sites are uniform and limited, so that a saturation value is attained beyond which no more adsorption occurs (Langmuir *et al*., 1916).

$$Qe = \frac{Qm\ KL\ Ce}{1+kL\ Ce} \qquad \textbf{(Eq.7)}$$

Where: Qe (mg/g) is the adsorption capacity of the adsorbent at equilibrium
Qm (mg/g) is the maximum adsorption capacity
Ce (mg/L) is the concentration of the dye solution at equilibrium
KL (L/mg) is the Langmuir equilibrium adsorption constant related to the adsorption energy which quantitatively reflects the affinity between the adsorbent and the adsorbate.

The Freundlich isotherm assumes that the adsorption is multilayered and that the surface of the adsorbent is heterogeneous (Freundlich *et al*., 1906). It is expressed by the following relation **(Eq.8)**:

$$Qe = K_F\ Ce^{1/n} \qquad \textbf{(Eq.8)}$$

Where: KF (L/g) is the Freundlich constant related to the adsorption capacity
1/n is the adsorption intensity or surface heterogeneity indicating the relative energy distribution and heterogeneity of the adsorption sies.

The Temkin isotherm, on the other hand, states that the adsorbent surface is heterogeneous, and the heat of adsorption decreases linearly with the amount of adsorption, its non-linear form is described by equation **(Eq.9)**:

$$Qe = \frac{RT}{b} \ln KTCe \qquad \textbf{(Eq.9)}$$

Where: KT is the equilibrium binding constant (L/g),
b is the Temkin constant related to the heat of adsorption (J/mol),
R is the universal gas constant (8.314 J/mol/K) and T is the absolute temperature (K).

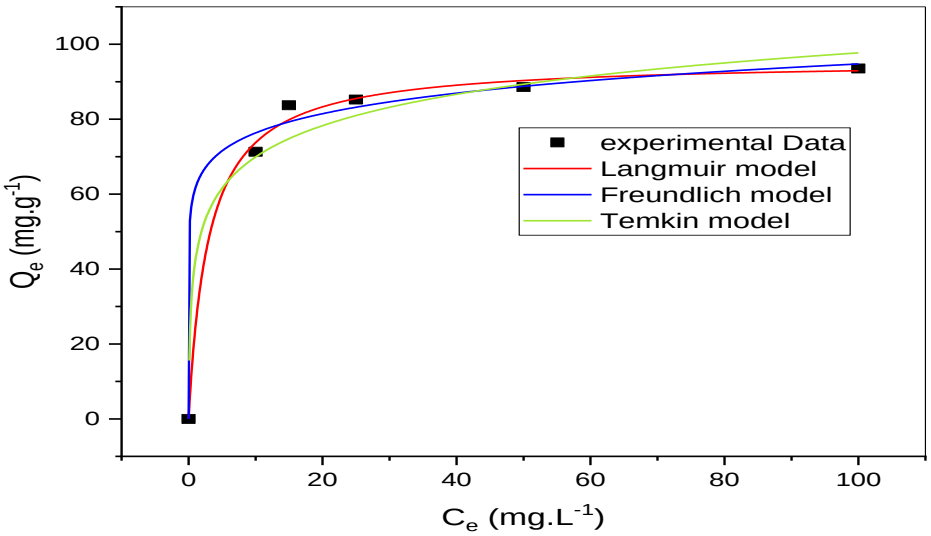


Figure 9: Non-linear fitting of the adsorption isotherms of the CI dye RB203 on the bioadsorbent *Lemna gibba* (T = 25°C, V = 25 mL, bioadsorbent mass = 0.25g).

The adsorption of RB203 IC on the powder *Lemna gibba* plant is better described by the Langmuir (R^2 = 0.996) and Freundlich (R^2 = 0.991) models than the Temkin (R^2 = 0.898) model, according to the data shown in **Figure 9** and **Table 4**, and by comparing the R^2 of the three models represented, but the maximum capacity of the sites is better described by the Langmuir model (Qm = 95.71 mg/g), which means that the adsorption sites are uniform and finite and still the adsorption is monolayer. This corresponds perfectly to the results obtained on Cd^{2+} adsorption by the genus *Lemna Aequinoctialis*. (Halaimi *et al.*, 2014).

Table 4: Langmuir, Freundlich and Temkin isotherm parameters.

isotherm	parameters	value
Langmuir	Qm (mg/g)	95.71
	KL (L/mg)	0.33
	R^2	0.996
Freundlich	KF (L/g)	61.50
	1/n	0.09
	R^2	0.991
Temkin	KT (L/g)	32.64
	b (J/mol)	0.26
	R^2	0.898

Conclusion

The adsorption of CI Reactive Blue 203 on the plant *Lemna gibba* in powder form was studied in batch mode and was evaluated by various parameters such as the influence of the pH of the solution on adsorption, the mass of the bioadsorbent, the concentration of the solution and the contact time. The study results showed that the adsorption isotherm is best described by the Langmuir model and that the

adsorption kinetics follows a pseudo-first order model. In addition, the results demonstrated that the percentage of decolorization reaches 90% at pH 3 of the solution and that the adsorption capacity reaches its maximum of 96% with only 0.5 g of bioadsorbent. The purifying ability of this plant is demonstrated once more by all these encouraging results, and it can also be employed as a low-cost, environmentally friendly plant biomaterial.

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