Sorption Capacity Measurement of Chlorella Vulgaris and Scenedesmus Acutus to Remove Chromium from Tannery Waste Water

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Abstract. Tanning process is a polluting activity due to the release of toxic agents into the environment. One of the most important of those toxic chemicals is chromium. Different alternatives have been proposed for the removal of this metal from tanning waste water which include the optimization of the productive processes, physicochemical and biochemical waste water treatment. In this study, the biological adsorption process of trivalent chromium was carried out in synthetic water and tannery waste water through two types of native green microalgae, called Chlorella vulgaris and Scenedesmus acutus in Free State and immobilized in PVA state. This, considering that cellular wall of microalgae has functional groups like amines and carboxyl that might bind with trivalent chromium. Statistical significance of variables as pH temperature, chromium and algae concentrations was evaluated just like biosorption capacity of different types of water and kind of bioadsorbent was calculated to determine if this process is a competitive solution comparing to other heavy metal removal processes.

1. Introduction
For the last years, the concern about the pollution of air, water and soil has become bigger due to the side effects that have been seen like climate change, the increase of human diseases and human poisoning with food or beverage. Heavy metal residues in water released by industries, are one of the most relevant pollutants. They may endanger ecosystems and public health because of their high mobility, their no degradability and their high ability to accumulate inside all living being and participate in biological reactions ruining vital processes inside cells, tissues and organs and promoting illness even when metals are in low concentration in environment. [1] Chromium is a heavy metal, which is used in industries of electroplating and tanneries. Those activities produce enriched effluents with trivalent chromium which could oxidize to hexavalent chromium that is more toxic. To avoid this health risk and preserve environment from metal pollution, industry discharges must be treated to separate them. For metals in aqueous solution there are several solutions to remove them such as precipitation, ion exchange or sorption. Those are physical chemical processes which are highly effective in metal removal from water but they are expensive due to their high demand of reagents and they might produce greater amount of other toxic products that must also be eliminated from treated water [1]-[3].
Alternatively, there are biological processes which use live or dead, free or immobilized cells of bacteria, fungi or algae and farming waste as sorbents, because cells have in their walls carbohydrates and polypeptides with hydroxyl, aldehydes, ketones, amines, phosphates and carboxyl groups that are responsible for the sorption and chelation of the metal cations. [4]-[6] The use of bio sorbents overcomes some of the disadvantages of the physicochemical processes and are useful at low concentrations [1], [2], [5], [7]. But many studies have been done using synthetic water done with deionized water, buffer solution and salt with the metal(s) of interest. The result of those studies were high loading capacity of specific metals. [1]-[3], [5], [7]-[10].

In spite of, real condition of waste water is more complex because of the great variability of water composition due to industrial processes. The appearance of many other organic or inorganic compounds, might affect the performance of bioprocess as Murphy exposed [11] Also, Han et al [12] used the green microalgae *Chlorella miniata* for the removal of hexavalent chromium and found that the presence of other ions affect the adsorption capacity of chromium.

For that reason, in this study it was necessary to understand the behavior of metal’s bio sorption in real waste water. That was done, by definition of relevant variables like pH, temperature, microalgae and chromium concentration, algae particle size or bio sorbent (considering the mass of algae and the carrier), and others, during bioprocess. Other aspect to know is the estimation of bio sorption capacity for each microalgae specie. In addition, some studies have consider the immobilization of bio sorbent in bigger structures constructed in natural or synthetic materials as polymers. The benefits of this procedure is the ease and economical recovery of employed biomass in bio sorption. Due to the greater size of the immobilized biomass, it would be necessary a simple mechanist to separate bio sorbents like sedimentation or filtration chambers. If biomass where free in the bio sorption process, it would be necessary to employ more complex and expensive mechanisms like ultrafiltration for example. However, there are some disadvantages like the loss of active side of biomass cell wall or drain of biomass cell from carrier material with the consequence of loss of bio sorption effectiveness [13]. In this study, a part of microalgae biomass was immobilized in a resistant material called Polyvinyl alcohol PVA and the bio sorption studies proposed for free microalgae where done for new bio sorbent material.

2. Materials and methods

2.1. Evaluation of tannery waste water treatment

Tannery waste water was taken from different tanneries inside Bogotá city and surrounding area. Waste water quality was determined by evaluation of several physical chemical factors that were recommended in literature [14]-[16]. Analysis was done following the Standard Methods for the Evaluation of Water and Wastewater APHA, AWWA and WEF at Environmental Engineering Laboratory at National University of Colombia [17]

2.2. Microalgae culture

The strains *C. vulgaris* and *S. acutus* were obtained from a natural water body in Bogotá. Each strain was isolated and grown in the Algae Cultivation Laboratory LAUN (maximum culture volume reached: 10 L) and Estación La Terraza (maximum culture volume reached: 1 m$^3$). Both laboratories of National University of Colombia. The following table present the culture conditions for each volume.

| Table 1: Culture parameters to produce microalgae biomass |
|---------------------------------|-----------------|----------------|
| **Culture variables**             | **Volume of 10 L** | **Volume of 1 m$^3$** |
| **Culture Media**           | **Liquid BBM** | **MK10-30-10 FERTITEC** |
|                              | [18]            | @**              |
| Temperature                   | $24 \pm 2 ^\circ C$ | $18 \pm 3 ^\circ C$ |


| Light source         | Artificial | Natural |
|---------------------|------------|---------|
| Culture period      | 8 d – 10 d | 8 d     |
| Illuminance         | 4500 ± 50 lux | ---    |
| Photoperiod         | 16 h with light | 12 h with light |
| CO₂ supply          | From pumped and filtered air (Millipore filter of 0.2 µm) |

* Volume of ten litters was used as a seed for 1 m³ volume of culture media.
** Massive biomass production was done with a liquid fertilizer called MK 10-30-10 FERTITEC ® (Tecnoquimicas SA). Biomass production results were very similar to those obtained using BBM medium, but at a fraction of the cost. [19]

2.3. Harvesting of microalgae
After biomass cultivation, each microalgae was harvested by centrifugation at speed of 4000 rpm for a period of 10-15 minutes. The recovered biomass was washed once with deionized water and separated again by centrifugation. The supernatant was removed and the biomass was dried at a temperature of 60 °C in ovens until a humidity value of 3% (established with a Sartorius MA 35 humidity balance). Dried biomass was manually macerated and separated by a 32 mesh Tyler screen (average particle size of 0.05 cm)

2.4. Microalgae immobilization
Immobilization of microalgae was made by encapsulation according to the methods described by Cheng and Houng [20], Pramanik and Khan [21]. With the result of previous experiments of chemical resistant and compression test, it was selected the following microalgae bead formulation: 15% ± 0.3 WW⁻¹ of PVA, 1% ± 0.1 WW⁻¹ of sodium alginate and 3.3% ± 0.2 WW⁻¹ of microalgae. PVA monomer and sodium alginate was dissolved in water at boiling point. Then, solution was cooled to 40 °C and microalgae powder (C. vulgaris or S. acutus separately) was dissolved in monomer solution to obtain a homogeneous dispersion. Solution of monomers and microalgae was fed in the form of dropwise in the saturated solution of boric acid and 2% WV⁻¹ of calcium chloride that was magnetically stirred at a speed of approximately 600-700 rpm during polymerization and immobilization process (calcium chloride destroys sodium alginate bonds and it could provide more porosity to PVA beads).

The feed rate was 5 drops per minute to prevent polymer agglomeration during formation of spheres. Immobilization time was 2 hours. Then, beads were collected and washed quickly to remove residual boric acid. The spheres were transferred to 2 M sodium orthophosphate solution for 5 hours (more than suggested by Cheng and Houng [20], Pramanik and Khan [21]) at room temperature with magnetic stirring plate to harden the bio sorption beads. Phosphorylated and hardened spheres were filtered, washed with water, allowed to room temperature to remove some of their moisture and then they were stored in a plastic container in the refrigerator at 4°C. (To evaluate if polymer beads would uptake chromium, the same immobilization process was done without algae). Structure of bio sorption beads was observed by electron microscopy SEM.

2.5. Bio sorption essays
The removal of chromium from synthetic water and tannery waste water with free an immobilized algae, would be affected by some variables. The choice of them was suggested by some authors. [5], [11], [22], [23] Two Experimental Response Surface design were selected. The rotatable Draper & Lin experimental design was applied for trivalent chromium removal in synthetic and tannery waste water and both free microalgae. Considering the results obtained in the experimental design of Draper & Lin, the factor(s) which did not have statistical significance was (were) eliminated from the following experimental design executed. Box Benhken experimental design was conducted for both species of immobilized microalgae and tannery waste water. Table 2 shows the statistical parameters for each experimental design.
Table 2: Statistical parameters applied on each experimental design.**

| Parameter              | Draper & Lin | Box Benhken |
|------------------------|--------------|-------------|
| Number of experiments  | 16           | 14          |
| Number of replicates   | 6*           | 6*          |
| Confidence level       | 95%          | 95%         |
| F value                | 3.53         | 3.02        |

* Replicates were done at central point of experimental design.
** The distribution of the levels and determination of ANOVA’s response surface was performed using Statgraphics Centurion XV® software (StatPoint Technologies, Warrenton, VA, USA).

The choice of the experimental design was performed under the criterion of the smallest number of experiments needed to obtain statistically reliable results. The relevant of selected factors was determined by ANOVA and the comparison between the amounts of chromium removed by both microalgae was studied using Student's T test and Fisher Test. Table 3 shows the selected factors and their value in which was executed both experimental design.

Table 3: Selected factors and their levels.

| Factor | Code | Levels          | -2 | -1 | 0  | 1  | 2  |
|--------|------|-----------------|----|----|----|----|----|
| T      | X₁  | 23.3            | 25 | 27.5| 30 | 31.3|
| pH     | X₂  | 3.6             | 4.0 | 4.5 | 5.0 | 5.3 |
| MC     | X₃  | 0.6             | 1.0 | 1.5 | 2.0 | 2.3 |
| CCS    | X₄  | 58.0            | 70  | 100 | 130 | 153 |
| CCW    | X₅  | 11.3            | 15  | 20  | 25  | 28.3|

* Where T is temperature (°C), MC is microalgae concentration (gL⁻¹), CCS (mgL⁻¹) and CCW (mgL⁻¹) is chromium concentration in synthetic water and tannery waste water respectively.
**This factor was proved only on Draper & Lin experimental design

Tannery waste water has trivalent chromium, thus synthetic water was prepared by reduction of hexavalent chromium from potassium dichromate K₂Cr₂O₇ with sulfuric acid and ethanol. The quantity of trivalent chromium in solution must reach a similar concentration according to the metal in tannery waste water. Reduction was verified by spectrophotometry measurement of hexavalent chromium [24], [25]. After precipitation and verification were completed, pH adjustment was done with solutions of sodium hydroxide and sulfuric acid 1M and 0.1M according to experimental designs. The supernatant with chromium was separated and its metal concentration was determined by Atomic Absorption Spectrometry AAS [17]. Then, dilutions were done to reach the metal concentration according to experimental designs. For tannery waste water, a volume of 500 ml was treated first by precipitation and determining the concentration of trivalent chromium was done in the same way as for synthetic water.

Experimental designs were done in 100 ml conical flasks with a volume of 30 ml of each solution, for a time of 24 hours and stirring of 120 rpm at different temperature (see Table 3) y using the Heidolph incubator shaker. For pH adjustment it was seen that after washing the microalgae, this retained traces of salts of the nutritive medium that are basic in nature so the microalgae increases the pH of the essay solution, which rather favors precipitation than bio sorption. The maintenance of the pH to the desired values was done using buffer solution of citric acid - disodium phosphate.

The amount of microalgae that should be added to the solution of 30 ml in each flask to meet the predefined concentration in the experimental design was recommended by different authors [21]-[23], [26]. It was calculated and weighted by a four-decimal digit balance Mettler Toledo brand. For essays
of chromium removal by immobilized microalgae, the following calculation was performed to obtain the approximate weight of PVA – Microalgae beads to assure the microalgae quantity according to experimental designs:

\[
\frac{X \text{ g free microalgae}}{L \text{ solution}} \times \frac{100 \text{ g polymer solution}}{3.3 \text{ g microalgae}} \times \frac{0.03 \text{ L solution}}{}
\]

Where X is the amount of free microalgae required in Box Behnken experimental design. Besides biosorption essays, test was done with the negative control (essays with no microalgae and essays with white polymer under the same experimental conditions to evaluate the effect of precipitation and adsorption of chromium by the polymer used in the immobilization of microalgae).

2.6. Sorption capability measurement

In clean and metal free metal flasks of 100 mL, six solutions with different chromium concentration in synthetic water (0 mgL\(^{-1}\); 32.1 ± 0.3 mgL\(^{-1}\); 74.2 ± 0.4 mgL\(^{-1}\); 110.2 ± 0.4 mgL\(^{-1}\); 154.4 ± 0.2 mgL\(^{-1}\); 200 ± 0.2 mgL\(^{-1}\)), citric acid - disodium phosphate buffer solution and 2.34 gL\(^{-1}\) and 1.5 gL\(^{-1}\) of free C. vulgaris and S. acutus respectively (according to optimal factor values obtained as results in experimental designs) were prepared. Biosorption essays were executed in Heidolph shaker at 120 rpm and at temperature where maximum chromium removal was presented for each microalgae, and for 24 hours.

Chromium concentration for bio sorption essays in tannery waste water were: 0 mgL\(^{-1}\); 20.1 ± 0.1 mgL\(^{-1}\); 40.2 ± 0.4 mgL\(^{-1}\); 60.2 ± 0.3 mgL\(^{-1}\); 80.3 ± 0.3 mgL\(^{-1}\) and 100.4 ± 0.3 mgL\(^{-1}\). Solutions were prepared with pH adjustment and dilution with deionized water. Concentration of free microalgae used for the residual water tests was 2.34 gL\(^{-1}\) of C. vulgaris and 2.0 gL\(^{-1}\) of S. acutus. The amount of immobile biomass used for the residual water tests was 40 gL\(^{-1}\) of immobile C. vulgaris and 46.8 gL\(^{-1}\) of immobile S. acutus. Stirring, bio sorption temperature and bio sorption time was the same as for synthetic water.

3. Results and discussion

3.1. Observed structure of bio sorption beads

The Figure 1 shows the observed structure of a blank bead. In this, it is seen a cavernous structure in the middle of beads. But outside, it is seen a smooth surface (Part B). Tiny pore may drive chromium solution inside bead. Because of the size of these pores, diffusion possibly is the controlling step.

Figure 2 shows a polymer – microalgae bead. Also in this bead, a cavernous structure is in the middle, but is bigger comparing to the blank bead (Part B) and it could be seen heterogeneous surface and black points which could be the pores of the bead. C and D present the microalgae distribution inside the bead. Some of them are available to be in contact with chromium solutions, but some of them might be totally or partially covered by PVA polymer.

3.2. Evaluation of bio sorption performance

From tannery waste water quality, it was seen that chromium concentration was from 3000 mgL\(^{-1}\) to 3200 mgL\(^{-1}\). After precipitation and pH adjustment it was seen that chromium concentration in synthetic water was higher although the initial chromium concentration was similar to tannery waste water. That is the reason for the evaluation of higher chromium concentration in the Draper and Lin experimental design.

Although higher concentration of chromium in synthetic water, it was seen that higher chromium removal percentages was reached by sorption for each free microalgae in synthetic water in comparison with bio sorption of chromium by free microalgae in tannery waste water. This might be because of the presence of many other compounds in tannery waste water that compete with chromium for active site of each microalgae as Murphy and Hang suggested [11] [12]. This is not the case in synthetic water which has less compounds. According to the comparison between chromium removal by free and immobilized microalgae in tannery waste water, it is seen that there is a
significant difference in bio sorption percentages. This can be explained by a decrease in cell wall activity after immobilization. This would happen when immobilization process is so harsh that could break cell wall or cover surface sites during the crosslinking procedure as Wijffels exposed [13].

Figure 1: Structure of blank bead obtained by SEM

Figure 2: Structure of polymer - microalgae bead obtained by SEM

Table 4 shows the optimal values of factors and the chromium removal percentage. It shows that C. vulgaris remove more chromium in synthetic water than S. acutus. However with tannery wastewater, free and immobile S. acutus made a higher chromium adsorption than C. vulgaris. To evaluate this difference in removal response between the two microalgae, there were performed the T-Student and Fisher tests. These tests assume that population means and variances are equal.

| Operative parameters | Optimal values |
|---------------------|----------------|
|                     | Free microalgae | Immobilized microalgae |
| Synthetic water     | C.v            | S.a                |
| Tannery waste water | C.v            | S.a                |
| T (°C) | 27.5 | 27.5 | 27.5 | 25  | 27.5 | 25  |
|-------|------|------|------|-----|------|-----|
| pH    | 4.5  | 5.34 | 4.5  | 4.0 | 4.0  | 4.5 |
| MC (g L⁻¹) | 2.34 | 1.5  | 2.34 | 2.0 | 2.0  | 2.34|
| CC (mg L⁻¹) | 93.9 | 93.9 | 20   | 25  | 15   | 20  |
| Stirring (rpm) | 120  | 120  | 120  | 120 | 120  | 120 |
| Max. removal % | 88.2 | 87.1 | 61.7 | 64.1| 32.6 | 35.3|

*Where T is temperature (°C), MC is microalgae concentration, CC is chromium concentration.
*C.v is *C. vulgaris* microalgae and S.a is *S. acutus microalgae*

### Table 5: Obtained values of t-student and fisher tests.

| Removal system* | Fisher test for equal variances | t Student test for equal simple means | standardized kurtosis** |
|-----------------|---------------------------------|--------------------------------------|------------------------|
|                 | F value | P value | T value | P value | C.v | S.a | C.v |
| FM – SW         | 0.85    | 0.71    | 1.92    | 0.06    | 1.164 | -   | -   |
|                 | 3       | 9       | 6       | 1       | 1.609 | -   | -   |
| FM – TW         | 0.78    | 0.58    | 0.28    | 0.77    | 1.155 | -   | -   |
|                 | 7       | 7       | 8       | 5       | 1.067 | -   | -   |
| IM – TW         | 1.24    | 0.64    | 0.77    | 0.44    | 0.124 | -   | -   |
|                 | 2       | 1       | 1       | 5       | 0.953 | -   | -   |

*Chromium removal was done by FM-SW free microalgae in synthetic water, FM-TW free microalgae in Tannery waste water and IM-TW immobilized microalgae in Tannery waste water.

**C.v is *Chlorella vulgaris* and S.a is *Scenedesmus acutus microalgae*

Population variance for the different type of bio sorption (with free or immobilized biomass and synthetic or tannery waste water) is equal because the relation between variances were less than the critical F value (For FM-SW and FM-TW = 4.35 for IM-TW = 4.41). Also P significant level (α = 0.05) shows higher values that validate null hypothesis. Thus, comparison between population means was done. In all cases is greater than the critical t value (for FM-SW and FM-TW: 2.086; for IM-TW is 2,101). The T value in all cases is lower and the P value for the t test is always higher than the level of significance. Thus, for the three chromium removal systems, null hypothesis is approved. There is no statistical evidence that any of the two species of microalgae as chromium biosorbents is more effective and can be used both without preference (See values in Table 5).

### 3.3. Factors affecting bio sorption analysis

Significance of chromium bio sorption variables were evaluated by Statgraphics Centurion XV® software (StatPoint Technologies, Warrenton, VA, USA). Figure 3 and Figure 4 show four diagrams of studied factors which may interact and affect bio sorption process of chromium in synthetic water and tannery waste water for both free microalgae. The vertical line represents the significance level of 5% and the bars which exceed the line have statistically relevance.
*Factor A: Temperature °C. Factor B: pH, Factor C: Concentration of biomass, Factor D: Concentration of chromium. Other bars are combined effects that may affect chromium bio sorption process.

Figure 3: Factor´s behavior in chromium bio sorption in synthetic water

Figure 4: Factor´s behavior in chromium bio sorption in tannery waste water
Therefore, those diagrams express that pH, chromium and microalgae concentration are important factors in bio sorption process in synthetic water and tannery waste water. Concentration of microalgae (both species) and pH have positive effects because when they increase, higher amount of chromium is adsorbed in synthetic water as Onyancha had explained before [23]. But in Tannery waste water, pH has a negative effect on the chromium removal. This ambiguous behavior might be described for the ionic charge of the active sites on the surface of the microalgae due to pH changes. That can attract or repel the metals in solution. At low pH values, carboxyl, hydroxyl and sulfonates groups, are deprotonated and thereby attracts the metal cations as it was explained by different authors [5], [11], [26], [27]. However, the increase of pH increases the importance of precipitation of metal which reduces the amount of chromium which adheres to the microalgae. Besides, it may rise the amount of sodium and other compounds which are soluble a higher pH and that increases competition for active side in cell wall. The chromium concentration has a negative effect in bio sorption process because when it increase, less amount of chromium is adsorbed, despite the increasing of the driving force [28], [29] which may lead the mass transfer and favors bio sorption only a fraction of them is adsorbed and the rest can repel each other when approaching the active sites.

The temperature in no one of the Draper and Lin experimental designs presented significant effect according to ANOVA analysis. However, the temperature has a negative effect on the chromium removal in synthetic water for both microalgae, while the temperature effect is opposite in chromium removal in tannery waste water. This is due to the increase on the metal diffusivity in the solution and this effect improves the chromium bio sorption if the controlling step is the diffusion [30]. But, up to the 27 °C (25 °C for removal of chromium from waste water by S. acutus), the reduction in adsorption capacity can be caused by a possible tendency of chromium to escape to the aqueous phase, disabling or damaging the active sites of the bio sorbent by breaking bonds discussed before by Sari and Tuzen [9]. Removal of chromium from synthetic water and tannery wastewater by both microalgae had other significant effects. The double effect of temperature (AA), is negative and is the most significant among the effects evaluated in the experimental design. It indicates that the breaking of bonds between chromium and the active site because of the increase in temperature is much more important than the increase in the diffusivity of chromium in the solution.

The dual effect of pH (BB) is negative due to the formation of chromium hydroxide Cr(OH)3 precipitates and cannot be adsorbed by the microalgae. This effect, decrease the concentration of chromium in the solution and let other free and dissolved ions to be adsorbed like sodium, potassium and magnesium which are found in higher proportion in the solution. Consequently those metals can be more likely to the microalgae active sites than chromium as Murphy explained [11]. The dual effect of pH is important in synthetic water because of precipitation but less for metal competition. For both species of microalgae and in chromium removal from tannery wastewater by S. acutus as shown in Figure 3 and Figure 4, while for C. vulgaris is not significantly important, possibly for a greater selectivity of the functional groups at chromium ions compared to S. acutus.

Other important factors in the removal of chromium from synthetic water and tannery wastewater for both microalgae were the combined factors. Combined factor of temperature-pH (AB) may be explained because with the temperature increase and pH values under 6, the hydrogen ions are easily detached from the functional groups of the surface and allow the microalgae to adsorb chromium ions. More microalgae concentration (CC) might lead to higher bio sorption because there is more active sites available. But it also could lead to minor removal of chromium if electrostatic interactions in cells that make them agglomerate with the loosening of bio sorption capability. This behavior was seen by Haeng et al and Vaghetto et al [5], [10] if chromium concentration (DD) upraise, the ratio between chromium and other cations also do, upholding chromium bio sorption. Thereby there is not enough adsorption site which could lead less adsorption capacity.

The temperature and concentration of microalgae effect (AC) for C. vulgaris is significant negative on the chromium removal in tannery wastewater, possibly because it stimulates electrostatic interactions between the functional groups of the microalgae. Likewise, with temperature rising, the chromium and compounds solubility and diffusion increase and that promote the rise in repulsion between ions. This hinders the arrival of chromium to the active sites on the surface of the microalgae. The effect between
pH and chromium concentration (CD) is explained by the proportional increase of other ions with increasing chromium concentrations; however chromium decreases by precipitation with the increasing of pH and lower amount of chromium that adheres to the microalgae.

Figure 3 and Figure 4 show the positive and negative effect of each principal factor evaluated in Draper and Lin experimental design. Bio sorption by free microalgae in synthetic and tannery waste water temperature has reached a maximum value where percentages of bio sorption is the highest.

![Figure 3 and Figure 4](image)

**Figure 5:** pH. Factor B: Microalgae concentration, Factor C: Chromium concentration.

Microalgae concentration has always a positive effect while chromium concentration has negative effect. The ambiguous behavior has seen clearly in this diagrams.

The Figure 5 shows for both immobilized microalgae in polyvinyl alcohol 15% + 1% alginate, that the three principal factors are significantly important. In the case of chromium removal from wastewater and immobile C. vulgaris, the most important variable is the concentration of chromium and this has a negative effect on the bio sorption, while microalgae concentration has a positive effect and this factor is the most important variable in the removal of chromium from wastewater by immobilized S. acutus.

By elimination of temperature as a variable, it also eliminate the importance of combined factors associated to temperature that explained most of the variation in the chromium removal by free algae. This consideration would be important because bio sorption statistical model would not represent the exact behavior. The twin factors of pH (AA) concentration of microalgae (BB), chromium concentration (CC) and pH - concentration of chromium explained before, also appear as statistically significant variables in the biological process of adsorption. Other combined factors are not statistically significant.

From essays of chromium bio sorption in tannery waste water by both immobilized algae, the twice pH factor (AA) has always positive effect, because the increase in pH promotes the deprotonation of the functional groups of microalgae and makes them very reactive and selective to heavy metal but environmental conditions for immobilized microalgae are milder than for free biomass. Therefore the basic precipitation and the presence of other cations have important effect, but functional groups of cell wall would maintain deprotonated, for that bio sorption process would be feasible. For immobilized C. vulgaris, the increased chromium concentration has a positive effect, it would be explained partially by the increase of the driving force and the bio sorption of chromium. But, for S. acutus, the increase in the concentration of chromium is negative due to the presence of functional
groups that are more selective to other ions presented in the waste water. This increase of the combined driving force with the deprotonation of the active site explains the positive effect of the combined factors of pH - chromium concentration (AC). A high microalgae concentration has a positive effect on the adsorption of chromium, because immobilized microalgae cells does not present the same effect of agglomeration by electrostatic effects as with free microalgae.

3.4. Chromium bio sorption capability determination
The following Figure 6 and Figure 7 shows isotherms for chromium removal from synthetic and tannery waste water with free and immobilized microalgae. To determine the type of adsorption of chromium by biomass, the evaluation of several models presented in the literature as Freundlich, Langmuir, Flory-Huggins, Dubinin - Radushkevich and Temkin was done [2], [5], [23], [31]-[33]. Table 6 presents the summary of models that best fit the experimental behavior of the bio sorption in synthetic water by both microalgae. The regression of the parameters, the coefficients of determination and the sum of squares (mse) for free microalgae and synthetic water are presented. According to Table 6 Freundlich and Dubinin - Radushkevich fitted with a correlation coefficient close to or greater than 90% and a sum of squares smaller than 0.1 Freundlich model is the one that best fits the experimental data. This model assumes that the surface is heterogeneous [31] and that the affinity and the energy of the active sites is different.

![Figure 6: Chromium bio sorption isotherms in synthetic water](image)

**Table 6: Isoterm models to determine bio sorption capability in synthetic water**

| Model* | Unit  | C. vulgaris | S. acutus |
|--------|-------|-------------|-----------|
| FM     | KF L g⁻¹ | 0.973 ± 0.281 | 1.173 ± 0.227 |
| R2     |       | 1.534 ± 0.876 | 2.073 ± 0.718 |
| mse    |       | 0.9594      | 0.9653     |
| n      |       | 0.0033      | 0.0027     |
| DRM    | QD mg g⁻¹ | 57.005 ± 5,513 | 68.851 ± 8,638 |
| KD     | mol2J⁻² | 6,30E-6 ± 3,68E-6 | 4,66E-6 ± 3,70E-7 |
| E      | J mol⁻¹ | 281.73 ± 81,536 | 327.67 ± 12,104 |
| R2     |       | 0.830       | 0.913      |
| mse    |       | 0.0725      | 0.0357     |
*MF is Freundlich model and DRM is Dubinin – Radushkevich model.

The values of the constant adsorption intensity n for *C. vulgaris* and *S. acutus* are close to one therefore it can be said that the adsorption tends to be linear and the effect of affinity for the solvent is not important on the adsorption of chromium. [2], [34]. For *C. vulgaris*, the value of n less than one. It indicates that adsorption may be chemical, whereas for *S. acutus*, the value of n is greater than one and therefore the adsorption of chromium might be physical according to Nemr Et al [35] and based on the work of Crini et al. [36] The adsorption capacity of Freundlich KF for *C. vulgaris* and *S.acutus* is 1.534 ± 0.876 Lg⁻¹ and 2.073 ± 0.718 Lg⁻¹ respectively and this indicates that *S. acutus* can adsorb 1.35 times more chromium than *C. vulgaris*.

*Figure 7:* Chromium bio sorption isotherms in tannery waste water by free microalga (a) and immobilied microalgae (b)
Where $q_{i}$; the maximum metal adsorption, adsorption capability, KF$_i$, mean adsorption intensity $n$, adsorption constant $b_i$, for each metal in aqueous solution and sum of concentration and affinities of other metals $S$.

Table 7 shows the isotherms models which better describe bio sorption behavior. The models of Langmuir and Freundlich presented a high coefficient of correlation for $S$. acutus and only Freundlich for $C$. vulgaris, the adsorption of chromium on the surface of the microalgae is a combination of both processes. This implies same affinity for the chromium in certain places of the surface of the microalgae although of the repulsion that can generate cations in solution [35], [36].

| Parameter | Free algae | Immobilized algae |
|-----------|------------|-------------------|
|           | $C$. vulgaris | $S$. acutus | $C$. vulgaris | $S$. acutus |
| n         | 2,311 ± 0,54  | 2,083 ± 0,37  | 3,127         | 3,129        |
| KF        | 1,214 ± 0,73  | 1,258 ± 0,59  | 0,2432        | 0,2781       |
| R$^2$     | 0,9001        | 0,9513        | 0,9089        | 0,835        |
| mse       | 0,0021        | 0,0012        | 6,07E-4       | 0,0014       |
| Q$_{max}$ | 14,78 ± 3,64  | 20,08 ± 7,42  | 0,1782        | 0,240        |
| b         | 0,029 ± 0,02  | 0,027 ± 0,01  | 0,067         | 0,071        |
| RL        | 0,21-0,57     | 0,11-0,39     | 0,10–0,36     | 0,09 – 0,33  |
| R2        | 0,8429        | 0,9097        | 0,9689        | 0,9385       |
| mse       | 2,812         | 1,117         | 0,00457       | 0,0093       |
| A         | 0,462 ± 0,02  | 0,385 ± 0,06  | 0,8135        | 0,9043       |
| b         | 911,52 ± 100  | 662,094 ± 240 | 6,77E4        | 5,11E4       |
| R$^2$     | 0,831         | 0,8842        | 0,941         | 0,8793       |
| mse       | 0,801         | 0,9600        | 2,65E-5       | 1,14E-4      |

For that reason more chromium can be adsorbed in the vacant active sites generating a chromium monolayer. In other places on the surface it is not like that, the adsorption is physical and favorable as predicted by the constant of adsorption intensity n but there were other compounds that can compete with chromium for active sites on the surface of microalgae.

Thus, the effect of the affinity of the solvent should be considerable and n value should be less than one [2], [34]. The higher affinity sites are occupied first and as the surface is saturated with metal and other ions, after that the surface of the microalgae reduces the adsorption energy and its affinity until it stops adsorbing more chromium as it happens on the heterogeneous surface [2], [31]. These models have adequately represented the chromium adsorption model by both microalgae, however, these models have not taken into account the effect of the presence of the other ions present in the wastewater. The multicomponent Langmuir model presents the same adjustment as the simple Langmuir model together with the maximum Q$_{max}$ adsorption capacity.
The combined model of Langmuir - Freundlich multicomponent has also presented a good fit and the value of n, indicates that the adsorption is physical and favorable [34]. However the values of the maximum capacity of adsorption are higher than those predicted for adsorption of chromium in synthetic water for which these models do not represent correctly the reality in spite of its good adjustment. The Temkin model presents a coefficient of determination close to 90%. This might validate some of the assumptions of the Langmuir model however the decrease of the power of the adsorption no other models can predict it like this model The adsorption energies are 0.911 ± 0.10 KJmol⁻¹ and 0.662 ± 0.24 KJmol⁻¹, which are low and the prediction that the interaction between the chromium and the cell surface is physical as assumed by Freundlich model [34].

A difference in chromium bio sorption between synthetic water and tannery waste water is that models of Flory Huggins and Dubinin - Radushkevich presented high coefficients of determination. Even the model of Dubinin - Radushkevich is the model that better adjusts in comparison with the others and indicate that the chromium adsorption chromium is carried out on a heterogeneous surface in the pores of the bio sorbent [2], [31], [35]. This is partially true for microalgae immobilized in PVA beads. Flory Huggins model has a high coefficient of determination. However, this model must be discard since the calculation of the free power of the adsorption presents a positive value, therefore the adsorption does not occur spontaneously and is not feasible.

4. Conclusion

Statistical experimental design allowed to identify that pH, microalgae and chromium concentration had statistical importance in the bio sorption of chromium by microalgae in contrast to temperature, but combined factors of last variable were important. For that temperature must be taken in count in the bio sorption process. The experimental design showed that the major chromium removal was done in synthetic water with a percentage of 88,2% with C. vulgaris as a sorbent at a pH of 4,5, 2,34 g/L of microalgae, temperature of 27°C and a chromium concentration of 20 mg/L, while for the bio sorption by S. acutus was 87,1% and a pH of 5,34, 2,0 g/L of microalgae, temperature of 27°C and a chromium concentration of 20 mg/L.

The removal of chromium of tannery waste water by the microalgae was reduced to 30,01% for C. vulgaris and 26,41% for S. acutus. The explanation of this efficiency drop was a major interaction between functional groups of the microalgae and other compounds present in wastewater which are competitor to the chromium removal. The removal of chromium from tannery wastewater comparing free microalgae and immobilized microalgae is reduced by 57% for C. vulgaris and 50% for s. acutus. This may be explained by the cover and destruction of a part of the active sites of the microalgae surface because of the process immobilization. Isotherm models which explained bio sorption of chromium for both type of microalgae in synthetic were Freundlich model and Dubinin - Radushkevich model, but it changed in tannery waste water where it was found that chemical bonds were weak and deactivation of free microalgae would occur.

5. References

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