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Removal of aniline blue dye using live microalgae *Chlorella vulgaris*

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Resumen

En este trabajo se evaluó la eficiencia de la microalga *Chlorella vulgaris* en la remoción del colorante azul de anilina, uno de los colorantes de mayor demanda en la industria de curtiduría; evaluando el efecto de la concentración inicial de 25, 50, 75 y 100 mg/L de colorante sobre el porcentaje de remoción de Azul de Anilina. La remoción del colorante se evaluó midiendo la absorbancia en un espectrofotómetro UV-VIS de las soluciones decoloradas hasta completar 11 días de tratamiento. Todos los tratamientos presentaron remoción de colorante. Mediante análisis ANOVA se demostró que la remoción del colorante depende de la concentración inicial y el tiempo de remoción; los mejores resultados en los 11 días de tratamiento se obtuvieron a una concentración inicial de 75 mg/L de azul de anilina.

Palabras clave: Biosorción, Azul de Anilina, microalga C*hlorella v*.

Abstract

In this work, the efficiency of the microalgae *Chlorella vulgaris* was evaluated in the removal of aniline blue dye, one of the most demanded dyes in the tanning industry; evaluating the effect of the initial concentration of 25, 50, 75 and 100 mg/L of dye on the percentage of removal of aniline blue. The removal of the dye was evaluated by measuring the absorbance in a UV-VIS spectrophotometer of the bleached solutions until 11 days of treatment were completed. All treatments presented dye removal. By means of ANOVA analysis it was demonstrated that the removal of the dye depends on the initial concentration and the removal time; the best results in the 11 days of treatment were obtained at an initial concentration of 75 mg/L of aniline blue.

Keywords: Biosorption, Aniline Blue, microalgae *Chlorella v*.

1. Introduction

Synthetic dyes are water-soluble organic compounds that are complex in nature due to the presence of aromatic molecular structures that make them much more stable to light, heat and oxidizing agents, being difficult to be removed from wastewater from various industries such as textiles, tannery, paper, printing and dyeing, food and cosmetics. The effluents from the tanning industry constitute one of the main problems to be treated due to the fact that they are characterized not only by their high chemical and biological demands for oxygen, suspended solids and content in toxic compounds; but also by color, so that the inadequate treatment or elimination of dyes can constitute a potential environmental impact for ecosystems significantly affecting photosynthetic activity in aquatic life, reducing light penetration and also being toxic to aquatic life due to the presence of aromatic compounds, metals, chlorides, etc., in them (Aksu and Tezer, 2005).

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Several physical and chemical methods such as electro-flocculation, membrane filtration, electro-kinetic coagulation, electro-chemical destruction and ion exchange are already known for the discoloration of dye wastewater, but the main disadvantage of these methods includes a high operational cost, limited versatility and less adaptability to a wide range of dye wastewater (Pathak, 2015; Kousha, 2013).

It has been shown that adsorption is the most promising option for non-biodegradable dyes for the removal of aqueous streams, with activated carbons being the most common adsorbent for this process due to their effectiveness and versatility, but it is quite expensive and the higher quality and costs. Several researchers have explored the application of low-cost adsorbents (peat, bentonite, Chinese clay, corn cob, wood chips and silica) to remove color, but failed to achieve high adsorption capacity (Pathak, 2015). This has led to the search for technologies that are efficient, economical and environmentally friendly. In recent years, several studies have focused on the biomass of some agricultural residues, bacteria, fungi, algae (Kousha, 2013).

Microalgae technology has proved to be a promising field in the treatment of dye effluents, being very low cost and having a high adsorption efficiency due to its large surface area allowing it to remove various types of contaminants from solutions. The use of microalgae for adsorption has been studied by several researchers, e.g. Mohan et al. (2002), investigated green algae belonging to *Spirogyra* species as viable biomaterials for the biological treatment of synthetic simulated azo dye effluents (Venkata et al, 2002). Edgardo R et al. (2012), studied the effect of the biomass amount and the presence of nutrients on the removal capacity of methylene blue dye in aqueous solution using the microalgae *Chlorella sp.* viva. (Pathak, 2015)

Farhadian et al. (2013), their study was carried out to optimize the various experimental conditions for the biosorption of the malachite green dye in the biomass of *Scenedesmus quadricauda* and *Chlorella vulgaris* by applying the surface response method. El-Sheekh et al. (2009), investigated the ability of *Chlorella vulgaris, Lyngbya lagerlerimi, Nostoc lincki, Oscillatoria rubescens* and *Volvox aureus* to discolor methyl red, orange II. However, there are no studies investigating the removal of aniline blue dye, one of the dyes commonly used in the tannery industry, so the main objective of this research is to study the ability of the microalgae *Chlorella vulgaris* to remove aniline blue dye in aqueous solution at four different initial concentrations.

2. Materials and Methods

Aniline blue dye biosorption experiments were performed with the microalgae Chlorella Vulgaris obtained from the Water Research Laboratory (WRL) of the National University of Trujillo (NUT). The dye was supplied by the Junior Curtiembre SAC, with the purpose of evaluating the efficiency of the microalgae in the removal of Anilina's Blue dye, the same one generated by the Tanneries in their effluents that emit to the domestic sewage system in the city of Trujillo - Peru.

Identification and Kinetics of the growth of the microalgae *Chlorella vulgaris*.

The identification of the microlaga *Chlorella vulgaris* was made observing in microscope and consulting the Taxonomic and Photographic Manual of Phytoplanktonic Genera of 7 lagoons of great Concepción, Concepción Chile; which indicate that the microalga *Chlorella vulgaris* is a unicellular green alga of fresh water; its cells are isolated, although eventually they can form aggregates, presenting a spherical morphology of green color and a size that oscillates between 2 and 6 µm. Figure 1 compares morphologically the microalgae isolated in the Water Research Laboratory with the bibliographic reference. (Gonzales and Inostroza, 2017)

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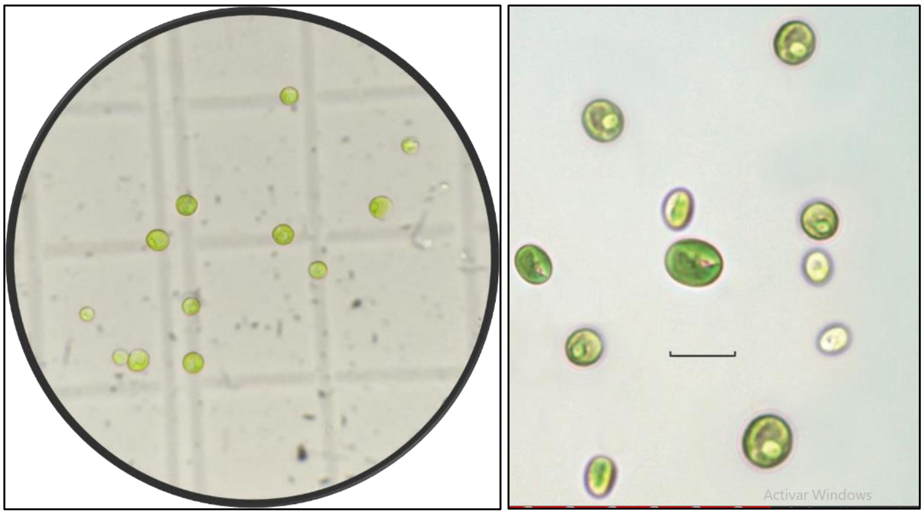

Figure 1: Morphological comparison of the isolated *Chlorella vulgaris* microalgae (left) vs. the bibliographic reference microalgae (right)

In order to evaluate the growth of the microalgae, a commercial fertilizer known as Hydroponic Solution provided by the Universidad Nacional Agraria la Molina was used as a culture medium.
The culture of the microalgae cells was carried out at room temperature, during a period of 14 days with constant agitation by bubbling of air in a photobioreactor of Bubble Column of 2 L of effective volume capacity, photoperiod day/night 12/12.
Growth kinetics assays were evaluated by cell count and mass absorbance measurement in UV-VIS Macherey Nagel spectrophotometer at a wavelength of 640 nm.

Calibration curve of aniline blue dye

For the calibration curve elaboration, a dye solution was prepared at 100 mg/L and its absorbance was measured at the maximum absorbance wavelength (λ), previously determined by a complete sweep performed in the UV-VIS spectrophotometer.

Preparation of the bioassays

In the photobioreactors of bubble column prior sterilization 600 ml of dye solution and microalgal biomass were colored in the absence of nutrients at room temperature 24°C, with constant agitation through air pumps. Dye removal was measured every 24 hours. The process consisted of taking aliquots from the bioreactors, then centrifuging them at 5,000 rpm for 10 minutes. The supernatant liquid was measured for absorbance at 596 nm by spectrophotometry and the percentage of the concentration of the dye removed with respect to the initial concentration was calculated using the following equation (Pathak et al., 2015).

$$\%\ Remoti\acute{o}n = \frac{C_0 - C}{C_0}\ x\ 100 \qquad (1)$$

Where Co is the initial concentration of the aniline blue dye and C is the concentration of the dye at time t.

3. Results

3.1. Growth kinetics of the microalgae *Chlorella vulgaris*.

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In Figure 2 and 3, we can see the similarity in the graphs of the growth kinetics of the microalga chlorella vulgaris evaluated by cell count and absorbance of the microalgal mass respectively, where the three characteristic phases of the growth of latency, exponential and stationary microalgae are observed.

The phase of adaptation of the microalgae to the conditions of the medium, latency phase is appreciated between day 0 to day 2, observing a slight decrease in cell concentration on the first day, adapting to the culture medium the next day to start its exponential phase.

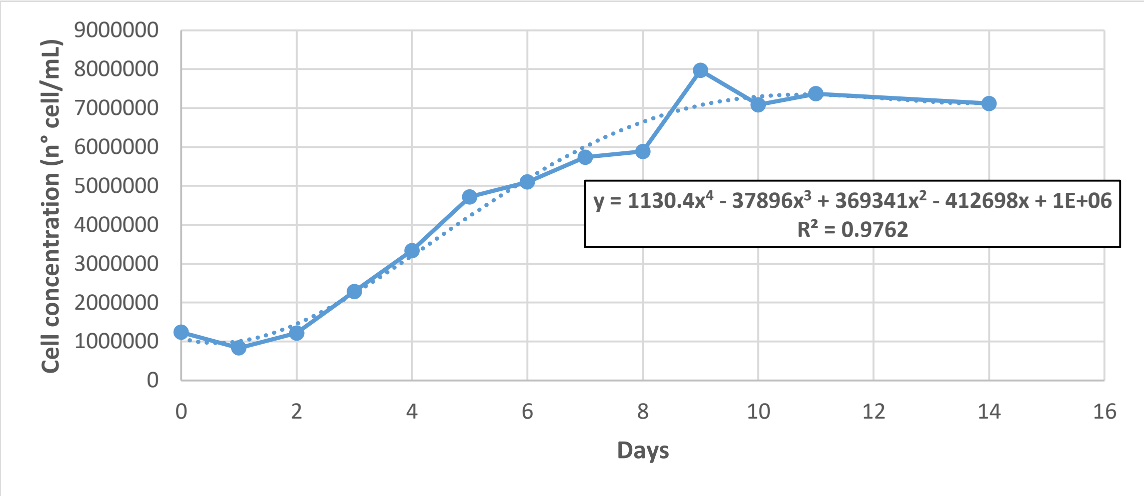

Figure 2. Cell concentration of microalgae *Chlorella Vulgaris* by time

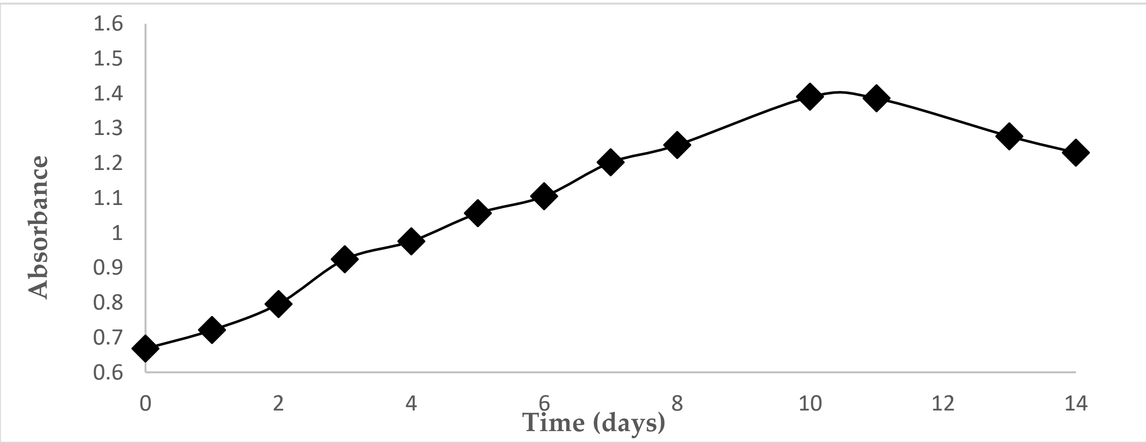

Figure 3. Growth kinetics of the microalgae *Chlorella vulgaris* through absorbance by time.

The exponential phase ranges from days 3 to 9, having a growth of approximately 7 days and a stationary phase that begins on day 10, a period in which the growth ends due to the absence of nutrients, evidencing the beginning of the phase of death of the microalgae, appearing a light yellow color. The study of the growth of the microalgae *Chlorella v.* allowed us to determine the

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harvest time of the microalgae for subsequent trials, choosing day 7 in full exponential phase period in which the microalgae is already adapted and in full growth, in addition to verifying that the culture medium is suitable for growth.

Calibration curve of the dye

A standard solution of aniline blue dye between 300 and 800 nm was swept to its maximum absorption at 596 nm, this wavelength being the wavelength at which the absorbance of the dye in the bioreactors was measured. Figure 4 shows the adjustment of the experimental data to a straight line, the correlation factor being 0.9997.

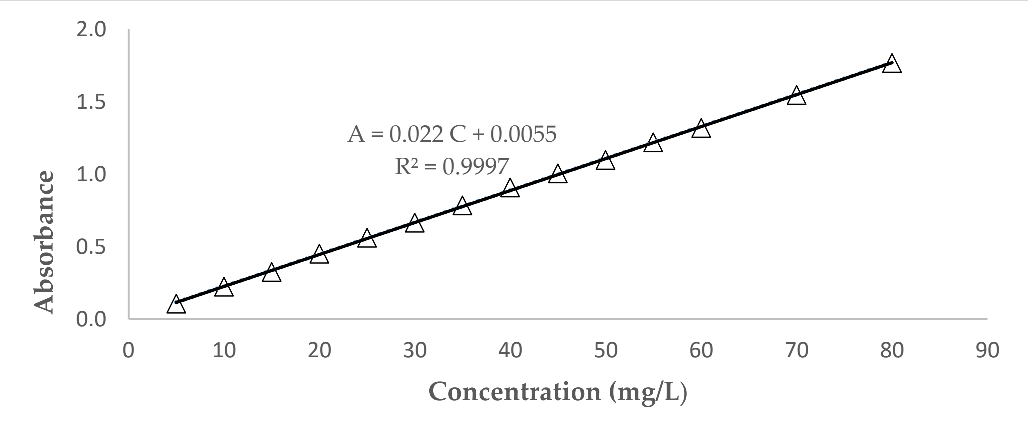

Figure 4. Colorant Calibration Curve

Removal of aniline blue dye using *Chlorella vulgaris*

Figure 5 shows the results obtained from bioassays to remove aniline blue dye with initial concentrations of 25, 50, 75 and 100 mg/L, for which the percentage of dye removed with respect to time is compared. It can be observed that the microalgae *Chlorella vulgaris* is able to reduce the concentration of aniline blue dye despite the fact that the presence of dye inhibits the normal growth of the microalgae by removing, after 11 days, 58, 53, 53 and 46.1%, obtaining the highest removal at the concentration of 25 ppm.

The algae cell wall contains different types of functional chemical groups such as carboxyl, amine, imidazole, phosphate, sulfate, sulfhydryl and hydroxyl that play a key role in the adsorption process Daneshvar et al. (2017). The adsorption efficiency of the anionic dye, aniline blue, is related to its acidic character because the number of positively charged sites in the biomass increases under acidic conditions and therefore the adsorption efficiency is increased by the attraction force between the positively charged sites in the mass of algae and the anionic dye molecules. Another reason could be due to the abundance of hydrogen ions in the acid solution and their ion exchange with the blue aniline dye.

On the other hand, Figure 5 also shows that as the concentration of dye increases, there is a decrease in removal efficiency which could be related to the saturation of the biomass of the microalgae after reaching equilibrium (Daneshvar et al, 2017)

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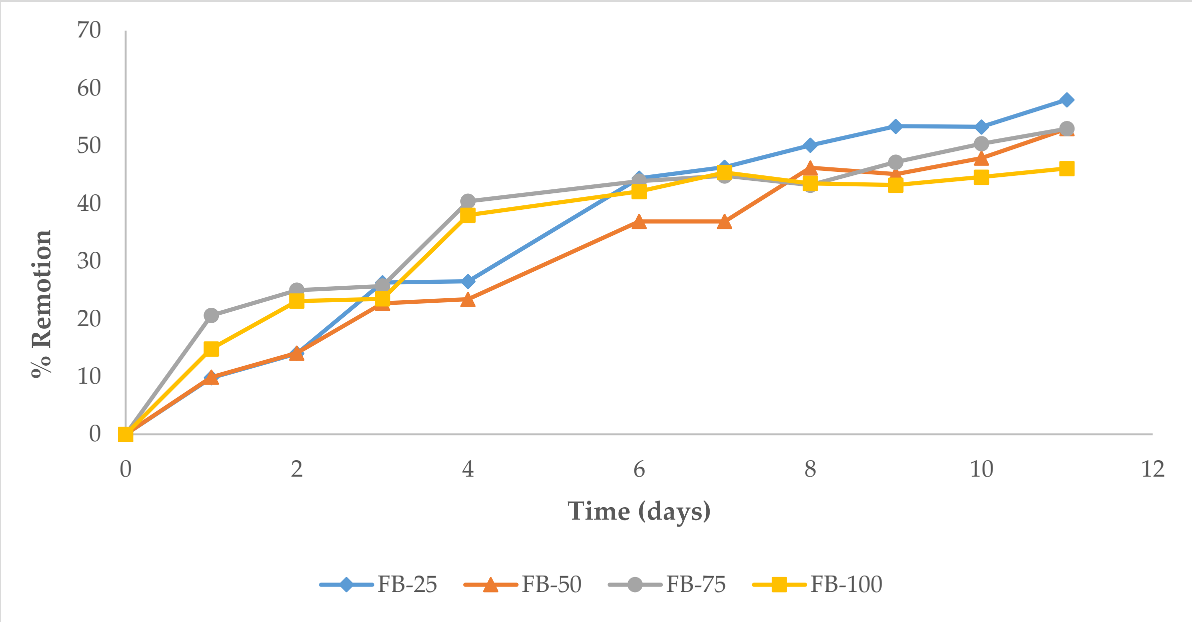

Figure 5. Percentage of aniline blue dye removal at different initial concentrations

Analysis of variance (ANOVA) of the Percentage of Removal of Aniline Blue dye at 4 levels of initial concentration.

The ANOVA table decomposes the variability of Colorant Removal into contributions due to several factors. Since the sum of Type III squares has been chosen (by omission), the contribution of each factor is measured by eliminating the effects of the other factors. The P-values test the statistical significance of each of the factors. Since the P-values are less than 0.05, these factors have a statistically significant effect on Colorant Removal with a 95.0% confidence level, indicating that the model used is valid for this study.

Table 1. Analysis of the variance for the efficiency of removal of aniline blue dye by the microalgae Chlorella Vulgaris

Source	Sum of squares	LG	Average square	ratio-F	Value-P
Principal effects					
A: Initial concentration	191.103	3	63.7009	3.42	0.0314
B: Days	6686.95	9	742.994	39.89	0.0000
Residues	502.945	27	18.6276		
Total (Corrected)	7381.0	39			

Figure 6 shows the aniline blue dye removal yields evaluated at 4 levels of initial dye concentration (25,50,75,100 ppm). Registering the best values of Percentage of yield the treatment evaluated at 75 ppm of initial concentration of dye.

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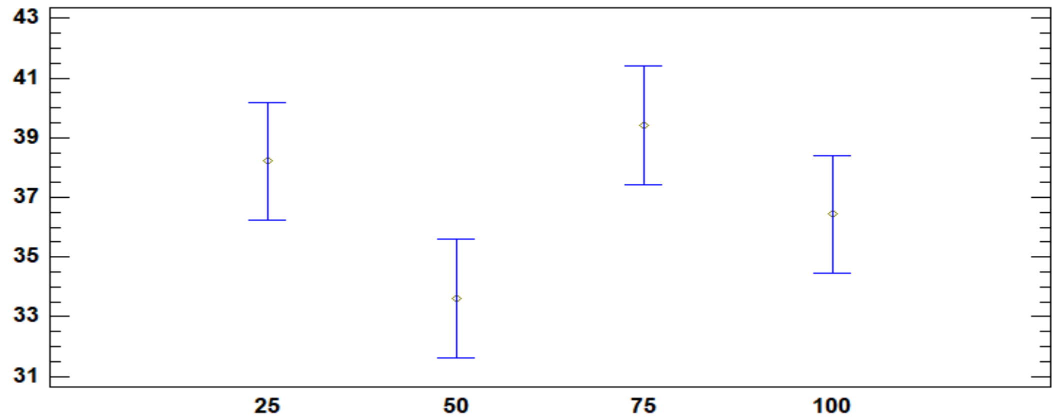

Figure 6: Dye removal yields at different initial concentration levels

4. Conclusions

According to the results obtained, it can be concluded that:

- The microalgae *Chlorella vulgaris* can be used in the bleaching processes of aniline blue, one of the dyes used in tannery.
- The removal of the dye depends on the initial concentration and the removal time; the best results in the 11 days of treatment are obtained at an initial concentration of 75 mg/L of aniline blue.

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