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Biodegradation of Erionyl Turquoise azoic textile dye from *Chlorella vulgaris* microalgae in simulated wastewater

Abstract

Bioremediation capacity of azo dyes in simulated characteristics of textile wastewater from the microalgae *Chlorella vulgaris* was determined. The evaluation of the percentage of removal of Erionyl Turquoise was carried out through the measurement of the change of the final and initial absorbance during 15 days. The microalgae were exposed to different concentrations of the azo dye (30, 70 y 130ppm) and an initial pH of 9. Concurrently, the behavior of the microalgae *C. vulgaris* was compared with a natural pH of 8.3. The highest percentage of removal was 91.7% and this was obtained with a concentration of 70 ppm and an initial pH of 9, while the lowest percentage of removal was 51.5% with a concentration of 130 ppm of the Erionyl Turquoise and an initial pH of 9. In this way, it can be established that the microalgae *C. vulgaris* has a considerable ability to remove azo dyes under some characteristics of textile wastewater.

Keywords: Azo dye, bioremediation, absorbance, *Chlorella vulgaris*

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Introduction

One of the industrial sectors with the greatest environmental impact is textiles. The contamination of bodies of water is part of its immense environmental footprint. Some of the most severe consequences of this problem are the toxic effects on the life of any aquatic organism followed by physical and genetic alteration; In addition, it prevents the adequate growth of plant life as a consequence of the limited penetration of light in the marine environment. On the other hand, it alters the physicochemical characteristics of the water such as pH, temperature, chemical oxygen demand, biochemical oxygen demand or amount of dissolved oxygen.[1]

In addition to this, the amount of dyes that are discarded after the wet processes of the textile industry exceeds two million tons per year.[2] The vast majority of these dyes are synthetic and difficult to biodegrade due to their complex aromatic structures (Brar et al, 2018). Azo dyes, which make up 70% of synthetic pigments and are the most resistant and recalcitrant, have in their molecular structure a double bond between two nitrogen atoms (RN=N-R'), which gives them stable behavior and consequently diversity of industrial uses,[3] but also its toxic, carcinogenic and mutagenic effects[4] The Erionyl Turquoise dye is mainly used for dyeing jeans and cotton (Figure 1).[5]

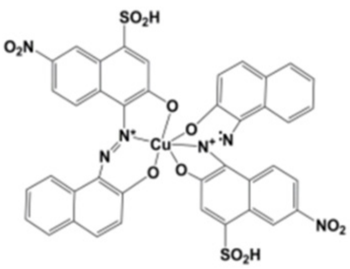

Figure 1 The figure shows the azoic structure of the Erionyl Turquoise dye. Fontalvo et al. Study of Colombian organic silk dyeing with dyes certified by the GOTS standard. Revista Investigaciones Aplicadas. 2014;8(1).

There are various physicochemical and biological mechanisms to degrade or separate azo dyes. Within the category of physicochemical treatments is membrane filtration, adsorption, coagulation/flocculation, precipitation, among others. Several of these treatments require high operational investments and long execution times to achieve their high removal efficiencies. For its part, the biodegradation of dyes, either by aerobic or anaerobic mechanisms, can achieve the same percentage of removal of these components and in some cases be less expensive (Selvaraj et al., 2021).[6]

Microalgae are usually a good alternative, regarding the improvement in wastewater treatment, due to their ability to reproduce in a wide variety of conditions, especially due to their ease of adsorbing and converting contaminants into nutrients for their growth. Microalgae employ different biological mechanisms such as biosorption, bioaccumulation and/or biodegradation, the latter being the most essential and driven by enzymatic activity.[7]

Chlorella vulgaris, is a species of unicellular microalgae, belonging to the protist kingdom that grows in fresh water, has been widely used in fields such as agrochemicals, biofuels, human nutrition and wastewater treatment, and more than anything for its different growth methods, whether autotrophic , heterotrophic or mixotrophic.[8] Its rigid wall allows it to withstand harsh conditions; on the other hand, its dry weight is composed of up to 58% of proteins that, among other functions, are involved in metabolic responses in the cell wall.[9]

Methodology

The active strain of the *Chlorella vulgaris* microalgae was provided by the research hotbed "Chemical Processes for the Integration of the Circular Economy in Industry" of the University of America Foundation. The sterilization of each material used was carried out through a JPinglobal horizontal laminar flow cabinet for 15 minutes.

a. Cultivation and adaptation of microalgae

The growth of the microalgae *C. vulgaris* was carried out from three 500mL solutions, of which 400mL correspond to the microalgae and 100mL to the culture medium. The culture medium was prepared by mixing 0.4mL of NPK complex fertilizer for foliar application diluted in 400mL of deionized water. After 15 days exposed to 12/12 light photoperiods, average room temperature of 17°C, pH of 8.3 and constant agitation by means of aquarium air compressors, 1.5L of the microalgae *C. vulgaris* were obtained.

b. Experimental development of dye biodegradation

The calibration curve of the concentration of the dye against the absorbance was made in a visible spectrophotometer GenesysTM 20 from Thermo Scientific, having obtained that the length of greatest absorption was 616nm, as indicated by Cardona, Osorio and Quintero,[5] with standard solutions of a minimum of 10ppm and a maximum of 130 ppm of the colorant, in duplicate. The dye concentrations for degradation were 30ppm, 70ppm and 130ppm. Regarding the pH, two values were evaluated: the initial pH of the culture medium (8.3) and a second value of 9 adjusted with NaOH [1M]. Follow-up was carried out for 15 days, under the same culture conditions (12/12 photoperiod, constant agitation, average temperature of 17°C). During the 15 days, a volume of 100 mL was maintained for each replica of each concentration and pH. For each centrifuged sample, the precipitate was observed in a Motic BA 210 microscope with a 40X and 100X lens during the 15 days of experimentation. In addition, pH and temperature measurements were made for each of the replicates carried out. The initial absorbance measurements (day 1) and for the following days until the end of the experiment were made at 616nm, with the supernatant from the centrifugation. The percentage of biodegradation, according to Kumar et al.[10] is calculated like this with the supernatant from centrifugation. The percentage of biodegradation, according to Kumar et al.[10] is calculated like this with the supernatant from centrifugation. The percentage of biodegradation, according to Kumar et al.[10] is calculated like this

$$\%Removal = \frac{ABS_i - ABS_f}{ABS_i} * 100$$

Where it refers to the initial absorbance and the final absorbance.

$ABS_i ABS_f$

Results and analysis

A decrease in the concentration of each simulated wastewater sample was evidenced, as shown in Figure 2, a maximum removal percentage (90.1%) is established at a concentration of 70ppm and a natural pH of 8.30. Similarly, the microalga *Chlorella vulgaris* was able to biodegrade 81% of the dye at an initial concentration of 130ppm with a natural culture pH, while at a concentration of 30ppm, a lower removal was reported compared to the others replicas (75.6%).

Figure 3 shows a maximum removal of 91.7% at a concentration of 70ppm and a pH of 9 initially adjusted. It can be established that, at a higher concentration of the dye and pH, biodegradation is inhibited,[11] for which the lowest percentage of biodegradation (51.5%) was obtained with the concentration and higher pH. Although it was the lowest value obtained in relation to the other replicates, this concentration exceeds the maximum experienced in previous studies,[12] such as Cardona et al.[5] where the biodegradation of the Erionyl Turquoise dye is analyzed and other similar azo dyes at a maximum concentration of 100 ppm, reaching a removal of 80% from ligninolytic fungi. In the case of studies carried out with the microalgae *C. vulgaris*,

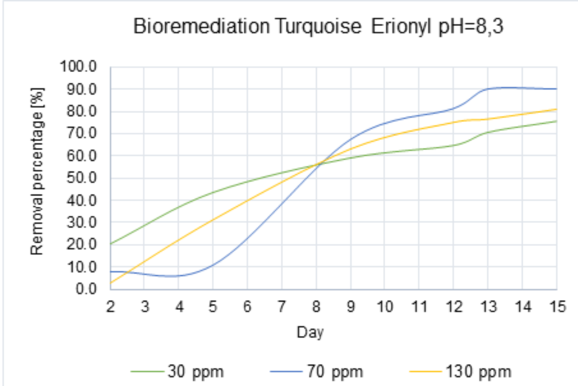

Figure 2 Removal percentage against the test day for each concentration (30, 70 and 130ppm) at pH 8.30.

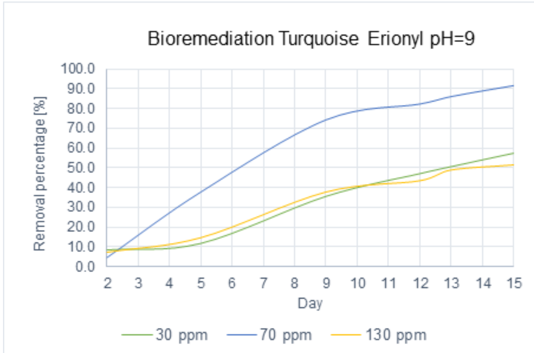

Figure 3 Removal percentage against the test day for each concentration (30, 70 and 130ppm) at pH 9.

On the other hand, during the first 10 days of experimentation, the speed of biodegradation of the dye was higher and constant, while from day 11 the speed of removal of the dye decreases in the case of both pH scenarios, maintaining a relationship with studies previously performed.[13,14] Therefore, the more complex the structure and the higher the molecular weight of the dye, the biodegradation will take longer and will not be as effective. Therefore, for azo dyes with less heavy functional groups (malachite green, crystal violet, etc.) from the microalgae *C. vulgaris*, a removal of up to 93% can be achieved in a time not exceeding 24h.[15]

However, during the experimentation, the pH of the samples, at different concentrations and initial pH, decreased, as shown in Table 1. The lower final pH values coincide with the higher percentages of removal of the Erionyl Turquoise dye.

Table 1 Average final pH of the replicates

Initial pH	Final pH		
	30ppm	**70ppm**	**130ppm**
8.3	7.82	7.8	7.98
9	8.02	7.69	7.87

This decrease in pH is directly related to the decrease in the surface charge of *C. vulgaris* cells;[13] Biosorption is benefited by working with low surface charges, allowing rapid diffusion of the dye through the cell wall, also due to its high surface area/volume ratio, because there is a storage of the dye inside the vacuole and a possible contribution of processes of photoconversion and bioconversion.[16] In addition to the above, studies attribute biodegradation to the enzymatic activity mainly responsible for azoreductase.[17,18]

Figure 4 shows the increase in size and change from intense green to aquamarine in the microscopic structure of the microalgae. First, the blue outline seen around some of the cells reflects the bioaccumulation mechanism through the cell wall, where there is an adherence on the surface biopolymers saturating the cells.[19]

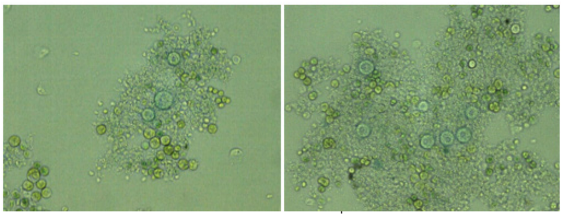

Figure 4 Microscopic view of the microalgae C. vulgaris on day 15 at a concentration of 30ppm (left) and 70ppm (right).

On the other hand, the number of turquoise cells is proportional to the concentration used: it can be said that for biodegradation of this azo pigment there is an optimal concentration of 70ppm. In the case of a lower concentration, the microalgae requires a greater amount of nutrients, which can be obtained directly by breaking the azo bonds present in the dye, while, if it works at higher concentrations, the presence of high amounts of dye can have a toxic effect and inhibit the growth of the microalgae, demonstrated by Hernández-Zamora et al.,[20] where negative physiological responses of *C. vulgaris* to concentrations greater than 400ppm of the Congo Red dye are evidenced.[21,22]

Conclusion

The highest percentage of Turquoise Erionyl dye removal in simulated wastewater, by *Chlorella vulgaris*, which was reached under the conditions of this work was 91.7% with an initial concentration of 70 ppm of dye and an initial pH of 9. This concentration is the optimum among the three analyzed: 30, 70 and 130 ppm, because the percentage of removal is similar, and in some cases higher, to that found in the literature for other dyes, or with the same dye, but by another microorganism. The remarkable thing about this optimal result is that it was achieved under minimal laboratory conditions (without any temperature, lighting or pH control system) in a maximum time of 15 days. Another result to highlight is that high removal (greater than 80%) is observed for a high initial concentration of the contaminant: 130 ppm. On the other hand, it was observed that the pH of the medium decreases progressively with all the samples throughout the treatment up to approximately 7.8, which could be related, in addition to the cellular morphological changes observed by means of the microscope, with the degradation processes carried out by the *Chlorella vulgaris* microalgae. After having demonstrated the high removal capacity of Turquoise erionyl in this simulated wastewater, a second stage will consist of the removal of different colorants in natural wastewater, by means of *Chlorella vulgaris*. with the degradation processes carried out by the microalgae *Chlorella vulgaris*. After having demonstrated the high removal capacity of Turquoise erionyl in this simulated wastewater, a second stage will consist of the removal of different colorants in natural wastewater, by means of *Chlorella vulgaris*. with the degradation processes carried out by the microalgae *Chlorella vulgaris*. After having demonstrated the high removal capacity of Turquoise erionyl in this simulated wastewater, a second stage will consist of the removal of different colorants in natural wastewater, by means of *Chlorella vulgaris*.

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Conflicts of interest

Author declares that there is no conflict of interest.

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