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Original paper

Comparative study of water discoloration by phytoremediation using *Pistia stratiotes* and *Chlorella vulgaris*

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Abstract Phytoremediation of wastewater from textile industry could be less expensive alternative techniques compared to the physical and chemical methods. *Pistia stratiotes* and *Chlorella vulgaris* have been evaluated regarding the potential for remediation of waters contaminated with 2 textile azotic dyes, AZUL MARINO and NEGRO, produced by Iberia, Spain. The characteristic absorption spectra in 380-800 nm range were recorded for these compounds and the absorbances for basal peaks were determined after 11 days in cultures of Pistia and Chlorella containing an initial concentration of 50 μg/mL of each azo dye. The results demonstrated that both alga and macrophyte plant are effective in this process, but the calculated percentage of discoloration was higher(90% for the blue dye and 70% for the black one) for Pistia than for Chlorella (only 23% for the blue dye and 21% for the black dye). Reported to the value of dry biomass, the phytoremediation using Pistia was more efficient. The ratio between the discoloration and the dry biomass in culture of Pistia was 58% /g for AZUL MARINO and 52%/g for NEGRO. In the cultures of Chlorella, this ratio was significantly lower, 16%/g for AZUL MARINO, and 15%/g for NEGRO dye.

Keywords Textile azo dyes, percentage of discoloration, biomass.

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Introduction

The discharge of dangerous and toxic effluents from various industries adversely affects water resources, soil fertility, aquatic organisms and ecosystem integrity. One of the major problems in global pollution is the presence of dyes in textile industry wastewaters. The increasing demand of textile materials resulted in increasingly water consumption in specific processes of dyeing and finishing (W. PRZYSTAŚ & al [1]). The textile waste water is considered one of the most polluting in the industrial sectors, containing a complex and variable mixture of substances like inorganic, organic, elemental and polymeric products and high quantities of dyes wastes (E.S. PRIYA & al [2]).

Azo dyes group is one of the most important classes of synthetic dyes used in the textile industry, representing more than 60-70% of dyes used in the industry (M.S. KHEHRA & al [3]). During the dyeing textiles processes, the amount of dye that is not retained on the fibre varies depending on its nature, ranging between 2% and 50% (N.T. SHETH & al [4]; R.Y. WATANAPOKASIN & al [5]). The presence of dyes in water effluents reduces the sunlight passing through water, reaching with difficulty in lower volumes; it affects the photosynthesis activities of aquatic flora, lowering the concentration of dissolved oxygen, with negative effects on aquatic flora and fauna (I. NILSSON & al [6]).

The physical and chemical methods such as coagulation, flocculation, adsorption, membrane filtration and irradiation (T. ROBINSON & al [7]; K.N. ARCHNA LOKESH & al [8]) traditionally used in effluent treatment processes have a good efficiency but they have two main constraints: high cost and the production of the significant amount of sludge material (E.S. PRIYA & al [2]). Biological treatments of wastewater from textile industry could be less expensive alternative techniques compared to the physical and chemical methods. These methods are based on treatment processes of polluting agents by biosorption, bioaccumulation or by enzyme biotransformation.

Nowadays, phytoremediation, one of a plant based green technologies has received great interest due to the discovery of hyperaccumulating plants which are able to accumulate, translocate, and concentrate high amount of certain toxic elements (M.A. RAHMAN & al [9]). Phytoremediation can be accomplished through phytoextraction, phytodegradation, rhizofiltration, phytostabilization and phytovolatilization.

The aquatic plants have been evaluated for the remediation of toxic contaminants such as As, Zn, Cd, Cu, Pb, Cr, Hg, etc (M.A. RAHMAN & al [9]) and textile dyes. Plants like water hyacinth (*Eichhorniacrassipes*), duckweeds (*Lemnagibba*), water spinach (*Ipomoea aquatica*), water ferns (*Azollacaroliniana*), water cabbage (*Pistia stratiotes*), and other have been studied (M.A. RAHMAN & al [9]) in order to evaluate their potential in phytoremediation.

A large number of experiments were conducted for the study of phytoremediation of wastewater contaminated with chromium (K. SANKAR GANESH & al [10]), cadmium (L. SANITA DI TOPPI & al [11]; S. REZANIA & al [12]), lead, copper (R.S. PUTRA & al [13]), arsenic (M.A. RAHMAN & al [9]; N.K. SINGH & al [14]), mercury (K. SKINNER & al [15]), silver nanoparticles (N.A. HANKS & al [16]), pharmaceuticals and personal care products (Y.L. LIN & al [17]).

Laboratory tests were performed in order to examine growth characteristics of floating plants in the presence of urban sewage (Y. ZIMMELS & al [18]; A. TEWARI & al [19]). Many studies were designed to determine the efficiencies and mechanisms of water nutrient removal via different aquatic plant species such as water lettuce and water hyacinth as a potential remediation strategy for polluted rural rivers (B. LU & al [20]).

Microscopic monocellular algae belonging to genus *Chlorella* were used for the treatment of wastewater contaminated with swine manure (X.Y. DENG & al [21]; L. CAO & al [22]), for phytoremediation of phenolic effluent (S.D. PRIYADHARSHINI & al [23]; S.D. PRIYADHARSHINI & al [24]), domestic wastewater (P. RAMSUNDAR & al [25]; X. LIU & al [26]), Congo red dye,(M. HERNÁNDEZ-ZAMORA & al [27]), for removal and biodegradation of triclosan in water (S. WANG & al [28]), and for the treatment process for settled municipal wastewater (L. EVANS & al [29]; H. ZNAD & al [30]; P. RAJASULOCHANA & al [31]). *Chlorella* species can be also used in the phytoremediation of wastewater contaminated with arsenic (M.S. PODDER & al [32]), and for zinc removal (G. FERRARO & al [33]).

The aim of this study was to evaluate the ability of aquatic plants *Pistia stratiotes* and monocellular alga *Chlorella vulgaris* to degrade the textile dyes in wastewater.

Materials and Methods

Plant samples and growth conditions

The plant material was represented by two different types of aquatic plants: microscopic algae *Chlorella vulgaris* strain AICB 555, and *Pistia stratiotes* (water lettuce), purchased from the market. *Chlorella vulgaris* was cultivated in the Bold's Basal Medium, an inorganic salts medium widely used for the culture of free-living planktonic freshwater algae. *Pistia stratiotes* was acclimatized under the laboratory conditions and kept in tap water. The plants were grown in natural light, an average of 10 hours daily, near the window, in December, at 20-22°C.

Textile dyes

Two textile azo dyes commercially available have been used:AZUL MARINO, code 24317 and NEGRO, code 16616 produced by IBERIA, Spain. The two dyes both contain, in varying proportions a mixture of colorants, as mentioned on the packaging: Reactive black 5; Blue der 2108; Blue 203; Red 123; Red 264; Red 278; Orange der 8068; Orange der 8638; Brown 49; Yellow azo; 2,4-diamino-5-[4-[(2-sulfoxylethyl)sulfony]phenylazo]benzene-sulfonic acid; 4-amino-3-[[4-[[2-(sulfooxy)ethyl]sulfonyl] phenyl]azo]-1-naphtalene-sulfonic acid. The solutions of the azo dyes were prepared in distilled water and then added in the two plant cultures.

Methods

Daily, samples were taken from *Chlorella* and *Pistia* cultures and analysed for visible absorption spectra in the 380-800 nm range, on a UV-VIS spectrophotometer T92+, PG Instruments. The peak values were recorded and compared with the control that is the initial culture, immediately after the dye has been added. The dry matter was analysed on a moisture analyzerKern RH 120-3.

The percentage of discoloration was calculated using the following formula:

$$Discoloration\ (\%) = \frac{Ai - Af}{Ai} \times 100 \qquad (1)$$

where Ai is the initial absorbance, after adding the dye and $Af$is the final absorbance of sample. This percentage was reported to the determined dry mass in order to compare the discoloration activity of the two plants.

Changes related to plant appearance in the dye solution was assessed on a microscope FLUO3 Bell Photonics. The number of algal cells was determined with a microscope counting chamber.

Results and Discussion

The plant biomass that was in contact with the azo dye solutions was analysed by determining the total quantity and humidity (table 1). In the culture of *Pistia stratiotes*, were used 6 rosettes of different sizes, some connected through stolons that had many roots with a length between 5 and 20 cm. The alga *Chlorella vulgaris*was analysed for biomass and cell number/mL.

Table 1. Wet and dry biomass in the cultures of *Pistia*and *Chlorella*

Plant / Textile dye	**Wet biomass**	**Dry biomass**	**Number of cells/mL**
Pistia / AZUL MARINO dye	17.1 g	1.53 g	-
Pistia / NEGRO dye	18.9 g	1.71 g	-
Chlorella / AZUL MARINO dye	-	1.41 g/L	$1.2x10^7$
Chlorella / NEGRO dye	-	1.41 g/L	$1.2x10^7$

The two dyes were analysed by recording the absorption spectra in the visible range of 380-800 nm. For both azo dyes, several absorption maxima were observed, as shown in figure 1 b) and d).The absorption spectra for the textile dye AZUL MARINO had a base peak at 596 nm and another, with lower absorbance, at 391 nm, and for NEGRO dye a base peak at the same wavelength and two smaller ones at 482 and 395 nm. To calculate the change in dye concentration over time as a result of plant action, the calibration curves representing absorbance vs. concentration, were plotted (figure 1 a) and c)).

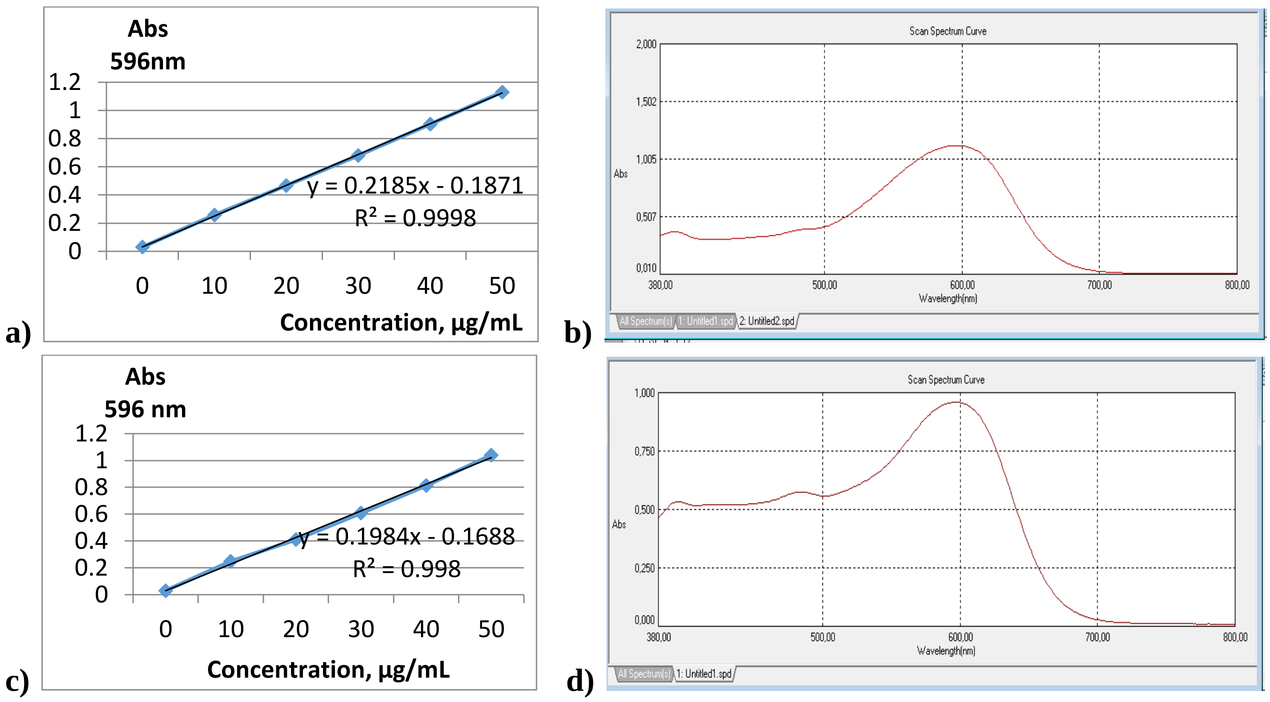


Figure 1. Calibration curves and absorption spectra for the textile dyes AZUL MARINO a), b) and NEGRO c), d)

To monitor the discoloration of water in cultures of *Pistia* and *Chlorella*, the absorbances were daily analysed(figure 2). The initial concentration of the textile dyes was 50 µg/mL, in order to obtain initial absorbance values in the domain of linearity of standard curve and to avoid dilution errors. The results are shown in figure 2 for the culture of *Pistia stratiotes* and in figure 3 for *Chlorella vulgaris*, and demonstrated significant differences.

In the cultures of *Pistia* there was a strong and continuous decrease of absorbance in the first week and a slower drop in the next 4 days, for both AZUL MARINO and NEGRO azo dyes.

In the case of AZUL MARINO dye, the characteristic absorbance at 596 nm for the blue components decreased from 1.09 to 0.45 and 0.11 after 5 and 11 days, respectively. The absorbance for the yellow compounds, recorded at 391 nm, decreased from 0.31 to 0.0.21 and 0.11 after 5 and 11 days, respectively. It was found that the decrease is more pronounced for the wavelength of 596 nm than 391 nm. In addition, it was observed that there was a displacement of the maximum values of absorbance to the lower values of wavelength; thus, in the first days, the maximum value of absorbance was recorded for wavelength values higher than 590 nm, and at the end of the experiment the wavelength for the maximum was at 554 nm, to the red colour. This could suggest the blue component was more easily degraded than other compounds. After 11 days, the concentration of this textile dye decreased from the initial value of 50 µg/mL to a concentration of 5 µg/mL. Although no measurements were made during this time, the water was completely clear after about 3 weeks, when the absorbance was lower than 0.03 and the appearance of the entire plant was as at the initial time.

For the second dye, NEGRO, the characteristic absorbances at 596, 482 and 395 nm were recorded (figure 1d). There was a similar decrease in absorbance with the first dye, which is explained by the similar composition but in different proportions of the components. As in the first case, the decrease was higher, from 1.09 to 0.29, for the compound with maximum of absorbance at 596 nm. In this case, the final dye concentration reached 14.5 µg/mL.

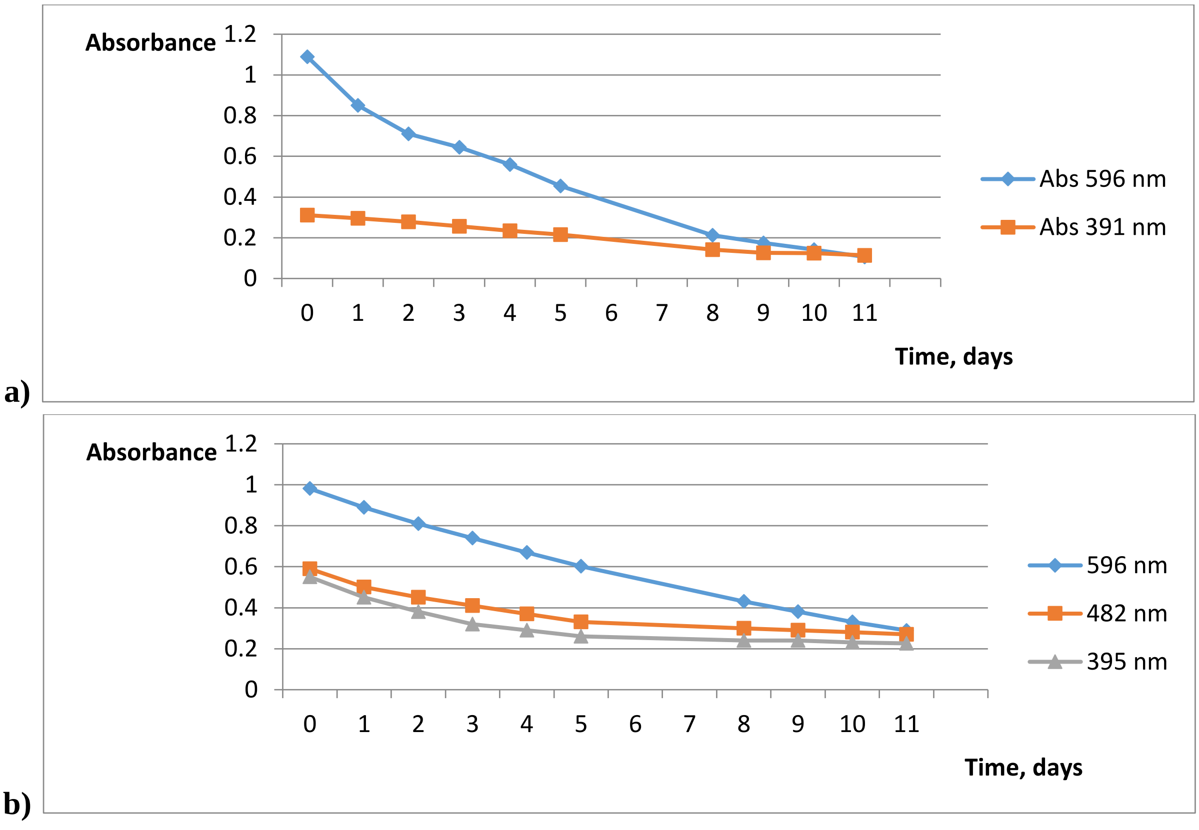


Figure 2. Absorbances values in the culture of *Pistia stratiotes* containing the textile dyes a) AZUL MARINO and b) NEGRO

The second experiment was performed with *Chlorella vulgaris*, using the same dyes at the same concentration. Absorbances were analyseddaily in filtered samples, for 11 days, using the two textile dyes, as shown in figure 3.Different from *Pistia*, in this case, the decrease of characteristic dye absorbances was much lower for the two dyes. Thus, for the culture containing AZUL MARINO, the absorbance at 596 nm decreased from 1.02 to 0.87 and 0.79 after 5 and 11 days respectively, and the culture remained colouredat the end (figure 3a), with a concentration of 39.5 µg/mL. The same phenomenon was also observed when using NEGRO dye under the same conditions (figure 3b).

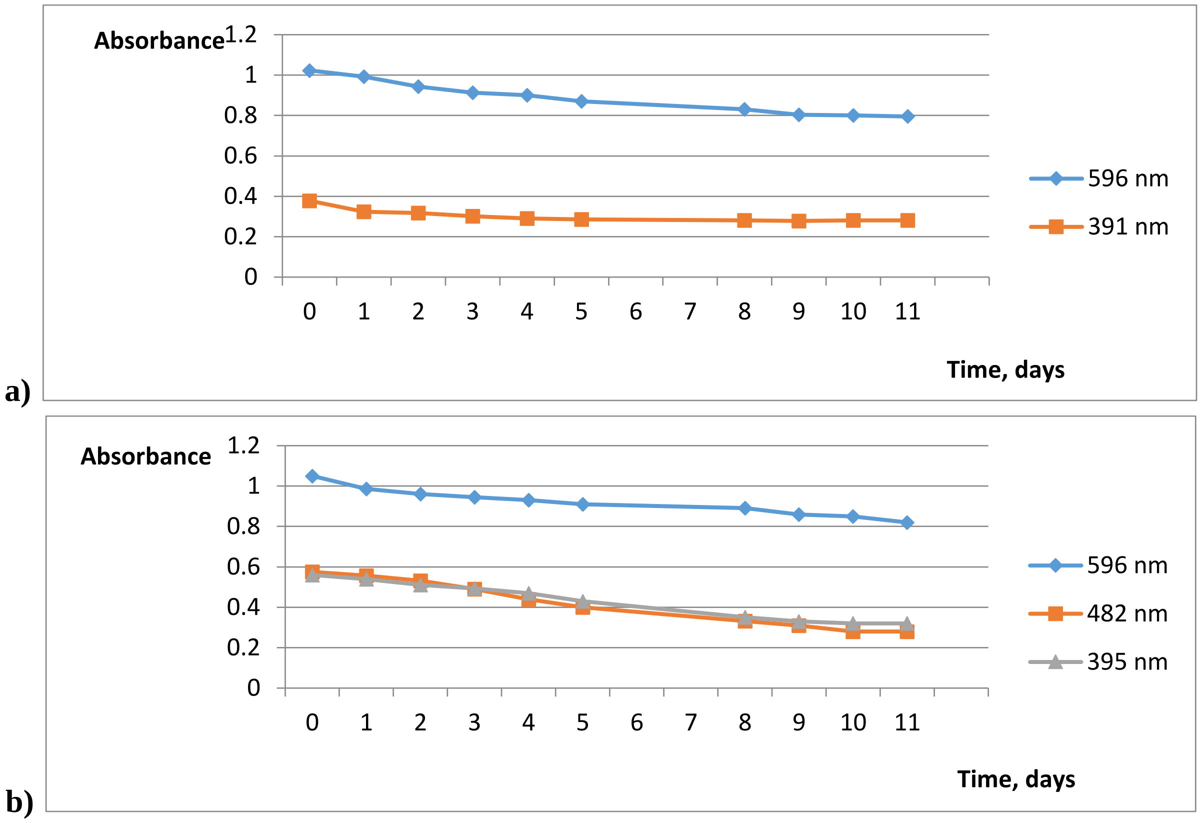


Figure 3. Absorbances values in the culture of *Chlorella vulgaris* containing the textile dyes a) AZUL MARINO and b) NEGRO

The percentage of discoloration for all cultures after 11 days is illustrated in figure 4. The discoloration depends on the species and the type of dye, the greatest values being determined in the cultures of *Pistia*, for both AZUL MARINO and NEGRO textile dyes. The ratio between the discoloration and the dry biomass in culture of Pistia was 58% /g for AZUL MARINO and 52%/g for NEGRO. In the cultures of *Chlorella*, this ratio was significantly lower, 16%/g for AZUL MARINO, and 15%/g for NEGRO dye.

At the end of experiment, the appearance of cultures was assessed macroscopically and microscopically. For *Pistia stratiotes*, it can be mentioned that after 3 weeks, the entire plant remained unchanged. The roots appeared blue-coloured at microscope (figure 5) in the first 3 weeks, but after this period they became colourless. The alga *Chlorella vulgaris* appeared to be much more affected by the presence of dyes in culture, as observed on microscope. Deformed cells in associations were observed in samples taken after 3 weeks (figure 6), the cell structure being severely altered. These aspects can be explained by the drastic action of azo dyes on the algae cells, even causing their death.

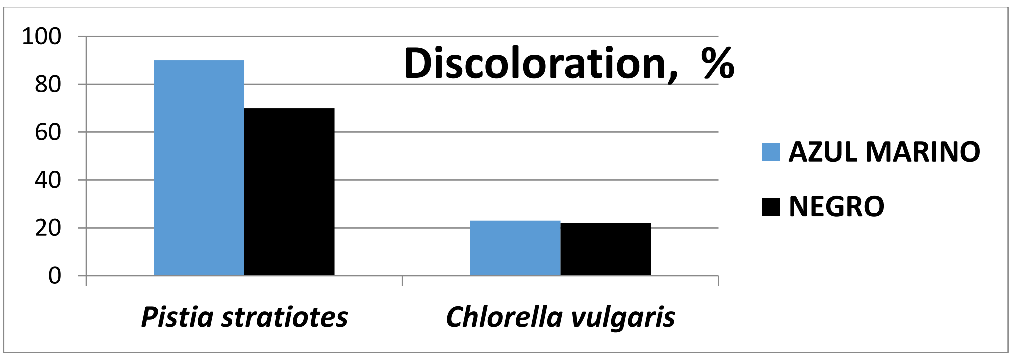


Figure 4. The percentage textile dyes discoloration in cultures of *Pistia* and *Chlorella*

a) 

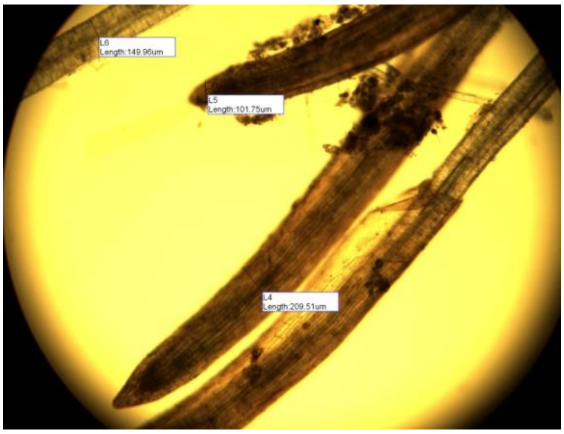
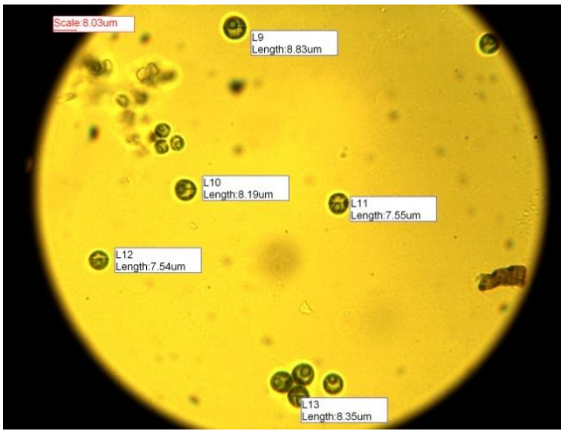
b) 

Figure 5. The appearance of plant *Pistia* stratiotes a) in culture with AZUL MARINO dye; b) microscopic image of roots after 2 weeks in culture with azo dye

a) 

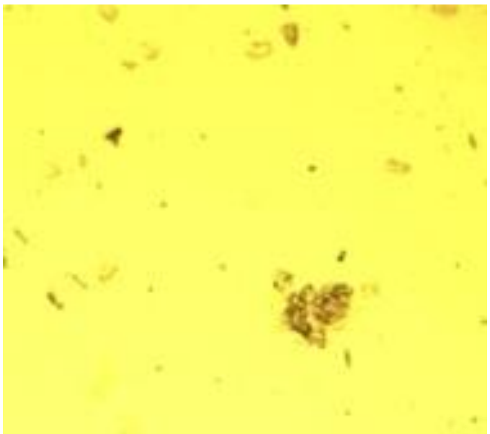
b) 

Figure 6. Microscopic image of *Chlorella* cells a) before adding azo dyes; b) after 3 weeks in culture with azo dye

The obtained results are in accordance with other results, although there are not many studies on the techniques of phytoremediation of waters contaminated with textile dyes. Most research has investigated the possibility of treating waters polluted with heavy metals and municipal organic matter, although textile dyes are a major and topical issue.In recent years it was demonstrated that the phytoremediation of textile wastewater using algae and aquatic Macrophytes is a well-established environmental protective technique. M. RIZWANA& al [34] tested the efficiency of several wetland plants and demonstrated that water lettuce has a great potential in removing N and P from eutrophic storm waters. In 2015, PATEL & al [35] studied the removal of textile dyes by using *Pistia* spp. and *Eichornia* spp. by Aquatic Macrophytes Treatments Systems and observed a high-colour reduction of two solutions containing Royal blue dye and HD blue dye. M. EL-SHEEKH & al [36] investigated the biodegradation of methyl red, orange II, G-Red (FN-3G), basic cationic, and basic fuchsine by some green algae and cyanobacteria. It was found that the percentage of discoloration of these dyes by *Chlorella vulgaris* was higher than 40%.The process of discoloration of wastewater from a paint industry by the microalgae *Chlorellasp.* was evaluate in 2017 by E. ANGULO & al [37] and the results showed that the discoloration percentage was higher than 70%. Through biosorption and biodegradation processes, *Chlorella vulgaris* was able to remove 83 and 58% of Congo red dye at concentrations of 5 and 25 mg/L, respectively (M. HERNÁNDEZ-ZAMORA & al [27]).

Conclusions

The bioremediation of wastewater contaminated with textile dyes remains an issue of interest and the use of algae and aquatic macrophytes can be an efficient and cost-effective solution.

Pistia stratiotes and *Chlorella vulgaris* were evaluated for the discoloration of water containing two textile azo dyes, namely AZUL MARINO and NEGRO produced by IBERIA. The obtained results demonstrated that both alga and macrophyte plant are effective in this process, but the calculated percentage of discoloration was higher (90% for the blue dye and 70% for the black one) for *Pistia* than for alga *Chlorella* (only 23% for the blue dye and 21% for the black dye). Reported to the value of dry biomass, the phytoremediation using *Pistia* was more efficient. The ratio between the discoloration and the dry biomass in culture of *Pistia* was 58% /g for AZUL MARINO and 52%/g for NEGRO. In the cultures of *Chlorella*, this ratio was significantly lower, 16%/g for AZUL MARINO, and 15%/g for NEGRO dye.

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