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Evaluation of the Environmental Factors Impact on the Bioremediation Efficiency of a Mono-azo Dye, Acid Blue 92 (AB92), by the Algae *Tetradesmus obliquus*

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Abstract

The Dyeing and textile industries are among the sectors that hold a significant global presence in Iran and worldwide. These industries are major water consumers; consequently, substantial quantities of wastewater containing toxic compounds, including synthetic dyes, are released into the environment at various stages of the process within these industries. The treatment and purification of wastewater generated by these industries is of great importance to reduce their associated risks, and a range of physical, chemical, and biological methods are employed to remove pollutants from industrial wastewater. Meanwhile, the use of microalgae for bioremediation is one of the primary eco-friendly treatment methods due to its low cost, reliance on natural processes, and reduced risk of toxic substance accumulation. In this study, the microalgae *Tetradesmus obliquus* were utilized to investigate the removal rate of Acid Blue 92 dye under various environmental conditions, including temperature, pH, and initial concentration of the dye, initial cell number, and reaction duration. The treatments encompassed temperature (25, 10, 5 °C), pH (8.5, 7.5, 6.5, 5.5, 4.5), initial dye concentration (5, 10, 20, and 50 mg/L), initial cell number (5, 10, 20, and 30 $\times 10^6$ cells/mL), and reaction duration (every 24 hours for 4 days). Furthermore, the reusability of individual algal biomass in the continuous purification of the dyes was investigated through several consecutive decolorization experiments. Based on the results, the removal efficiency of Acid Blue 92 dye increased with increasing cell number and increasing temperature. However, the removal efficiency decreased with increasing initial concentration of the dye. In addition, the optimal pH for the dye decolorization process was determined to be in the pH range of 6-7. The results of the pollutant removal reproducibility tests showed that this alga can repeatedly remove the dye from the contaminated wastewater. Therefore, the algae probably absorb and degrade the pollutant from

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the environment to an acceptable extent by utilizing the biodegradation process. In conclusion, it can be stated that *T. obliquus* algae have a significant capacity for the removal and biotreatment of acid blue dye 92, particularly under optimal conditions, and may be considered as a viable option for eliminating colored pollutants in aquatic environments.

Keywords: Bioremediation, *Tetradesmus obliquus*, algae, Organic pollutants, mono-azo dye, Acid Blue 92

Introduction

The contamination of drinking water globally presents one of the main environmental challenges we face today. A significant portion of water pollution occurs due to the discharge of sewage containing waste as well as improper disposal of industrial wastes, which include dyes, heavy metals, pharmaceutical products, and more. These substances disrupt the ecological cycle of species and change the availability and quality of vital elements for living organisms. It is predicted that the world will face a 40% water shortage by 2030, which poses a serious challenge to sustainable development (Sun et al., 2016).

The dyeing and textile sectors are among the industries that have with extensive potential in Iran and globally. These industries are recognized as the largest consumers of water, consequently leading to the discharge of significant quantities of wastewater containing toxic compounds, including synthetic dyes, are released into the environment at different stages of the process in these industries. Currently, over one hundred thousand types of commercial dyes are utilized in various industries, with approximately 10-15% of these dyes being released into the environment. Furthermore, reports indicating that around 2.5×10^5 tons of dyes are discharged into the environment annually (Singh and Singh, 2017).

Due to their wide application and ease of production, synthetic dyes are widely used in industries such as paper printing, food products, pharmaceuticals, cosmetics, and as additives in petroleum products, in addition to the textile industry. The stability of these dyes is also much higher than that of natural dyes. Therefore, they can cause significant environmental problems by creating an imbalance in the chemical and organic content of aquatic ecosystems (Sarkar et al., 2017).

Discharging wastewater containing these compounds without treatment into aquatic environments will have harmful effects on aquatic life and subsequently on the health of aquatic consumers. Therefore, it seems necessary to take effective measures to treat water and wastewater contaminated with these dyes. Water sources contaminated with such toxic wastewater can, over time, contaminate the surrounding soil ecosystems and cause serious environmental problems for the growth of living organisms, both plants and animals. In this context, reports show that some azo dyes can severely affect plant growth by obstructing seed germination, diminishing seedling survival rates, reducing photosynthesis, and preventing branch and root elongation (Baena-Baldiris et al., 2020). Consequently, the treatment of these substantial volumes of wastewater is of great importance. At present, the management of water monitoring and treatment processes is dominated by physical and chemical treatment methods along with traditional infrastructures, which are characterized by classical control and treatment systems. Current techniques, such as physical and biological adsorption, membrane filtration, oxidation, ozonation, reverse osmosis, ion exchange, photocatalysis, and electrochemical oxidation are frequently used for the treatment of pollutants. These methods for treatment and removal do not always followed by the prevailing standards, which ultimately leads to serious pollution due to the production of new toxic intermediate compounds. These methods are also expensive, energy-intensive, and not affordable for small industries and low-income processors. This financial burden is likely the main reason for the deceleration in pollution control efforts, particularly in less developed and developing countries (Khandare et al., 2013; Ummalyma et al., 2018).

The technology of using algae to remove pollutants, called algae purification (Moradi et al., 2020), is superior to other technologies due to its low cost, being based on natural processes, and reducing the risk of toxic accumulation (Cepoi and Zinicovscaia, 2020). The process of bioremediation of pollutants can be carried out through biosorption or biodegradation. Biosorption refers to the removal of pollutants from the liquid phase, such as wastewater or culture medium and their transfer to the solid phase (biosorbent surface) (Rasolzadeh et al., 2019). Sometimes, the algae involved in bioremediation, after biodegrading the pollutant compounds, use them as a source of carbon, nitrogen, or energy (Moradi et al., 2020).

Bioremediation methods that utilize bacteria, fungi, yeast, and even their consortium designs have been effective in removing dyes, but they face implementation problems in the practical and field management of wastes. The use of plants and green algae to clean up pollutants in situ can be done with much lower remediation costs and also offers a carbon-neutral and therefore environmentally friendly approach to removing toxic pollutants from the environment (Dietz and Schnoor, 2001). The use of microalgae as a bioremediation agent for colored wastewater has attracted much attention because these microalgae play an important role in carbon dioxide fixation. In addition, the biomass produced by algae is very efficient as a raw material for the production of biofuels (Huang et al., 2018). Algae are considered potential biosorbents due to the diversity of functional groups, such as hydroxyl, carboxyl, amino, phosphate, and other groups present on the cell surface. In addition to the characteristics of the cell wall, the process of biosorption of pollutants into the algal cells is also of great importance (Moradi et al., 2020). The process of degradation and biodegradation of pollutants inside living cells is carried out through various enzymatic oxidation and reduction reactions (Zohoorian et al., 2020). In this study, the microalga *Tetradesmus obliquus* was utilized to investigate the removal rate of Acid Blue 92 dye and the effect of some biotic and abiotic factors on bioremediation efficiency.

Material and methods

T. obliquus alga was obtained from the Artemia and Aquaculture Research Institute of Urmia University and cultured in BBM medium at 25 °C. This alga was identified by Asal Pishe et al. (2012) and registered in NCBI with the number (Accession Number OR393092).

T. obliquus was cultured in Bold's Basal Medium (BBM), a standard culture medium for freshwater algae. The culture medium was prepared according to standard protocols and sterilized by autoclaving at 121°C and 15 psi for 20 min (Asghari et al., 2023). To ensure uniformity and reproducibility, a fresh culture medium was used in all experiments (Torbati, 2019). Color removal experiments were performed in 250 mL Erlenmeyer flasks containing 100 mL of BBM culture medium by inoculating with *T. obliquus*.

Temperature (5, 10, and 25 °C), pH (4.5, 5.5, 6.5, 7.5, and 8.5), initial dye concentration (5, 10, 20, and 50 mg/L), initial cell number in the medium (5, 10, 20, 30 $\times 10^6$ cells. mL^{-1}), and reaction time (every 24 hours for 4 days) were adjusted and investigated as the main variables. Each time, the effect of just one factor on removal efficiency was determined, and other effective parameters were kept constant. The Erlenmeyer flasks were placed on a circular shaker at a speed of 150 rpm to provide uniform suspension of algal cells and proper aeration. Dilute KOH and H_2SO_4 solutions were also used to adjust the initial pH of the solution, and the pH was measured by a pH meter (Hanna Instrument Inc.).

The number of cells in the culture medium was counted using a hemocytometer slide (Kennari et al., 2008). These experiments were conducted over a 4-day period, with samples collected at specific time intervals (0, 24, 48, 72, and 96 hours). The samples were centrifuged at 4000 rpm for 10 minutes to separate the algal cells and leave a clear solution for analysis. The percentage of dye removal was calculated using the following equation at the maximum wavelength (λmax = 571 nm) using a UV-Vis spectrophotometer (Camspec M330 model, UK) (Torbati, 2019).

Dye Removal (%)

A_0: Initial absorption of dye solution

A: Solution absorption at the time of measurement.

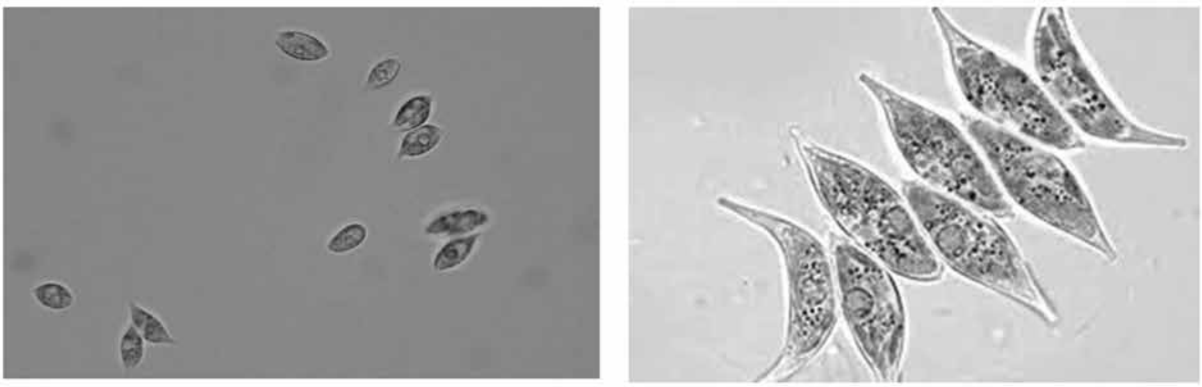

Fig. 1. Spindle cells of the algae *Tetradesmus obliquus*

Results

The results of the effect of the initial number of *T. obliquus* algae cells on the efficiency of AB92 dye removal over 4 days are shown in Figure 2. According to the results, with an increase in the number of algae cells, the percentage of dye removal from the medium also increased. So in the treatments of 5 ×10^6 to 30 ×10^6 cells mL^{-1}, respectively, 55 and 95 % of the dye with an initial amount of 10 mg/L was removed from the medium culture after 4 days. The initial concentration of the dye can play an important role in the efficiency of dye removal. The results of the effect of the initial concentration of the AB92 dye on the efficiency of dye removal in *T. obliquus* algae cells for 4 days are shown in Figure 3. According to the results, the efficiency of the dye removal process decreases with increasing initial concentration of the dye. However, the amount of dye removed per unit time increases with increasing initial concentration. These results probably indicate a complex relationship be-

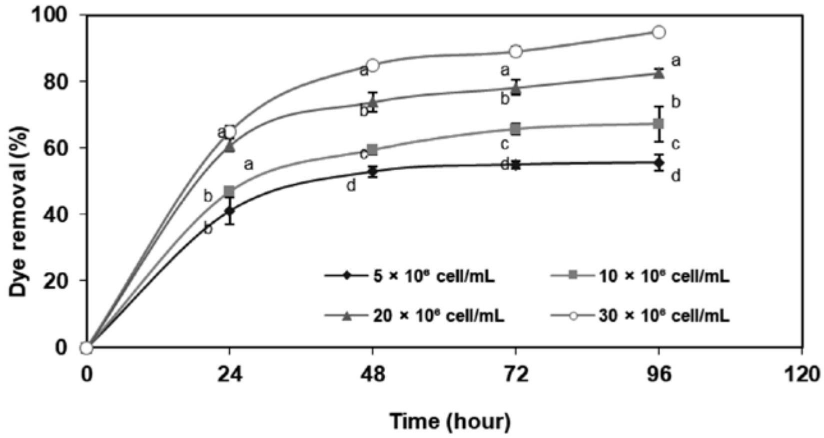

Fig. 2. Effect of cell number on the dye removal efficiency of T. *obliquus* algae for 4 days (data reported as Mean ± SD, T=25 °C, pH=6.5, [AB92]0=10 mg/L; different letters in the statistical analysis for each 24 hours indicate significant differences in dye removal efficiency)

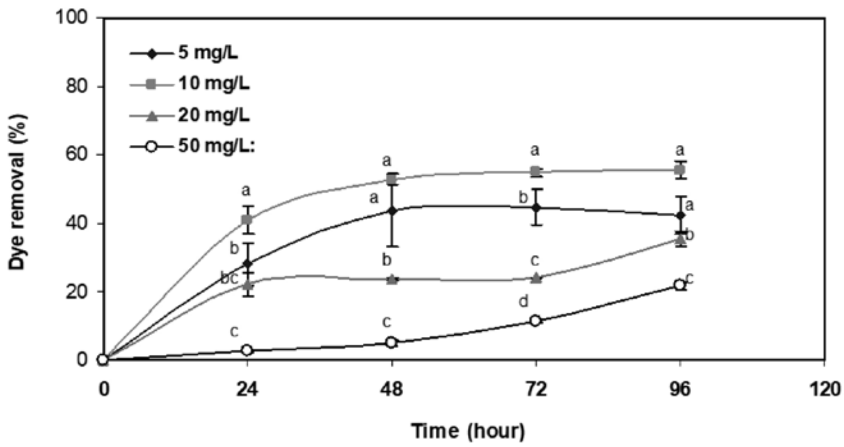

Fig. 3. Effect of initial dye concentration on dye removal efficiency of *T. obliquus* algae for 4 days (data reported as Mean ± SD, T=25 °C, pH=6.5, Cell No.= 5×10^6 cell/mL; different letters in statistical analysis for each 24 hours indicate significant differences in dye removal efficiency)

tween the ability of the algae to decompose and the concentration of the dye.

The results of the effect of different pH levels of the *T. obliquus* algae culture medium on the efficiency of removal of AB92 dye by microalgae for 4 days are shown in Figure 4. The optimal efficiency of removal of AB92 dye was determined after 4 days of algae treatment at pH 6.5.

The results of the effect of different temperatures (25-5 ºC) of the *T. obliquus* algae culture medium on the efficiency of removing the AB92 dye in the microalgae for 4 days are shown in Figure 5. The results show that the efficiency of the dye removal percentage increases with increasing temperature.

Figure 6 shows the results of the evaluation of the repeated removal of dye (10 mg/L) by the same algal mass with an initial cell count of 5 × 10^6 alga during 4 consecutive trial pe-

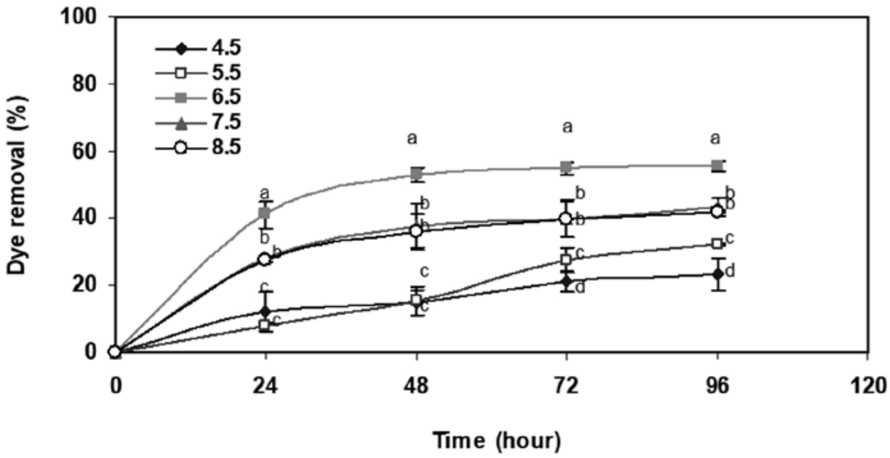

Fig. 4. Effect of different pH on the dye removal efficiency of *T. obliquus* algae for 4 days (data reported as Mean ± SD, T=25 ºC, Cell No. = 5×10^6 cell/mL, $[AB92]_0$=10 mg/L; different letters in the statistical analysis for each 24 hours indicate significant differences in dye removal efficiency)

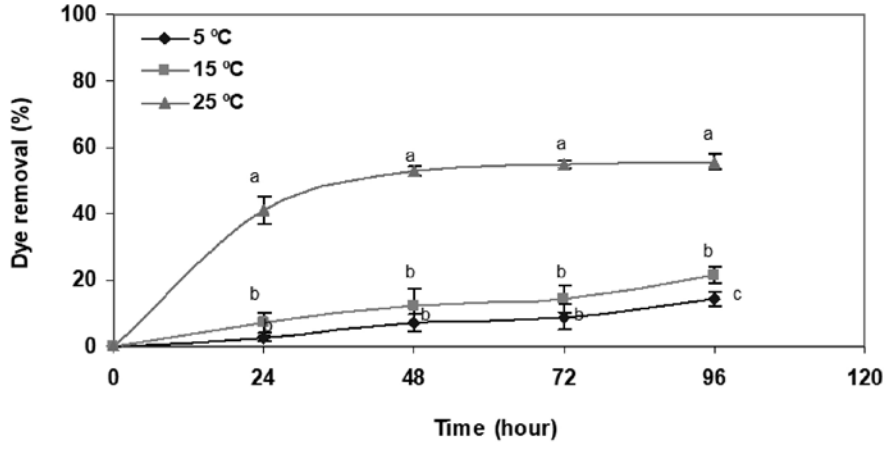

Fig. 5. Effect of different temperatures on the dye removal efficiency of *T. obliquus* algae for 4 days (data reported based on Mean ± SD, pH=6.5, Cell No. = 5×10^6 cell/mL, $[AB92]_0$=10 mg/L; different letters in the statistical analysis for each 24 hours indicate significant differences in dye removal efficiency)

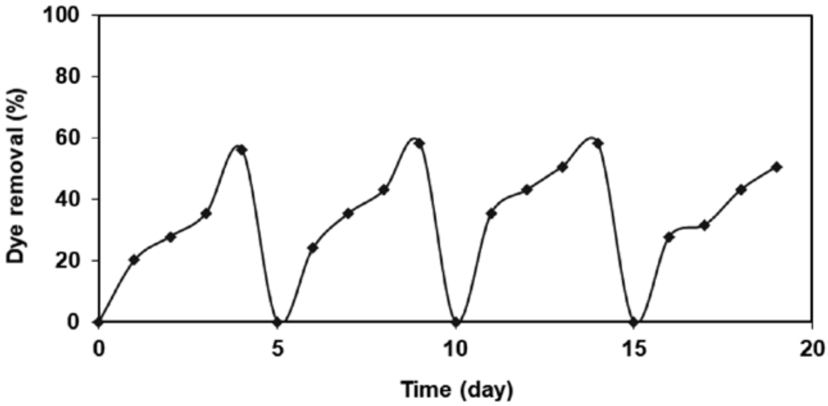

Fig. 6. Effect of successive iterations on the dye removal efficiency of AB92 in *T. obliquus* algae for 4 days (T=25 ºC, pH=6.5, Cell No. = 5×10^6 cell/mL, $[AB92]_0$=10 mg/L; average data reported per 24 hours)

riods. In this study, sampling was performed at intervals of every 24 hours, and the percentage of removal of this material was calculated. This process was repeated 4 times. According to Figure 5, the ability of the algal mass to repeatedly purify the dye was confirmed. The acceptable ability of the single algal mass to repeatedly remove the dye indicates that the biodegradation process is one of the main mechanisms involved in the removal of AB92 by algae.

Discussion

This study confirms the potential of *T. obliquus* as an effective bioremediation agent for synthetic dyes such as AB92. According to the results, the dye removal efficiency of this algae is significantly affected by environmental factors. The efficiency of the dye removal percentage increases with increasing initial algae number. According to the results of Torbati, 2019, the number of initial cells in the test medium also played a positive role in the efficiency of dye removal. It seems that with an increase in the number of algae cells in the medium, the number of pollutant biosorption sites increases, and subsequently, the pollutant removal rate increases (Ayele et al., 2021). Furthermore, according to the literature review, it has been determined that a threefold increase in the algal biomass of *Caulerpa scalpelliformis* and *Pithophora* sp. has resulted in a 60% and 33% increase in the removal efficiency of cationic dye, respectively (Aravindhan et al., 2007; Kumar et al., 2006).

The pH parameter is one of the most important factors affecting the efficiency of biosorption of dyes by algae (El-Naggar et al., 2018). In addition, the pH of the environment can affect the solubility of some dyes as well as the interaction between dye molecules and algae. The cell surface charge of algae depends on the pH of the environment due to the presence of functional groups such as carboxyl, hydroxyl, amine, etc. For example, at acidic pH, the surface of algae has a higher positive charge and is favorable for the absorption of anionic dyes (Sun et al., 2019). According to the literature review, the optimal pH for the growth of most microalgae has been determined to be in the neutral range for the genus *Scenedesmus* (El-Sheekh et al., 2017). Based on our results, the optimal pH for the removal of AB92 is also in this range. This pH is probably ideal for the balance between algal health and dye absorption capacity. Also, the AB92 dye is an anionic dye compound, and the pH that is optimal for algal growth and activity, and also creates a more positive charge on the algal surface, which can be determined to be optimal for the absorption of this dye (Ayele et al., 2021).

Khataee et al. (2012) noted that as temperatures drop, the movement and absorption of water decline, and as a result of the reduced permeability of the algal membranes, the uptake of solvent molecules in water decreases, leading to a reduction in the effectiveness of the absorption process. In this study, temperatures higher than ambient temperature were not investigated due to the biological nature of the adsorbent and the negative role of high temperatures in the structure and activity of algae. The negative role of high temperatures in the bioremediation process has also been confirmed in previous reports. It has been found that the absorption of Golden Yellow C-2g dye in *C. scalpelliformis* algae decreases significantly with an increase in temperature from 20 to 60 ºC (Aravindhan et al., 2007). The viscosity of the dye-containing solution decreases, and its absorption by the biosorbent and subsequent removal at temperatures exceeding ambient levels is reduced. It is likely that at high temperatures, the rate of dye absorption decreases due to changes in the active sites of the biosorbent (Ayele et al., 2021).

This study confirms the effect of temperature (up to ambient temperature), while the efficiency of the removal process decreases with increasing initial dye concentration. Furthermore, the optimum pH for achieving the highest dye removal percentage was determined at pH 6.5. The integration of algal bioremediation strategies can play an important role in sustainable environmental management practices and, simultanously provide complementary benefits for the economic perspective through the utilization of biomass. These applications can help reduce the negative impacts of industrial pollution on the environment and lead to the development of sustainable and environmentally friendly solutions for the treatment of industrial wastewater.

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