

## Original Article

# Aspects of the growth of *Microcystis aeruginosa* on dyes and its bioremediation potential in textile effluents

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**Abstract:** Given the textile sector's intense economic activity, its effluents are known to contribute to water quality degradation, with ecological and ecotoxicological implications. This study aimed to evaluate the influence of pure textile dyes (tartrazine yellow and indigo blue) on the growth of the cyanobacterium species *Microcystis aeruginosa* through direct and indirect methods. Furthermore, it aimed to verify whether this species has bioremediation potential in effluents contaminated with dyes through ecotoxicological evaluation and color reduction. Therefore, we added an inoculum of  $3 \times 10^3$  cells/mL of *M. aeruginosa* to a concentration of 500 mg/L of each pure dye in solutions with WC medium. They remained in direct and indirect light for seven days, and the number of cells was evaluated over 168 hours. Then, we collected two types of effluents representative of the dyes studied (jeans factory blue dye and carpet factory yellow dye), which remained raw and diluted by 50%. We added an inoculum of  $8.64 \times 10^3 \pm 93$  cells/mL of this cyanobacterium to each effluent. Moreover, we added a control without inoculum for each condition. Aeration remained constant for 25 days. We conducted ecotoxicological evaluations of the effluents (before and after 25 days of bioremediation) through seven-day assays with the larvae of *Danio rerio*, verifying survival and growth parameters. The results showed that the cyanobacterium grew significantly in direct contact with the tartrazine yellow dye ( $1.49 \times 10^5 \pm 1910$  cells/mL). However, growth was reduced with both dyes in indirect contact, and there was total inhibition with indigo blue in direct contact, significantly differing from the control. After 25 days, the number of cells in the effluents diluted by 50% decreased compared to the inoculated amount. Considering the color reduction in the carpet factory effluent (100%), the presence of cells enabled a 43% reduction, and in the only aerated condition, 34.6% decolorization. Thus, considering the greater color degradation in the presence of cyanobacteria in this effluent, we believe that cell growth and active metabolism occurred before the 25 days tested. The color removal percentages in the presence of cells did not reduce toxicity. Therefore, we concluded that *M. aeruginosa* has the potential to grow in high concentrations of tartrazine yellow (500 mg/L).

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## Introduction

Aquatic environments are the targets of diffuse and point sources of pollution that compromise the multiple uses of these resources. Point source pollution is characterized by directly discharging pollutants into the water body. It is easy to see and quantify. Main examples include domestic and industrial effluent discharges. While domestic effluents stand out for their microbiological problems, industrial effluents are characterized by the large

quantity and diversity of organic, inorganic, natural, and synthetic contaminants. These components result from the processing of raw materials, generating by-products that are often highly complex and ecotoxic. These residues can persist in aquatic environments, harming the viability of organisms in the short or long term, considering the concentrations of contaminants released and the length of time the biota is exposed to them (Merten and Minela, 2002; Silva and Fracacio, 2021).

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In the Brazilian economy, the textile sector generates large-scale employment and large volumes of production and exports (Lopes Fujita and Jorente, 2015). However, the lack of treatment, or its inadequacy, can cause severe environmental contamination problems (Kunz et al., 2002). In this context, one of the main active ingredients in textile effluents that can impact water quality includes the dyes used to dye textile fibers. These dyes are generally resistant to biological treatment, which implies releasing highly colored liquid waste. These effluents also contain chemical auxiliaries that make treatment difficult. Estimates point to approximately 20-30% of the dye load being released in dye waste during textile processing (Silva et al., 2012; Sözen et al., 2020).

The literature reports toxic effects on reproduction, cytotoxic and teratogenic effects, and biochemical alterations for the species *Danio rerio* when exposed to the textile dyes tartrazine yellow (50 mg/L) and disperse red (1 mg/L). In addition to the direct interaction with biological systems, the dye in the water compromises the penetration of light and, consequently, the photosynthetic process of the producing organisms (Ventura-Camargo and Marin-Morales, 2013; Meireles et al., 2018; Silva and Fracacio, 2021). According to Pagga and Taeger (1994), in conventional aerobic sewage treatment plants, most textile dyes are not degraded by bacteria. Therefore, around 40 to 80% of the dyes are physically adsorbed to the sewage sludge.

The textile industry uses activated sludge to remove high effluent COD levels. However, it alone cannot treat highly colored waste (Holkar et al., 2016; Sözen et al., 2020). However, the current need does not only require technologies for color removal. It also involves producing reusable water, mineralizing aromatic compounds or recovering dyes, removing toxicity, not producing toxic sludge, or possibly not producing sludge (Holkar et al., 2016).

Conventional treatment methods (aerobic-anaerobic combination) manage to decolorize a wide variety of dye types, with a removal percentage of over 76%. They are cheap methods and do not form

foam. However, they do not eliminate all the dye particles, forming methane and hydrogen sulfide as potentially toxic by-products. Moreover, they are inflexible methods, requiring a large area of land to produce sludge and taking a long time (Katheresan et al., 2018).

Furthermore, according to the literature, the concentration of dyes such as indigo blue, usually found in textile effluents, ranges from 300 to 740 mg/L (Conceição et al., 2013; Couto et al., 2018). According to Beach et al. (2011), the approximate concentration of the yellow tartrazine dye in fabric dyeing baths is around 16,509 mg/L. Nevertheless, this concentration decreases after manufacturing, generating effluents of around 500 mg/L (information obtained through personal communication with Dynatech®). Furthermore, according to Albuquerque (2009), the concentration of dyes corresponding to 100 mg/L<sup>-1</sup> is common in textile effluents.

Therefore, degrading these dyes, constantly entering the receiving water bodies, becomes essential. Among the possible treatments, bioremediation is a natural process in which microorganisms detoxify or remove pollutants. It is a promising method for removing and degrading many environmental contaminants, such as oil, dyes, insecticides, and herbicides (Raghukumar et al., 2001; Forlani et al., 2008; Koul and Fulekar, 2013; Dellamatrice et al., 2017). According to the literature, there are several studies evaluating the degradation of dyes by cyanobacteria (Jinqi and Houtian, 1992; Kalavathi et al., 2001; Shah et al., 2001; Vijayakumar and Manoharan, 2012; Henciya et al., 2013; Dellamatrice et al., 2017).

Bioremediation with cyanobacteria is a natural process in which microorganisms use polluting compounds as carbon sources in their metabolism, transforming them into substances with little or no toxicity, such as carbon dioxide (CO<sub>2</sub>), water, biomass, and mineral salts. Bioremediation with cyanobacteria offers a better treatment solution than conventional methods. It reduces the environmental impact due to its CO<sub>2</sub> capacity and is more economical than using other groups of microorganisms (Idi et al.,

2015). Thus, considering the economic and environmental benefits, cyanobacteria, which can grow in environments with wide environmental variation (excess nutrients and low oxygen concentrations), are highly recommended as an alternative in the bioremediation of dyes and textile effluents.

Cyanobacteria species, such as the genera *Oscillatoria*, *Plectonema terebrans*, *Phormidium tenue*, and *Microcystis aeruginosa*, commonly found in aquatic environments, have shown potential for bioremediation of paper mill effluents, oil spills (Raghukumar et al., 2001; Cohen, 2002; Burger et al., 2021), and dye industry effluents (Vijayakumar and Manoharan, 2012). Among the dominant cyanobacteria in Brazil, the genus *Microcystis* stands out (Soares et al., 2013). Its proliferations are identified practically everywhere (Zurawell et al., 2005; Harke et al., 2016). *Microcystis aeruginosa* is frequently reported in tropical Asia, Africa, and South and Central America (Mowe et al., 2015). Thus, this study aimed to evaluate the potential decolorization of different textile effluents by a species of cyanobacteria, *Microcystis aeruginosa*. This species is resistant and dominant in many ecosystems and has yet to be reported in the literature for this purpose. We used an additional approach to the toxic effects of these cyanobacteria on the survival of *Danio rerio* larvae exposed to ecotoxicological tests before and after the decolorization treatment.

## Materials and Methods

**Cultivation of cyanobacteria:** The cyanobacterium *Microcystis aeruginosa* (Kützing) was isolated from the Barra Bonita reservoir (Barra Bonita, São Paulo) and kept in axenic cultures in the freshwater microalgae culture collection of the Phycology Laboratory of the Botany Department of the Federal University of São Carlos (WDCM: UFSCar CC 835). For the tests, the strain was grown in WC medium (Guillard and Lorenzen, 1972), pH 7, in 500 mL flasks following the recommendations of ABNT NBR 12648 (2011). Cell growth was analyzed by counting in Neubauer chambers under a Carl Zeiss Axiostar Plus

optical microscope.

Analysis of the cell growth of cyanobacteria exposed to pure dye standards: To assess the growth potential of cyanobacteria, we carried out tests using a concentration of 500 mg/L for the synthetic textile dye indigo blue, 95% pure (Sigma Aldrich®), and for the tartrazine azo textile dye (100% pure standard), provided by Dynatech®.

Due to the insolubility of the indigo blue dye, we prepared a stock solution of the dye in a 1000 mL glass flask, to which we added 0.575 mg of the dye, 1.5 mg of sodium dithionite, and 500 mL of WC medium at pH 9 (adjusted with 0.1 N sodium hydroxide solution). The flask was autoclaved at 121°C for 15 min, adapting the methodologies of Manu and Chauhari (2003) and Meksi et al. (2012) so that the dye was reduced to its leucoindigo (soluble) form in basic solution. Then, we added the indigo dye stock solution and WC medium at pH 7 to 40 mL cell culture flasks, keeping the concentration at 500 mg/L.

We prepared a stock solution with 2,500 mg of tartrazine and 500 mL of previously autoclaved WC medium at pH 7 for the tartrazine yellow dye. We added it to 40 mL cell culture flasks at a 500 mg/L concentration.

Each treatment was done in triplicate with three replicates: control (only 40 mL of WC medium + inoculum), indigo blue dye, and tartrazine dye. We inoculated the initial concentration of  $3 \times 10^3$  cells/mL of cyanobacteria separately in each flask in a laminar flow chamber. The flasks were kept at  $23 \pm 1^\circ\text{C}$ , with constant illumination of approximately 108  $\mu\text{mol}$  photons (Quantameter QSL-100, Biospherical Instruments) on a light box for seven days (Fig. 1). Every 24 hours, each flask was shaken, and the positions were reversed. Samples for counting were taken at the following time intervals: 24, 72, 120, and 168 hours.

**Evaluation of the influence of dye coloration on cell growth:** To assess the impact of the coloring of textile effluents on photosynthesis and cell growth, we carried out tests without direct contact with the dyes. Therefore, we added the previously dissolved dyes at a concentration of 500 mg/L separately to 40 mL cell

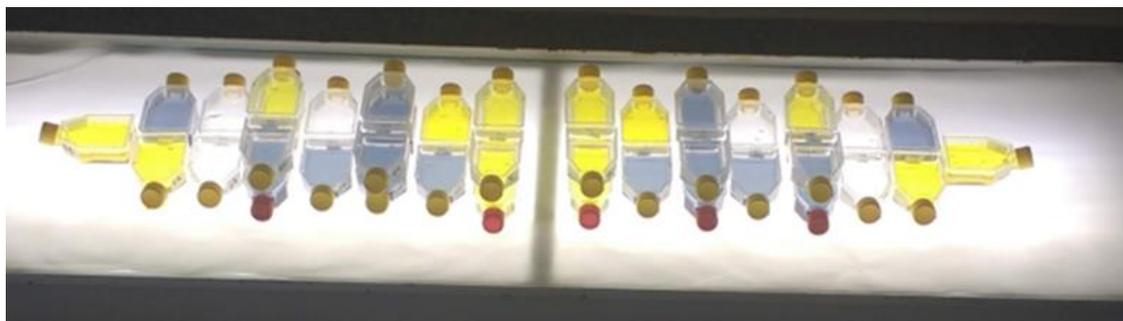


Figure 1. Image of the tests with the pure dye standards through direct and indirect contact (overlap). Flasks were placed on a light box with tartrazine yellow dye, indigo blue, and control groups (transparent).

culture flasks. As shown in Figure 2, these flasks were overlapped by other flasks containing WC medium and the inoculum of each cyanobacterium. We followed the same procedures described in the paragraph above.

**Chemical characterization of raw effluents:** The textile effluents were collected from a jeans factory (Araguari, Minas Gerais State, Brazil) and a carpet factory (São Carlos, São Paulo State, Brazil). Metals were determined by plasma emission spectrometry: the inductively coupled plasma method (ICP-OES) according to method 3120B (APHA, 2012). The samples were prepared following EPA US 3010A (1992). Following the recommendations of the Standard Methods for the Examination of Water and Wastewater (APHA, 2012), we determined the Biochemical Oxygen Demand (BOD) using method 5210 B, Chemical Oxygen Demand (COD) according to method 5220 D, total nitrogen concentrations using method 4500 A, nitrate using method 4110 B, and nitrite using method 4500 B.

**Bioremediation of textile effluents:** The bioremediation experiments were done in 1.5 L glass bottles with raw effluent samples diluted 50% with ultrapure water. The test volume was 1 L. It contained 950 ml of the effluent (raw or diluted) and 50 mL of WC medium. We added the inoculum of  $8.64 \times 10^3 \pm 93$  cells/mL of *M. aeruginosa* in each experimental condition mentioned above. At the same time, we filled transparent 1.5L flasks with 1L of effluent (raw and diluted by 50% separately) to assess degradation in the absence of cyanobacteria. Both the bioremediation and natural degradation assays were kept at  $25 \pm 2^\circ\text{C}$ , 12 hours of light (600 lux), and

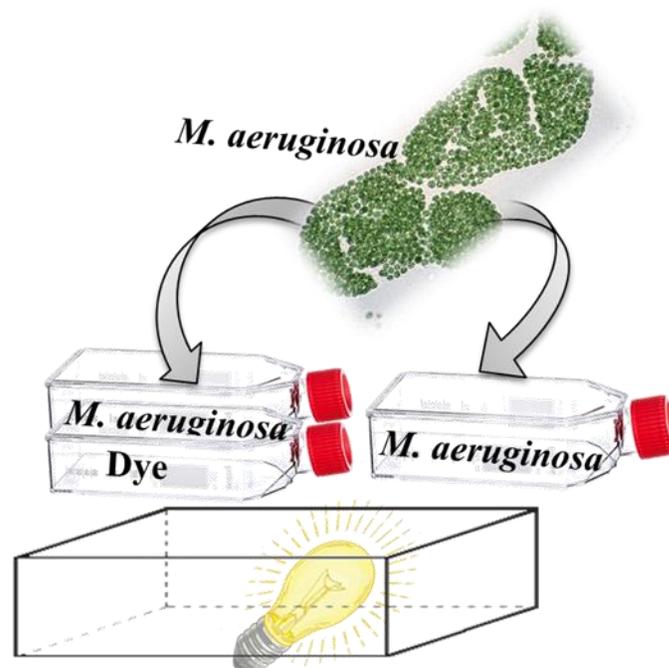


Figure 2. Experimental design to evaluate the cell growth of *Microcystis aeruginosa* exposed to pure dye standards indirectly.

constant aeration.

We evaluated decolorization in both experiments (bioremediation with cyanobacteria and natural degradation without the microorganism) through spectrophotometry in the UV-visible region (Hach model DR3900 spectrophotometer) every 48 hours for 25 days. We used the bioremediation times reported by Parikh and Madamwar (2005) and Vijayakumar and Manoharan (2012), with estimated degradation times of 26 and 30 days, respectively. We used the 425 nm wavelength for the carpet factory effluent and the 667 nm wavelength for the jeans factory effluent since these are the bands with the highest absorbance.

**Ecotoxicological tests before and after**

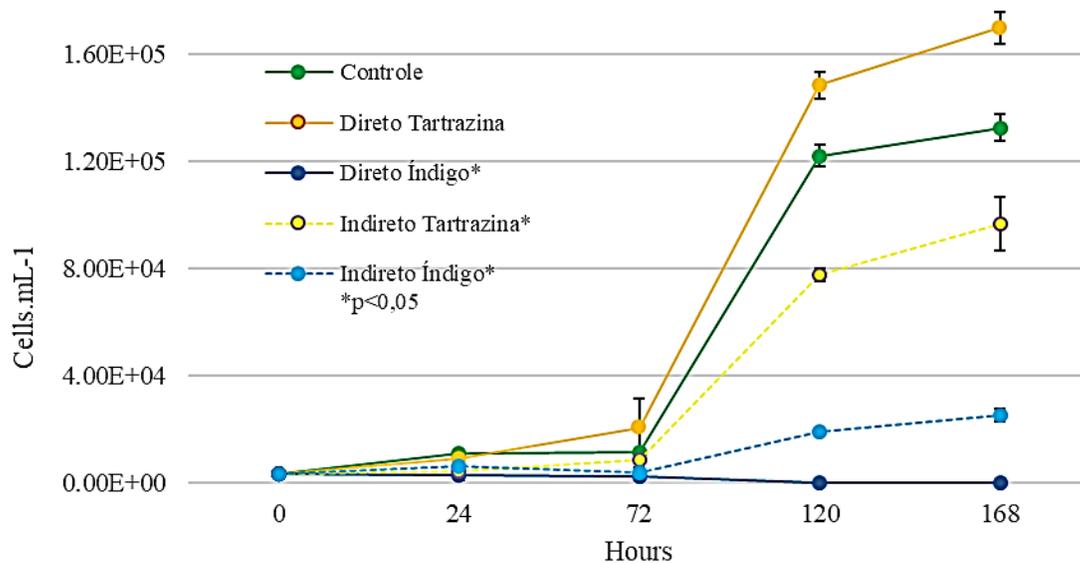


Figure 4. Growth curves of *Microcystis aeruginosa* exposed directly and indirectly to the dyes, constructed using averages over 168h.

**bioremediation:** We performed the ecotoxicological tests by exposing larvae of *D. rerio* to the raw effluent (100%) and to 5 dilutions (75, 50, 25, 12.5, and 6.25%) prepared with culture water. This water was prepared from the water supply previously filtered with activated carbon filters and reconstituted with macro and micronutrients, following the recommendations of ABNT NBR 15499 (2016). The Control was maintained only with culture water.

For each experimental condition, we prepared four replicates, each containing ten larvae ( $n = 40$ ) aged between 0 and 24 hours. The larvae were exposed to the effluents described above for 168 hours, and the survival and growth endpoints were evaluated. The hardness, conductivity, pH, and dissolved oxygen (DO) parameters were monitored daily, and the test solution was renewed every 48 hours.

The results were expressed in CL15 (168h) (the nominal concentration of the chemical agent that causes lethality to 15% of the larvae after 168 hours of exposure). The estimated chronic value ( $V_{cest}$  (168h)), defined as the nominal concentration of the chemical agent that has no significant effect on larval survival and growth after 168 hours of exposure, was calculated by multiplying CL15 (168h) by the correction factor 0.3 (ABNT, 2016). According to Bertolotti (2009), in short-term chronic assays with cyprinids, multiplication by a 0.3 factor is suitable for

extrapolating the effects on growth based on the survival of *D. rerio* larvae.

The bioremediation effluents, raw and diluted to 50% with and without the cyanobacteria, were used to carry out the toxicity tests with larvae. The larval survival results were averaged and compared with those of the control group (culture water only).

**Statistical treatment:** After ecotoxicological tests with raw and diluted effluents (75, 50, 25, 12.5, and 6.25%), the CL15 (168h) was calculated using the linear interpolation method available in the ICPIN statistical software (Norberg-King, 1993). Fisher's exact test verified the significance of the survival assessments. We also used the Mann-Whitney test to compare the differences in the means of the growth curves of *M. aeruginosa*, both using the Toxstat 3.5 software (West and Gulley, 1995).

## Results and Discussions

Analysis of the growth of *Microcystis aeruginosa* exposed to pure dye standards: After 120 hours of growth; the pure tartrazine standard showed  $1.49 \times 10^5 \pm 1910$  cells/mL in direct contact, a greater number of cells when compared to the control group with  $1.22 \times 10^5 \pm 93$  cells/mL. However, when in indirect contact with the dye, there was less growth,  $7.78 \times 10^4 \pm 2410$  cells/mL, with a significant difference compared to the same control group ( $P \leq 0.05$ ; Fig. 3). This result

Table 1. Results of the analysis of metals, total nitrogen, nitrate, nitrite, BOD, and COD in the raw effluents (LQ - Limit of Quantification).

Parameters (mg.L <sup>-1</sup> )	LQ	Jeans factory	Carpet factory	CL(I)15 (168h)*
Aluminum	0.01	1.73	0.150	-
Antimony	0.005	<0.005	0.263	-
Arsenic	0.01	<0.01	0.0192	-
Barium	0.01	0.023	0.0479	-
Beryllium	0.01	<0.01	<0.01	-
Bismuth	0.01	<0.01	<0.01	-
Boron	0.01	0.058	0.0112	-
Cadmium	0.001	< 0.001	< 0.001	0.054
Calcium	0.5	6.48	13.6	-
Lead	0.01	<0.01	<0.01	-
Cobalt	0.01	<0.01	<0.01	-
Copper	0.005	0.0300	0.0474	0.030
Chrome	0.01	<0.01	<0.01	9.7
Tin	0.01	<0.01	<0.01	-
Strontium	0.01	0.103	0.0638	-
Iron	0.01	0.737	0.657	-
Phosphorus	0.01	9.04	3.29	-
Lithium	0.01	<0.01	0.0835	-
Magnesium	0.5	1.96	1.96	-
Manganese	0.01	1.32	0.0885	-
Molybdenum	0.01	<0.01	<0.01	-
Nickel	0.01	< 0.01	<0.01	5.9
Potassium	0.5	9.27	8.39	-
Silver	0.01	<0.01	<0.01	0.021
Selenium	0.008	<0.008	<0.008	-
Silicon	0.5	7.52	13.8	-
Sodium	0.5	977	30.9	2
Thallium	0.01	<0.01	<0.01	-
Titanium	0.01	<0.01	<0.01	-
Uranium	0.01	<0.01	<0.01	-
Vanadium	0.01	<0.01	<0.01	-
Zinc	0.01	0.248	0.183	3.6
Total Nitrogen	1	6.46	2.27	0.84
Nitrate (as N)	5	<5	<5	-
Nitrite (as N)	0.2	0.81	<0.2	-
BOD	90	138	488	-
COD	100	550	1240	-

suggests that the strong color of the tartrazine dye inhibited cell growth. However, when in direct contact, the opposite occurred, probably due to the strong presence of nitrogen, which favors the growth of *M. aeruginosa* (Paerl and Otten, 2013).

Cell growth decreased directly and indirectly when exposed to the pure indigo blue standard (Fig. 3). At 120h of exposure to direct contact, the pure indigo blue standard completely inhibited cell growth. However, indirect contact resulted in  $1.91 \times 10^4 \pm 1000$  cells/mL, differing significantly from the control group ( $1.22 \times 10^5 \pm 93$  cells/mL). Dellamatrice et al. (2017) reported 91% removal of the indigo dye at 200

mg/mL by another cyanobacterium species, *Phormidium valderianum*, after 14 days of incubation. Nonetheless, the authors did not report the concentration of cells in the initial inoculum and whether the dye had been solubilized before the tests since the dye showed a pronounced darkening of the solution when solubilized.

**Characterization of raw effluents:** The amount of total nitrogen in the jeans factory effluent was higher ( $6.46 \text{ mg.L}^{-1}$ ) than in the carpet factory effluent ( $2.27 \text{ mg.L}^{-1}$ ) (Table 1). The opposite occurred with BOD and COD. The carpet factory effluent showed the highest concentrations (Table 1). According to Yaseen

and Scholz (2019), there is a wide variety of textile factories, which makes it challenging to estimate typical concentration ranges for parameters such as metals, nutrients, and organic and inorganic compounds.

Thus, it is worth noting that not every microorganism will be efficient at bioremediating and growing in any textile effluent due to its metabolic requirements. Therefore, although cyanobacteria can proliferate under adverse conditions, they may not be adapted to the many effluents generated. It corroborates the bioremediation data obtained in this study, in which the cell growth of *M. aeruginosa* was observed in direct contact with the tartrazine dye. However, the same behavior was not observed in the effluents from the carpet factory, which also included the same dye.

**Bioremediation of textile effluents:** The effluent from the jeans factory showed an intense blue color, attributed to indigo dyes. Regarding removing this color over 25 days, the jeans factory effluent, 100% aerated only, showed 73.6% color removal. Meanwhile, the same effluent (100%) with *M. aeruginosa* showed only 48% decolorization. Both treatments showed the formation of precipitates, probably because a fraction of the indigo dyes returned to their original insoluble form. According to Manu and Chauhari (2003), dyes become soluble after being reduced in alkaline solution. However, it is worth noting that this study used effluents generated after the manufacturing process. Therefore, it is a much more complex matrix than indigo dye as the only active ingredient. Dellamatrice et al. (2017), after a shorter bioremediation time of textile effluent, 14 days of treatment, obtained 23.8% decolorization with *Anabaena* sp. and 28.4% with *Phormidium* sp.

Regarding the carpet factory effluent, whose visual color was yellow due to the presence of the tartrazine azo dye (personal communication at the carpet factory), the removal rate of this color over the 25 days for the aerated effluent was only 34.6 and 43.5% in the raw effluent (100%) in the presence of *M. aeruginosa*.

None of the pure dye standards showed discoloration, contrary to what Parikh and Madamwar

(2005) reported. Although they observed that the synthesis of chlorophyll-a by the cyanobacteria *Gloeocapsa pleurocapsoides*, *Phormidium ceylanicum*, and *Chroococcus minutes* was strongly inhibited by cyclic azo dyes, they verified the degradation of the dyes through visible spectroscopy.

The color removal efficiency of both the dyes and the effluents evaluated here was lower than that reported in the literature for different pure dyes. As Shah et al. (2001) observed, the cyanobacterium *Ph. valderianum* achieved 90% efficiency in decolorizing the Acid Red, Acid Red 119, and Direct Black 155 dyes.

Kalavathi et al. (2001) state that the color removal mechanism is due to the production of molecular oxygen, hydroxyl anions, and hydrogen peroxide during photosynthesis. The use of cyanobacteria in the degradation or accumulation of xenobiotic compounds has been frequently reported. Examples include the removal of potentially toxic metals (Cd, Mn, Cr, and Ni) and dyes (Asthana et al., 1995; Khattar et al., 1999; Matsunaga et al., 1999; Mohamed, 2001; Dellamatrice et al., 2017).

The initial concentration of *M. aeruginosa* in the effluents was  $4.1 \times 10^2$  cells/mL. There was a significant reduction in this cell concentration after 25 days. In the 50% diluted effluents from the jeans factory, this concentration was  $1.32 \times 10^2$  cells/mL. In the carpet factory, it was  $2.47 \times 10^2$  cells/mL. This result suggests that the effluents showed characteristics that inhibited cell division for *M. aeruginosa*, despite several studies reporting the ability of cyanobacteria to grow in pharmaceutical, textile, industrial (Vijayakumar et al., 2005; Dubey et al., 2011; Burger et al., 2021), pesticides (El-Bestawy et al., 2007), and tannery effluents (Nanda et al., 2010).

The genus *Microcystis* depends on nitrogen and phosphorus (macronutrients) for its growth. Thus, the concentrations of nutrients and other metals in the effluents in this study (Table 1) tend to favor its development, as observed in the growth curve of *M. aeruginosa* in direct contact with the tartrazine dye (Fig. 3). Paerl and Otten (2013) relate excessive N<sub>2</sub>

Table 2. Survival of *Danio rerio* larvae exposed for 168h to effluents after 25 days of bioremediation with *Microcystis aeruginosa* (MIC) and aerated effluents only.

	Jeans factory			
	Control	100%	100% + MIC	50% + MIC
Survival 168h(%)	100	80	70*	80
pH	8.49±0.44	8.38±0.41	8.08±0.33	8.13±0.21
Conductivity (mS/cm)	0.15±0.01	2.78±0.94	4.94±0.39	2.53±0.37
DO (mg/L)	5.35±0.13	5.91±0.71	5.21±0.52	5.24±0.19
	Carpet factory			
	Control	100%	100% + MIC	50% + MIC
Survival 168h(%)	100	60*	60*	90
pH	8.79±0.08	8.16±0.78	7.82±0.17	7.57±0.07
Conductivity (mS/cm)	0.158±0.01	0.210±0.04	0.210±0.03	0.382±0.03
DO (mg/L)	5.54±0.56	5.26±0.55	5.14±0.30	5.37±0.4

\* $P \leq 0.05$ 

loads to the growing dominance and geographical expansion of genera of non-N<sub>2</sub>-fixing cyanobacteria, specifically those of the genus *Microcystis*.

Phosphorus can also ensure cell maintenance since this genus can persist even under nitrogen-limited conditions. According to Paerl et al. (2014), even under limited nitrogen and phosphorus conditions, the genus *Microcystis* maintained dominance over the other genera analyzed in a hypertrophic lake in China.

In this sense, reducing the number of cells may not be related to nitrogen and phosphorus concentrations in the raw effluent. It is probably related to the reduction in transparency and consequent impairment of the passage of light in the ecotoxicological tests. The absence of proliferation in highly colored effluents from paper mills has also been associated with limiting light penetration into the water column and restricting photosynthetic capacity (Mahmood and Paice, 2006; Burger et al., 2021).

Polyak et al. (2013) observed toxicity and growth inhibition of two strains of *M. aeruginosa* with 50 µg.L<sup>-1</sup> of copper and 250 µg.L<sup>-1</sup> of zinc. Although zinc is considered an essential element for biological metabolism, there is evidence of negative morphological and physiological effects on exposed organisms and their offspring (Schamphelaere et al., 2007; Carvalho et al., 2017).

In this study, the copper concentrations detected may also have reduced cell numbers, 30 and 47.4 µg.L<sup>-1</sup> of copper for the jeans and carpet factories, respectively. Another hypothesis is that the excess sodium in both effluents or the metals' interaction

could harm growth by reducing metabolism.

**Ecotoxicological tests before and after bioremediation:** Lethality tests with larvae for the jeans factory effluent resulted in CL15(168h) = 7.19% and V<sub>cest</sub> (168h) = 2.16 (v/v) of the raw effluent, indicating a significant potential for toxicity to both the organism's survival and growth. We observed that 25, 50, 75, and 100% of the jeans factory's raw effluent caused 100% mortality within 48 hours of exposure. Similarly, the raw effluent from the carpet factory showed CL15(168h) = 11.88% and V<sub>cest</sub> (168h) = 3.56% of the raw effluent. Notably, the copper, nitrogen, and especially sodium concentrations in the raw effluent exceeded the CL15 values for the larvae (Table 1). In other words, these elements alone have ecotoxicity potential (Bertoletti, 2009). The CL15 and V<sub>cest</sub> obtained here for both effluents ratify the potential ecotoxicity of untreated textile effluents for aquatic biota.

After 25 days of testing, the jeans factory effluent, which was only aerated, showed no toxicity to the larvae, with 80% of the exposed organisms surviving (Table 2). In the effluent with *M. aeruginosa*, 70% of the larvae survived, differing significantly from the control with culture water. In the carpet factory effluent, toxicity persisted, as only 60% of the larvae survived both in the aerated effluent and with *M. aeruginosa*, differing significantly from the control group (culture water) (Table 2). The effluent diluted by 50% had 80% larval survival for aerated effluent and 90% with *M. aeruginosa* (Table 2). It is worth noting that the cyanobacteria did not increase the

ecotoxicity of the effluents.

Dellamatrice et al. (2017) observed a reduction in the toxicity of textile effluents and fewer morphological changes in the *Hydra attenuata* organisms induced by three strains of cyanobacteria, concluding that they were able to reduce the effluent's pollutant load. Vijayakumar et al. (2005) confirmed these results. They observed the decolorization of effluents from a dye industry, the complete removal of nitrites and phosphates, and the reduction of BOD and COD after 30 days of bioremediation with *Oscillatoria brevis*.

However, although the use of cyanobacteria is viable, it is known that the cyanobacteria in question are potential producers of microcystins (Ferrão-Filho and Kozłowsky-Suzuki, 2011; Moreira et al., 2014; Zastepa et al., 2015). In this sense, analyzing the possible cyanotoxins generated after the process is necessary.

### Conclusion

The growth of the cyanobacterium *M. aeruginosa* was stimulated in direct contact with the pure standard of the tartrazine dye. However, the dye inhibited this behavior. On the other hand, the indigo blue dye compromised cell growth in both direct and indirect contact. After 25 days of bioremediation, the number of cells in the effluent diluted by 50% compared to the initial inoculum was reduced. The coloration of the carpet factory effluent (100%), only aerated, was reduced by 34.6% and by 43% with the presence of cells. Thus, cell growth and active metabolism probably occurred before the 25 days tested, considering the greater degradation of color with the presence of cyanobacteria in the effluent. Ecotoxicological tests with 100% carpet factory effluent revealed persistent ecotoxicity for larvae, with and without *M. aeruginosa*. The bioremediation effluent from the jeans factory also contained ecotoxicity. The cyanobacteria did not increase the effluents' ecotoxicity. Therefore, we conclude that *M. aeruginosa* has the potential to grow in high concentrations of tartrazine yellow (500 mg/L), and shorter times in analyzing cell growth in effluents

should be investigated to better evaluate its bioremediation performance in complex matrices with dyes.

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