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Bioremediation of Tartrazine Dye Using the Green Microalga *Chlorella vulgaris* in Aqueous Systems

Siham Mohaisen Jiheel*, Buthaina Al-Magdamy

Department of Biology, College of Education for Pure Science (Ibin Al-Haitham), University of Baghdad, Baghdad 10053, Iraq

Corresponding Author Email: seham.jheel2202@ihcoedu.uobaghdad.edu.iq

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ABSTRACT

The present study investigated the efficacy of the green microalga *Chlorella vulgaris* in the bioremediation of the carcinogenic synthetic dye tartrazine from aqueous systems. *Chlorella vulgaris*, a unicellular green alga, was obtained from an Iraqi water body, cultivated under controlled laboratory conditions, and exposed to three initial dye concentrations (25, 50, and 100 ppm) over varying contact periods (1, 3, 6, 9, and 12 days). High-performance liquid chromatography (HPLC) analysis was used to determine the dye removal efficacy of *Chlorella vulgaris*. The removal efficiency increased with time but decreased with increasing dye concentration. HPLC results revealed that the highest removal efficiency (100%) was achieved after 9 days at the lowest concentration (25 ppm). The findings indicate that both the initial dye concentration and the exposure duration significantly affect the dye removal performance of *Chlorella vulgaris*. This study highlights the remarkable potential of *Chlorella vulgaris* for the sustainable bioremediation of tartrazine-contaminated water. The alga demonstrated the ability to tolerate and thrive in environments with high dye concentrations, offering an eco-friendly solution for mitigating synthetic dye pollution.

1. INTRODUCTION

Water is an essential element for the existence of human civilization. Water and life are closely intertwined, as humans have historically been drawn to water bodies and have depended on them for various aspects of life [1]. However, rapid population growth and technological advancements have exacerbated global water pollution. One of the major contributing factors is the widespread use of synthetic dyes in various industries, including textiles, food, leather tanning, paints, printing, paper manufacturing, cosmetics, and pharmaceuticals [2]. These industries consume large volumes of water and often discharge untreated or partially treated wastewater into rivers, thereby contributing significantly to water pollution. These pollutants alter the physicochemical characteristics of water, posing serious threats to aquatic ecosystems and their inhabitants, which ultimately impacts human health [3].

Synthetic dyes are complex chemical compounds with carcinogenic, toxic, and mutagenic properties, which accumulate within the food chain [4]. Textile dyes fall under the category of azo dyes, which break down into aromatic amines that can cause cancer and skin sensitization in people [5]. Tartrazine is a synthetic dye with the chemical formula $C_{16}H_9N_4Na_3O_9S_2$, a molecular weight of 534.36 g/mol, and consists of two benzene rings. It is classified as a yellow azo dye and is used as a food additive to enhance flavor and color in foods. Tartrazine is a biologically active substance that is soluble in water [6]. As soon as an artificial food coloring agent is consumed, the body begins to experience a number of detrimental health effects. In addition to heart disease and cancer, studies show that these additives cause respiratory illnesses like asthma, neurological disorders such as attention deficit hyperactivity disorder (ADHD), and metabolic issues like obesity [7]. Cancer research has shown that prolonged exposure to tartrazine induces toxicity in eukaryotic cells, leading to mutations and cancer [8]. Beyond its application in food, tartrazine is also widely used in non-food industries, including pharmaceuticals, cosmetics, soaps, shampoos, stamp dyes, and coloring pens [9].

Algae are a large group of living organisms, most of which are eukaryotic and a few of which are prokaryotic. They play an important role in maintaining the balance of the ecosystem [10, 11]. There are many different types of algae. Microalgae are unicellular microscopic organisms that can perform photosynthesis. They are visible under a microscope and typically range in size from 1 to 400 µm [12]. Protein, carbohydrates, fats, and vitamins are all abundant in algae, and the majority of species exhibit high growth efficiency in contaminated waters [13]. Waste from human activities has a significant impact on how different aquatic organisms, such as algae, behave and are used as indicators of water quality [14]. Most local studies focus on the classification of algae in diverse environments [15-18]. *Chlorella vulgaris* is considered a green unicellular alga that ranges in diameter from 2 to 10 µm. It is characterized by a regular spherical

shape that primarily spreads in freshwater environments, such as rivers and lakes, in addition to moist soil. It has a thick cellular wall and contains chlorophyll a and b pigments, which give it a distinctive green color. *Chlorella vulgaris* is identified based on morphological characteristics using a light microscope [19].

Chlorella vulgaris is an efficient, sustainable, and low-cost remediation agent, outperforming chemical and biological methods. It grows rapidly, tolerates diverse conditions, and removes pollutants via functional groups and enzymatic degradation [19, 20]. Additionally, it yields bioresource-rich biomass [21]. Recent studies emphasize *C. vulgaris* as an effective agent in dye removal via biosorption, bioaccumulation, and enzymatic degradation. Their cell walls, rich in polysaccharides, proteins, and functional groups, aid dye binding and uptake [20, 22]. *Chlorella vulgaris* withstands high pollutant levels through antioxidant defenses that counter oxidative stress from reactive oxygen species [23]. It has shown strong potential in removing azo dyes, including malachite green, tartrazine, and carmoisine [24]. Additionally, assimilated compounds are converted into protein-, lipid-, and carotenoid-rich biomass [25]. Despite these advances, there remains limited work on the efficiency of locally isolated *C. vulgaris* strains in the bioremediation of synthetic dyes such as tartrazine, which this study addresses.

This study is innovative in utilizing a locally isolated strain of *C. vulgaris* from the Tigris River, Iraq, for the bioremediation of the carcinogenic dye tartrazine. This study investigates the adaptive capacity of native microalgae under controlled laboratory conditions, in contrast to previous research utilizing commercial strains. The study methodically assesses the efficacy of tartrazine removal across different concentrations (25–100 ppm) and exposure durations (1–12 days), achieving total elimination at 25 ppm within nine days. A major step forward is the use of high-performance liquid chromatography (HPLC) to accurately measure how much dye has broken down. The study also shows that algae can adapt physiologically, such as by grouping cells together, storing lipids, and becoming more tolerant to oxidative stress. This is a long-term, eco-friendly way to control dye pollution and learn more about how algae-based bioremediation works.

The present study aimed to quantify the removal efficiency of tartrazine by *C. vulgaris* under different initial concentrations (25, 50, and 100 ppm) and contact times (1-12 days) and to elucidate the potential mechanisms involved.

2. MATERIALS AND METHODS

2.1 Material

2.1.1 Source of *Chlorella vulgaris*

Chlorella vulgaris used for the study was obtained from the Iraqi environment, specifically from the Tigris River adjacent to the Textile Factory.

2.2 Methods

2.2.1 Cultivation of *Chlorella vulgaris*

Chlorella vulgaris was cultivated in BG11 culture medium under optimized laboratory conditions with a light intensity of 3000 lux, a photosystem of 8:16 (light/dark), and a temperature of 25 ± 2°C, with daily shaking (Figure 1). The number of algal cells was estimated using the transect method by employing the hemocytometer (red blood cell counting chamber), according to the method described by Vembadi et al. [26]. A drop of the well-mixed sample was placed on the two designated chambers of the hemocytometer and covered with a cover slip. The cells were then counted daily, and the results were expressed as cells × 10^6/mL, based on the following equation:

$$Sample\ volume\ in\ one\ transect\ (mL) = L \times W \times D$$

where, L is the length of the transect, W is the width of the transect, and D is the chamber depth.

$$Number\ of\ transects\ in\ 1\ mL\ of\ sample = \frac{1000}{SV}$$

where, SV is the sample volume in one transect.

The number of cells per milliliter was calculated from the equation below [27]:

$$No.\ of\ cells\ per\ mL = \frac{Total\ counted\ cells - Dilution\ factor \times 10^4}{No.\ of\ counted\ squares}$$

where,

Total counted cells: The total number of cells counted under the microscope.

Dilution factor: If the sample was diluted (for example, 1:10 → factor = 10).

10^4: A constant derived from the known volume of the hemocytometer square (0.1 mm^3).

Number of counted squares: The number of small squares that were counted (usually 4 or 5).

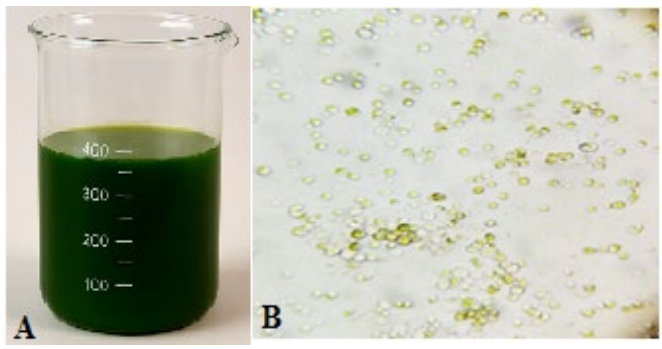

Figure 1. (A) The BG-11 culture medium containing the algal culture in a laboratory flask, (B) Morphology of the locally isolated *Chlorella vulgaris* strain under normal growth conditions (control group, 40x magnification)
The cells exhibit a typical spherical shape and are predominantly dispersed as single cells or small groups.

2.2.2 Bioremediation of tartrazine dye by *Chlorella vulgaris*

The experiment was conducted to evaluate the efficiency of *Chlorella vulgaris* in the sustainable bioremediation of tartrazine dye in water at varying concentrations. Tartrazine was introduced into the medium at concentrations of 0, 25, 50, and 100 ppm before inoculation with the algae. The cultures were grown in 250 mL flasks containing 100 mL of BG-11 medium, supplemented with 30 mL of *Chlorella vulgaris* culture (cell density: 3.0 × 10^8 cells/mL). Algal cultures were incubated under the previously described conditions, and tartrazine removal was assessed using high-performance liquid chromatography (HPLC) on day 12, across five time

points of three-day intervals [28].

2.2.3 HPLC analysis

Tartrazine concentrations were analyzed using an HPLC system (SYKAM, Germany) equipped with a UV-Vis detector. Prior to analysis, culture samples were centrifuged at 5,000 rpm for 10 min to remove algal biomass, and the supernatant was filtered through 0.45 µm syringe filters to eliminate residual particulates. The mobile phase consisted of acetonitrile and 0.1 M phosphate buffer (60:40, v/v), delivered at a flow rate of 1.0 mL/min. Separation was achieved on a C18–ODS column (25 cm × 4.6 mm, 5 µm particle size) maintained at 30°C. An injection volume of 20 µL was used for all samples, and the detection wavelength was set at 254 nm. Each chromatographic run was completed within 10 minutes, with tartrazine eluting at a retention time of approximately 3.8 minutes, identified by comparison with an authentic standard. Chromatograms were recorded and analyzed using ClarityChrom software [29].

2.2.4 Statistical analysis

Data were analyzed using GraphPad Prism with Dunnett's test and two-way analysis of variance (ANOVA). The results were expressed as mean ± standard error of the mean (SEM), and differences were considered statistically significant at $p \leq 0.05$.

3. RESULTS AND DISCUSSION

As shown in Table 1, the experimental results demonstrated a clear increase in the absorbance of *Chlorella vulgaris* during the treatment period, indicating enhanced cell density, chlorophyll content, and photosynthetic activity, which directly influenced the efficiency of tartrazine removal. At a low concentration of 25 ppm, the residual dye concentration decreased from 23.55 ppm on day zero to 7.00 ppm on the sixth day, and reached undetectable levels by the ninth day, corresponding to a removal efficiency of 100% on day nine and complete removal thereafter [30, 31]. This rapid removal is attributed to the high metabolic activity during the exponential growth phase, when cells exhibit optimal enzymatic function and nutrient assimilation, thereby facilitating effective dye uptake and transformation. The correlation between increased absorbance and dye removal suggests that active growth and photosynthetic efficiency are key determinants in the early stage of bioremediation.

Table 1. Residual concentration and removal efficiency of tartrazine by *Chlorella vulgaris*

Time (Day)	Residue Conc. (ppm)			Removal Percentage (%)		
	25	50	100	25	50	100
0	23.55	48.77	97.01	5.80	2.46	2.99
3	18.25	35.08	71.45	27.00	29.84	28.55
6	7.00	21.0	55.7	72.00	58.00	44.30
9	UDL	5.01	17.09	100.00	89.98	82.91
12	UDL	UDL	6.98	100.00	100.00	93.02

UDL: under detection limit.

At an intermediate concentration of 50 ppm, the residual tartrazine concentration decreased more gradually from 48.77 ppm at day zero to 35.08 ppm on day three, and 21.00 ppm on day six, ultimately reaching 5.01 ppm by day nine, with a corresponding removal efficiency of 89.98%. This slower initial removal reflects the time required for cells to adapt to higher pollutant concentrations, triggering biochemical defense mechanisms such as the production of unsaturated fatty acids and antioxidant enzymes to mitigate oxidative stress [32, 33]. The gradual increase in removal over the treatment period highlights the importance of cellular adaptation in overcoming moderate stress conditions, although the decline in absorbance toward the later stage indicates that nutrient depletion and waste accumulation begin to limit growth and photosynthetic activity.

At the highest tested concentration of 100 ppm, the residual dye concentration decreased from 97.01 ppm on day zero to 71.45 ppm on day three, 55.70 ppm on day six, and 17.09 ppm by day nine, corresponding to removal efficiencies of 26.33%, 42.58%, and 82.91%, respectively. Maximum removal efficiency of 93.02% was observed on day twelve (Figure 2). This pattern is attributed to the aggregation of algal cells and their encapsulation in lipid layers, which enhances the adsorption and retention of tartrazine molecules [34]. However, prolonged exposure to high dye concentrations induced cellular stress and cytotoxicity, leading to cell death and a subsequent decline in absorbance, which resulted in a temporary plateau in removal rates [35, 36].

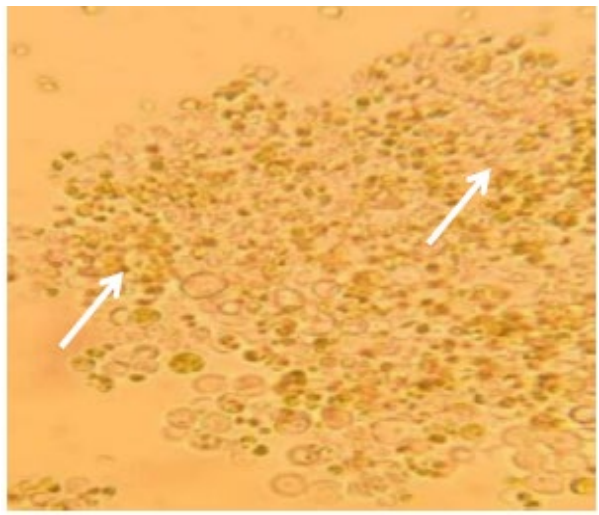

Figure 2. Morphological changes in *Chlorella vulgaris* after 12 days of exposure to 100 ppm tartrazine (40x magnification)
White arrows indicate the formation of cell aggregates and the presence of extracellular substances, potentially lipids, which are hypothesized to enhance dye adsorption and stress tolerance.

Overall, these data confirm a strong positive correlation between algal growth, photosynthetic activity, and tartrazine removal efficiency, particularly during the early and exponential growth phases. Figure 3 illustrates this relationship by plotting residual dye concentration and removal percentage alongside the absorbance of *C. vulgaris* over the treatment period. As the experiment progressed, limiting factors such as nutrient depletion, waste accumulation, and cellular stress gradually reduced algal growth and photosynthetic efficiency, which in turn decreased the removal rate. These findings underscore the importance of optimizing initial dye concentrations, nutrient availability, and cultivation conditions to maximize bioremediation efficiency.

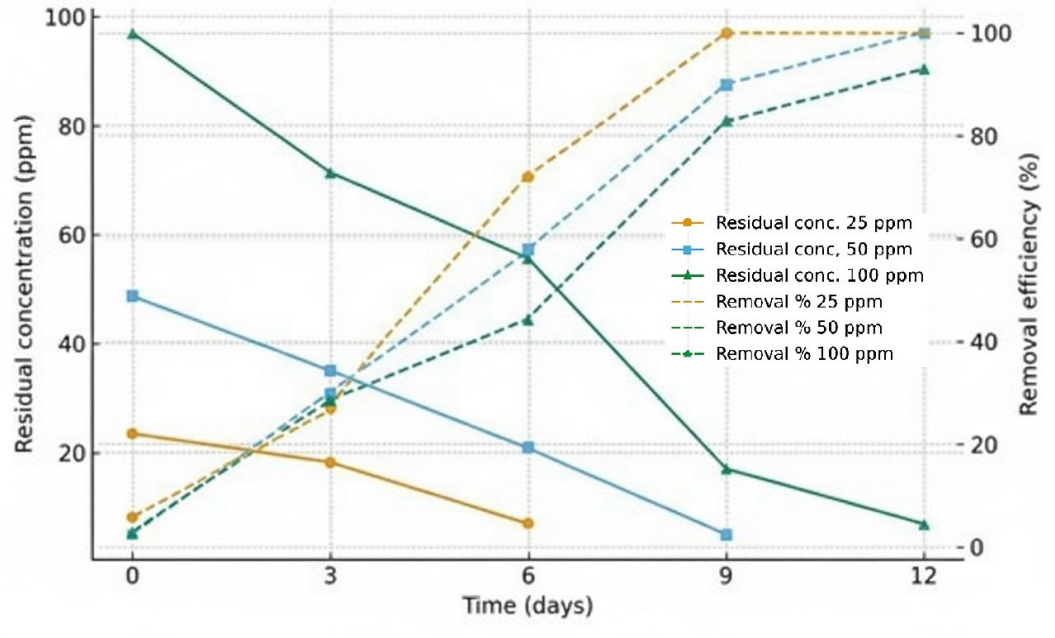

Figure 3. Residual tartrazine concentration, removal percentage, and *Chlorella vulgaris* absorbance over time at initial concentrations of 25, 50, and 100 ppm

Since there were notable variations between the first day and the third, sixth, and ninth days, the statistical analysis's findings demonstrated a significant difference ($p \leq 0.05$) between the treatment days for all three dye concentrations. The third day showed significant differences ($p \leq 0.05$) with the sixth day of the experiment at a concentration of 25 ppm. Significant differences ($p \leq 0.05$) were found between the first day and the third, sixth, and ninth days, as well as between the third and the twelfth days, when the tartrazine concentration was 50 ppm. Additionally, there were notable variations between the sixth and ninth days of the experiment and the twelfth day. At 100 ppm, significant differences ($p \leq 0.05$) were observed between day 1 and days 3, 6, 9, and 12, as well as between day 3 and days 9 and 12. Further significant differences ($p \leq 0.05$) were noted between day 6 and days 9 and 12, and between days 9 and 12. The faster decomposition of tartrazine can be attributed to its simple structure and the absence of metal complexes, which makes it more susceptible to enzymatic degradation. However, at higher concentrations, the decomposition rate decreases due to the toxic effects on microorganisms [37]. Table 1 shows that on the first day of the experiment, the processing rate for all concentrations dropped, indicating that the algae had become acclimated to tartrazine. On the third day, it evidently increased, demonstrating the algae's biological activity during the three days. Because the algae use tartrazine as a carbon source, the concentration of tartrazine causes the concentration of nutrients in the medium to rise, which explains the increase in processing rate [20]. The removal rate varies slightly among the three concentrations, but the treatment rate keeps increasing steadily until the sixth day. Due to the lower concentration of the contaminant and the smaller cell size of the algae, which helped remove the contaminant more quickly than the other concentrations, complete treatment of tartrazine was observed on the ninth day at a concentration of 25 ppm. The size of the cells influences how well the algae remove pollutants from the environment, and one of the most significant factors affecting the biological treatment efficiency of microalgae is the ratio of surface area to volume. This is consistent with a study [38], which reported that smaller cells, having a higher surface area-to-volume ratio, exhibit greater pollutant absorption capacity and are less prone to damage at low contaminant concentrations. In contrast, at 50 ppm, complete removal was delayed until day 12.

According to a study by Ghosal et al. [23], true green algae have membrane defense systems against oxidation that help them fend off pollutants and eliminate free radicals that the algae produce. High pollutant concentrations result in the generation of reactive oxygen species (ROS) in significant quantities, including O^-, OH, and H_2O_2, which oxidize and damage membrane lipids. Consequently, it was observed that the treatment rate was postponed until the experiment's twelfth day. Conversely, a higher density of accessory pigments like carotenoids, beta-carotene, and triacylglycerols results from a nitrogen shortage and an increase in carbon sources in the medium. Because carotenoids have antioxidant qualities, their buildup in cells shields against the effects of free radicals [39]. As the concentration of pollutants decreases, microalgae regulate the concentration of carbon, phosphorus, and nitrogen by internally accumulating fats, carbohydrates, and proteins in their biomass [24, 40]. The highest processing rate was recorded on the ninth day at a concentration of 100 ppm, which may have been caused by cell aggregation and the fatty material that surrounded them, as depicted in Figure 3. Environmental pollutants cause noticeable changes in the qualitative characteristics of water, which in turn affect the living things that are present in it [38, 41]. This was supported by certain investigations that discovered the presence of pollutants and their distribution within the cells has an impact on the fat content of algae. Furthermore, since the majority of pollutants can be retained in the fatty structures of the algal cells or their surroundings, the presence of fatty substances inside or around the algae increases their consumption and resistance to pollutants (Figure 4) [25]. However, Table 1 also demonstrates that the processing rate decreased as the dye concentration increased, which could be connected to the algal cells becoming saturated once they achieve equilibrium [22]. As observed in Figure 3, high concentrations of tartrazine prevented eukaryotic cells from dividing by causing toxicity,

mutations, and cancer if exposed for extended periods of time [42]. Physiological changes were observed in the algal cells, including increased cell size, reduced aggregation, and evidence of cell death. By day 12 at 100 ppm, the algal population had declined to 10.11, reflecting the toxic impact of elevated tartrazine levels.

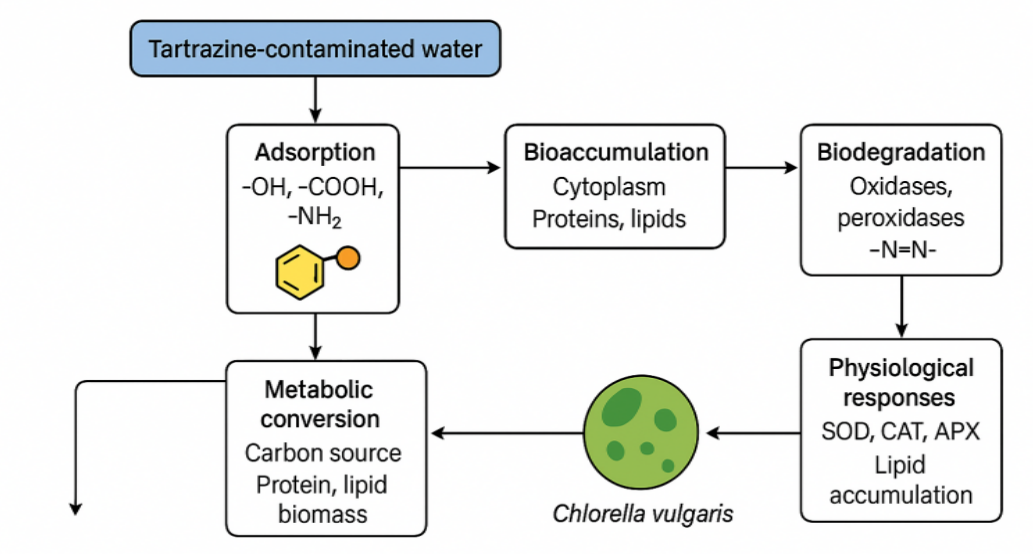

Figure 4. Schematic diagram illustrating the potential mechanisms by which *Chlorella vulgaris* removes and tolerates tartrazine dye

Mechanisms include biosorption onto functional groups on the cell wall, intracellular uptake, enzymatic degradation, antioxidant and lipid-based defense responses, and the formation of detoxified end products.

4. CONCLUSIONS

This study showed that *Chlorella vulgaris* is very effective at getting rid of the carcinogenic azo dye tartrazine from water systems. It completely broke down the dye in nine days at lower concentrations. The findings show that how well the algae can remove the dye depends on both the starting concentration and how long it is exposed to it. This shows that the algae can adapt and stay healthy under stress. HPLC was used to get an accurate count of how much dye had broken down, which made the results more reliable. The research underscores the viability of locally isolated *C. vulgaris* as a sustainable, environmentally friendly, and economical bioremediation agent for synthetic dye contamination.

While the study highlights the strong potential of *Chlorella vulgaris* in tartrazine bioremediation, it has notable limitations. The proposed mechanisms, like reducing oxidative stress and building up lipids, were based on what other researchers had found in the literature rather than being tested in the lab with biochemical or molecular assays. The toxicity and biodegradability of degradation products were not evaluated, leaving uncertainties about the treated water's safety. The experiments were done in a controlled lab setting that might not be like how real wastewater works. The study also didn't look at whether the algal biomass could be used again or how stable it would be over time. Mechanistic validation, toxicity analysis, and pilot-scale trials for real-world use should all be part of future work. Future research should concentrate on clarifying the biochemical and molecular mechanisms responsible for the degradation of tartrazine by *C. vulgaris*, evaluating the toxicity and biodegradability of the degradation products, and performing pilot-scale experiments with actual wastewater to assess efficiency, biomass reusability, and environmental safety for large-scale industrial wastewater treatment applications.

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