BIODEGRADATION OF RHODAMINE-B TEXTILE DYE USING CYANOBACTERIA UNDER OLIGOTROPHIC CONDITIONS

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The persistence and resistance of Rhodamine-B and other analogous synthetic dyes in aquatic ecosystems are a cause of serious environmental and public health concern. Their persistence against degradation by conventional wastewater treatment methods urges the pursuit of new, environmentally friendly alternatives. The present study investigated the potential of a filamentous cyanobacterial strain, CB4, isolated from a freshwater body in Sri Lanka, to degrade Rhodamine-B under oligotrophic (nutrient-deprived) conditions. A 10 ppm of Rhodamine-B solution was inoculated with 6 mL of CB4 culture in the exponential phase and incubated at room temperature under light illumination of 2000–3000 lux for 28 days. The medium was not supplemented with any nutrients, simulating indigenous effluent conditions. Decolourization was quantified spectrophotometrically at 563 nm, while growth was measured at 680 nm. Although the dye received a lower decolourization efficiency (6.16%), CB4 exhibited a good adaptability to the dye medium with 89.81% growth efficiency and 10 mg/day biomass accumulation. High-Performance Liquid Chromatography (HPLC) analysis revealed a 10.26% reduction of peak area compared to the control, implicating partial degradation of Rhodamine-B. The phytotoxicity assay using green gram (*Vigna radiata)* seedlings revealed enhanced mean shoot and root lengths in the CB4-treated sample (9.65 cm and 9.92 cm, respectively), compared to the untreated control. These findings demonstrate that CB4 can tolerate and bring about partial biodegradation of Rhodamine-B under oligotrophic conditions. Despite incomplete decolourization, the reduction of phytotoxicity and detectable dye degradation highlighted CB4 as a promising candidate for low-cost, sustainable bioremediation of dye-contaminated textile wastewater.

Keywords: biodegradation, cyanobacteria, HPLC, oligotrophic, phytotoxicity, Rhodamine-B dye

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INTRODUCTION

The discharge of synthetic dyes into water bodies by textile industries is one of the major environmental issues, particularly in third-world nations, where effluents are generally released without treatment directly into water bodies. One such dye, being a derivative of xanthene with intense colour and chemical stability, is Rhodamine-B. Yet, the same properties make it environmentally persistent and resistant to degradation, leading to the accumulation of toxic residues in the environment. Rhodamine-B exerts several adverse health effects, which involve cytotoxicity, carcinogenicity, and genotoxicity, posing great danger to aquatic life and human populations on long-term exposure *(Hossain Talukder et al., 2015; Saxena et al., 2020).*

Conventional methods employed in dye remediation, such as adsorption, coagulation-flocculation, chemical oxidation, and membrane filtration, have found extensive application in the removal of industrial wastewater. Such processes are, however, marred with humongous capital expenses, energy-consuming procedures, partial degradation, and secondary pollution issues like sludge development *(Touliabah et al., 2022)*. In compliance, biological alternatives have been utilised in recent years, with greater emphasis put on phycoremediation, i.e., the application of photosynthetic microorganisms such as cyanobacteria and microalgae to remove, destroy, or convert toxic contaminants into less toxic products *(Tripathi et al., 2023)*. Cyanobacteria are particularly ideal bioremediation agents due to their metabolic diversity, enzymatic capabilities, and ability to grow in low-nutrient (oligotrophic) environments. They break down dyes by enzymatic action, biosorption, and phototrophic growth with no requirement for supplemental nutrients *(Al-Tohamy et al., 2022; Carneiro et al., 2010)*. They also contain enzymes like azoreductases, laccases, and peroxidases, which are likewise reported to be participating in chromophoric group cleavage in dyes, hence the process of detoxification *(Patel et al., 2022)*.

CB4 was chosen as it is an indigenous cyanobacterial isolate from Sri Lanka, with good adaptability to low-nutrient conditions, making it a suitable candidate for oligotrophic biodegradation studies. For this purpose, the present work studies the potential of decolourization and detoxification through the unicellular cyanobacterium CB4 under oligotrophic conditions. The main aim is to explore the dye decolourization, yield of biomass, and minimise the phytotoxicity, consistent with phytotoxicity tests by CB4. The research justifies the implementation of low-cost, eco-friendly bioremediation technologies to treat textile dye effluents, particularly in those areas where it is not reasonable to use traditional wastewater treatments.

METHODOLOGY

An aqueous solution of 10 ppm Rhodamine-B was prepared and inoculated with 6 mL of the CB4 filamentous cyanobacterial culture in sterile 250 mL Erlenmeyer flasks containing 100 mL of the dye solution. The inoculum represented an actively growing culture, identified under the microscope and by visual observation of increased blue-green colour. The cultures were incubated under laboratory conditions at 25 ± 2°C for a period of 28 days, with continuous illumination (2000–3000 lux) from white fluorescent lamps, without external nutrients. A control flask containing Rhodamine-B solution without CB4 inoculation was maintained in parallel. This was a preliminary experiment conducted without replicates, aimed at establishing the feasibility of CB4-mediated Rhodamine-B degradation. Decolourization potential was assessed by measuring the absorbance of cell-free supernatant at 563 nm, the λ max of Rhodamine-B, in a UV-Visible spectrophotometer at 7-day intervals. CB4 growth performance was also assessed spectrophotometrically at 680 nm, on the basis of chlorophyll-a content, which is indicative of photosynthetic biomass. All the biomass was harvested at the end of the experiment, oven-dried at 60°C, and gravimetrically measured for total daily biomass accumulation. For estimating detoxification capacity, a phytotoxicity test on *Vigna radiata* seeds (mung bean) was conducted. Seeds were surface sterilised and supplied with treated or untreated dye solution for watering over 3 days. Root and shoot growth were estimated after incubation, and growth variation was used to predict decreased toxicity following biotreatment.

RESULTS AND DISCUSSION

CB4 strain displayed remarkably high recovery and significant growth efficiency (89.81%) in dye-stressed and oligotrophic conditions with a mean biomass yield of 10 mg/day. The growth confirms that CB4 maintained photosynthetic functions and metabolism after cytotoxic treatment with Rhodamine-B, possibly by using stored reserves or trace organics within the dye matrix.

Although the decolourization efficiency attained (6.16%) was lower than anticipated, it was calculated using the following equation:

$$\text{Decolorization efficiency (\%)} = \frac{A_0 - A_t}{A_0} \times 100$$

Where A_0 = Initial absorbance at 563nm, and A_t = absorbance at time t

High-performance liquid chromatography (HPLC) provided some insight into the degradation process. Reduction in peak area of Rh-B of 10.26% and contribution to peak area of 72.49% indicated partial degradation or cleavage of the dye molecule into lower molecular weight intermediates by CB4. This result suggests enzymatic activity, potentially via oxidoreductases or dye-degrading enzymes, although biochemical evidence is needed.

The phytotoxicity assay also validated the detoxifying efficacy of CB4. Treated seeds with the CB4 biotreated solution for the dye possessed longer roots and shoots (9.92 cm and 9.65 cm, respectively) compared to those treated using the untreated dye, demonstrating reduced toxicity and improved bioavailability of critical water parameters. Although no statistical tests were applied due to the absence of replicates, the consistent improvement across seedlings suggests a reproducible detoxification effect. These results are consistent with other remediation research conducted using cyanobacteria, in which it has been established that biodegradation reduces the environmental toxicity of synthetic dyes and results in the restoration of ecological safety *(Hossain Talukder et al., 2015).*

CONCLUSIONS/RECOMMENDATIONS

The findings demonstrated that the cyanobacterium CB4 can endure and partially break down Rhodamine-B in nutrient-deficient environments. Even though the decolourization was inefficient, both HPLC and phytotoxicity tests supported the partial biodegradation and remarkable detoxification of the dye.

Consistent with these observations, CB4 is a prime candidate for incorporation into cost-effective, environmentally friendly wastewater treatment systems, particularly where there is no advanced infrastructure. Further work should seek to define more rigorously the enzymic pathways involved in the degradation of the dye by more extensive enzyme profiling and activity assays. Genomic and transcriptomic analysis would shed additional light on the genetic mechanisms involved in stress response and pollutant metabolism. It is also suggested that pilot-scale experiments be performed with real industrial effluents to confirm the efficiency of the strain in real wastewater matrices. Exploration of the synergistic advantage of CB4 as a blend with other microbial species or organic co-substrates can further extend its application. In general, CB4 is vast as a biological asset for the design of greener remediation technologies for dyes in water.

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