Bio Decolourization and Bio-Degradation of Rhodamine B Dye Reactive by Marine Waste Products

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**Abstract:** The rapid pace of urbanization and industrialization has led to the release of large volumes of toxic sludge into the environment, posing significant health risks due to contaminants like synthetic dyes. Rhodamine B (RhB), a widely used dye in the textile and leather industries, is known for its carcinogenic and mutagenic properties. This study focuses on evaluating the efficacy of marine waste-derived chitosan in removing Rhodamine B from aqueous solutions. FTIR analysis revealed the presence of various functional groups within the chitosan structure, including halo compounds, primary alcohols, sulfate groups, α,β-unsaturated ketones, and esters. The degradation efficiency of RhB was assessed at different chitosan concentrations and time intervals. Results showed a concentration-dependent increase in dye removal, achieving up to 82.5% removal at 1000 mg chitosan. Time-based experiments indicated that extending the treatment duration enhanced removal efficiency, reaching up to 89.4% after 24 hours. These findings underscore the potential of chitosan as a viable and eco-friendly solution for wastewater treatment and environmental remediation.

**Keywords:** Rhodamine B; Chitosan; Dye removal; Wastewater treatment; Environmental remediation

# Introduction

The environment is being exposed to massive volumes of toxic sludge due to the fast urbanization and industrialization processes (Garg et al. 2022). All elements of the ecosystem are contaminated by the improper handling of this hazardous waste. Since very early times, dyes have been used to color practically every kind of material [(Ajay et al., 2023; Chokkattu et al., 2023; Padarthi et al., 2023)](https://paperpile.com/c/A7qp5K/CXeOR+bJ94v+iK4HZ). Dye has the potential to trigger cancer, cause genetic mutations, and inhibit the actions of enzymes (Roy et al. 2018). Using various microplasma media made from oxygen, air, nitrogen, & argon gases at atmospheric pressure, rhodamine B was degraded in an aqueous solution [(Yin et al., 2023)](https://paperpile.com/c/A7qp5K/oSSll). An aqueous solution containing 10 parts per million of rhodamine B (RhB) was produced and subjected to various treatment times and applied potentials in a microplasma reactor [(Meiyazhagan et al., 2020)](https://paperpile.com/c/A7qp5K/Jckgq). Rhodamine B, a cationic dye containing aromatic compounds & xanthene rings, is commonly used in the dyeing and leather industries due to its high-water solubility and inexpensive cost [(Dharman et al., 2023; S. Sindhu et al., 2023; Sreenivasagan et al., 2023)](https://paperpile.com/c/A7qp5K/yHuVB+umyLQ+ZTJr5). Rhodamine B has carcinogenic and mutagenic qualities, which pose health concerns to humans and the environment [(Ramakrishnan et al., 2023; Shenoy & Maiti, 2023; J. S. Sindhu et al., 2023)](https://paperpile.com/c/A7qp5K/BedEt+NhK0s+VMAiC)As a result, developing and implementing effective treatment strategies for rhodamine B elimination is critical to reducing its negative effects [(Elhami et al., 2023)](https://paperpile.com/c/A7qp5K/gMjhb), [(Karthik et al., 2023)](https://paperpile.com/c/A7qp5K/OZHvD). The textile industry releases a significant amount of wasted synthetic dyes into wastewater, which increases pollution in the environment and puts human health at risk [(Kasabwala et al., 2021; Rajeshkumar & Lakshmi, 2021; Varghese et al., 2023)](https://paperpile.com/c/A7qp5K/ZYOM0+ANWc8+eLWfR). While many physicochemical techniques have been used to effectively remove color and dyes from textile effluents, these techniques have certain disadvantages, including the need for more costly chemicals, limited sensitivity, and the creation of extra sludge that presents a secondary disposal issue [(Dhruv Patel & Bhatt, 2022)](https://paperpile.com/c/A7qp5K/b4rQX). Bioremediation has been deemed a promising and emerging active topic of research for the treatment of undesirable color and target substances from the contaminated environment [(Kour et al., 2021)](https://paperpile.com/c/A7qp5K/HVG3M);Sai et [(“Comparative Study of Green Synthesized Silver (Ag) Nanoparticles for Wastewater Treatment over Filtration Technique for Bioremediation Application,” 2022)](https://paperpile.com/c/A7qp5K/TlG2)There is an increasing need for less expensive biological methods that employ microorganisms, fungi, algae, and plants as well as other environments[(Pranati et al., 2021; Sakthi et al., 2021)](https://paperpile.com/c/A7qp5K/4yat+myqO)). Numerous processes, including biosorption, biodegradation, and biomineralization, are used in these biological procedures [(Singh et al., 2024)](https://paperpile.com/c/A7qp5K/rB38g). Rectorite (REC), a layered silicate mineral, is used as the strengthener to obtain the necessary mechanical strength for recovery, and chitosan (CS) as the polymer substrate [(Keerthana & Ramesh, 2021; Murugesan, 2021; Tiwari & Jain, 2021)](https://paperpile.com/c/A7qp5K/Fn75u+QFYS8+o977B)[(Subramanian et al. 2021)](https://paperpile.com/c/A7qp5K/UKTdy). Because of its functional groups on the polymer chain, CS, a polysaccharide derived from fungi, insects, and shellfish, is recognized as a natural adsorbent. It is very effective, environmentally friendly, and biodegradable [(Chen et al., 2021)](https://paperpile.com/c/A7qp5K/tfsEe), (Duraisamy [(Chen et al., 2021)](https://paperpile.com/c/A7qp5K/tfsEe)). The current study aims to evaluate the removal capacity of Rhodamine B dye using marine waste product Chitosan.

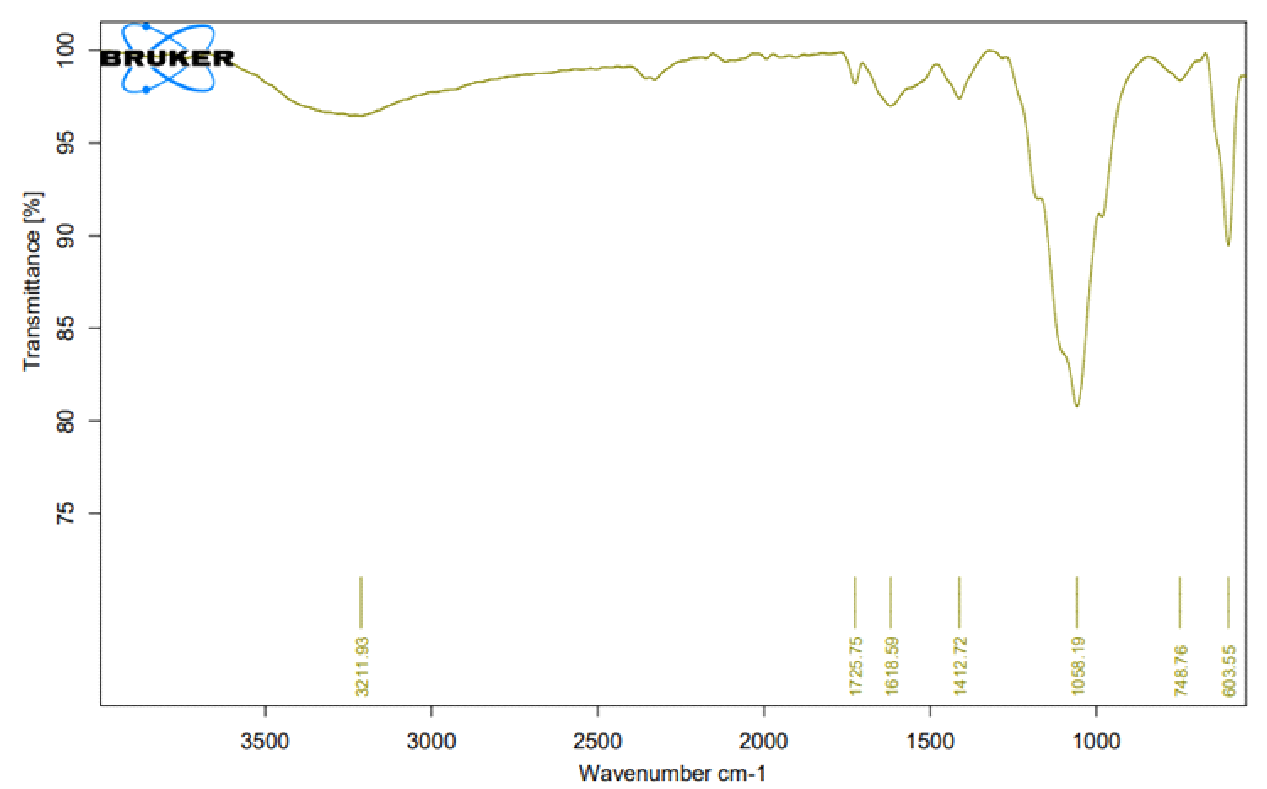
# Materials and Methods

The squid bones were collected at the local fish market of kolathur, chennai and refrigerated overnight. Around 1000 g of squid bones were then washed thoroughly and crushed into smaller pieces. These crushed pieces were air dried for 72 hours. Samples that had been air dried were placed in a hot air oven at 65°C for 3 days to extract the dried material. The dry weights of the samples were obtained prior to extracting chitin and preparing chitosan.For chitin extraction, 25 g of crustacean shell powder was used. 2 M HCl was used to demineralize the mixture for 150 minutes at 60°C while stirring continuously. The shell powders were decalcified, and then they were filtered and carefully washed with distilled water until the pH was balanced. 3M NaOH was used to deproteinize the protein (for 120 minutes at 80˚ C). The demineralized and deproteinized products were decoloured using 1:2:4 ratios of chloroform, methanol, and distilled water, and the decoloured products were maintained in a hot air oven at 60°C for 24 hours. The extracted chitin was kept in the refrigerator for future usage [(Mohan et al., 2021)](https://paperpile.com/c/A7qp5K/XFzQy).Deacetylation process was done by treating the obtained chitin with 65% NaOH for 72 hours at 30˚c to convert it to chitosan. Later, it was centrifuged at 5000 rpm for 15 minutes to remove excess alkali. The supernatant was discarded and the pellets were washed with d. H2O water until pH is neutral. The end product is chitosan and it was dried overnight and stored at room temperature for further use.Adsorption experiments were conducted by adding chitosan in four concentrations (250, 50, 750 and 1000 mg) to 50 mL solutions of Rhodamine B in four conical flasks while stirring. The mixtures were kept in an orbital shaker at 400 rpm & 35˚c for durations ranging from 8 to 24 hours. To determine the optimal contact time for complete decolorization of the dyes, the effect of contact time between the adsorbent and adsorbate was tested over this period. UV absorption of Rhodamine B dye wavelength is 558nm.The chitosan sample was characterized using a FT-IR analysis. The IR spectrum of the chitosan was recorded with a Bruker Alpha II spectrophotometer. The IR spectrum of the nanofiber was recorded over the range of 500 to 3500 cm⁻¹ at a scanning speed of 1 μm/min.

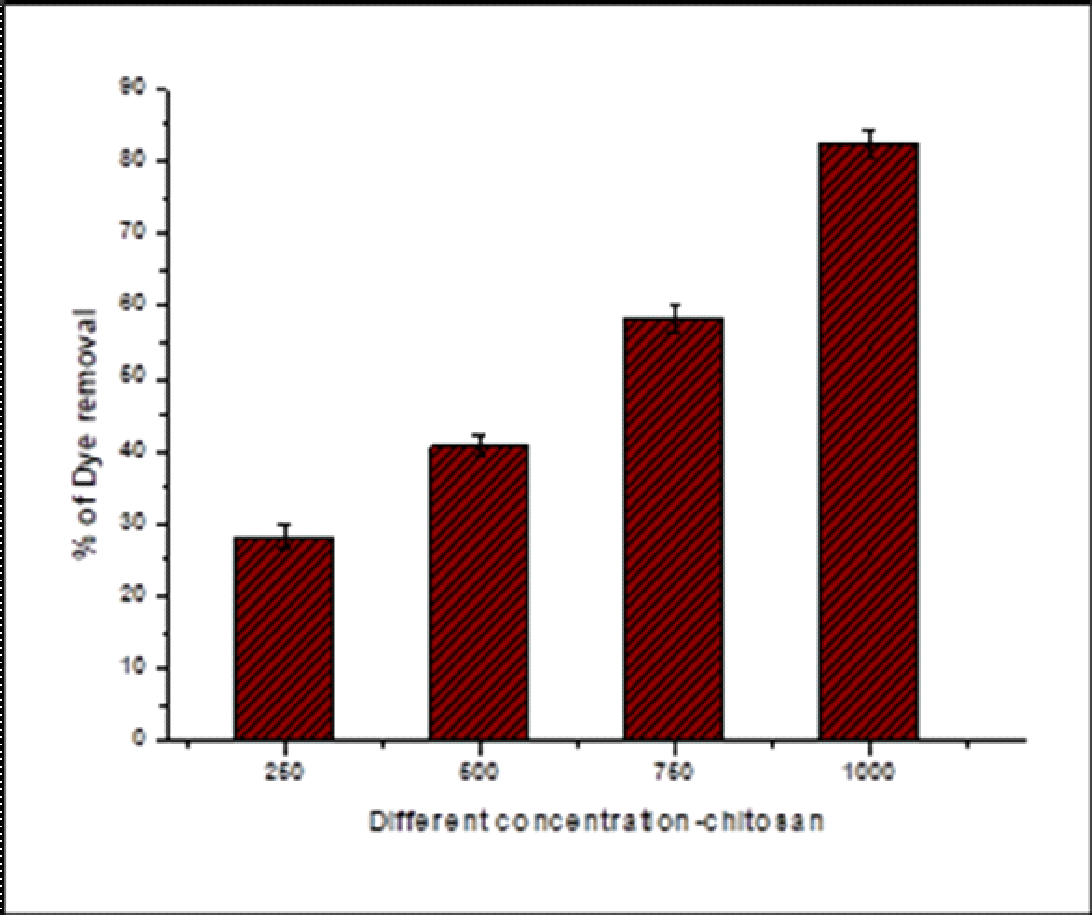
# Results and Discussion

The FTIR analysis of the chitosan sample provided detailed insights into its chemical structure through the identification of various functional groups (Fig.1). A strong absorption band at 603.55 cm⁻¹ was attributed to C-Br stretching, indicating the presence of halo compounds. A significant peak at 748.76 cm⁻¹ corresponded to C-H bending in 1,2-disubstituted compounds. The presence of primary alcohols was confirmed by a prominent C-O stretching band at 1058.19 cm⁻¹.

In this study, the degradation of Rhodamine B dye was investigated using varying concentrations of chitosan and different time intervals to assess removal efficiency(Saadh et al., 2024). Chitosan concentrations of 250, 500, 750, and 1000 mg were tested, revealing distinct trends in dye removal efficiencies (Fig. 2). At 250 mg, chitosan demonstrated a modest yet significant 28.2% removal of Rhodamine B, suggesting its initial efficacy in lower dosage scenarios (Almatrafi et al., 2024). Increasing the chitosan concentration to 500 mg notably enhanced dye removal to 40.8%, indicating a concentration-dependent improvement in effectiveness. Subsequent concentrations of 750 mg and 1000 mg demonstrated even greater efficacy, achieving removal percentages of 58.4% and 82.5% respectively. These results underscore chitosan's capability to effectively bind and degrade Rhodamine B dye molecules from aqueous environments, highlighting its potential as a viable solution for wastewater treatment and environmental remediation efforts.

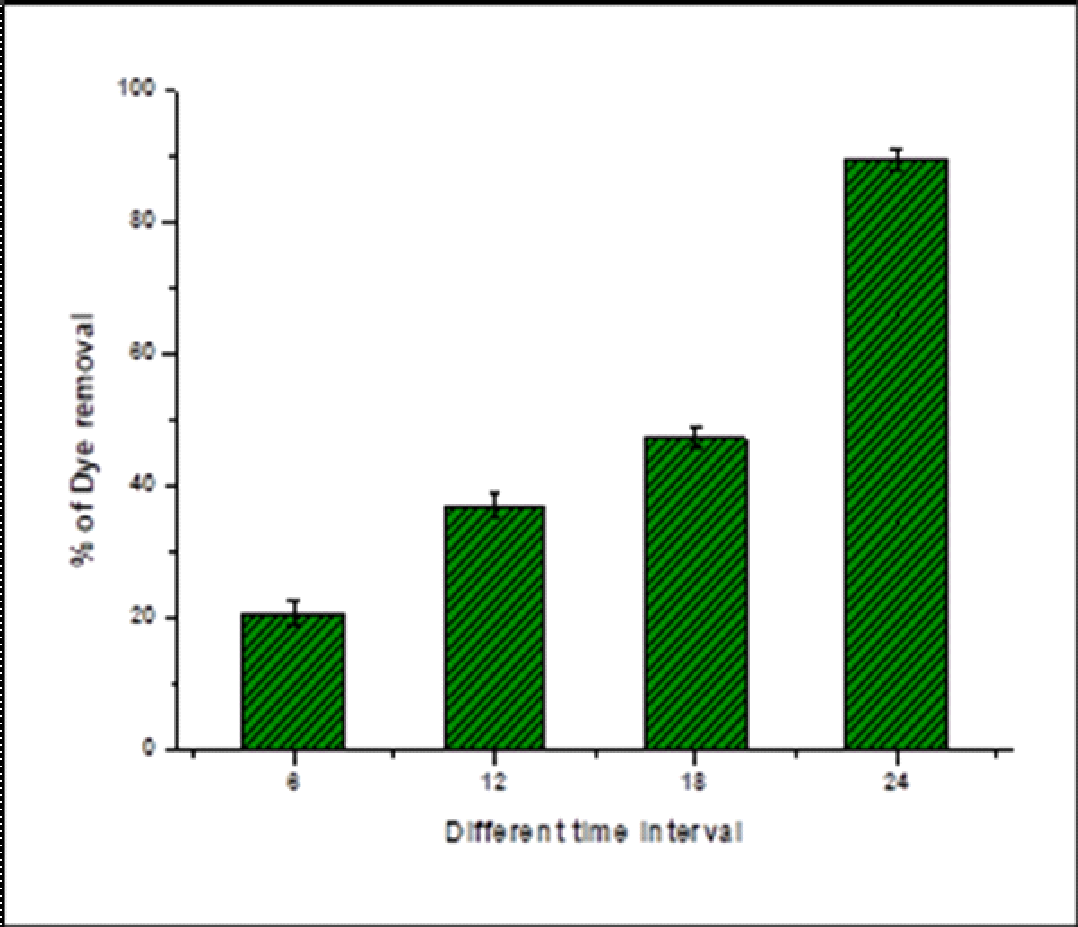


**Figure 1.** FTIR spectrum of chitosan showing its functional groups



**Figure 2.** Absorption of Rhodamine b using chitosan at various concentration

Also, the degradation of Rhodamine B dye was investigated over varying time intervals. The results showed a progressive increase in dye removal efficiency with extended treatment durations (Fig. 3). After 6 hours, 20.9% of the dye was removed, which significantly improved to 37.2% after 12 hours. Continuing the treatment for 18 hours further enhanced removal to 47.2%, with the most substantial removal observed after 24 hours, reaching 89.4%. These findings indicate a time-dependent efficacy in degrading Rhodamine B dye, suggesting potential for effective treatment in wastewater containing dye pollutants.



**Figure 3**. Absorption of Rhodamine b using chitosan at different time interval

# Discussion

Additionally, a notable absorption at 1412.72 cm⁻¹ was assigned to S=O stretching, suggesting the incorporation of sulfate groups within the chitosan structure (Moraes [(Saito et al., 2022)](https://paperpile.com/c/A7qp5K/W8yD)). The spectrum further revealed a strong peak at 1618.58 cm⁻¹, indicative of C=C stretching in α,β-unsaturated ketones, a result of the conjugation of the carbon-carbon double bond with the carbonyl group (Samreen[(Saito et al., 2022)](https://paperpile.com/c/A7qp5K/W8yD) 2023). A pronounced C=O stretching band at 1725.75 cm⁻¹ corresponded to α,β-unsaturated esters, confirming the presence of ester functional groups [(Saito et al., 2022)](https://paperpile.com/c/A7qp5K/W8yD). A broad and intense band at 3211.93 cm⁻¹ was observed, typically associated with O-H stretching vibrations, indicative of hydroxyl groups or water molecules, characteristic of hydrogen bonding interactions within the chitosan structure (Chen [(Saito et al., 2022)](https://paperpile.com/c/A7qp5K/W8yD)11). The chitin-derived Schiff base adsorbent effectively removes Rhodamine B dye from aqueous solutions, with a maximum removal capacity of 233.4 mg g1 (25°C) at pH 8 [(Alakhras et al., 2022)](https://paperpile.com/c/A7qp5K/jQ8OQ). The nanocomposite demonstrated high remediation capacity, as 82% of the rhodamine B dye was remediated within 3 hours of procedure [(Sharma et al., 2020)](https://paperpile.com/c/A7qp5K/8cYMQ). The decolorization rates for the degradation of rhodamine B (RhB) by magnetic chitosan/TiO2 composite were 86.4% [(Zhang et al., 2023)](https://paperpile.com/c/A7qp5K/fd55a)[(G. & Ganapathy, 2022; Kumar & Ramesh, 2021)](https://paperpile.com/c/A7qp5K/hddoQ+36RNk)). Fe-doped biochar derived from waste sludge efficiently breaks down Rhodamine B in wastewater, providing a viable way to repurpose waste sludge and turn it into an inexpensive catalyst for breaking down refractory organic contaminants in aquatic settings [(Zang et al., 2020)](https://paperpile.com/c/A7qp5K/4h2as). Rhodamine B waste dye effluent may be efficiently degraded by hydrodynamic cavitation using carbon tetrachloride, hydrogen peroxide, and Fenton's reagent, yielding degradation rates of 99.9% and 82%, respectively [(Mishra & Gogate, 2010)](https://paperpile.com/c/A7qp5K/A3wNU).

# Conclusion

This study demonstrates the significant potential of chitosan derived from marine waste as an excellent adsorbent for removing the Rhodamine B dye from aqueous solutions. The FTIR analysis revealed the existence of functional groups conducive to adsorption processes. Experimental results highlighted a clear concentration- and time-dependent improvement in dye removal efficiency, with the highest removal rates observed at 1000 mg chitosan concentration and 24 hours of treatment. These outcomes suggest that chitosan is a promising, cost-effective & environmentally friendly material for mitigating dye pollution in wastewater, offering a sustainable approach to environmental protection and public health safety. Further research and optimization could enhance its application in large-scale wastewater treatment systems.

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