# Bioremediation of polluted water bodies using algae and microbial consortia

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## Abstract

Water scarcity is an inescapable dilemma resulting from the swift depletion of Earth's resources and adverse environmental effects. The primary objective of this study is to examine the removal efficacy of indigenous microalgae in conjunction with bacteria under diverse settings and to assess the patterns of algae development in actual wastewater. This study evaluates the viability of using actual textile effluent as an incubator for microalgal cultivation and wastewater remediation. Textile effluent comprises a mixture of diverse inorganic and organic molecules, predominantly including dyes and fertilizers. The microalgal-bacterial consortia demonstrated potential as an effective method for the biological remediation of waste textiles. This study employed fed-batch reactors to treat actual textile wastewater during a 24-hour cycle for one week. The experiment was executed in three distinct phases: single phase, two-phase, and connection, aimed at nutrient removal. The findings show that a microalgae-bacteria consortia achieves optimal removal efficiencies of 56.6% for nitrate (NO3-), 87.5% for phosphorus (PO4), and 92.6% for chemical oxygen demands (COD), with optimum concentrations of 13.9 mg/L of chlorophyll and 1.8 g/L for bacteria dry cell pounds, correspondingly. Optimal color removal was attained in a singular phase (exclusively algae), 42.6%. The findings demonstrated that this technology efficiently addresses actual

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textile effluent cost-efficiently and generates wood suitable for use as a biological fertilizer and in energy-effective initiatives.

**Keywords:** Bioremediation, Polluted water, Algae, Microbial consortia

## Introduction

Accelerated industrialization and a growing population pose significant dangers to the environment and assets, particularly water (Khan *et al.*, 2024; Escobedo *et al.*, 2024). Water shortage is projected to increase by at least 42% by 2035, primarily owing to the mismanagement of water supplies and insufficient access to innovative technology. The diminishing water supplies are critically connected to comprehensive industrial advancement, including economic expansion, nutrition, wellness for humans, energy generation, and ecological restoration. Resources from the earth, both renewable and non-renewable, are being rapidly depleted owing to catastrophic events, overexploitation, and other human activities (Xu and Zhao, 2023). Humans and other creatures are susceptible to sickness, decline, and extermination due to the improper handling and overexploitation of resources from nature, posing a significant threat to current ecosystems.

Numerous sectors, including textiles, livestock, and agriculture, utilize various deleterious compounds (Khan *et al.*, 2023). These substances are ultimately discharged into the natural world as the principal source of pollution from industry, resulting in detrimental effects on ecosystems. The natural input is inferior to human origin due to a temporal equilibrium between manufacturing and consumption.

The textile business is among the earliest and most intricate sectors (Salmi and Kaipia, 2022). This sector is fundamental and significantly contributes to economic growth. Its foundations can be assessed by its ability to produce and the development of the nation's economy. The country's textile sector is the largest global producer and exporter of textile products, accounting for 59% of the world's cotton-based apparel. It accounts for 70% of exports and 43% of Gross Domestic Product (GDP). The textiles sector uses organic materials like cloth, nylon, acrylic paint, polyester, jute, and velvet, in conjunction with different substances as adsorbents throughout the production process. In the fiscal year 2021, the textile sector made an income of 4.6 billion USD. The manufacturing of textiles in South Asia has developed and enhanced, boosting the economy through this sector and establishing connections on global stages.

The increase in these textile-based goods generates a substantial volume of wastewater (Gogoi *et al.*, 2023). Un-treated textile waste can jeopardize aquatic and terrestrial creatures by negatively impacting the natural environment (Unisa and Gowda, 2022). The absence of treatment primarily results from inadequate wastewater treatment facilities. Treating wastewater from the textile sector is essential, necessitating rehabilitation to fulfill home and commercial requirements.

The inundation of aquatic ecosystems with Heavy Metals (HMs) is a pressing concern due to society's rapid development and industrialization (Uddin *et al.,* 2021). Various sectors, including leather, electrolysis, steel, clothing, storage batteries, and mineral extraction, emit elements such as cadmium (Cd), magnesium (Mn), nickel (Ni), copper (Cu), cobalt (Co), argent (Ag), chrome (Cr), and lead (Pb). These HMs are very hazardous, even at minimal concentrations, and primarily contribute to water contamination (Kapoor and Singh, 2021). Water pollution generates significant issues, including limitations of potable water for consumption and essential services and operations in both industrial and domestic contexts (Morin-Crini *et al.,* 2022). The carrying metals adversely impact the condition of groundwater and ecosystem-wide wildlife and vegetation. Certain metallic substances, such as Ni, Hg, Cd, Cr, and Pb, are more toxic in their elemental or mixed states, as these pollutants seep into lakes and rivers and swiftly accumulate in human cells or organs (Ilić, Nešković Markić and Stojanović Bjelić, 2018). Metals reach an individual's body via food consumption, breathing, and water intake. The elevated toxicity, buildup, retention in biological cells, and facile translation in aquatic ecosystems lead to significant deleterious impacts on human well-being and aquatic organisms. Prior research has documented the presence of HMs in the gills, skeletal muscles, and liver cells of many fish species under polluted marine environments, and these metals accumulate in numerous human organs once they enter the food web (Shakir *et al.,* 2024).

The hazardous materials exceeding allowed limits adversely affect living organisms and the environment (Hassan and Saleh, 2022). The allowable threshold of HMs in food products has been associated with reducing risks to human health. The extraction of metals from industrial wastewater is essential due to its potential to induce diseases, disrupt ecosystems, and maintain high environmental and nutritional standards (Majdanishabestari and Soleimani, 2019).

Many investigators have investigated the purification of textile waste using algae and bacterial strains to evaluate their efficacy in removing dyes and pollutants before disposal, thereby mitigating the impact on aquatic ecosystems (Kusumlata *et al.,* 2024). The photosynthesis of the algae genus Spirogyra has effectively eliminated nutrients and dyes that react from textile effluent. Extensive research has been conducted on microalga-based wastewater management in conjunction with strains of bacteria. This research aimed to examine the efficacy of algae C. vulgaris combined with the Enterobacter species for removing chemicals, hue, and micronutrients from textile effluent. Several microalgal species exhibit the capacity for the biosorption of organic compounds and basic colors. Current research indicates that the biological remediation of textile waste utilizing microalgae and bacteria effectively eliminates a significant amount of color and nutrients from the effluent.

The study utilized a micro-algal-bacterial group to create an efficient textile wastewater removal process that concurrently eliminates nutrients (nitrates and phosphates) and organic pollutants (color and oxygen-based request) through a three-stage methodology. A study was conducted between axenic vulgaris and axenic Staphylococcus strains in a two-stage approach aimed at reducing nutrients and Chemical Oxygen Demands (COD) over a week in an ongoing way. Numerous benefits can be identified in an algal-bacterial consortium structure: (1) optimal removal effectiveness in algae and bacterial consortia, (2) superior O2 and CO2 exchanges in the consortia relative to the sole usage of organisms, and (3) heightened biomass and chlorophyll development.

## Material and Methods

### *Microorganisms and Cultivation Environments*

The freshwater algae cells were sourced, and the batch-scale test was performed in a 1200-mL open Duran (Schott) container. The incubation occurred at 25°C for 24 hours under a light intensity of 120 µmol $m^2s^1$, utilizing fluorescent lamps with constant aeration provided by an aeration pump. The daily provision of nutrients (nitrates, phosphates, and dextrose) as a feeding resource for algae resulted in accelerated development, measured at 660-nm Optical Density (OD) using a UV spectrophotometer.

The microorganisms were extracted from actual textile wastewater. The specimen was inserted into nutrient agar using pouring plates and incubated for 24 hours. After 24 hours of sufficient growth, select one colony using the vaccination procedure. Extract 25 mL of the nutrition broth into a Falcon tube for continued growth. The population was in axenic culture on a shaker-type incubator for 48 hours at 38°C. Upon achieving the appropriate inoculum and verifying growth using its OD at 650 nm using an Ultraviolet (UV) spectrophotometer, the broth containing the bacteria was centrifuged and introduced into the vessel to exclude nutrients.

This aquatic microalga was chosen due to its extensive application in sewage treatment. It has been demonstrated to break down phosphorus and nitrogen from diverse wastewater streams effectively. Staphylococcus aureus was selected because it is an anaerobic and culturable bacterial species commonly implicated in sewage treatment.

### *Authentic Textile Effluent*

The actual textile wastewater (both primary wastewater) was utilized in the batch-scale research. The specimen was obtained. Karachi has a longitude of 25.9° and a latitude of 68.3°. To avert contamination by bacteria and chemical alterations, the material was stored at 5°C. Before its application in the batch-scale test, the specimen was employed to assess its various physicochemical properties (mg/L). The physicochemical assessment of textile effluent was conducted using the criteria for the Examination of Waters and effluent and the EPA techniques. The subsequent parameters are determined using the following methodologies: nitrate (NO3-) via the Salicylic Acid (SA) technique, phosphorus (PO4) through the application of Ascorbic Acid (APHA), and Chemical Oxygen Demand (COD) utilizing the open reflux technique.

### *Experimental Configuration*

Biological remediation of textile waste is regarded as the most viable and efficient technique for nutrient removal. The whole test was performed in a 1100-mL open Duran (Schott) bottle, containing a sterilized 550 mL operational volume of actual textile waste to be processed over one week. For the treatment process of Textile Wastewater (TWW), a continuous lighting of 60 µmol $m^2s^1$ (12:0 light/dark) was supplied using white Light Emitting Diode (LED), with a reported temperature of 28°C. An aeration pump exclusively facilitated the process of mixing.

It illustrates the test setup executed in three phases:

Stage 1: a pure algae cultivation with an addition of 0.6 g/L of algae.

Stage 2: Initially infused with bacteria cells, micro-algae are added for enhanced elimination. One gram per liter of bacterial inoculum was introduced into the vessel for five days; it was cleaned out with bacterial wastewater; thereafter, an additional feedstock of 0.6 grams per liter of algae was incorporated into the same reactors.

Stage 3: A grouping of algal and bacterial was established by introducing 0.6 g/L of algae biomass and 1 g/L of bacteria into the inoculum into an independent reactor.

## Results and Discussion

### *The Alteration in Growth Kinetics of Algae and Bacteria*

The symbiotic relationship among microalgae and the bacterial consortia is the most effective way to augment microalgal biomass output, including chlorophyll. Bacteria can enhance the development of microalgae by supplying carbon dioxide and receiving oxygen from the algae. The generation of biomass attained optimal nutrient removal efficiency in wastewater. In treating actual textile sewage, algae proliferation was assessed by quantifying the overall amount of chlorophyll (Chl; mg L-1), encompassing chlorophyll a and b, utilizing an 85% ethanol fluid solution. The chlorophyll content was assessed using a modified approach, which was proposed. It illustrates the assessment of microalgal development via chlorophyll concentration: (1) a single-stage treatment with microalgae yielded 6.9 mg/L; (2) a two-stage approach, pretreatment with microbes, resulted in 7.2 mg/L; and (3) a microalgal-bacterial consortia exhibited 13.9 mg/L of chlorophyll. The collaboration attained a peak chlorophyll output of 13.9 mg/L. A 10 mL portion of processed textile sewage, filtered through 0.6-µm paper filters via the manager, was taken to assess bacterial growth. Bacterial proliferation was monitored via the Dry Cell Weight (DCW) method during three phases of textile wastewater processing. In the two-stage management, the growth rate of bacteria was 1.8 g/L, whereas in the microalgal-bacterial connection, a bacterial dry cell weight of 1.8 g/L was reported. The group of cells reached a maximum DCW yield of 1.8 g/L. The variation in the proliferation of algae and bacterial organisms during TWW removal was investigated at different points. The proliferation of algae (chlorophyll) was first sluggish, thereafter accelerating as it acclimated to a novel cultural context by the final day of therapy. The proliferation of the bacteria attained their peak dry cell weight on the third day and plateaued. Figure 1a and b illustrate the findings from morphological characterisation.

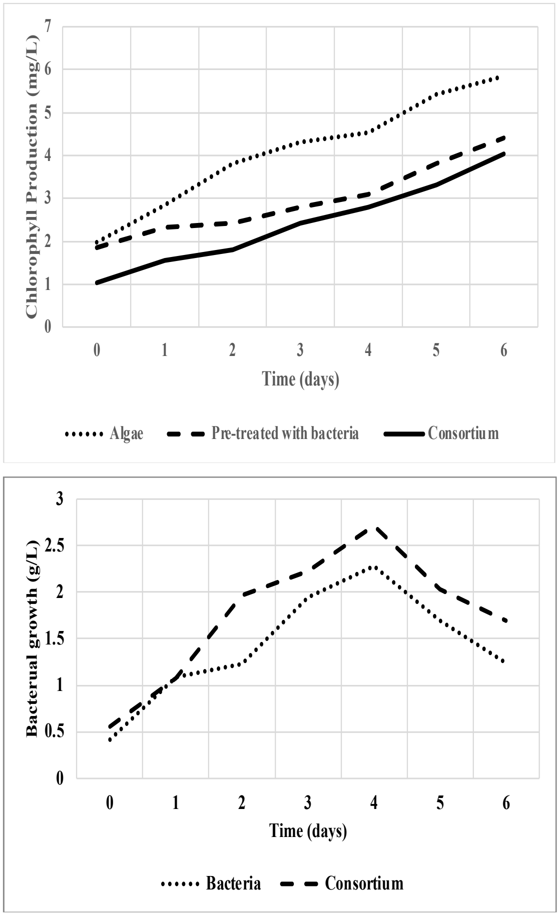


**Figure 1: Microalgae and Bacteria growth analysis.**

*Nitrate Elimination*

Nitrogen can be categorized into two types: organic nitrogen and inorganic nitrogen. Organic nitrogen is an essential nutrient necessary for the development of all species. Organic nitrogen can be synthesized from inorganic forms of nitrogen, such as nitrates (NO3), nitrites (NO2), and ammonium (NH4). Wastewater contains multiple types of nitrogen, such as NO3, NO2, and NH4, inside aquatic habitats. Excessive quantities might adversely affect water quality. The effectiveness of eliminating nitrates is significantly influenced by the amount and level of nitrate concentration, attained via absorption routes. During photosynthesis, bacteria utilize oxygen dissolved by microalgae, facilitating the degradation of organic contaminants in sewage. In sewage treatment, the symbiotic interaction between algae and bacteria facilitates the exchange of calories, dietary supplements, and chemical compounds, promoting rapid growth.

*Elimination of Phosphate*

Wastewater runoff presents a potential risk of eutrophication due to elevated phosphorus levels. Phosphate is a micronutrient vital for synthesizing lipids, proteins, and nucleic acids. Organic phosphorus is a crucial ingredient facilitating the breakdown of energy in the growth of microalgae and microorganisms. This element is frequently present in DNA, protein, lipid, and glucose metabolism. In microalgal digestion, phosphorus absorption predominantly occurs in HPO4 (phosphorus acid) and HPO2- (hydrogen phosphate). Both of these types are integrated into organic compounds via the phosphorylation cycle.

*Removal of COD*

CODs were substantially eliminated in all three phases at both acid and fundamental pH levels. The pH significantly influences the activity of bacteria and microalgae in the oxidation of organic molecules, such as carbon monoxide, in textile effluent. Figure 2 indicates that the elimination of COD in single-stage algae was recorded at 54.4% at a pH of 9.2. In a two-stage method including microbes, 37.7% of COD was eliminated, following a pretreatment with bacteria that achieved a 79.6% removal of algae biomass at a pH range of 4-10. At a pH of 7 to 10, the microalgal-bacterial consortia had the maximum clearance rate of 92.6%.

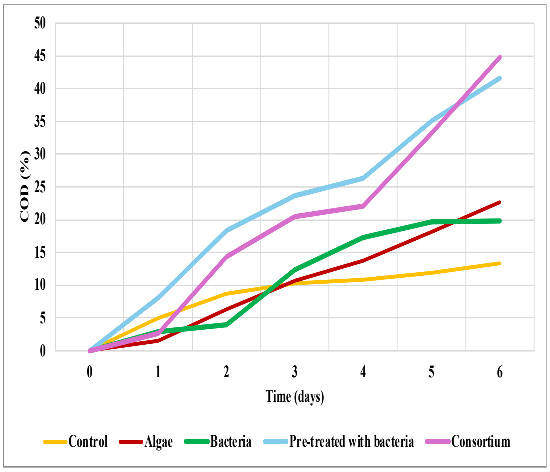


**Figure 2: COD analysis.**

A minor alteration in control occurred, with a 0.5% reduction in COD accompanied by a modest variation in pH. The mixture's ratio of algae and bacteria does not influence COD elimination, as a uniform ratio was employed across every phase. The microalgal-bacterial consortia utilize oxygen in the water and carbon from plants, specifically COD, for photoautotrophic activity. Algae in sewage emit Dissolved Oxygen (DO), which bacteria use to decompose organic matter.

Microalgae generate a significant quantity of oxygen, catalyzing bacterial metabolism. Thus, a mutually beneficial relationship is demonstrated, as the group effectively eliminates COD. Conversely, pH is essential for assessing the CO2 concentration in wastewater to destroy nutrients. Therefore, a beneficial relationship between algae and microorganisms is crucial for the sustainable elimination of COD in sewage and the elimination of nutrients. In wastewater, the reciprocal exchange of compounds like CO and O between species was associated with eliminating nutrients.

*Decolorization*

Microalgal and bacterial species significantly contribute to the decolorization of textile wastewater through an environmentally friendly and cost-effective adsorption technique in textile sewage treatment. The decolorization by algae is elucidated by a mixture of processes, including biosorption, biodegradation, and biocoagulation. In the preliminary phases of the research, colour from the wastewater is absorbed by algae cells, which is termed absorption. The outermost layer of the microalgal cells serves as the principal mechanism for absorption of color in textile wastewater.

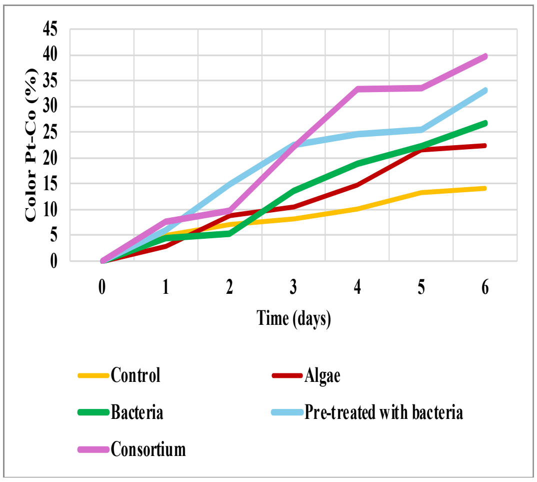


**Figure 3: Color-Pt-Co analysis.**

Figure 3 illustrates the elimination of color. These ingested pigments are transported into the algae cells and kept in a vacuole, serving as a carbon source and consumed by the conversion. Textile effluent comprises several hazardous effluents that can inhibit algal cell proliferation in an aquatic environment. Algae that produce exopolymers into their surroundings to adapt to harmful and stressful situations. These exopolymers function effectively in chelation and complicated reactions via biocoagulation. Dye removal integrates absorption, transformation, and biocoagulation reactions.

*Variation in pH*

Numerous parameters, including light, nutrient focus, and absorption time, affect microalgal development; pH is among these. Analogous to nutrient removal effectiveness, distinct trends in pH variations were noted across three different treatment methods. Specific microalgal taxa have acclimatized to thrive in acidic environments, but most favor neutral and alkaline settings. This research illustrates the variations in pH across all phases of actual textile effluent involving microalgal and different kinds of bacteria. At the outset, the pH reading of 13.1 was uniform throughout all treatment units. Following five days of cultivation, the ultimate pH values vary across all stages of textile sewage, ranging from 4 to 12. In the single-stage microalgae management, the pH was potentially lowered to 9.2 due to H ion release during nitrate ions (NH4+) ingestion in the nitrification procedure, enabling algae development. The primary cause of the pH decrease during algae therapy is likely the removal of nitrate from textile sewage.

## Conclusion

In contrast to the waste stabilizing treatment procedure, symbiotic consortia systems effectively achieved the simultaneous elimination of nutrients, phosphorus, and COD from actual textile effluent. The algae Chlorella vulgaris and Staphylococcus aureus group demonstrated optimal removal efficiency of nitrate, phosphate, COD, and color at pH 9.1 throughout a brief treatment duration of just 5 days. The findings show that a microalgae-bacteria consortia achieves optimal removal efficiencies of 56.6% for nitrate (NO3-), 87.5% for phosphorus (PO4), and 92.6% for chemical oxygen demands (COD), with optimum concentrations of 13.9 mg/L of chlorophyll and 1.8 g/L for bacteria dry cell pounds, correspondingly. The hue removal efficiency was 42.6% in one stage using micro-algal species that exhibited enhanced color elimination through an adsorption process. The collaboration system facilitated an increase in the development of bacteria, as seen by 13.9 mg/L generated chlorophyll and 1.8 g/L DCW, suggesting the presence of mutually beneficial interactions between the two organisms.

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