Research Article

Harnessing Bacterial Consortia and Green-Synthesized Metal Oxide Nanoparticles for Photocatalytic Dye Degradation

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ABSTRACT

This study introduces an innovative approach to degrade hazardous dyes by leveraging eco-friendly nano photocatalysts and microbial consortia. The research assesses the effectiveness of this integrated system in dye degradation for wastewater treatment and environmental remediation. Utilizing a bacterial consortium isolated from drains in the textile dyeing industry, alongside a photocatalytic process (Metal oxide/UV), the study demonstrates significant results. When applied individually, both biological and photochemical methods displayed limited decolorization efficiency. However, after 5 days under specific conditions (37°C, pH 7, and 200 mg/L dye concentration), bacterial degradation achieved a noteworthy 72.38% decolorization rate. In comparison, UV photocatalytic treatment with zinc oxide nanoparticles alone yielded a modest 23.5% decolorization. The combined approach, integrating UV-metal oxide treatment with bacterial consortia, showcased the most promising outcome. Notably, the highest bacterial degradation rate of 83.8% was observed when the dye mix sample underwent pretreatment with zinc oxide nanoparticles synthesized using leaves of *Parmentiera cereifera*, followed by 77.46% with *Hibiscus schizopetalus*, and 73.9% with *Combretum rotundifolium*. These previously unexplored plants exhibit potential as sources for green-synthesized metal oxide nanoparticles for dye degradation applications. Further optimization of degradation parameters could unlock the full potential of these nanoparticles.

Keywords: Environmentally hazardous dyes, degradation, Metal oxide green synthesis, bacterial consortia, Decolorization, Combined effects

INTRODUCTION

Over the past century, the environment has faced a significant influx of harmful chemicals, posing a threat to ecosystems worldwide. Among the industries contributing to this environmental burden, textile manufacturing stands out as a major player. Textile factories consume vast amounts of water, chemicals, and dyes in their production processes, leading to the discharge of effluents into the

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environment (1, 2). The diversity of dyes and pigments used in the industry is vast, with approximately 10,000 different types employed across various applications. Annually, over 7×10^5 tons of synthetic dyes are produced worldwide. The quantity of dye that becomes fixed to fibers depends on the type of dye, fiber, and dyeing process used. However, a substantial amount of dye often remains in the used dye bath. Consequently, up to 200,000 tons of these dyes are discharged into effluents every year, highlighting inefficiencies in industrial dyeing processes (3). Azo dyes, widely employed to impart permanent color to fabrics due to their resistance to external factors, dominate the textile dyeing landscape, with over 70% of textile industries relying on them. However, the effluents containing azo dyes released into the environment contribute significantly to pollution (4). Azo dyes contain a chromophore azo group (N=N), which provides the characteristic color to fabrics (5). Unfortunately, these dyes exhibit strong resilience to degradation, persisting in the environment despite conventional wastewater treatment methods (6, 7). Their presence in the environment poses serious health risks, including carcinogenic and mutagenic effects, affecting both aquatic life and human health (8, 9, 10). Efforts to mitigate the impact of azo dyes on the environment have explored various treatment methods, including physical, chemical, and biological approaches. While physical and chemical methods have shown efficacy, they often come with environmental risks due to their toxic nature (8). In contrast, biological methods offer an eco-friendlier alternative, involving the aerobic and anaerobic degradation of azo dyes by microorganisms. However, the effectiveness of microbial degradation alone is often limited, leading to incomplete dye degradation.

Recent interest has emerged in utilizing heterogeneous photocatalytic oxidation processes for dye degradation, leveraging metal nanoparticles synthesized from plant extracts. These nanoparticles, particularly copper and zinc oxide, exhibit catalytic properties that facilitate the degradation of azo dyes under mild conditions, without generating harmful byproducts (11). Additionally, the use of nanosized materials offers advantages in terms of surface area and catalytic properties, enhancing the efficiency of dye degradation (12). The plants selected for the synthesis of metal oxide nanoparticles are *Combretum rotundifolium*, *Parmentiera cereifera* and *Hibiscus schizopetalus* which were never explored for similar kind of studies.

In light of these challenges and opportunities, our study focuses on screening potential dye-degrading bacterial isolates from textile dyeing industry effluents. We aim to harness these isolates to establish a consortium for the decolorization of dye mixtures, integrating biological treatment with photocatalytic pretreatment using green-synthesized metal oxide nanoparticles. By combining these approaches, we seek to develop innovative and eco-friendly strategies for the effective removal of persistent dye mixtures from aqueous systems.

MATERIALS AND METHODS

Chemicals and dyes

In the present study, the dyes used were commercially available reactive dyes viz. Sky-blue FF, Pink FB, Violet 4BS, Congo red, Green BN, Lemon 6GS and Orange Indigo which were obtained from a local company (Sri Devi Dyes and Chemicals, Bangalore, India) and used without further purification. In India, these dyes are commonly used by the textile dyeing industries. Each dye was initially prepared at a concentration of 1 g/100 mL. Subsequently, 1 ml from each dye stock was combined, resulting in a total volume of 7 ml for the mixture containing seven different dyes. The concentration of each dye in this mixture remained consistent at 1 g/100 mL. This mixture served as the primary stock solution, from which working stocks were prepared at concentrations of either 20 mg/100 mL or 30 mg/100 mL and used for isolation and screening of potent dye-decolorizing bacterial isolates. The mixture of commonly used dyes were chosen in this study to replicate the waste containing different dyes produced during the textile dyeing and subsequent processing in textile dyeing industry. This choice closely resembles real-world scenarios, making our findings highly applicable in practical settings. All chemicals, reagents and media ingredients had the desired purity and were purchased from Hi-Media Laboratories, India and SRL chemicals.

BIOLOGICAL EXPERIMENTS AND DECOLORIZATION PROCEDURES

Sample collection

Effluent samples were obtained from textile industries situated in the Harohalli industrial area, along Kanakapura road, Karnataka, India. These samples were collected using sterile bottles and promptly transported to the laboratory, where they were refrigerated at 4°C. Within 24 hours of collection, the samples were inoculated to isolate the dye-degrading bacteria.

Isolation and screening of dye degrading bacteria

For all degradation studies, a nutrient broth or agar medium was prepared with the following composition:

glucose (1 g/L), peptone (1 g/L), beef extract (1 g/L), and yeast extract (1 g/L), with a pH of 7.0. The dye mixture (1 g/100mL) was filter-sterilized using a sterile syringe filter (Make: Millex, Model: GV PVDF, Pore size: 0.22 microns) and then added to sterilized nutrient agar to achieve a concentration of 400 mg/L. (10). The effluent was used to isolate the potential isolates that could degrade the dyes. This was not used as an ingredient in the media. The dyes are used in the media to screen the potential bacterial isolates by observing zone of decolorization around the colony so that the screening becomes easy.

The screening and isolation of dye-decolorizing bacteria were conducted using all three effluent samples. Each effluent sample (dilutions: 10°, 10-1, and 10-2) was inoculated onto sterile nutrient agar plates using the spread plate technique, with 1 ml of each sample. These plates were then incubated at 35°C for 3 days. Bacterial colonies showing zones of decolorization on the nutrient agar plates were identified as potential dye-degrading bacteria and labeled as isolates 1 to 18. Purification of the selected isolates was achieved through repeated subculturing on selective nutrient agar media. The isolates were streaked onto sterile nutrient agar plates and allowed to propagate at 37°C for 24 hours. Propagated bacterial colonies were subcultured on slope nutrient agar medium and preserved in a refrigerator at 4°C for further testing.

Bacterial decolorization in liquid media

For degradation studies in liquid media, all the eighteen bacterial isolates selected were inoculated into test tubes containing approximately 15 ml of Nutrient broth with a dye mixture concentration of 200 µg/ml. Usually, textile wastewater contains dye concentrations between 100 to 200 µg/ml (10). Therefore, we chose a concentration of 200 µg/ml for our study. These tubes were then incubated at 37°C for 3 days. After incubation, 2 ml of the solution was centrifuged at 1,957 × *g* (Make: Thermofisher, Model: Sorvall ST Plus) and the supernatant was used to obtain UV-Vis spectrophotometer (Make: Shimadzu, Model: UV 1780) at the λ_{max} of the dye mixture to assess degradation efficiency. This measures absorbance at a single wavelength, which may not correspond to the lambda max of each dye present in the mixture. However, by selecting a wavelength that is sensitive to changes in absorbance due to dye degradation, we can effectively monitor overall decolorization trends in the mixture. Furthermore, control samples containing the same medium without inoculum were also incubated to observe the abiotic decolorization. At regular time intervals, an aliquot (5 mL) of the culture media was harvested from the flasks, centrifuged at 1,957 × *g* at 10°C and the supernatant was examined for the residue dye concentration.

By measuring the absorption of culture supernatant at λ_{max}, dye decolorization was determined. The decolorization percentage was determined according to the formula:

$$D = (A1-A2)/A1 \times 100\%$$

where A1 represented the absorbance of the control, A2 represented the absorbance of the corresponding treated sample and D was the dye decolorization efficiency (Janaki V, 2015).

The chosen bacterial isolates displaying notable decolorization were utilized to establish the consortium. The isolates, cultured individually in nutrient broth under shaking conditions for 24 hours, were combined to form consortia by blending them in equal proportions. These isolates were combined in various permutations (Isolate 10 and 14, Isolate 14 and 18, Isolate 10 and 18, Isolate 10, 14, and 18) to evaluate the impact of their collective metabolism on decolorization efficiency at a dye mixture concentration of 200 mg/L in both static and shaking conditions (150 RPM). Subsequently, the decolorization efficiency of all the consortia was assessed and calculated. These consortia were preserved at 4°C for subsequent utilization. To scale-up the process, a 6% (v/v) of the bacterial consortia culture suspension from inoculums grown was inoculated into 350 mL of medium in 500 mL flasks and incubated in a shake flask incubator at 37°C and 150 rpm for 3 days and the decolorization efficiency was assessed.

Synthesis of Eco-Friendly Nano Photocatalysts from plant extracts

Synthesis of ZnO/CuO Nanoparticles. Eco-friendly nano photocatalysts, such as copper and zinc oxide (CuO and ZnO) nanoparticles, were synthesized using environmentally friendly methods by utilizing leaves of the plants *Combretum rotundifolium* (Monkey brush vine), *Hibiscus schizopetalus* (Spider hibiscus) and *Parmentiera cereifera* (Candle tree) which were never explored for similar kind of studies. Fresh leaves of these plants were collected, washed, rinsed, chopped and then dried at 50°C for 3 days, and then powdered. Distilled water was added to each leaf powder in 1:10 ratio, and heated at 80°C for 3 hours. 50 ml of the resultant mixture was centrifuged at 7826 × *g*. The supernatant was separated and named as extract. The use of chopped leaves in this study was

primarily aimed at extracting bioactive compounds and phytochemicals present in the leaves, which could potentially contribute to the synthesis and characteristics of the nanoparticles. The process of chopping will also allow the drying process fast and easy powdering. Leaves contain a variety of bioactive compounds beyond chlorophyll, such as flavonoids, polyphenols, terpenoids, sugars, ketones, aldehydes, carboxylic acids, and amides, and other secondary metabolites which are responsible for bioreduction of nanoparticles (13). These compounds can play crucial roles in the synthesis and stabilization of nanoparticles, influencing their size, morphology, and surface properties (14).

The extracts obtained were used for the synthesis of metal oxide nanoparticles. To 50 ml of each plant extract, 2 g of Zinc acetate dihydrate ($Zn(CH_3COO)_2 \bullet 2H_2O$) was added and 8.5 g of Cupric chloride (CuCl) was added to 50 ml of plant extract in another batch. The mixture was stirred at 60° C for 2 hours, and 15 ml of the resultant was centrifuged at 7826 × *g*. The supernatant was removed and the nanoparticle pellet was redispersed in distilled water. The centrifugation-redispersion process was done thrice, followed by ethanol (3-4 times) to remove impurities. The purified samples were transferred to a petri plate and kept in a hot air oven at 65°C for 8 hours and brown colored powders were obtained. The physicochemical properties of the photocatalysts were characterized using techniques such as UV-vis spectrophotometer and scanning electron microscopy (SEM).

UV-Photocatalytic degradation. In a batch system with a total capacity of 300 ml, photocatalytic experiments were conducted. In the photocatalytic reactor (Make: Heber, Model: Mild Steel Annular Type) the total volume of the dye solution was 100 mL. In order to provide proper mixing, a reactor containing a mixture of dye solution and metal oxide photocatalyst (reaction solution) was installed on a magnetic stirrer. The light source used is mercury lamp with a power of 150 W (UV-C, with a maximum emission wavelength of 254 nm). Inside the glass tube, the UV lamp was installed in the center and parallel to the length of the reactor. In order to avoid the surrounding ultraviolet radiation, the reactor is in an enclosed chamber. 300 ml of 200 µg/ml of dye mix was prepared and poured into quartz tubes of the photocatalytic reactor. The respective ZnO nanoparticles were added to each tube at a concentration of 20 µg/ml, and the samples were exposed to UV light of wavelength 254 nm, for 2 hours. In the same manner, samples containing Cu nanoparticles were also exposed to UV light of 254 nm for 2 hours. The UV-pretreated treated dye samples were used to prepare the nutrient broth media which was further autoclaved at 121°C, 15 psi for 15 min (Autoclave Make: Supertek, Model:CH10027D) before inoculating with the bacterial consortia.

Combined biological-photochemical process. The combined effects of studies were carried out to investigate the efficiency of UV radiation process as a pre-treatment of dye before microbial degradation to make the recalcitrant dye molecules biodegradable and to attain maximum degradation. To carry out these experiments, the UV pretreated dye mixture sample (200 mg/L) was used to prepare nutrient broth media. The medium was sterilized at autoclaved at 121°C, 15 psi for 15 min (Autoclave make: Supertek, Model:CH10027D) and inoculated with the developed bacterial consortia. 100 µL of inoculum (consortia) was added and then incubated for 3 days at 37°C. The degradation achieved solely by the bacterial consortia, without any UV pretreatment, served as a control or reference point for evaluating the effectiveness of UV-mediated degradation of textile dyes. By comparing the degradation results between the bacterial consortia alone and the combined UV pretreatment followed by bacterial degradation, we aimed to assess whether UV pretreatment enhances the degradation process. 5 ml of treated sample was centrifuged at 1957 × *g*, the supernatant was collected and UV-Vis Spectra was taken to measure the degradation efficiency.

Data Analysis

All data from dye decolorization assays were evaluated for statistical significance using one-way analysis of variance (ANOVA). Results are presented as the mean ± standard error of triplicate samples. Statistical significance was determined at a p-value of less than 0.05, indicating a significant difference.

RESULTS AND DISCUSSION

Isolation of dye-decolorizing bacteria

The dye-degrading bacteria were selected by examining the area of decolorization surrounding the colony. Around 18 bacterial isolates showed a zone of decolorization, suggesting their ability to degrade dye (Fig. 1). These isolates were selected, cultured, and labeled from Isolate 1 to Isolate 18. The lambda max of the mixture of dyes was found to be 502.5 nm, so the absorbance of the sample was read at this wavelength to measure the degradation efficiency. Filtrates obtained from the control cultures grown in the absence of dye mixture were used as blanks while obtaining these spectra.

Bacterial dye degradation analysis

The highest degradation was observed in dyes degraded by isolates 10, 14 and 18 (Fig. 2). Decolorization of 51.3% was shown by isolate 10 followed by isolate 14 (59.36%) and isolate 20 (63.54). The UV visible spectrums indicated that the major peak corresponding to the lambda max disappeared whereas other two peaks didn't disappear completely (Fig. 3). This could be due to the presence of different dyes in the mixture and all the newly formed molecules in the dye mixture may not be getting degraded equally. The absorbance throughout the spectrum reduced indicating the degradation of all the dyes in the mixture. A new peak appeared at 420 nm indicating the formation a new compound after degradation.

Based on the effect of single isolates, four different types of the consortium were developed (Fig. 2). When all four consortia were tested in 200 ml of media, the consortium containing isolates 10, 14, and 18 showed the highest degradation under static conditions (72.38%) and shaking conditions (50.62%). This suggests that bacteria degraded the dye more effectively under static conditions compared to shaking conditions (Fig. 4). Further scale-up studies conducted in 350 ml of media confirmed that degradation was higher under static conditions (75.14%) compared to shaking conditions (52.36%) (Figs. 5 and 6), and the degradation efficiency slightly improved.

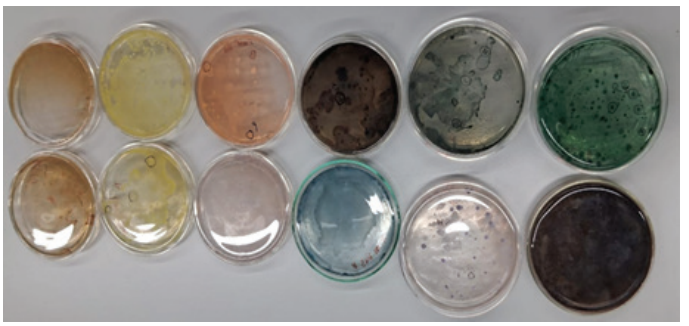

Figure 1. Screening potential dye-degrading bacterial isolates on nutrient media containing dyes: observation of decolorization zone surrounding colonies.

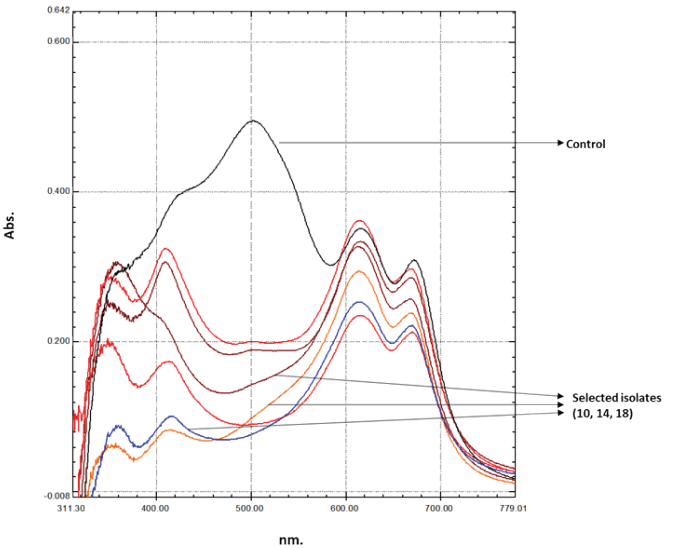

Figure 3. UV-Vis spectra of dye mixture in the presence (Test) or absence (Control) of bacterial isolates.

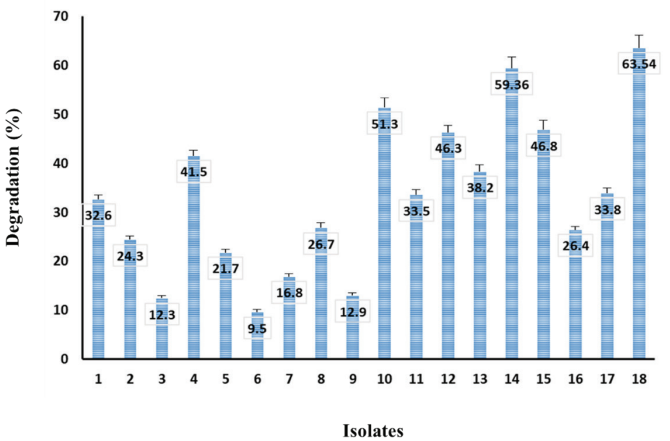

Figure 2. Degradation activities (%) of dye mixture by the selected bacterial isolates (1 to 18) (p<0.05).

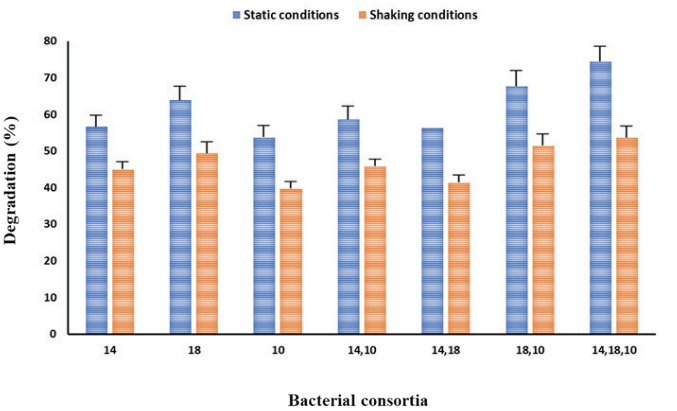

Figure 4. Degradation activities (%) of selected bacterial isolates (10, 14,18) and the consortia in static and shaking conditions (p<0.05).

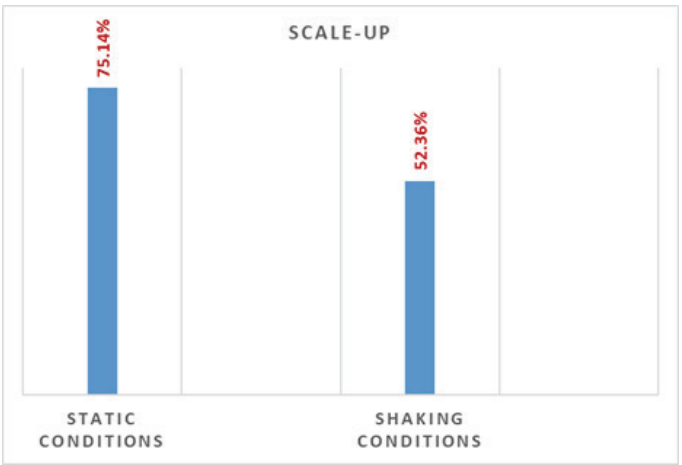

Figure 5. Decolorization efficiency after scaling up the treatment process in static and shaking conditions (p<0.05).

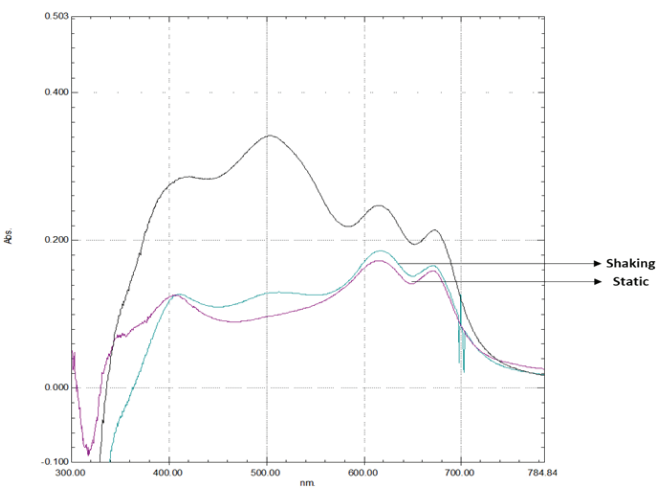

Figure 6. UV-Vis spectra of dye mixture in the shaking or static (Test) or absence (Control) of bacterial consortium.

Additionally, the UV-vis spectrum analysis indicated a slightly different degradation pattern between shaking and static cultures.

In general, the impact of a single bacterial isolate on a single dye tends to be more pronounced compared to its effect on a mixed dye (15, 16). However, the consortium displayed varying levels of dye degradation, leveraging its broader enzymatic capabilities, and mitigating the formation of toxic intermediary metabolites through the selection of dead-end products primarily produced via co-metabolism processes (17). Decolorization of the dye mixture by the consortium commenced approximately 24 hours post-incubation, with maximal decolorization observed around the 72-hour mark. Similar findings have been documented across various literature sources (18). The efficacy of the decolorization process hinges on factors such as the survival, adaptability, and enzymatic activities of microorganisms present within the mixed cultures (19).

The gram staining and motility test results indicated that isolate 10 and 14 are gram-positive, motile bacilli arranged in chains and clusters, respectively. In contrast, isolate 18 is gram-positive, non-motile cocci arranged in clusters (Fig. 7). Motility was determined by observing growth radiating from the stab inoculation line. Additionally, cell morphology and arrangement were observed from the same gram staining observation slide. Gram-positive bacteria, such as cocci and bacilli, are known to produce a variety of enzymes involved in dye degradation processes, including oxidoreductases and hydrolases. These enzymes may act on different classes of dyes through various mechanisms, such as reduction, oxidation, or hydrolysis. The varying colony morphologies observed on the nutrient agar media for all three isolates confirm their distinct strain identities.

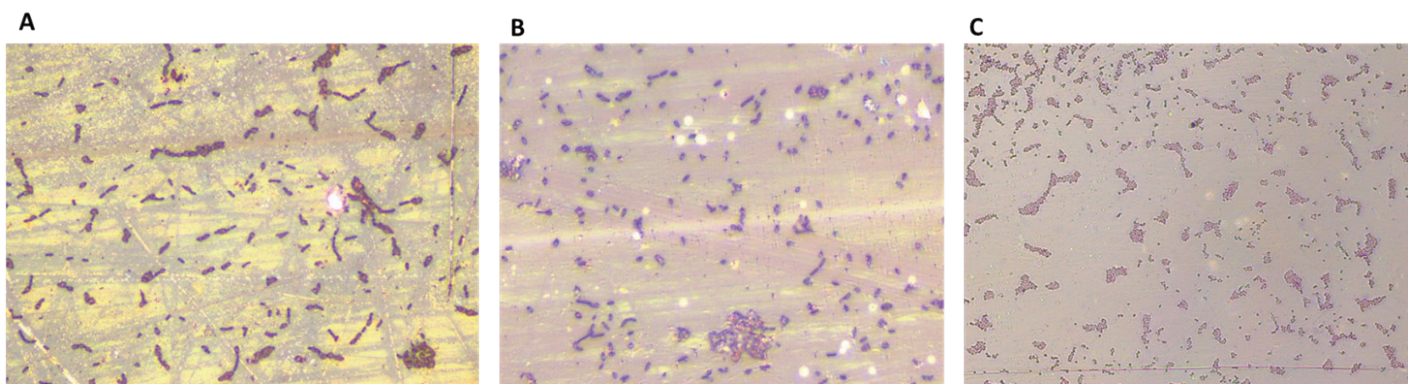

Figure 7. Gram staining results: (A) Gram positive bacilli (Isolate 10), (B) Gram positive bacilli (Isolate 14) and (C) Gram positive cocci (Isolate 18).

Degradation using UV-photocatalytic mediated process and integrated approach

Metal oxide nanoparticles, known for their large surface area and numerous active sites, accelerate reactions and boost product yield. In our study, we utilized these nanoparticles as catalysts to enhance dye degradation. Copper and zinc oxide nanoparticles were synthesized using leaf extracts from *Combretum rotundifolium*, *Parmentiera cereifera*, and *Hibiscus schizopetalus*. These extracts, when mixed with metal salts, changed color, indicating nanoparticle formation. The nanoparticles were then employed as catalysts (20 µg/ml) in dye degradation. When exposed to high-energy UV photons, these nanoparticles absorb the energy, promoting electrons to higher energy levels and causing chemical changes in the material, aiding in dye degradation. In our experiments, zinc oxide nanoparticles proved to be superior catalysts compared to copper oxide nanoparticles. Among the three plants, *Parmentiera cereifera* yielded metal oxide nanoparticles that showed the highest degradation efficiency. Zinc and copper oxide nanoparticles derived from *Parmentiera cereifera* exhibited degradation efficiencies of 23.5% and 18.2%, respectively, while other treatments showed lower degradation rates. (Fig. 8). While the nanoparticles themselves are indeed a product of the metal salts and leaf extracts, the specific characteristics of the nanoparticles, including their size, shape, and surface chemistry, can be influenced by the constituents present in the leaf extract (20, 21). Therefore, the differences in decolorization efficiencies observed with different plants may indeed be attributed to the unique composition of the leaf extracts, which in turn affect the properties of the synthesized nanoparticles.

The pretreated dye samples were then subjected to bacterial degradation. The highest degradation occurred in the presence of zinc oxide nanoparticles synthesized from *Parmentiera cereifera* (83.8%), followed by *Hibiscus schizopetalus* (77.46%). Photo-catalytic treatment combined with bacterial consortia also showed better degradation compared to samples treated only with bacterial consortia. Different classes of dyes might show varying rates of decolorization. Bacterial degradation and UV-photolysis may target different components or pathways within the dye molecule, resulting in complementary rather than synergistic effects. Since all dyes have some level of absorbance across the spectrum, the degradation of all dyes in the mixture can be confirmed by observing a decrease in absorbance across the spectrum in both control and test samples using UV-Vis spectrometry. While it's possible that not all dyes degrade at the same rate, our approach closely reflects real-world scenarios. Further investigation into the specific enzymatic pathways involved and their substrate specificities would provide valuable insights into the mechanisms underlying dye decolorization. The degradation percentage of dye samples their respective UV-visible spectrums are shown in Fig. 8 and 9. The degradation efficiency was highest on the third day of cultivation, reaching 83.8% with zinc oxide nanoparticles from *Parmentiera cereifera*. Subsequent cultivation did not yield further degradation.

Future research endeavors could certainly explore the combined approach involving bacterial remediation followed by UV photolysis to provide a more

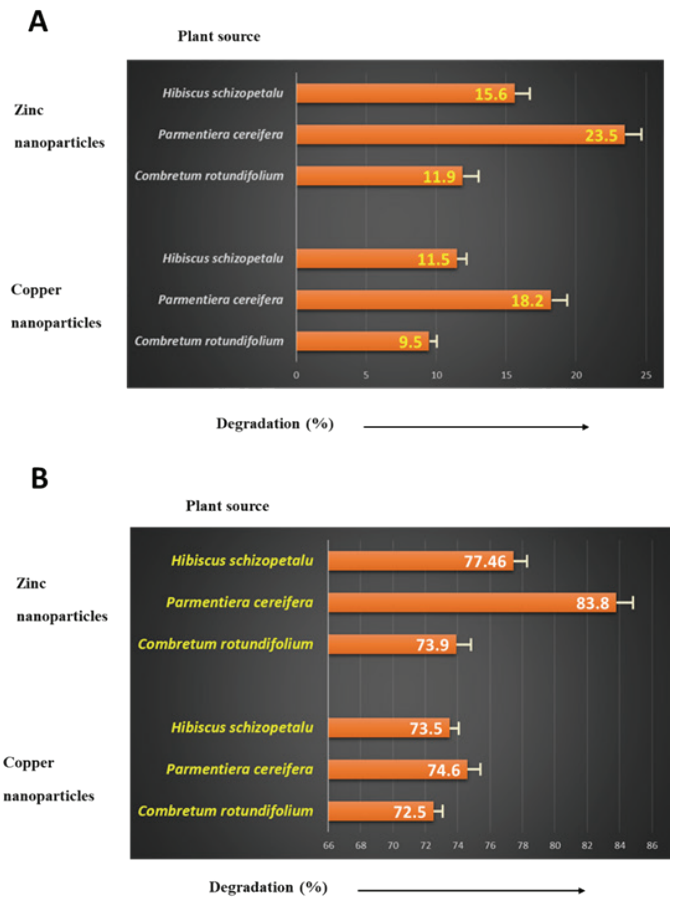

Figure 8. Bar graph showing decolorization percentage using (A) only metal oxide nanoparticles combined with UV treatment (B) UV-metal oxide nanoparticles as pretreatment to microbial degradation. Note that error bars indicate the standard deviations for the triplicates (p<0.05).

comprehensive understanding of the remediation process. Overall, the integrated system combining photocatalysts and microbial consortia demonstrated significant improvement in dye degradation compared to individual treatments (Fig. 10). This combined approach enhances degradation rates and facilitates better dye removal.

Characterization of Eco-Friendly Nano Photocatalysts

The scanning electron microscopy (SEM) images in Fig. 11A present representative results of the morphology of Zinc oxide nanoparticles synthesised using *Parmentiera cereifera* leaves. The nanoparticles were characterized by homogeneity of shape and size. The obtained ZnO

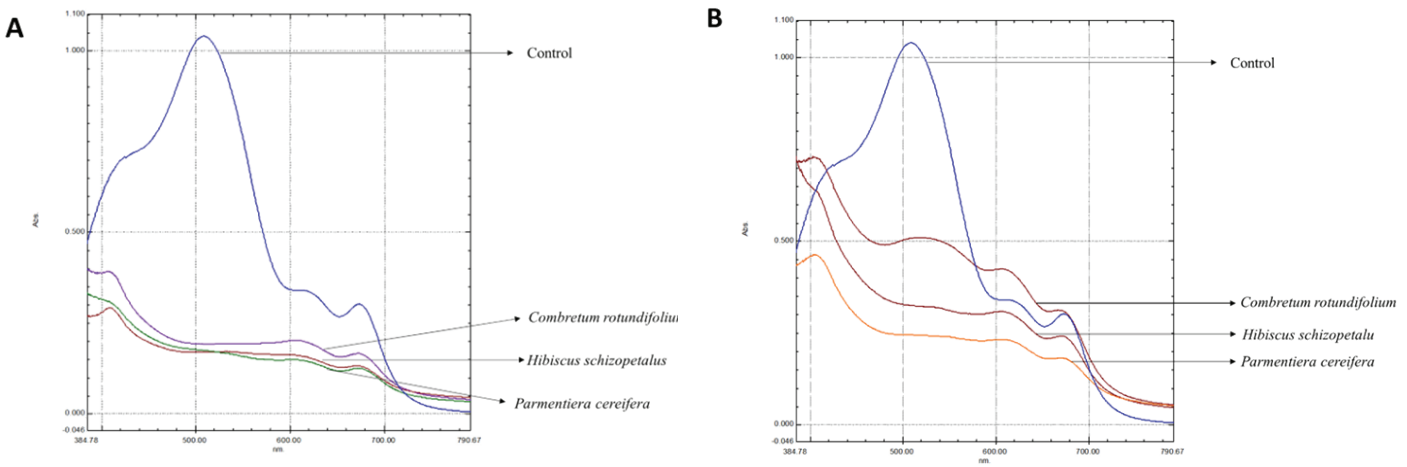

Figure 9. UV-Vis spectra comparison of the dye mixture with (Test) and without the bacterial consortium (Control). In the Test analysis, the dye mixture was pretreated with (A) UV-zinc oxide nanoparticles and (B) UV-copper oxide nanoparticles prepared using three plant extracts before bacterial degradation.

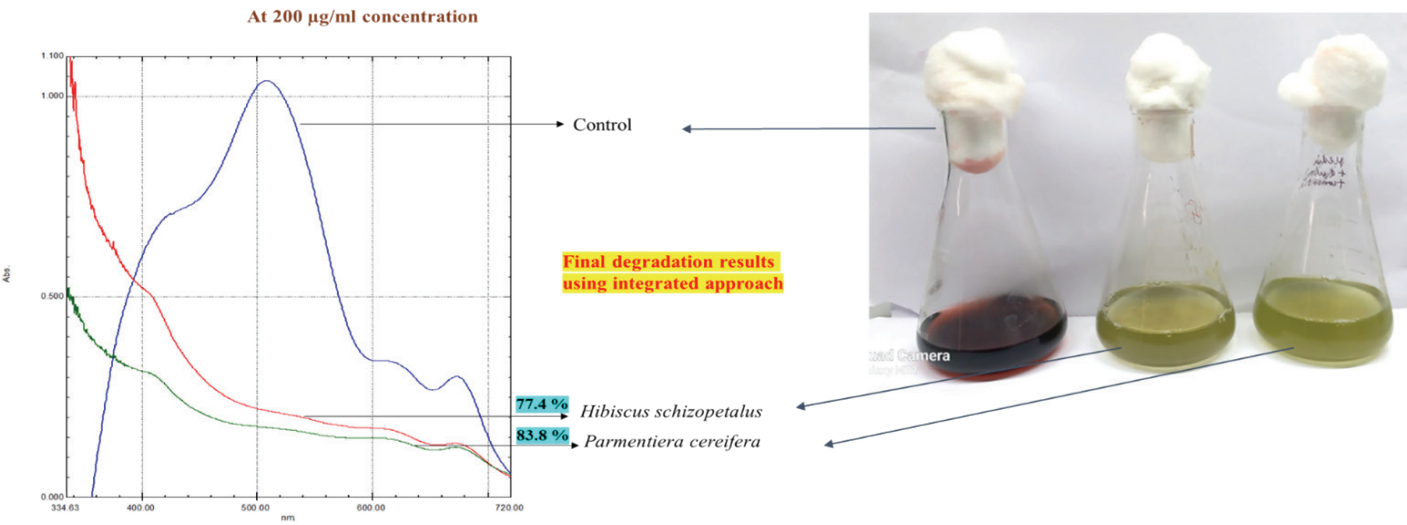

Figure 10. Left: UV-Vis spectra comparison between the untreated dye mixture (Control) and the dye mixture treated with UV-zinc oxide nanoparticles synthesized using *Parmentiera cereifera*-consortia and UV-zinc oxide nanoparticles synthesized using *Hibiscus schizopetalus*-consortia (Test). Right: Visual comparison of the corresponding control and test flasks, indicating visible degradation.

NPs ranged from 300 nm to 500 nm with the shape of nanorods and oval irregular bodies. The scanning electron microscopy (SEM) images in Fig. 11B present representative results of the morphology of copper oxide nanoparticles synthesised using *Parmentiera cereifera* leaves. The obtained CuO NPs ranged from 500 nm to 700 nm with the shape of nanorods and oval irregular bodies (Fig. 11).

Thin Layer Chromatography (TLC) was performed on four samples to validate the degradation process by observing the resulting products. The samples analyzed included the dye mixture, dye degraded by bacteria, dye treated with zinc nanoparticles, and dye treated with copper nanoparticles. A mobile phase consisting of Ethanol, Hexane, Ethyl acetate, and Dimethyl Sulfoxide in a 1:1:1:1 ratio was utilized. Silica coated on aluminum sheets served as the stationary phase. Notably, bands present in the untreated dye were absent in the treated dye samples, indicating degradation. Additionally, new bands were observed, suggesting the formation of by-products during degradation (Fig. 12).

Limitations of the study

We recognize some limitations of our current methodology, and we will address it in future studies to ensure a more comprehensive exploration of bacterial diversity and their potential for dye decolorization.

1. The molecules that resulted from the decolorization process were not assessed for their potential toxicity. It is essential to conduct toxicity studies on the degraded end products to ensure safety.

2. The experimental conditions used for the combined treatment have not been optimized to maximize synergy between bacterial degradation and UV-photolysis.

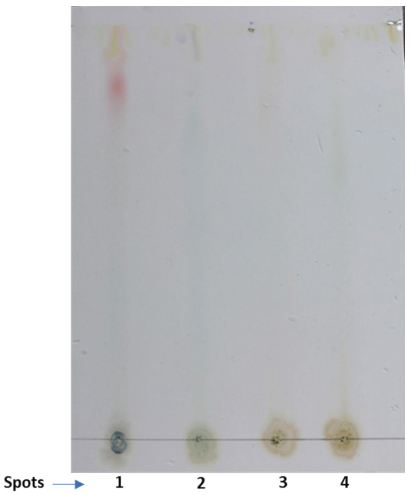

Figure 12. Separation of untreated dye mix (1), bacterial consortia-degraded dye mix (2), dye treated with UV-zinc oxide nanoparticles-consortia (3), and dye treated with UV-copper oxide nanoparticles (4) using the solvent system ethanol, hexane, ethyl acetate and dimethyl sulfoxide in the ratio 1:1:1:1.

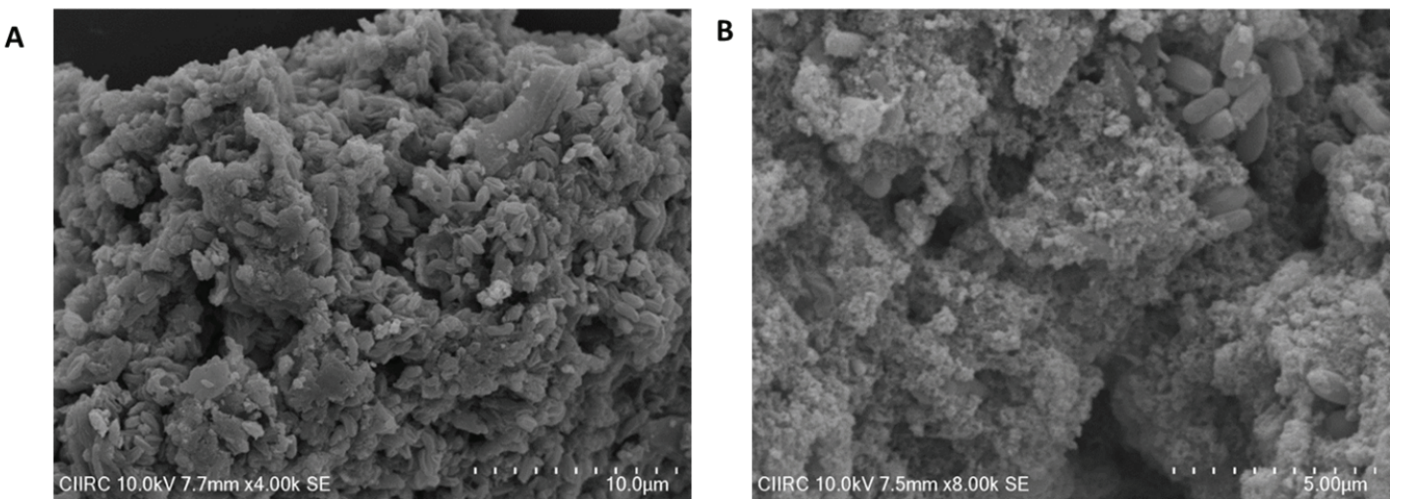

Figure 11. Scanning electron microscopy (SEM) images of (A) Zinc oxide nanoparticles synthesized using *Parmentiera cereifera* leaves. Nanoparticles and nanorods, oval with an average size of 300 - 500 nm. (B) Copper oxide nanoparticles synthesized using *Hibiscus schizopetalus* leaves. Nanoparticles and nanorods with an average size of 500-600 nm.

Factors such as pH, temperature, media optimization, UV treatment time, and substrate concentration could influence the effectiveness of the combined approach.

CONCLUSION

The integrated approach employing eco-friendly nano photocatalysts and microbial consortia demonstrated notable efficacy in degrading environmentally hazardous dyes. This combined method enhanced dye removal showcasing promising implications for wastewater treatment and environmental remediation. The consortium, comprising three bacterial strains, exhibited a 1.3-fold increase in decolorization efficiency compared to individual strains, efficiently tackling complex dye mixtures. UV–vis analysis confirmed the efficacy of this approach, which significantly outperformed singular treatments in decolorization and aromatic ring removal. Notably, the combined strategy achieved a remarkable 83.8% decolorization of a high-concentration textile dye mixture within 72 hours, economically and efficiently. Photocatalytic pre-treatment followed by microbial degradation proved superior, with zinc oxide nanoparticles from *Parmentiera cereifera* exhibiting the highest degradation rate. SEM analysis revealed rod-shaped green nanoparticles, correlating with UV-Visible absorption spectra changes indicative of dye degradation. Further optimization of the combined system is warranted, given its superior performance and cost-effectiveness compared to individual treatments, with future studies focusing on its potential application in textile processing industries.

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