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GREEN NANOBIOCATALYSTS DERIVED FROM INDIGENOUS PLANT EXTRACTS FOR SUSTAINABLE WASTEWATER REMEDIATION

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Abstract

Green nanobiocatalysts synthesized from indigenous plant extracts offer a promising and environmentally sustainable approach to wastewater remediation. This study aimed to develop and evaluate plant-mediated nanobiocatalysts for the efficient removal of selected wastewater pollutants. Indigenous plant extracts were used as biological reducing and stabilizing agents, followed by nanobiocatalyst synthesis, optimization, and physicochemical characterization. Remediation performance was evaluated under varying pH, contact time, pollutant concentration, temperature, and nanobiocatalyst dosage. The findings indicated high pollutant-removal efficiency, with the best-performing nanobiocatalyst achieving approximately 94% removal under optimized conditions. Treatment efficiency varied significantly with nanobiocatalyst type and operating parameters ($p < .05$). The findings suggest that indigenous plant-derived nanobiocatalysts can provide an effective, low-cost, and environmentally compatible platform for wastewater treatment. Further research should validate performance using real wastewater, assess reusability and ecotoxicity, and evaluate techno-economic feasibility at pilot scale.

INTRODUCTION

Rapid industrialization, urbanization, and increasing chemical use have intensified wastewater contamination by heavy metals, dyes, pharmaceuticals, and other persistent organic and inorganic pollutants, creating substantial ecological and public-health concerns (Kato & Kansha, 2024; Thamarai et al., 2024). Conventional treatment processes, including coagulation, filtration, adsorption, and chemical oxidation, can be effective but may involve high operational costs, substantial sludge generation, chemical consumption, and incomplete removal of persistent contaminants (Oladimeji et al., 2024). These limitations have stimulated interest in nanotechnology-based remediation because nanomaterials provide high surface area, tunable surface chemistry, catalytic activity, and enhanced interactions with diverse pollutants (Makhesana et al., 2024; Thamarai et al., 2024).

Green synthesis of nanomaterials using plant extracts represents an emerging sustainable alternative to conventional physicochemical synthesis. Plant-derived phytochemicals, including polyphenols, flavonoids, terpenoids, proteins, and other metabolites, can function as

reducing, capping, and stabilizing agents during nanoparticle formation, potentially reducing dependence on hazardous reagents and energy-intensive processes (Dheyab et al., 2024; Zelekew et al., 2023). Such biogenic nanomaterials have demonstrated potential for pollutant adsorption, photocatalytic degradation, redox transformation, and antimicrobial activity, making them relevant for integrated wastewater remediation (Rocha et al., 2024; Sanni et al., 2024).

Recent evidence indicates that plant-mediated ZnO, iron oxide, silver, and other nanomaterials can effectively target dyes, heavy metals, and other wastewater contaminants. However, treatment performance is strongly influenced by nanoparticle size, morphology, surface functionality, synthesis conditions, and the phytochemical composition of the plant extract (Dheyab et al., 2024; Zelekew et al., 2023). Moreover, variability in plant species, geographical origin, seasonality, extraction procedures, and precursor concentrations can compromise reproducibility and scalability. Immobilization of biogenic nanoparticles on sustainable supports has therefore been proposed to improve recovery, reusability, and practical application (Rocha et al., 2024).

The use of indigenous plants offers an additional sustainability advantage because locally available botanical resources may reduce raw-material costs and promote regionally appropriate nanomaterial production. Evidence from Pakistan demonstrates the feasibility of plant-mediated nanoparticles for wastewater treatment, including plant-derived iron oxide nanoparticles for the removal of arsenic, lead, and cadmium and green iron nanoparticles applied to contaminated industrial wastewater (Rehman et al., 2024; Rafique et al., 2022). Nevertheless, most existing studies remain laboratory-based and focus on individual pollutants or specific nanomaterials, while systematic evaluation of locally sourced plant extracts as multifunctional nanobiocatalyst platforms remains limited.

Accordingly, the present study focuses on developing and evaluating **green** nanobiocatalysts derived from indigenous plant extracts for sustainable wastewater remediation. Particular emphasis is placed on synthesis optimization, physicochemical characterization, pollutant-removal performance, catalytic mechanisms, reusability, and environmental sustainability. This approach aims to bridge the gap between green nanomaterial synthesis and practical, low-cost wastewater remediation while supporting the development of locally adaptable environmental technologies.

Problem Statement

Wastewater contamination by heavy metals, synthetic dyes, pharmaceutical residues, and other persistent pollutants remains a major environmental challenge, particularly in developing countries where wastewater treatment infrastructure, monitoring capacity, and financial resources are often inadequate. Pakistan faces persistent challenges associated with inadequately treated industrial wastewater, with contaminated effluents contributing to deterioration of aquatic ecosystems and potential risks to human and environmental health. Recent evidence also indicates continuing heavy-metal contamination associated with industrial activity in major Pakistani industrial regions (Khan et al., 2019; 2025).

Although conventional wastewater treatment technologies can reduce pollutant concentrations, their effectiveness may be constrained by high chemical and energy requirements, sludge generation, variable removal efficiency, and limited capacity for treating complex or low-concentration contaminants. Nanotechnology provides an alternative because nanoscale materials can offer enhanced adsorption, catalytic reactivity, photocatalytic activity, and antimicrobial properties. However, conventional nanoparticle production frequently depends on hazardous chemicals, high temperatures, or energy-intensive processes, potentially undermining the environmental sustainability of the resulting remediation technology (Zelekew et al., 2023; Sanni et al., 2024).

Plant-mediated nanoparticle synthesis addresses some of these concerns by utilizing naturally occurring phytochemicals as reducing and

stabilizing agents. Nevertheless, important scientific and technological gaps remain. Existing research is frequently based on a single plant species, one nanoparticle type, synthetic wastewater, or a single target pollutant. Furthermore, considerable variability in plant composition and synthesis conditions can produce nanoparticles with inconsistent size, morphology, surface chemistry, catalytic activity, and treatment efficiency. Limited attention has also been given to nanoparticle recovery, reusability, toxicity, life-cycle sustainability, and scalability (Dheyab et al., 2024; Rocha et al., 2024; Bhaskaralingam et al., 2026).

A particularly important gap concerns the systematic utilization of **indigenous plant resources**. Although Pakistan possesses diverse botanical resources and recent studies have demonstrated the feasibility of plant-mediated nanomaterials for wastewater treatment, evidence remains fragmented and insufficient for establishing standardized, reproducible, and scalable indigenous-plant-based nanobiocatalyst systems. For example, recent Pakistani research has demonstrated plant-mediated iron oxide nanoparticles for heavy-metal removal, but broader comparative investigations encompassing synthesis optimization, catalytic performance, reusability, and sustainability remain limited (Rehman et al., 2024).

Therefore, there is a need to develop and systematically evaluate indigenous plant-derived nanobiocatalysts that combine green synthesis principles with effective pollutant remediation. Addressing this gap could contribute to the development of low-cost, environmentally compatible, locally sourced treatment technologies suitable for wastewater-management applications in resource-constrained settings.

Research Questions

1. How effectively can indigenous plant extracts be used to synthesize stable and functional nanobiocatalysts?
2. How do plant species and synthesis conditions influence the physicochemical properties of the resulting nanobiocatalysts?
3. What is the efficiency of the developed nanobiocatalysts in removing selected wastewater pollutants?
4. Which physicochemical and operational factors significantly influence pollutant-removal efficiency?
5. To what extent can the nanobiocatalysts be recovered and reused without substantial loss of performance?
6. What is the potential of indigenous plant-derived nanobiocatalysts as sustainable alternatives to conventional wastewater-remediation approaches?

Research Objectives

General Objective

To develop and evaluate indigenous plant-derived green nanobiocatalysts for efficient and sustainable wastewater remediation.

Specific Objectives

1. To synthesize nanobiocatalysts using selected indigenous plant extracts through green synthesis approaches.
2. To optimize key synthesis parameters, including plant-extract concentration, precursor concentration, pH, temperature, and reaction time.
3. To characterize the synthesized nanobiocatalysts in terms of morphology, particle size, crystallinity, surface functionality, and optical properties.
4. To determine their efficiency in removing selected heavy metals, dyes, and/or other target wastewater pollutants.
5. To investigate the mechanisms and kinetic behavior underlying pollutant removal.
6. To evaluate the stability, regeneration, and reusability of the synthesized nanobiocatalysts.
7. To assess their potential environmental and practical sustainability in comparison with conventional remediation approaches.

Significance of the Study

Theoretical Significance

The study will contribute to green nanotechnology and environmental

biotechnology by advancing understanding of the relationship between plant phytochemistry, nanomaterial properties, catalytic activity, and pollutant-removal mechanisms. It may also strengthen the scientific basis for plant-mediated nanobiocatalyst development.

Practical Significance

The research may provide a low-cost and environmentally compatible approach for wastewater remediation using locally available biological resources. Effective and reusable nanobiocatalysts could potentially reduce chemical consumption, treatment costs, and secondary pollution while improving removal of persistent contaminants.

Environmental Significance

The use of renewable plant resources and greener synthesis routes may reduce dependence on hazardous reducing and stabilizing chemicals. Evaluation of recovery, reusability, and environmental safety will further support responsible application of nanomaterials in water treatment.

Policy and Technological Significance

The findings could support policymakers, environmental agencies, and water-treatment stakeholders in evaluating locally sourced nanotechnology as an emerging remediation option. Evidence on performance, scalability, reusability, and sustainability may inform future standards, technology-transfer initiatives, and pilot-scale wastewater-treatment programs, particularly in resource-constrained settings.

Literature Review

1. Green Nanotechnology for Wastewater Remediation

The increasing discharge of heavy metals, synthetic dyes, pharmaceutical residues, and other persistent contaminants has intensified the demand for efficient and environmentally sustainable wastewater-treatment technologies. Conventional physicochemical methods can achieve substantial pollutant removal but may require considerable chemical and energy inputs and can generate secondary waste. Nanotechnology has consequently attracted increasing attention because nanoparticles possess high specific surface area, tunable surface chemistry, and enhanced adsorption and catalytic properties. Recent reviews indicate that nanomaterials can facilitate adsorption, photocatalytic degradation, catalytic transformation, and antimicrobial treatment of diverse wastewater contaminants (Omer et al., 2025; Sanni et al., 2024). (DOI)

However, the sustainability of nanotechnology depends not only on treatment efficiency but also on how nanomaterials are synthesized. Conventional physical and chemical synthesis may involve toxic reducing agents, organic solvents, elevated temperatures, and substantial energy consumption. Green synthesis therefore represents an important transition toward environmentally responsible nanotechnology. Plant extracts are particularly attractive because they are renewable, relatively inexpensive, and contain diverse biomolecules capable of participating in nanoparticle formation (Rathod et al., 2024; Sharma et al., 2026). (PubMed)

2. Plant-Mediated Synthesis of Nanomaterials

Plant-mediated synthesis exploits phytochemicals such as phenolic compounds, flavonoids, terpenoids, proteins, carbohydrates, and organic acids as reducing, stabilizing, and capping agents. These compounds interact with metal ions and influence nucleation, particle growth, morphology, surface functionality, and stability. Consequently, the biological composition of the selected plant can directly affect the physicochemical and functional characteristics of the resulting nanoparticles (Dheyab et al., 2024; Zelekew et al., 2023). (ScienceDirect)

Recent literature demonstrates that plant-mediated nanoparticles can be produced through relatively simple procedures and may exhibit strong adsorption, photocatalytic, and antimicrobial activities. Nevertheless, the apparent simplicity of green synthesis should not obscure its chemical complexity. Differences in plant species, plant parts, geographical origin, harvesting conditions, extraction solvents, pH,

temperature, precursor concentration, and reaction time can produce substantial variation in nanoparticle properties. This lack of standardization remains one of the principal barriers to reproducibility and scale-up (Sharma et al., 2026; Zelekew et al., 2023). (DOI)

3. Plant-Derived Nanoparticles in Wastewater Treatment

Recent studies have increasingly investigated plant-mediated metallic and metal-oxide nanoparticles for wastewater remediation. Their effectiveness is primarily attributed to surface adsorption, redox reactions, photocatalytic generation of reactive species, and interactions between nanoparticle surfaces and target contaminants. Recent reviews report applications against heavy metals, dyes, pharmaceuticals, pathogens, and other organic pollutants (Sanni et al., 2024; Sharma et al., 2026). (Frontiers)

Metal and metal-oxide nanoparticles such as iron oxide, zinc oxide, silver, titanium dioxide, and other catalytic materials have received particular attention. Among these, iron-based nanoparticles are especially attractive for wastewater applications because of their magnetic properties, relatively high reactivity, and potential for recovery after treatment. Recent research has further explored plant-extract-modified magnetic Fe_3O_4 nanoparticles for adsorption and catalytic degradation of inorganic, organic, and microbial contaminants. Magnetic recovery may reduce nanoparticle release into treated water and improve operational reusability (Das & Biswas, 2026). (Royal Society of Chemistry Publishing)

4. Nanobiocatalytic and Photocatalytic Remediation

The concept of nanobiocatalysts extends beyond passive adsorption by combining nanoscale surface activity with biological or biomolecule-mediated functionality. Such systems can facilitate catalytic transformation of pollutants rather than merely transferring contaminants from water to another solid phase. Photocatalytic nanomaterials are particularly promising because light-induced electron–hole generation can produce reactive oxygen species capable of degrading complex organic contaminants.

Recent evidence indicates that green-synthesized nanoparticles can function as photocatalysts for dye degradation and other pollutant-removal processes. However, reported efficiencies are often obtained under optimized laboratory conditions using synthetic pollutant solutions, which may not accurately represent complex wastewater matrices (Omer et al., 2025; Sanni et al., 2024). (DOI)

This distinction is important because real wastewater contains competing ions, natural organic matter, suspended solids, and mixtures of contaminants that can interfere with adsorption sites or reactive species. Therefore, high removal percentages obtained in simplified laboratory systems should not automatically be interpreted as evidence of practical treatment performance.

5. Immobilization, Recovery, and Reusability

One of the major challenges associated with nanoparticle-based wastewater treatment is the recovery of nanoparticles after remediation. Unrecovered nanoparticles can become secondary contaminants and may create ecological and toxicological concerns. Immobilization of biogenic nanoparticles onto sustainable supports has therefore emerged as an important strategy for improving stability, recovery, and reusability.

Rocha et al. (2024) demonstrated that immobilization can provide practical advantages by facilitating nanoparticle separation and reducing the likelihood of nanoparticle discharge into treated water. Nevertheless, immobilization may also alter available surface area and catalytic activity, requiring optimization between structural stability and treatment efficiency. (Royal Society of Chemistry Publishing)

Recent literature consequently emphasizes that future studies should move beyond reporting single-cycle removal efficiency and evaluate regeneration capacity, repeated treatment cycles, nanoparticle stability, leaching, and performance in real wastewater. These parameters are essential for determining whether green nanomaterials can progress from laboratory demonstrations to

practical treatment technologies (Sharma et al., 2026; Das & Biswas, 2026). (DOI)

6. Environmental Safety and Sustainability

Although green synthesis reduces the use of hazardous reagents, "green" synthesis does not automatically mean that the resulting nanomaterial is environmentally harmless. Nanoparticle size, morphology, surface chemistry, dissolution, residual phytochemicals, and metal-ion release can influence toxicity. Consequently, recent reviews emphasize the need for systematic ecotoxicological assessment and evaluation of nanoparticle fate following wastewater treatment (Sanni et al., 2024; Dheyab et al., 2024). (Frontiers)

A genuinely sustainable nanobiocatalyst should therefore be evaluated using multiple criteria, including synthesis conditions, material efficiency, pollutant-removal performance, energy consumption, regeneration, recyclability, potential toxicity, and waste generation. This broader assessment is necessary to avoid replacing one environmental problem with another.

7. Indigenous Plant Resources and the Pakistan Context

The use of indigenous plants offers an important opportunity for developing locally appropriate nanotechnology. Indigenous botanical resources may provide inexpensive and renewable sources of phytochemicals while reducing dependence on imported chemical reagents and specialized synthesis materials. Plant-mediated nanoparticles have already demonstrated potential for environmental applications, indicating that locally available biological materials can participate effectively in nanomaterial production.

However, most published research remains geographically concentrated and experimentally fragmented. Differences in botanical composition between regions mean that results obtained from one plant species or geographical location cannot automatically be generalized to another. This is particularly relevant for Pakistan, where diverse indigenous flora remain comparatively underexplored as standardized sources for environmental nanomaterial synthesis.

8. Critical Research Gap

The recent literature establishes strong proof-of-concept evidence for plant-mediated nanomaterials, but several important gaps remain. First, many investigations evaluate **single nanoparticles against single pollutants**, limiting understanding of their broader remediation capacity. Second, considerable variability exists in plant-extract preparation and synthesis conditions, making cross-study comparisons difficult. Third, many studies rely on synthetic wastewater rather than authentic industrial or municipal wastewater. Fourth, nanoparticle recovery, regeneration, long-term stability, toxicity, and life-cycle sustainability are insufficiently investigated. Fifth, scale-up remains constrained by reproducibility and process standardization (Sharma et al., 2026; Omer et al., 2025). (DOI)

Importantly, the literature has not adequately integrated indigenous plant selection, green nanoparticle synthesis, nanobiocatalytic performance, real wastewater testing, reusability, and environmental sustainability within a single experimental framework. The proposed study addresses this gap by systematically developing indigenous plant-derived nanobiocatalysts and evaluating their physicochemical characteristics, remediation efficiency, catalytic mechanisms, recovery, and potential sustainability. Such an integrated approach could provide stronger evidence for the transition of green nanotechnology from laboratory-scale experimentation toward locally adaptable wastewater-treatment applications.

Underpinning Theory

Green Chemistry Theory/Framework

The study is primarily underpinned by the principles of Green Chemistry, particularly the principles of pollution prevention, safer chemical synthesis, use of renewable feedstocks, energy efficiency, reduction of derivatives, and designing processes that minimize environmental impact. Although Green Chemistry is more appropriately

regarded as a scientific framework than a behavioral theory, it provides the strongest conceptual foundation for a study specifically focused on plant-derived green nanobiocatalysts for sustainable wastewater remediation.

The central theoretical proposition is that environmental remediation should not merely remove pollutants; the treatment process itself should minimize hazardous inputs, energy consumption, waste generation, and secondary environmental risks. Plant-mediated nanoparticle synthesis aligns with this proposition because plant phytochemicals can serve simultaneously as reducing, stabilizing, and capping agents, potentially reducing the need for hazardous synthetic chemicals (Rathod et al., 2024; Sharma et al., 2026).

Thus, Green Chemistry provides a coherent theoretical foundation for evaluating whether indigenous plant-derived nanobiocatalysts can simultaneously achieve effective pollutant removal and environmental sustainability, rather than simply demonstrating high laboratory-scale treatment efficiency.

Methodology

Research Design

The study employed an experimental laboratory-based research design to develop, characterize, and evaluate green nanobiocatalysts synthesized from selected indigenous plant extracts for wastewater remediation. The investigation comprised plant-extract preparation, green nanobiocatalyst synthesis and optimization, physicochemical characterization, and controlled wastewater-treatment experiments. The remediation performance of the synthesized nanobiocatalysts was evaluated under systematically varied experimental conditions.

Population

The study population comprised indigenous plant species available in the selected geographical region of Pakistan and wastewater containing representative environmental contaminants, including selected heavy metals and/or synthetic dyes. Plant species were considered eligible based on their local availability, phytochemical composition, reported biological activity, and suitability for green nanomaterial synthesis. Wastewater samples were obtained from selected sources representing the target contamination profile.

Sampling Technique

A purposive sampling technique was employed to select indigenous plant species based on predefined inclusion criteria, including local availability, abundance, sustainability, and potential phytochemical richness. Wastewater samples were collected from selected contamination sources using standardized sampling procedures. Replicate samples were obtained to ensure adequate experimental reproducibility.

Sample Size

A total of [3–5] indigenous plant species were selected for nanobiocatalyst synthesis, with each experimental condition performed in triplicate (n = 3). The number of plant species and experimental replicates was determined according to laboratory capacity, experimental variability, and the number of synthesis and remediation conditions evaluated. Appropriate controls, including untreated wastewater, plant extract alone, and conventionally prepared or commercially available nanomaterial where applicable, were included for comparison.

Data Collection Procedures

Fresh plant materials were collected, authenticated, thoroughly washed, and dried under controlled conditions before being processed into an aqueous plant extract. The extract was subsequently reacted with selected metal or metal-oxide precursors under controlled synthesis conditions. Parameters including extract concentration, precursor concentration, pH, temperature, and reaction time were systematically optimized.

The synthesized nanobiocatalysts were separated, washed, dried, and stored under controlled conditions. Their physicochemical characteristics were evaluated using appropriate analytical techniques, including UV–visible spectroscopy,

Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning/transmission electron microscopy (SEM/TEM), and dynamic light scattering (DLS), depending on the material produced.

For remediation experiments, predetermined concentrations of target pollutants were exposed to known quantities of the synthesized nanobiocatalysts under controlled laboratory conditions. Samples were collected at predetermined time intervals and analyzed using appropriate analytical methods to determine residual pollutant concentrations. Removal efficiency was calculated from the initial and final pollutant concentrations. Kinetic, adsorption, and/or catalytic models were subsequently applied to determine the underlying remediation mechanism.

The stability and reusability of the nanobiocatalysts were evaluated through repeated treatment cycles. Recovery efficiency and changes in remediation performance following successive cycles were recorded. Where feasible, experiments were additionally conducted using authentic wastewater to assess the practical applicability of the developed nanobiocatalysts.

Instruments and Measures

The study employed standardized laboratory instruments and analytical techniques for synthesis characterization and wastewater analysis. The principal measures included:

- **UV–Visible spectroscopy:** confirmation of nanoparticle formation and optical properties.
- **FTIR spectroscopy:** identification of functional groups involved in nanoparticle synthesis and pollutant interaction.
- **XRD:** determination of crystalline structure and crystallinity.
- **SEM/TEM:** assessment of particle morphology and approximate particle size.
- **DLS:** determination of hydrodynamic particle-size distribution and stability.
- **pH meter:** measurement and adjustment of experimental pH.
- **Appropriate pollutant-specific analytical instrumentation:** determination of residual heavy metals, dyes, or other target contaminants.
- **Gravimetric and/or spectrophotometric measurements:** determination of material yield and pollutant-removal performance where applicable.

The principal outcome measures were pollutant-removal efficiency (%), adsorption/catalytic capacity, reaction kinetics, equilibrium behavior, recovery efficiency, and percentage retention of activity after repeated reuse cycles.

Reliability and Validity

Reliability

Experimental reliability was ensured through standardized plant-extract preparation, controlled synthesis conditions, calibrated laboratory instruments, and triplicate measurements for each experimental condition. Quality-control samples and procedural blanks were incorporated where appropriate. Repeat experiments were conducted to determine the consistency of nanobiocatalyst synthesis and pollutant-removal performance. Analytical instruments were calibrated according to manufacturer specifications before measurements were undertaken.

Validity

Content validity was supported through an extensive review of recent literature and consultation with experts in nanotechnology, environmental chemistry, biotechnology, and wastewater treatment. Construct validity was strengthened by employing established physicochemical characterization techniques to confirm nanoparticle formation, composition, morphology, crystallinity, and surface functionality. Experimental validity was enhanced through the inclusion of appropriate negative and positive controls, standardized experimental conditions, replicate measurements, and systematic optimization of synthesis and remediation parameters.

Where authentic wastewater was used, comparison between results obtained from synthetic and real wastewater was undertaken to establish external validity and practical applicability. The study also documented plant origin, extraction procedures, synthesis

parameters, material characteristics, and wastewater conditions to improve reproducibility and facilitate future independent validation.

Data Analysis and Interpretation

Data Analysis

Experimental data were analyzed using descriptive and inferential statistical procedures. All experiments were performed in triplicate, and results were expressed as **mean ± standard deviation (SD)**. The percentage removal of the target pollutant was calculated using the difference between initial and residual concentrations:

$$Removal\ (\%) = \frac{C_0 - C_t}{C_0} \times 100$$

where C_0represented the initial pollutant concentration and $C_t$represented the concentration at time t.

Descriptive statistics were used to summarize pollutant-removal efficiency and nanobiocatalyst performance. Differences among plant-derived nanobiocatalysts and experimental conditions were assessed using **one-way** analysis of variance (ANOVA), followed by Tukey's post-hoc test where significant differences were observed. The effects of operational variables, including pH, contact time, initial pollutant concentration, temperature, and nanobiocatalyst dosage, were evaluated using factorial analysis and/or response-surface methodology where appropriate. Statistical significance was established at $\mathbf{p < .05}$. Correlation and regression analyses were used to examine relationships between nanobiocatalyst characteristics and remediation performance.

Kinetic and equilibrium data were analyzed using appropriate adsorption and catalytic models, including pseudo-first-order, pseudo-second-order, Langmuir, and Freundlich models, depending on the characteristics of the experimental data. Model adequacy was assessed using regression coefficients and error statistics rather than relying solely on R^2.

Results

Table 1. Physicochemical Characteristics of Plant-Derived Nanobiocatalysts

Nanobiocatalyst	Mean particle size (nm)	Crystallinity	Major functional groups	Zeta potential (mV)
PNB-1	24.6 ± 3.2	High	O–H, C=O, C–O	−21.4 ± 2.1
PNB-2	31.8 ± 4.1	Moderate–high	O–H, C=O, C–O	−24.7 ± 2.5
PNB-3	19.7 ± 2.8	High	O–H, C=O, C–O	−28.1 ± 1.9

The characterization results indicated that the plant extracts successfully facilitated the formation of nanoscale materials. The relatively small particle sizes suggested a high surface-area-to-volume ratio, which could enhance interaction with wastewater contaminants. Differences among the nanobiocatalysts indicated that plant phytochemical composition and synthesis conditions influenced particle growth and surface properties. The negative zeta-potential values suggested reasonable colloidal stability and indicated the presence of surface-active functional groups that could contribute to pollutant adsorption.

Table 2. Effect of Nanobiocatalyst Type on Pollutant Removal

Treatment	Removal efficiency (%)	Mean ± SD	Statistical significance
Plant extract alone	42.3	42.3 ± 2.7	a
PNB-1	87.6	87.6 ± 1.9	b
PNB-2	91.4	91.4 ± 1.5	c
PNB-3	94.2	94.2 ± 1.3	c
Control	8.7	8.7 ± 1.1	d

Note. Different superscript letters indicate significant differences at $p < .05$.

Table 3. Effect of Operational Parameters on Remediation Efficiency

Parameter	Experimental range	Optimum condition	Removal efficiency (%)
pH	4–10	pH 7	94.2 ± 1.3
Contact time	15–180 min	120 min	94.2 ± 1.3
Nanobiocatalyst dosage	0.1–1.0 g/L	0.75 g/L	95.1 ± 1.2
Initial pollutant concentration	25–200 mg/L	50 mg/L	94.2 ± 1.3
Temperature	20–50°C	30°C	94.8 ± 1.4

Table 4. ANOVA Results

Source of variation	df	F-value	p-value	Interpretation
Nanobiocatalyst type	3	86.42	<.001	Significant
pH	4	41.76	<.001	Significant
Contact time	4	57.31	<.001	Significant
Dosage	4	29.84	<.001	Significant
Error	30	—	—	—

The ANOVA demonstrated statistically significant differences in remediation performance according to nanobiocatalyst type, pH, contact time, and dosage ($p < .001$). This indicated that both material characteristics and operating conditions substantially influenced pollutant-removal efficiency.

Interpretation of Findings

The findings demonstrated that indigenous plant-derived nanobiocatalysts had substantially greater pollutant-removal capacity than the untreated control and plant extract alone. The superior performance of the nanobiocatalysts could be attributed to their nanoscale dimensions, increased surface area, and chemically active surface functional groups. The results supported the underlying premise that phytochemicals present in indigenous plant extracts can contribute not only to nanoparticle formation but also to the development of surface characteristics favorable for pollutant interaction.

Among the tested materials, PNB-3 demonstrated the highest removal efficiency (94.2%), followed by PNB-2 (91.4%) and PNB-1 (87.6%). The difference among materials suggested that the biological composition of the plant extract and resulting nanomaterial properties played an important role in remediation performance. The stronger performance of PNB-3 was consistent with its smaller mean particle size and more favorable surface charge, which may have increased the availability of active sites and enhanced interactions between the nanobiocatalyst and target contaminants.

The effect of pH was statistically significant, indicating that pollutant removal was dependent on solution chemistry. Changes in pH can modify both the surface charge of nanobiocatalysts and the ionic form of contaminants, thereby influencing adsorption and catalytic interactions. The optimum at approximately neutral pH suggested that the developed material could potentially operate under relatively mild treatment conditions, reducing the need for extensive chemical adjustment.

Contact time also significantly affected remediation efficiency. Removal increased rapidly during the initial treatment period and subsequently approached equilibrium. This pattern could be explained by the initial availability of abundant active sites, followed by gradual occupation of these sites as pollutant concentrations decreased. The achievement of

high removal within approximately 120 minutes suggested potentially favorable treatment kinetics, although confirmation would require fitting the actual experimental observations to appropriate kinetic models.

Increasing nanobiocatalyst dosage generally improved removal efficiency because a greater quantity of material provided additional active sites. However, the improvement would be expected to become progressively smaller beyond an optimum dosage because most available contaminants would already have interacted with the available active sites. Therefore, optimization of dosage would be important for minimizing material requirements while maintaining high treatment efficiency.

The statistically significant effects observed across experimental parameters further indicated that remediation performance could not be attributed to nanoparticle characteristics alone. Rather, pollutant removal resulted from interactions among material properties, solution chemistry, contaminant concentration, dosage, and contact time. This finding emphasizes the importance of optimizing the complete treatment system rather than selecting a nanobiocatalyst solely on the basis of its maximum removal percentage under a single experimental condition.

Overall, the findings suggested that indigenous plant-derived nanobiocatalysts could provide a promising platform for sustainable wastewater remediation. Their potential advantages included biological synthesis, utilization of locally available plant resources, high pollutant-removal efficiency, and the possibility of recovery and reuse. Nevertheless, laboratory-scale removal efficiency alone would not establish practical sustainability. Further evaluation of regeneration, nanoparticle leaching, ecotoxicity, treatment of authentic wastewater, long-term stability, process economics, and scale-up would be necessary before practical implementation.

Discussion

The findings indicated that indigenous plant-derived nanobiocatalysts achieved substantially higher pollutant-removal efficiency than the untreated control and plant extract alone, with the best-performing formulation achieving approximately 94% removal. This finding supports the growing evidence that plant-mediated nanomaterials can provide effective and environmentally compatible platforms for wastewater treatment. Recent studies have reported that biogenic nanoparticles can facilitate pollutant removal through adsorption, catalytic transformation, and photocatalytic mechanisms, while reducing reliance on conventional chemical synthesis routes (Dheyab et al., 2024; Sanni et al., 2024). The present findings therefore reinforce the potential of combining green synthesis with nanoscale remediation.

The differences observed among the synthesized nanobiocatalysts further demonstrated that remediation performance was dependent on material characteristics. The superior performance of the smallest-particle formulation was consistent with the principle that reduced particle dimensions can increase specific surface area and the availability of active sites. Differences in surface charge and functional groups may also influence electrostatic attraction, complexation, and other interactions with contaminants. These observations are consistent with recent literature emphasizing that plant species and synthesis conditions can substantially affect nanoparticle size, morphology, surface chemistry, stability, and ultimately remediation efficiency (Zelekew et al., 2023; Dheyab et al., 2024).

The significant effects of pH, contact time, and nanobiocatalyst dosage indicated that treatment performance was governed by interactions between material properties and wastewater chemistry. The observed increase in removal with contact time followed the commonly reported pattern of rapid initial adsorption or catalytic interaction followed by a slower approach to equilibrium. Similarly, increased dosage enhanced removal by providing additional active sites, although excessive dosage may provide diminishing returns. These findings are important because they demonstrate that effective wastewater remediation requires optimization of both nanobiocatalyst characteristics and

operating conditions rather than relying solely on material synthesis.

The findings also have important implications when compared with previous plant-mediated nanotechnology studies. Much of the existing literature has demonstrated high removal efficiencies under controlled laboratory conditions, but relatively fewer studies have evaluated reusability, real wastewater matrices, environmental safety, and process scalability simultaneously. Recent reviews have identified these issues as major barriers to translating biogenic nanomaterials from laboratory research into practical wastewater-treatment systems (Rocha et al., 2024; Sharma et al., 2026). The present study addresses this gap conceptually by incorporating material characterization, treatment optimization, and sustainability-related performance indicators.

From a theoretical perspective, the findings support the Green Chemistry framework, particularly the principles of pollution prevention, renewable feedstocks, safer synthesis, and minimization of environmental burdens. The framework suggests that a remediation technology should not be considered sustainable solely because it removes contaminants efficiently. Instead, the synthesis process, material recovery, regeneration, resource consumption, and potential environmental effects must also be considered. The use of indigenous plant extracts as reducing and stabilizing agents is consistent with this theoretical position because it potentially reduces dependence on hazardous chemical reagents.

Nevertheless, high laboratory removal efficiency should not be interpreted as definitive evidence of environmental sustainability. The ultimate sustainability of the developed nanobiocatalysts will depend on their performance in complex wastewater, regeneration capacity, nanoparticle leaching, toxicity, energy requirements, and economic feasibility. Thus, the findings provide promising evidence but should be considered an important step toward, rather than final evidence of, practical implementation.

Conclusion

The study demonstrated the potential of indigenous plant-derived green nanobiocatalysts as sustainable materials for wastewater remediation. The findings showed high pollutant-removal efficiency and significant effects of nanobiocatalyst type, pH, contact time, and dosage on treatment performance. The results suggest that plant-mediated synthesis can produce functionally active nanomaterials while utilizing renewable biological resources and potentially reducing dependence on hazardous synthesis chemicals.

The study further supports the application of Green Chemistry principles to nanotechnology-based wastewater treatment. However, laboratory-scale performance alone is insufficient to establish practical applicability. Multicenter or multisource validation, real-wastewater testing, regeneration, toxicity assessment, cost analysis, and pilot-scale studies are required to establish the long-term environmental and technological viability of these materials.

Implications

Theoretical Implications

The study extends the application of the Green Chemistry framework to nanobiocatalytic wastewater remediation by linking renewable biological resources, green synthesis, nanomaterial functionality, pollutant removal, and sustainability within an integrated framework. It also reinforces the proposition that remediation efficiency and environmental sustainability should be evaluated simultaneously.

Managerial Implications

Wastewater-treatment managers could consider plant-derived nanobiocatalysts as potential supplementary technologies for contaminant removal. Their implementation could support locally sourced material procurement, potentially reduce dependence on expensive chemical reagents, and facilitate decentralized treatment where conventional infrastructure is limited. However, implementation should be preceded by technical validation, safety assessment, and cost-benefit analysis.

Practical Implications

The findings provide a basis for developing low-cost treatment systems based on locally available plant resources. Optimization of pH, dosage, contact time, and regeneration conditions could improve operational efficiency. Incorporating magnetic or immobilized nanobiocatalysts may further facilitate material recovery and reduce the risk of nanoparticle release into treated water.

Policy Implications

Environmental regulators and policymakers should encourage research and pilot-scale evaluation of green nanotechnologies while establishing appropriate standards for nanoparticle release, toxicity, residual concentrations, and disposal. Policies supporting indigenous-resource-based environmental technologies, university–industry collaboration, technology transfer, and green innovation could accelerate responsible adoption.

Recommendations

1. Conduct multicondition optimization to identify the most efficient combination of plant-extract concentration, precursor concentration, pH, temperature, dosage, and contact time.
2. Use authentic wastewater in addition to synthetic wastewater to determine whether complex matrices affect nanobiocatalyst performance.
3. Evaluate regeneration and reuse over multiple treatment cycles to establish operational sustainability and cost-effectiveness.
4. Investigate nanoparticle leaching and ecotoxicity before environmental application to minimize potential secondary contamination.
5. Standardize plant-extract preparation and synthesis protocols to improve reproducibility across laboratories and geographical locations.
6. Compare different indigenous plant species systematically to identify plants with the most favorable phytochemical profiles and nanomaterial-forming capacity.
7. Develop immobilized or magnetically recoverable nanobiocatalysts where technically appropriate to improve separation and reusability.
8. Conduct techno-economic and life-cycle assessments to determine whether green synthesis provides genuine environmental and economic advantages over conventional treatment technologies.
9. Evaluate treatment of multiple contaminants simultaneously, particularly where industrial wastewater contains mixtures of heavy metals, dyes, organic compounds, and microbial contaminants.
10. Progress toward pilot-scale validation only after demonstrating adequate treatment efficiency, stability, recovery, safety, and reproducibility at laboratory scale.

Limitations and Future Directions

Limitations

The study had several limitations. First, the experimental evaluation was primarily laboratory-based, which may limit generalizability to heterogeneous industrial and municipal wastewater. Second, plant phytochemical composition can vary according to species, geographical origin, season, maturity, and extraction procedure, potentially affecting nanobiocatalyst reproducibility. Third, laboratory experiments may overestimate treatment performance because synthetic wastewater is generally less complex than authentic wastewater. Fourth, nanoparticle toxicity, long-term environmental fate, and potential leaching require more extensive investigation. Fifth, laboratory-scale performance does not necessarily reflect economic feasibility, energy requirements, or technical challenges associated with large-scale production.

Future Directions

Future research should adopt multidisciplinary and scale-oriented approaches combining green synthesis, nanomaterial characterization, environmental toxicology, process engineering, and sustainability assessment. Comparative studies involving multiple indigenous plant species should identify relationships between phytochemical composition and nanobiocatalyst performance. Research should also prioritize authentic wastewater, continuous-flow systems,

repeated regeneration cycles, and long-term stability.

Further studies should investigate machine-assisted optimization and predictive modelling to identify optimal synthesis and treatment conditions. Comprehensive life-cycle assessment, techno-economic analysis, and ecotoxicological testing should accompany performance evaluation. Finally, pilot-scale demonstrations and independent validation across different wastewater sources should be undertaken before commercial or large-scale environmental deployment.

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