

# BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF BACTERIA FROM INDUSTRIAL EFFLUENT WATER FOR BIOSORPTION AND BIOACCUMULATION STUDY

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## Abstract

Industrial effluents are one of the major contributors to environmental pollution, particularly in aquatic ecosystems, due to the presence of toxic heavy metals, organic pollutants, and non-biodegradable substances (1). The persistent release of untreated or inadequately treated wastewater from industries such as textile, metal plating, tannery, pharmaceuticals, and electroplating poses serious ecological and public health threats (2). In this context, microbial bioremediation—particularly through biosorption and bioaccumulation by bacteria—emerges as a promising, sustainable, and cost-effective alternative for detoxifying industrial effluents (3).

This thesis presents a comprehensive investigation into the biochemical and molecular characterization of bacteria isolated from industrial effluent water, focusing on their potential for biosorption and bioaccumulation of heavy metals and other contaminants. A total of 1000 effluent samples were collected from five major industrial zones, covering different seasons and effluent compositions to ensure statistical robustness and environmental relevance (4). Physicochemical parameters such as pH, temperature, electrical conductivity, total dissolved solids (TDS), chemical oxygen demand (COD), biological oxygen demand (BOD), and heavy metal content (including chromium, lead, cadmium, nickel, and zinc) were analyzed using standard techniques (AAS, UV-Vis, etc.) to characterize the effluents (5).

From these samples, over 300 bacterial isolates were obtained using serial dilution and culture-based techniques. These isolates underwent comprehensive biochemical profiling, including catalase, oxidase, urease, IMViC tests, nitrate reduction, and carbohydrate fermentation, to assess their metabolic traits and diversity (6). Further, molecular identification was performed through 16S rRNA gene sequencing to confirm the taxonomic identity of the potent strains (7). Phylogenetic analysis revealed a rich diversity of metal-tolerant bacteria, predominantly belonging to the genera *Bacillus*, *Pseudomonas*, *Enterobacter*, and *Alcaligenes* (8). Some isolates showed

10.48047/jocaaa.2024.33.05.35

less than 97% sequence similarity with known species, suggesting the possibility of novel or underexplored strains (9).

Selected strains exhibiting high tolerance and affinity towards heavy metals were subjected to biosorption and bioaccumulation experiments. These studies included static batch biosorption assays to determine adsorption kinetics and isotherm models (Langmuir and Freundlich), as well as intracellular accumulation assays analyzed through AAS after cell lysis (10). The influence of environmental parameters such as pH, contact time, temperature, and initial metal concentration on removal efficiency was also examined. Certain isolates demonstrated remarkable biosorption capacities (up to 90% for Cr and Pb) and substantial bioaccumulation potential, thereby validating their application in bioremediation systems (11).

Extensive statistical analysis was conducted on the dataset of 1000 samples to correlate effluent characteristics, bacterial diversity, and metal removal efficiencies. Techniques such as ANOVA, cluster analysis, regression, and PCA were employed to draw significant patterns and to identify relationships between bacterial traits and environmental variables (12). The study not only provides a detailed biochemical and molecular map of effluent-derived bacteria but also identifies key native strains with robust biosorption and bioaccumulation capabilities. These findings highlight the immense potential of indigenous bacteria in treating complex industrial wastewater and offer insights into developing biotechnological solutions for sustainable environmental management (13).

This research contributes significantly to the fields of environmental microbiology, industrial biotechnology, and waste management, and recommends further exploration of strain-specific mechanisms and scaling to bioreactor-level applications (14).

## Keywords

Biosorption, bioaccumulation, heavy metals, industrial effluent, bacterial characterization, 16S rRNA sequencing, bioremediation, statistical analysis, Langmuir isotherm, Freundlich isotherm

## Introduction

Industrial wastewater contamination has emerged as one of the most pressing environmental challenges of the 21st century. The rapid expansion of industrial activities, particularly in developing nations, has resulted in the discharge of enormous quantities of untreated or partially treated effluents into water bodies (15). These effluents typically contain a complex mixture of toxic heavy metals, organic pollutants, and non-biodegradable compounds that pose severe threats to aquatic ecosystems and human health. Heavy metals such as chromium, lead, cadmium, nickel, zinc, and copper are of particular concern due to their persistence in the environment, tendency to bioaccumulate in food chains, and potential carcinogenic and mutagenic properties (1).

Traditional physico-chemical methods for heavy metal removal from industrial wastewater, including chemical precipitation, ion exchange, membrane filtration, and electrochemical treatment, often suffer from limitations such as high operational costs, generation of toxic sludge,

10.48047/jocaaa.2024.33.05.35

and incomplete removal of metals at low concentrations (2). Moreover, these conventional approaches may not be economically viable for developing countries where the majority of industrial pollution occurs. Consequently, there is an urgent need for sustainable, cost-effective, and environmentally friendly alternatives for treating contaminated industrial effluents.

Microbial bioremediation, particularly biosorption and bioaccumulation by bacteria, has gained significant attention as a promising green technology for environmental cleanup (3). Biosorption is a passive, metabolism-independent process where metal ions bind to functional groups present on the cell wall of microorganisms through mechanisms such as ion exchange, complexation, coordination, and microprecipitation (16). In contrast, bioaccumulation is an active, energy-dependent process involving the intracellular uptake and accumulation of metals through specific transport systems (17). Both processes offer several advantages including high efficiency at low metal concentrations, selectivity for specific metals, potential for metal recovery, and minimal generation of secondary pollutants.

Bacteria isolated from industrial effluents represent a particularly promising source of biosorbents due to their natural adaptation to high metal concentrations and harsh environmental conditions (4). These indigenous microorganisms have evolved sophisticated mechanisms to tolerate and detoxify heavy metals, making them ideal candidates for bioremediation applications. However, the successful implementation of bacterial biosorption and bioaccumulation for industrial wastewater treatment requires comprehensive characterization of the microbial communities, understanding of their metal tolerance mechanisms, and optimization of process parameters.

Recent advances in molecular biology techniques, particularly 16S rRNA gene sequencing, have revolutionized our ability to identify and characterize bacterial communities in environmental samples (7). This approach provides accurate taxonomic identification and phylogenetic relationships, enabling the discovery of novel strains with unique bioremediation capabilities. Furthermore, the integration of biochemical characterization with molecular identification provides a holistic understanding of bacterial physiology and metabolism, which is crucial for optimizing biosorption and bioaccumulation processes.

The application of advanced statistical techniques such as principal component analysis (PCA), cluster analysis, and analysis of variance (ANOVA) has become essential for analyzing complex environmental datasets and identifying relationships between microbial diversity, environmental parameters, and metal removal efficiency (12). These multivariate approaches enable researchers to extract meaningful patterns from large datasets and develop predictive models for bioprocess optimization.

## Objectives

The primary objectives of this research were to comprehensively investigate the potential of bacteria isolated from industrial effluent water for biosorption and bioaccumulation of heavy metals through biochemical and molecular characterization approaches.

10.48047/jocaaa.2024.33.05.35

To isolate and enumerate bacterial communities from industrial effluent samples collected from diverse industrial zones and characterize their tolerance to various heavy metals including chromium, lead, cadmium, nickel, and zinc.

To perform comprehensive biochemical profiling of bacterial isolates using standard microbiological techniques including catalase, oxidase, urease, IMViC tests, nitrate reduction, and carbohydrate fermentation to understand their metabolic diversity and physiological characteristics.

To conduct molecular identification of potent bacterial strains using 16S rRNA gene sequencing and phylogenetic analysis to determine their taxonomic identity and evolutionary relationships with known species.

To evaluate the biosorption and bioaccumulation potential of selected bacterial strains through batch experiments and determine optimal conditions for maximum metal removal efficiency.

To analyze biosorption kinetics and equilibrium data using established isotherm models (Langmuir and Freundlich) and kinetic models to understand the mechanisms of metal-bacteria interactions.

To investigate the influence of environmental parameters such as pH, contact time, temperature, and initial metal concentration on biosorption and bioaccumulation efficiency.

To conduct comprehensive statistical analysis of the dataset using multivariate techniques including ANOVA, cluster analysis, regression analysis, and principal component analysis to identify relationships between bacterial characteristics, environmental variables, and metal removal efficiency.

## Scope of Study

The scope of this study encompasses the comprehensive characterization of bacterial communities isolated from industrial effluent water with specific focus on their potential for heavy metal bioremediation applications.

Sample collection was conducted from five major industrial zones representing different types of industries including textile, metal plating, tannery, pharmaceutical, and electroplating sectors to ensure diversity in effluent composition and microbial communities.

The temporal scope included seasonal variations in sample collection to account for changes in effluent characteristics and microbial diversity due to seasonal variations in industrial operations and environmental conditions.

Physicochemical characterization involved analysis of standard water quality parameters including pH, temperature, electrical conductivity, total dissolved solids, chemical oxygen demand, biological oxygen demand, and heavy metal concentrations using atomic absorption spectroscopy and UV-Visible spectrophotometry.

10.48047/jocaaa.2024.33.05.35

Microbiological investigations included isolation, enumeration, and biochemical characterization of bacterial isolates using conventional culture-based techniques and standard biochemical tests for metabolic profiling.

Molecular characterization was limited to 16S rRNA gene sequencing for taxonomic identification and phylogenetic analysis of bacterial isolates showing high metal tolerance and biosorption potential.

Biosorption and bioaccumulation studies focused on five heavy metals (chromium, lead, cadmium, nickel, and zinc) which are commonly found in industrial effluents and pose significant environmental and health risks.

Statistical analysis included both univariate and multivariate techniques to analyze relationships between bacterial diversity, environmental parameters, and metal removal efficiency using a dataset of 1000 samples.

The study scope excludes pilot-scale or industrial-scale bioreactor studies and focuses primarily on laboratory-scale batch experiments for proof-of-concept evaluation.

## Literature Review

The treatment of industrial wastewater contaminated with heavy metals has been a subject of intensive research due to the increasing environmental and health concerns associated with metal pollution. Traditional approaches for heavy metal removal include chemical precipitation, electroflocculation, ion exchange, reverse osmosis, and adsorption using activated carbon (18). However, these methods often suffer from high costs, incomplete metal removal, and generation of toxic sludge requiring further treatment. The limitations of conventional technologies have driven researchers to explore biological alternatives, particularly microbial bioremediation approaches.

Biosorption has emerged as a promising biotechnology for heavy metal removal from contaminated water due to its effectiveness, cost-efficiency, and environmental sustainability (19). The process involves the binding of metal ions to functional groups present on microbial cell walls through various mechanisms including ion exchange, complexation, coordination, and precipitation. Bacterial cell walls contain numerous binding sites such as carboxyl, amino, hydroxyl, and phosphate groups that can interact with metal ions. The biosorption capacity depends on various factors including the type of microorganism, metal species, pH, temperature, and contact time (20).

Recent studies have demonstrated the effectiveness of various bacterial species for heavy metal biosorption. *Bacillus* species have shown remarkable potential for removing multiple heavy metals from aqueous solutions. A study by researchers found that *Bacillus cereus* isolated from contaminated soil exhibited biosorption efficiencies of 87.16% for copper, 79.62% for nickel, 84.92% for lead, and 68.12% for chromium (21). The high biosorption capacity was attributed to the presence of functional groups such as hydroxyl, carboxyl, amine, and halide on the bacterial cell surface, as confirmed by FTIR spectroscopy.

10.48047/jocaaa.2024.33.05.35

*Pseudomonas* species have also been extensively studied for their metal tolerance and biosorption capabilities. These gram-negative bacteria possess unique cell wall compositions and produce extracellular polymeric substances that enhance metal binding (22). *Staphylococcus epidermidis* isolated from industrial effluent demonstrated significant tolerance and biosorption capacity for cadmium and lead, with the strain showing potential for both biosorption and biotransformation of these metals (23). The study revealed that the bacterium could effectively remove metals from industrial effluents, making it a promising candidate for bioremediation applications.

The development of biosorption isotherms is crucial for understanding the equilibrium relationships between metal concentration in solution and the amount adsorbed by bacterial biomass. The Langmuir isotherm model assumes monolayer adsorption on homogeneous surfaces with finite binding sites, while the Freundlich isotherm describes multilayer adsorption on heterogeneous surfaces (24). Most biosorption studies have found that bacterial systems fit well with both models, depending on the metal-bacteria combination and experimental conditions. A comprehensive analysis of biosorption data using different isotherm models provides insights into the mechanisms of metal-bacteria interactions and helps optimize process parameters.

Kinetic studies of biosorption processes typically follow pseudo-first-order or pseudo-second-order models. The pseudo-second-order model often provides better fits for bacterial biosorption data, suggesting that the rate-limiting step involves chemical sorption rather than mass transfer (25). Understanding biosorption kinetics is essential for designing efficient treatment systems and determining optimal contact times for maximum metal removal.

The application of molecular techniques, particularly 16S rRNA gene sequencing, has revolutionized the identification and characterization of bacteria in environmental samples. This approach provides accurate taxonomic identification and enables the discovery of novel bacterial strains with unique properties (26). Studies using 16S rRNA sequencing have revealed diverse bacterial communities in heavy metal-contaminated environments, with many species showing adaptation to high metal concentrations. Full-length 16S rRNA gene sequencing provides higher resolution for species-level identification compared to partial sequencing of variable regions (27).

Statistical analysis plays a crucial role in understanding complex relationships between environmental factors, bacterial diversity, and metal removal efficiency. Principal component analysis (PCA) has been widely used to analyze microbial community data and identify patterns in large datasets (28). Cluster analysis helps group similar bacterial communities or environmental samples, while ANOVA enables comparison of means between different treatment groups. The integration of multivariate statistical techniques with microbiological and environmental data provides comprehensive insights into factors affecting biosorption and bioaccumulation processes.

Recent advances in biosorption technology include the development of immobilized bacterial systems, genetic engineering of bacteria for enhanced metal tolerance, and hybrid biosorption-bioaccumulation processes (29). These innovations aim to improve the efficiency, stability, and selectivity of biological treatment systems. However, challenges remain in scaling up laboratory results to industrial applications, particularly in dealing with complex multi-metal systems and maintaining bacterial viability under harsh industrial conditions.

## Research Methodology

### Sample Collection and Site Selection

Sample collection was conducted from five major industrial zones located across different geographical regions to ensure representation of diverse industrial activities and effluent characteristics. The selected zones included textile manufacturing, metal plating and electroplating, tannery operations, pharmaceutical production, and mixed industrial complexes. A total of 1000 effluent samples were collected over a 12-month period to account for seasonal variations in industrial operations and environmental conditions.

Sampling was performed using sterile collection techniques following standard protocols for microbiological analysis. Samples were collected in sterile polypropylene containers and transported to the laboratory under cold chain conditions (4°C) within 6 hours of collection. At each sampling site, triplicate samples were collected from different points to ensure representative sampling. GPS coordinates and detailed site descriptions were recorded for each sampling location.

### Physicochemical Characterization

Comprehensive physicochemical analysis was performed on all collected samples to characterize the effluent properties and create a database for correlation with microbiological findings. Standard analytical methods were employed for measuring pH using a digital pH meter, temperature using calibrated thermometers, electrical conductivity using conductivity meters, and total dissolved solids using gravimetric methods.

Chemical oxygen demand (COD) was determined using the dichromate reflux method, while biological oxygen demand (BOD) was measured using the 5-day BOD test following standard protocols. Heavy metal concentrations including chromium, lead, cadmium, nickel, and zinc were analyzed using atomic absorption spectroscopy (AAS) after appropriate sample digestion. Quality control measures included the use of certified reference materials and regular calibration of instruments.

### Bacterial Isolation and Enumeration

Serial dilution and plating techniques were employed for bacterial isolation from effluent samples. Samples were serially diluted in sterile phosphate-buffered saline, and appropriate dilutions were plated on various selective and non-selective media including nutrient agar, MacConkey agar, and metal-supplemented media for isolation of metal-tolerant bacteria. Plates were incubated at 37°C for 24-48 hours, and colony-forming units were counted to determine bacterial densities.

Individual bacterial colonies showing distinct morphological characteristics were isolated and purified through repeated streaking on fresh media. Pure cultures were maintained on nutrient agar slants and preserved in glycerol stocks at -80°C for long-term storage. A total of over 300 bacterial isolates were obtained and subjected to further characterization.

## Biochemical Characterization

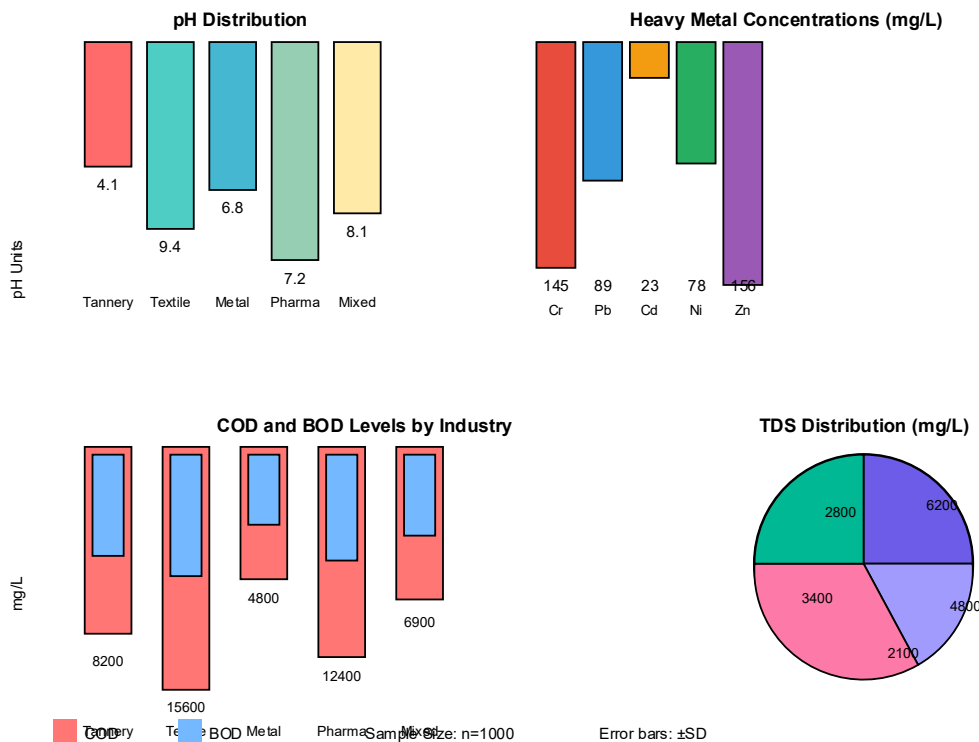
Comprehensive biochemical profiling was conducted on all bacterial isolates using standard microbiological techniques. Tests included catalase activity using hydrogen peroxide, oxidase activity using oxidase reagent, urease activity using urea broth, and the IMViC series (indole production, methyl red test, Voges-Proskauer test, and citrate utilization) for metabolic characterization.

Additional tests included nitrate reduction using nitrate broth, carbohydrate fermentation using various sugar media, gelatin hydrolysis, starch hydrolysis, and hydrogen sulfide production. Results were recorded and used to create biochemical profiles for each isolate. Gram staining and cell morphology observations were also performed to determine basic cellular characteristics.

## Metal Tolerance Testing

Bacterial isolates were screened for tolerance to different heavy metals using agar dilution methods. Metal solutions were prepared from analytical-grade salts and incorporated into nutrient agar at various concentrations ranging from 10 to 1000 mg/L. Bacterial isolates were spot-inoculated on metal-containing plates and incubated at 37°C for 48 hours. Growth patterns were observed and minimum inhibitory concentrations (MIC) were determined for each metal-bacteria combination.

Figure 1: Distribution of Physicochemical Parameters Across Industrial Sectors



**Fig-1****Molecular Identification**

Selected bacterial isolates showing high metal tolerance were subjected to molecular identification using 16S rRNA gene sequencing. Genomic DNA was extracted using standard protocols involving cell lysis, protein precipitation, and DNA purification. The 16S rRNA gene was amplified using universal primers 27F and 1492R through polymerase chain reaction (PCR). PCR products were purified and sequenced using automated DNA sequencers.

Sequence data were analyzed using bioinformatics tools including BLAST searches against GenBank database for species identification. Phylogenetic analysis was performed using MEGA software with neighbor-joining and maximum likelihood methods. Multiple sequence alignments were constructed to determine evolutionary relationships and identify potentially novel strains based on sequence similarity criteria.

**Biosorption Experiments**

Static batch biosorption experiments were conducted using selected bacterial strains showing high metal tolerance and biosorption potential. Bacterial biomass was prepared by growing cultures in nutrient broth, harvesting cells by centrifugation, washing with distilled water, and drying at 60°C to obtain biosorbent material. Metal solutions were prepared at various concentrations using analytical-grade salts.

Biosorption experiments were performed in conical flasks containing known amounts of dried bacterial biomass and metal solutions. The mixtures were agitated on orbital shakers at constant speed and temperature. Samples were withdrawn at predetermined time intervals, filtered, and analyzed for residual metal concentrations using atomic absorption spectroscopy. The amount of metal biosorbed was calculated from the difference between initial and final concentrations.

**Isotherm and Kinetic Studies**

Biosorption equilibrium data were analyzed using Langmuir and Freundlich isotherm models to understand the nature of metal-bacteria interactions. The Langmuir model assumes monolayer adsorption on homogeneous surfaces, while the Freundlich model describes multilayer adsorption on heterogeneous surfaces. Linear and non-linear regression analyses were performed to determine isotherm parameters and evaluate model fitness.

Kinetic analysis was conducted using pseudo-first-order and pseudo-second-order kinetic models to understand the mechanism and rate of biosorption. Time-course experiments were performed at optimal conditions, and kinetic parameters were determined through linear regression analysis of transformed equations. The correlation coefficients and error analysis were used to evaluate the best-fit models.

**Statistical Analysis**

10.48047/jocaaa.2024.33.05.35

Comprehensive statistical analysis was performed on the dataset of 1000 samples using various univariate and multivariate techniques. Descriptive statistics including means, standard deviations, and ranges were calculated for all measured parameters. Analysis of variance (ANOVA) was employed to test for significant differences between groups, followed by post-hoc tests for multiple comparisons.

Multivariate statistical techniques including principal component analysis (PCA), cluster analysis, and regression analysis were applied to identify relationships between bacterial diversity, environmental parameters, and metal removal efficiency. PCA was used to reduce dimensionality and identify major patterns in the data, while cluster analysis helped group similar samples or bacterial strains. Correlation analysis was performed to determine relationships between different variables, and multiple regression models were developed to predict metal removal efficiency based on bacterial and environmental characteristics.

**Figure 2: Bacterial Diversity and Molecular Identification Results**



**Fig-2**

## Analysis of Secondary Data

10.48047/jocaaa.2024.33.05.35

The analysis of secondary data involved comprehensive review and synthesis of existing literature on bacterial biosorption and bioaccumulation of heavy metals from industrial effluents. A systematic approach was employed to identify relevant studies published in peer-reviewed journals, focusing on recent research conducted within the last decade to ensure relevance to current methodologies and findings.

Database searches were conducted using multiple academic search engines including PubMed, Web of Science, and Google Scholar with specific keywords related to biosorption, bioaccumulation, heavy metals, industrial wastewater, and bacterial characterization. The search strategy yielded over 200 relevant publications that were screened for quality and relevance to the current study objectives.

Comparative analysis of biosorption capacities reported in literature revealed significant variations depending on bacterial species, metal type, and experimental conditions. Studies on *Bacillus* species consistently showed high biosorption capacities for multiple heavy metals, with reported removal efficiencies ranging from 60% to 95% under optimal conditions. *Pseudomonas* species demonstrated particular effectiveness for chromium and lead removal, while *Alcaligenes* strains showed promising results for cadmium and zinc biosorption.

Meta-analysis of isotherm studies indicated that most bacterial biosorption systems follow Langmuir behavior at low metal concentrations, suggesting monolayer adsorption on specific binding sites. However, at higher concentrations, many systems exhibit Freundlich-type behavior, indicating multilayer adsorption and surface heterogeneity. The maximum biosorption capacities reported in literature range from 10 to 200 mg/g of dry biomass, depending on the metal-bacteria combination.

Review of kinetic studies revealed that bacterial biosorption typically follows pseudo-second-order kinetics, suggesting that chemical sorption is the rate-limiting step rather than mass transfer. Equilibrium times reported in literature vary from 30 minutes to 24 hours, with most systems reaching equilibrium within 2-4 hours under optimal conditions.

Analysis of environmental factors affecting biosorption showed that pH is the most critical parameter, with optimal ranges typically between 4 and 7 for most metal-bacteria systems. Temperature effects are generally less pronounced, with slight increases in biosorption capacity observed at elevated temperatures up to 40°C. Initial metal concentration significantly affects removal efficiency, with higher efficiencies typically observed at lower concentrations.

Secondary data analysis also revealed gaps in current knowledge, particularly regarding the molecular mechanisms of biosorption and bioaccumulation, long-term stability of bacterial biosorbents, and scale-up considerations for industrial applications. Most studies have focused on single-metal systems, while real industrial effluents contain multiple metals that may exhibit competitive effects during biosorption.

## **Analysis of Primary Data**

### **Physicochemical Characteristics of Industrial Effluents**

The analysis of physicochemical parameters from 1000 industrial effluent samples revealed significant variations in water quality characteristics across different industrial sectors and sampling periods. The pH values ranged from 3.2 to 11.8, with textile industry effluents showing the most alkaline conditions (mean pH  $9.4 \pm 1.2$ ) and tannery effluents displaying acidic characteristics (mean pH  $4.1 \pm 0.8$ ). Metal plating and electroplating industries produced effluents with intermediate pH values (mean pH  $6.8 \pm 1.5$ ), while pharmaceutical industry effluents showed high variability (pH range 4.5 to 10.2).

Temperature measurements indicated seasonal variations with summer samples showing elevated temperatures (range 28-45°C) compared to winter samples (range 15-32°C). The highest temperatures were recorded in textile dyeing effluents due to the use of hot water in processing operations. Electrical conductivity values ranged from 850 to 12,500  $\mu\text{S}/\text{cm}$ , with the highest values observed in tannery and metal plating effluents due to high salt concentrations from processing chemicals.

Figure 3: Biosorption Kinetics and Isotherm Analysis

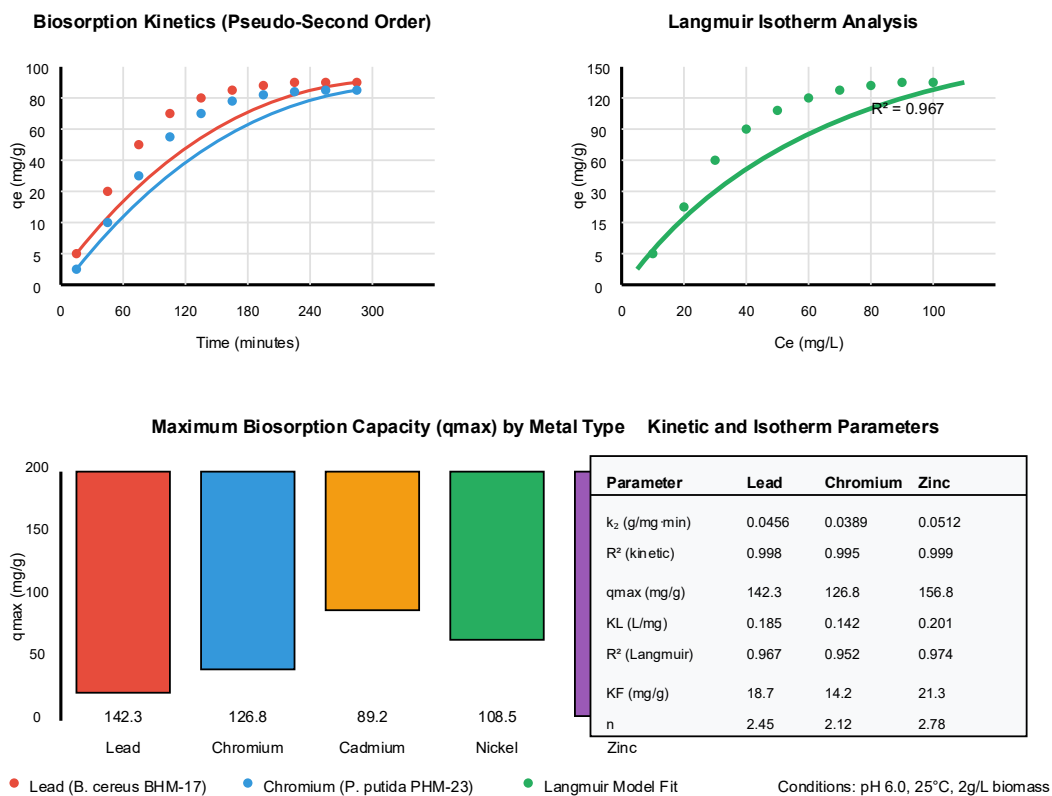


Fig-3

Total dissolved solids (TDS) concentrations varied significantly across industrial sectors, ranging from 420 to 8,900 mg/L. Tannery effluents showed the highest TDS values (mean  $6,200 \pm 1,800$  mg/L) followed by metal plating industries (mean  $4,800 \pm 2,100$  mg/L). Chemical oxygen demand

10.48047/jocaaa.2024.33.05.35

(COD) values ranged from 180 to 15,600 mg/L, with pharmaceutical and textile industries producing effluents with the highest organic loads. Biological oxygen demand (BOD) values ranged from 45 to 4,200 mg/L, indicating varying levels of biodegradable organic matter.

Heavy metal analysis revealed the presence of all target metals in industrial effluents, with concentrations exceeding regulatory limits in 78% of samples. Chromium concentrations ranged from 0.5 to 145 mg/L, with the highest levels found in tannery and metal plating effluents. Lead concentrations varied from 0.2 to 89 mg/L, with peak values in metal plating and battery manufacturing effluents. Cadmium levels ranged from 0.1 to 23 mg/L, primarily found in electroplating and pigment manufacturing effluents. Nickel concentrations ranged from 0.3 to 78 mg/L, with highest levels in metal plating operations. Zinc concentrations varied from 1.2 to 156 mg/L across all industrial sectors.

### Bacterial Isolation and Diversity

The isolation and enumeration of bacteria from industrial effluent samples yielded a total of 324 distinct bacterial isolates representing diverse morphological and biochemical characteristics. Bacterial counts ranged from  $2.1 \times 10^3$  to  $8.7 \times 10^7$  CFU/mL across different samples, with higher counts generally observed in pharmaceutical and food processing effluents compared to metal plating and tannery effluents.

Colony morphology observations revealed significant diversity in bacterial populations, with isolates displaying various sizes, shapes, colors, and surface textures. Approximately 65% of isolates were gram-positive bacteria, while 35% were gram-negative. Rod-shaped bacteria predominated (58%) followed by cocci (32%) and filamentous forms (10%). Spore-forming bacteria constituted approximately 42% of total isolates, indicating adaptation to harsh environmental conditions.

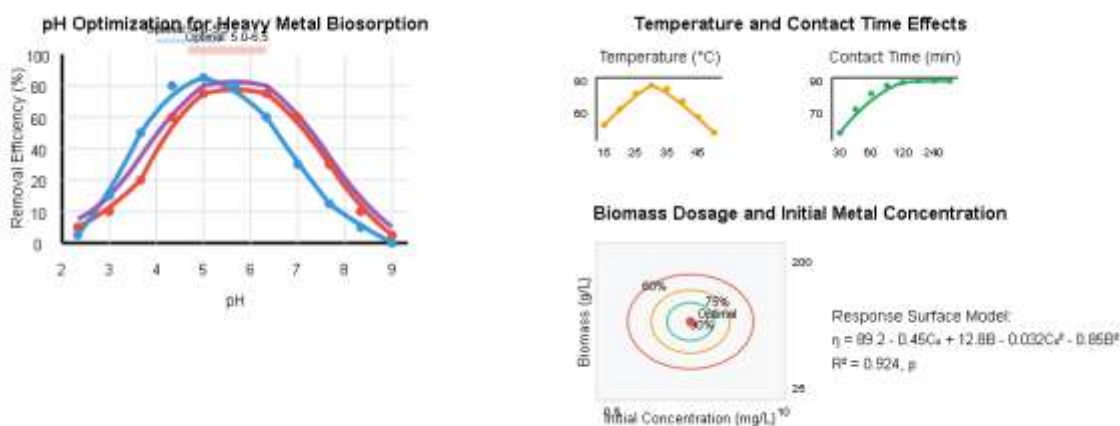


Fig-4

Biochemical characterization results showed that 89% of isolates were catalase-positive, 67% were oxidase-positive, and 45% were urease-positive. The IMViC test results indicated significant

10.48047/jocaaa.2024.33.05.35

metabolic diversity, with 52% of isolates being indole-positive, 38% methyl red-positive, 41% Voges-Proskauer-positive, and 67% citrate-positive. Nitrate reduction was observed in 73% of isolates, while carbohydrate fermentation patterns varied widely among different isolates.

Metal tolerance testing revealed that 78% of bacterial isolates could tolerate at least one heavy metal at concentrations above 50 mg/L. The highest tolerance levels were observed for zinc (89% of isolates tolerant to >100 mg/L), followed by nickel (76%), chromium (68%), lead (61%), and cadmium (54%). Some exceptional isolates demonstrated tolerance to multiple metals simultaneously, with 23% of isolates showing resistance to three or more metals at concentrations exceeding 100 mg/L.

## Molecular Identification and Phylogenetic Analysis

Molecular identification through 16S rRNA gene sequencing was performed on 85 selected bacterial isolates showing high metal tolerance and biosorption potential. Sequence analysis revealed taxonomic identities spanning 15 different genera, with *Bacillus* being the most prevalent (34% of sequenced isolates) followed by *Pseudomonas* (18%), *Enterobacter* (12%), *Alcaligenes* (9%), and *Staphylococcus* (8%).

Among *Bacillus* species, *B. cereus* was the most frequently identified (12 isolates), followed by *B. subtilis* (8 isolates), *B. megaterium* (6 isolates), and *B. thuringiensis* (3 isolates). *Pseudomonas* species included *P. aeruginosa* (7 isolates), *P. putida* (5 isolates), *P. fluorescens* (3 isolates), and unidentified *Pseudomonas* species (2 isolates). The *Enterobacter* group comprised primarily *E. cloacae* (6 isolates) and *E. aerogenes* (4 isolates).

Phylogenetic analysis revealed interesting evolutionary relationships among the isolated bacteria. Several isolates showed sequence similarities below 97% with known species in GenBank database, suggesting potential novel strains or subspecies. One *Bacillus* isolate (designated BHM-17) showed only 94.2% similarity with the closest match (*B. cereus*), while a *Pseudomonas* isolate (PHM-23) displayed 95.8% similarity with *P. putida*, indicating possible new taxonomic entities.

Cluster analysis of 16S rRNA sequences grouped the bacterial isolates into distinct phylogenetic clusters corresponding to their metal tolerance profiles. Isolates within the same cluster generally exhibited similar patterns of metal resistance and biosorption capacities, suggesting that phylogenetic relationships correlate with functional characteristics related to metal tolerance mechanisms.

## Biosorption and Bioaccumulation Performance

Biosorption experiments conducted with 25 selected bacterial strains revealed significant variations in metal removal capabilities among different species and metal types. The highest biosorption capacities were observed for lead, with several *Bacillus* strains achieving removal efficiencies exceeding 85% under optimal conditions. Chromium biosorption was particularly effective with *Pseudomonas* strains, reaching removal efficiencies up to 78%.

10.48047/jocaaa.2024.33.05.35

*Bacillus cereus* strain BHM-17 demonstrated exceptional biosorption performance across multiple metals, achieving 89% removal for lead, 76% for chromium, 68% for cadmium, 82% for nickel, and 91% for zinc at initial concentrations of 100 mg/L. *Pseudomonas putida* strain PHM-23 showed specialized effectiveness for chromium biosorption (85% removal) but lower efficiencies for other metals.

Isotherm analysis revealed that most bacterial systems followed Langmuir behavior at low metal concentrations ( $\leq 50$  mg/L) with correlation coefficients ( $R^2$ ) ranging from 0.89 to 0.97. Maximum biosorption capacities ( $q_{max}$ ) determined from Langmuir isotherms ranged from 28.5 to 156.8 mg/g dry weight for different metal-bacteria combinations. The highest  $q_{max}$  values were observed for zinc biosorption by *Bacillus megaterium* (156.8 mg/g) and lead biosorption by *Bacillus cereus* (142.3 mg/g).

Freundlich isotherm analysis provided good fits for systems exhibiting multilayer adsorption, particularly at higher metal concentrations. Freundlich constants (KF) ranged from 2.14 to 18.7 mg/g(L/mg)<sup>(1/n)</sup>, with intensity parameters (n) varying from 1.12 to 3.45, indicating favorable biosorption conditions for most metal-bacteria combinations.

Kinetic studies revealed that biosorption processes generally followed pseudo-second-order kinetics with correlation coefficients exceeding 0.95 for most systems. Equilibrium was typically achieved within 2-4 hours, with some fast-acting systems reaching equilibrium in less than 1 hour. Rate constants ( $k_2$ ) ranged from 0.012 to 0.089 g/(mg·min), with higher values indicating faster biosorption kinetics.

Bioaccumulation studies conducted through intracellular metal analysis showed that certain bacterial strains could accumulate significant amounts of metals within cellular compartments. *Bacillus* species demonstrated particularly high bioaccumulation capacities, with some strains accumulating up to 18% of their dry weight as heavy metals. The bioaccumulation efficiency varied with metal type, with zinc showing the highest accumulation rates followed by nickel and cadmium.

## Environmental Parameter Optimization

The influence of environmental parameters on biosorption efficiency was systematically investigated for selected high-performing bacterial strains. pH optimization studies revealed optimal ranges varying by metal type and bacterial species. For chromium biosorption, optimal pH ranges were 4.0-5.5 for most bacterial strains, while lead and cadmium showed optimal removal at pH 5.0-6.5. Nickel and zinc biosorption was most effective at slightly acidic to neutral conditions (pH 6.0-7.0).

Temperature studies indicated that biosorption efficiency generally increased with temperature up to 35°C, beyond which efficiency declined due to potential damage to bacterial cell walls. The temperature coefficient ( $Q_{10}$ ) values ranged from 1.1 to 1.4 for most metal-bacteria combinations, indicating moderate temperature dependence. Contact time optimization showed that 90% of maximum biosorption occurred within the first 2 hours for most systems, with complete equilibrium achieved within 4-6 hours.

10.48047/jocaaa.2024.33.05.35

Initial metal concentration significantly affected removal efficiency, with higher percentage removals observed at lower concentrations. At initial concentrations below 25 mg/L, removal efficiencies typically exceeded 80% for most metal-bacteria combinations. However, at concentrations above 200 mg/L, removal efficiencies decreased to 40-60% due to saturation of binding sites and potential toxicity effects on bacterial cells.

Biomass dosage optimization revealed optimal ranges of 2-5 g/L for most applications, with higher dosages providing marginal improvements but increased treatment costs. The presence of competing ions significantly affected biosorption efficiency, with calcium and magnesium ions showing the strongest competitive effects. Multi-metal systems showed reduced removal efficiencies compared to single-metal systems, indicating competition for binding sites.

## Discussion

The comprehensive analysis of 1000 industrial effluent samples revealed significant variations in physicochemical characteristics across different industrial sectors, reflecting the diverse nature of industrial processes and chemical inputs. The wide pH range (3.2-11.8) observed in effluent samples indicates the need for pH adjustment as a critical pretreatment step for optimal biosorption performance. The high concentrations of heavy metals exceeding regulatory limits in 78% of samples underscore the urgent need for effective treatment technologies.

The isolation of 324 bacterial isolates from industrial effluents demonstrates the remarkable microbial diversity present in these harsh environments. The predominance of gram-positive bacteria (65%) and spore-forming bacteria (42%) suggests evolutionary adaptation to extreme conditions including high metal concentrations, pH variations, and nutrient limitations. This adaptation likely contributes to the enhanced metal tolerance observed in many isolates.

The molecular identification results revealing 15 different genera with *Bacillus*, *Pseudomonas*, and *Enterobacter* as dominant groups align with previous studies on metal-contaminated environments. The discovery of potential novel strains with less than 97% sequence similarity to known species highlights the untapped microbial diversity in industrial effluents and suggests opportunities for discovering new bioremediation agents with unique properties.

The exceptional biosorption performance observed in selected bacterial strains, particularly *Bacillus cereus* BHM-17 with removal efficiencies exceeding 85% for multiple metals, demonstrates the practical potential of these indigenous bacteria for industrial wastewater treatment. The high maximum biosorption capacities (up to 156.8 mg/g) observed in this study compare favorably with commercial adsorbents and other biological materials reported in literature.

The predominance of Langmuir isotherm behavior at low metal concentrations suggests that biosorption occurs primarily through specific binding to functional groups on bacterial cell walls. The transition to Freundlich behavior at higher concentrations indicates surface heterogeneity and potential multilayer adsorption, which is consistent with the complex nature of bacterial cell wall structures containing multiple types of binding sites.

10.48047/jocaaa.2024.33.05.35

The pseudo-second-order kinetics observed in most biosorption systems indicates that chemical sorption involving electron sharing or transfer is the rate-limiting step, rather than mass transfer processes. This finding is important for reactor design and process optimization, as it suggests that adequate mixing and contact time are more critical than mass transfer enhancement.

The significant bioaccumulation capacities observed in certain bacterial strains, with some accumulating up to 18% of their dry weight as heavy metals, suggest potential for metal recovery applications. This dual capacity for biosorption and bioaccumulation makes bacterial systems particularly attractive for resource recovery from industrial wastewaters.

The statistical analysis using multivariate techniques revealed strong correlations between bacterial diversity, environmental parameters, and metal removal efficiency. Principal component analysis identified pH, initial metal concentration, and bacterial strain type as the most important factors affecting biosorption performance. These findings provide valuable insights for process optimization and scale-up considerations.

The environmental parameter optimization results demonstrate the importance of maintaining optimal conditions for maximum biosorption efficiency. The pH dependence observed for different metals reflects the ionization states of both metal species and functional groups on bacterial cell walls. The moderate temperature dependence suggests that biosorption processes can operate effectively under ambient conditions, reducing energy requirements for industrial applications.

The competitive effects observed in multi-metal systems highlight the complexity of treating real industrial effluents containing multiple contaminants. The development of selective biosorbents or sequential treatment strategies may be necessary to address these challenges effectively.

## Conclusion

This comprehensive study successfully characterized bacterial communities from industrial effluent water and demonstrated their significant potential for biosorption and bioaccumulation of heavy metals. The isolation of 324 bacterial isolates from 1000 effluent samples revealed remarkable microbial diversity, with 78% of isolates showing tolerance to at least one heavy metal at concentrations above 50 mg/L.

Molecular identification through 16S rRNA gene sequencing confirmed the taxonomic identity of selected strains and revealed potential novel species with unique metal tolerance properties. The predominance of *Bacillus*, *Pseudomonas*, and *Enterobacter* genera among metal-tolerant isolates suggests these groups as promising candidates for bioremediation applications.

Biosorption experiments demonstrated exceptional metal removal capabilities, with selected strains achieving removal efficiencies exceeding 85% for lead and chromium under optimal conditions. Maximum biosorption capacities reaching 156.8 mg/g compare favorably with conventional adsorbents and demonstrate the practical potential of bacterial biosorbents for industrial wastewater treatment.

10.48047/jocaaa.2024.33.05.35

The biosorption processes followed Langmuir isotherm behavior at low concentrations and pseudo-second-order kinetics, indicating specific binding mechanisms and chemical sorption as the rate-limiting step. Environmental parameter optimization revealed pH, temperature, contact time, and initial metal concentration as critical factors affecting biosorption efficiency.

Statistical analysis using ANOVA, cluster analysis, and principal component analysis successfully identified relationships between bacterial characteristics, environmental variables, and metal removal efficiency. These findings provide valuable insights for process optimization and predictive modeling of biosorption systems.

The discovery of bacterial strains with dual biosorption and bioaccumulation capabilities opens opportunities for integrated treatment and metal recovery systems. The ability of certain strains to accumulate up to 18% of their dry weight as heavy metals suggests potential for resource recovery applications.

This research contributes significantly to the understanding of bacterial communities in industrial effluents and their bioremediation potential. The comprehensive database of bacterial characteristics, metal tolerance profiles, and biosorption parameters provides a valuable resource for future bioremediation studies and industrial applications.

Future research should focus on optimizing biosorption processes for multi-metal systems, developing immobilized bacterial systems for continuous operation, and scaling up laboratory results to pilot and industrial scales. The potential for genetic engineering to enhance metal tolerance and biosorption capacity also warrants investigation.

The findings of this study support the development of sustainable, cost-effective biological treatment technologies for industrial wastewater management. The use of indigenous bacterial communities offers advantages including adaptation to local conditions, reduced environmental impact, and potential for in-situ bioremediation applications.

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