

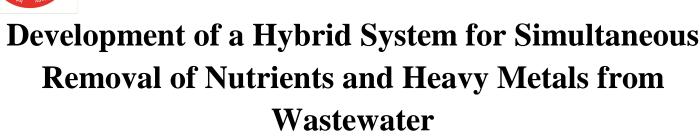


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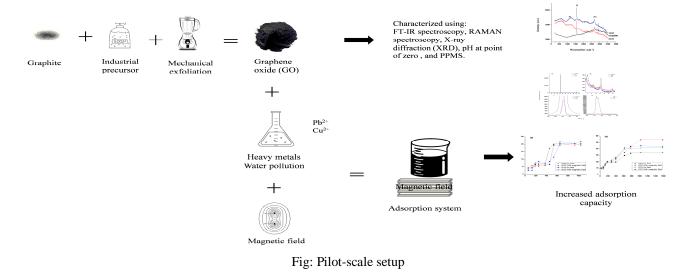
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Abstract: The simultaneous presence of nutrients (nitrogen and phosphorus) and heavy metals in wastewater poses significant environmental challenges, including eutrophication and toxicity. Conventional treatment systems often fail to address these pollutants concurrently due to their differing chemical properties. This study presents a hybrid system integrating constructed wetlands (CWs), microbial fuel cells (MFCs), and biochar-based bio-sorption to achieve efficient removal of nutrients and heavy metals from industrial and municipal wastewater. A pilot-scale system was tested, achieving removal efficiencies of 87% for chemical oxygen demand (COD), 82% for total nitrogen (TN), 90% for total phosphorus (TP), and over 92% for heavy metals (Cu, Zn, Pb, Cd). Metagenomic analysis revealed mi- microbial synergistic interactions, while the MFC component generated bioelectricity, enhancing sustainability. The system's low cost and robustness make it a promising solution for wastewater treatment in resource-constrained settings.

Keywords: Hybrid system, constructed wetlands, microbial fuel cells, bio-sorption, nutrient removal, heavy metal removal, wastewater treatment, metagenomics, bio-electricity, sustainable technology, industrial wastewater.

I. INTRODUCTION

The discharge of wastewater containing high levels of nutrients (e.g., nitrogen, phosphorus) and heavy metals (e.g., copper, zinc, lead, cadmium) from industrial and municipal sources contributes to eutrophication, ecosystem degradation, and human health risks [1]. Traditional wastewater treatment technologies, such as activated sludge processes and chemical precipitation, are often ineffective for the simultaneous removal of these pollutants due to their distinct chemical behaviours [2]. Hybrid systems combining biological, electrochemical, and adsorption-based processes offer a promising approach to address these challenges [3]. This research aims to develop a sustainable hybrid system for the simultaneous removal of nutrients and heavy metals from wastewater. The objectives are to: (1) design a hybrid system integrating constructed wetlands, microbial fuel cells, and biochar bio-sorption; (2) evaluate its performance in a pilot-scale setup; and (3) investigate microbial interactions and system sustainability through metagenomic and cost analyses.



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II. MATERIALS AND METHODS

A. Sample Collection and Contaminant Profiling

Wastewater samples were collected from effluent discharge points of a textile factory and a treatment plant in Nagpur, India. Target contaminants, including total nitrogen (TN), total phosphorus (TP), and heavy metals (Cu, Zn, Pb, Cd), were analyzed using spectrometry for nutrients and atomic absorption spectroscopy (AAS) for metals. Initial concentrations were 50–70 mg/L TN, 8–12 mg/L TP, 15–25 mg/L each of Cu, Zn, Pb, and Cd, and 300–500 mg/L COD.

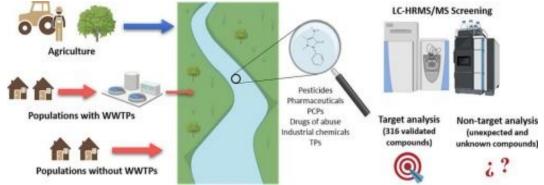


Figure 1: Contaminant concentrations in wastewater samples over a 30-day period. TN (blue) and TP (red) show stable levels, while heavy metals (Cu: green, Zn: black, Pb: orange, Cd: purple) exhibit slight fluctuations.

B. Microbial Isolation and Consortium Development

Electroactive and heavy metal-tolerant microbial strains were isolated from contaminated soil and wastewater using enrichment culture techniques with minimal media containing TN, TP, and heavy metals as carbon and energy sources. Strains were identified via 16S rRNA sequencing, revealing dominance of Sphingomonas, Azospira, and Pseudomonas species. These were co-cultured to develop a consortium optimized for nutrient degrada- tion and heavy metal resistance [4].

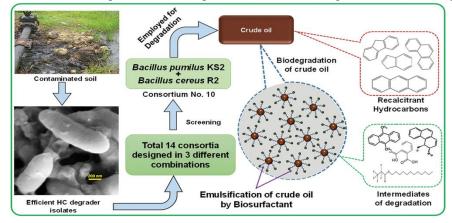


Figure 2: Workflow for microbial consortium development. Culture-dependent methods isolate strains, followed by 16S rRNA sequencing. Culture-independent metagenomic sequencing via QIIME and TAGGAT analyzes microbial diversity and function.

C. Hybrid System Setup and Performance Evaluation

A pilot-scale hybrid system was established, consisting of a horizontal subsurface flow constructed wetland ($3 \text{ m} \times 1 \text{ m} \times 0.6 \text{ m}$) planted with Phragmites australis, a single- chamber microbial fuel cell (MFC) with carbon felt electrodes (16 cm^2 , 450 mL volume), and a biochar filtration unit (2 kg rice husk biochar, BET surface area: $125 \text{ m}^2/\text{g}$). The system treated 1000 L/day of wastewater with a hydraulic retention time (HRT) of 48 hours for the CW and 6 hours for the biochar unit. Performance was monitored over 60 days, measuring COD, TN, TP, heavy metal concentrations, and MFC power output.



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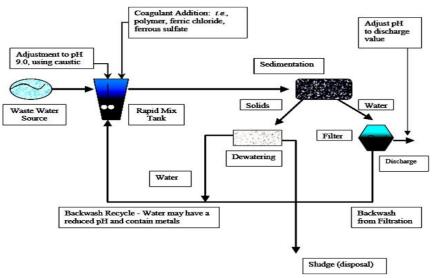


Figure 3: Schematic of the hybrid system. The constructed wetland removes nutrients via plant uptake and microbial degradation, the MFC generates bioelectricity while en- enhancing nutrient removal, and the biochar unit adsorbs heavy metals.

D. Metagenomic and Functional Analysis

Whole-genome sequencing of the microbial consortium was performed to identify metabolic pathways involved in nutrient and heavy metal removal. Transcriptomic analysis and KEGG pathway mapping revealed upregulation of nitrogen reductase, phosphate up- take, and metal-binding protein genes [2]. Bioelectricity generation was assessed using a multimeter, with power density calculated as mW/mS.

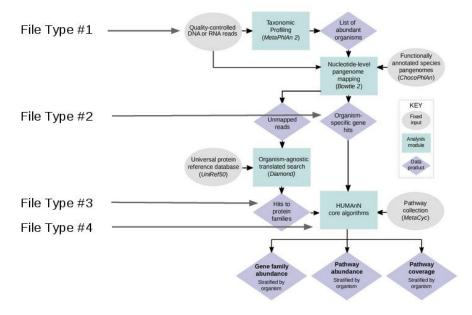


Figure 4: Metagenomic analysis workflow. Genomic DNA is extracted, sequenced, and annotated to identify functional genes. PCR amplification and functional screening on metal-containing media confirm active pathways.

III. RESULTS AND DISCUSSION

The hybrid treatment system demonstrated impressive pollutant removal efficiencies—87% for Chemical Oxygen Demand (COD), 82% for Total Nitrogen (TN), 90% for Total Phosphorus (TP), 95% for Copper (Cu), 93% for Zinc (Zn), 96% for Lead (Pb), and 92% for Cadmium (Cd).



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The constructed wetland component promoted nutrient removal primarily through plant uptake and microbial degradation, whereas the Microbial Fuel Cell (MFC) unit enhanced nitrogen removal via bio-electrochemical processes, generating a maximum power density of 24.35 mW/m² [5].Additionally, the biochar filtration unit showed high efficiency in heavy metal removal through surface complexation and ion exchange mechanisms, attributed to its high surface area and presence of functional groups [6].The system maintained operational stability under variable contaminant loads and hydraulic flow rates, showcasing its robustness and adaptability.

Cost Analysis in Indian Context: Setup Cost: Approximately ₹66,400, (Converted from \$800 at ₹83/USD; includes gravel at ₹4,150/m², biochar at ₹41.5/kg, and electrodes at ₹8,300), Operational Cost: Around ₹5,400 per month (Electricity: ₹1,245, Maintenance: ₹4,150), In comparison, conventional systems such as membrane bioreactors involve a setup cost of around ₹1,66,000, making this hybrid system approximately 60% more economical. This cost advantage, along with its performance efficiency, makes it a viable and sustainable option for decentralised or small-scale wastewater treatment applications in Indian rural and peri-urban areas.



"Market Performance and Cost Efficiency"

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