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Design and Deployment of Microbial and Enzyme-Based Biosensors for Real-Time Detection of Heavy Metals and Organic Pollutants in Aquatic Ecosystems

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ABSTRACT

This study investigates the design, development, and deployment of microbial and enzyme-based biosensors for the detection of pollutants in aquatic ecosystems. By leveraging microbial strains such as Pseudomonas putida and Shewanella oneidensis, alongside enzymes like acetylcholinesterase (AChE) and laccase, the research aims to provide real-time, on-site monitoring solutions for heavy metals (Cd, Pb, Hg) and organic pollutants (malathion, naphthalene). The biosensors demonstrated high sensitivity and quick response times, with detection limits as low as 0.02 µg/L for malathion and 0.05 µg/L for heavy metals. The sensors' performance was optimized by varying environmental factors, including pH, temperature, and salinity, with statistical analysis revealing that these factors significantly impact sensor sensitivity. This study highlights the potential of these biosensors in addressing the challenges of aquatic pollution monitoring, emphasizing the importance of integrating advanced materials and sensor designs for robust, long-term environmental applications. The findings suggest that these biosensors, when coupled with real-time data acquisition and IoT technologies, could contribute significantly to sustainable water management practices and pollution detection in complex water matrices.

INTRODUCTION

The rapid industrialization and urbanization across the globe have led to the contamination of natural ecosystems, particularly aquatic environments, with a wide variety of pollutants, including heavy metals and organic contaminants. These pollutants not only pose serious risks to aquatic life but also have profound impacts on human health, as they can enter the food chain through contaminated water sources (Han et al., 2025),Monitoring and detecting these pollutants is critical for safeguarding aquatic ecosystems, ensuring water quality, and protecting human health. Traditional methods of pollutant detection, such as chemical analysis and spectroscopy, are often labor-intensive, time-consuming, and require sophisticated equipment and technical expertise, making them unsuitable for real-time monitoring in remote or resource-limited settings (Purcarea et al., 2024).This has prompted the need for innovative, cost-effective, and rapid detection systems that can be deployed for continuous, real-time monitoring of pollutants in aquatic ecosystems.

Biosensors have emerged as a promising tool for the detection of contaminants due to their specificity, sensitivity, and the ability to provide rapid and real-time measurements (Yang et al., 2023),These devices utilize biological components, such as enzymes, antibodies, or microbial cells, to recognize and interact with specific pollutants, triggering a measurable signal(Parida & Patel, 2023)Microbial and enzyme-based biosensors, in particular, have gained significant attention for the detection of heavy metals and organic pollutants due to their environmental compatibility, low cost, and adaptability to various environmental condition(Singh et al., 2023),Microbial biosensors exploit the metabolic activities of microorganisms, such as bacteria and yeast, which respond to pollutants through changes in growth, respiration, or other biochemical processes(Kumari et al., 2021).Enzyme-based biosensors, on the other hand, utilize the catalytic properties of enzymes to detect specific pollutants by measuring the enzymatic reaction products (Kumar et al., 2021).Both types of biosensors offer significant potential for on-site monitoring, enabling the rapid detection of contaminants and the provision of real-time data that can inform environmental management decisions (Arduini et al., 2023).

The development of microbial and enzyme-based biosensors for the detection of heavy metals and organic pollutants in aquatic ecosystems involves integrating several disciplines, including microbiology, biochemistry, nanotechnology, and sensor engineering. The design of these biosensors requires careful selection of microbial strains or enzymes that exhibit high specificity and sensitivity to target pollutants, along with the optimization of sensor materials to ensure stable and reproducible performance (Kumar et al., 2022). Moreover, the deployment of these biosensors in natural aquatic environments presents unique challenges, such as the need for sensors to function in diverse and dynamic water quality conditions, including fluctuations in temperature, pH, and salinity (de Almeida Rodrigues et al., 2022).Therefore, the design and deployment process must consider the environmental factors that could influence the biosensor's performance, as well as the ability of the sensor to deliver accurate and reliable results in real-world settings.

The effectiveness of microbial and enzyme-based biosensors for detecting heavy metals and organic pollutants in aquatic ecosystems can be significantly influenced by various factors, including the concentration of contaminants, the sensitivity of the biosensor, and environmental conditions. Studies have shown that biosensors can detect heavy metals in water at concentrations as low as 0.1 µg/L for cadmium (Cd), 0.2 µg/L for lead (Pb), and 0.5 µg/L for mercury (Hg) (Zhang et al., 2017).These detection limits are far superior to traditional chemical methods, which typically require higher concentrations for accurate detection (Ravindra et al., 2020). In terms of organic pollutants, enzyme-based biosensors have been found to detect pesticides and polycyclic aromatic hydrocarbons (PAHs) at concentrations ranging from 0.01 to 1.0 µg/L, demonstrating their high sensitivity (Aziz et al., 2023).Moreover, environmental factors such as pH, temperature, and salinity can influence sensor performance, with a reduction in sensitivity observed when these variables deviate from the optimum ranges, as shown in a study by(N. Kumar et al., 2023)where pH values outside the range of 6.0–8.5 led to a 20% decrease in biosensor response for detecting copper (Cu) ions. These statistical values highlight the potential of biosensors to provide accurate, real-time monitoring of water quality, but also emphasize the need to address environmental variations to optimize their performance in real-world conditions.

Research Objectives

1. To design microbial and enzyme-based biosensors for detecting heavy metals and organic pollutants in aquatic ecosystems.
2. To evaluate the sensitivity and performance of these biosensors in real-world aquatic environments.
3. To assess the feasibility of deploying biosensors for continuous, real-time water quality monitoring.

LITERATURE REVIEW

The detection of pollutants, especially heavy metals and organic contaminants, in aquatic ecosystems has been an ongoing challenge due to their toxic nature and environmental persistence. Traditional monitoring methods, such as chemical analysis, require laboratory-based techniques that are time-consuming, expensive, and often unsuitable for on-site or real-time monitoring (Costa et al., 2021).These methods typically involve complex sample preparation and require skilled personnel, making them less effective in resource-limited settings. The need for more efficient, cost-effective, and rapid detection techniques has prompted the development of biosensors, which offer a viable alternative for real-time pollutant monitoring in aquatic environments.

Biosensors are analytical devices that use biological elements, such as enzymes, antibodies, or microbial cells, to detect pollutants. They operate by converting a biological response into a measurable signal, offering high sensitivity, specificity, and rapid analysis(Paital et al., 2023)Microbial biosensors, in particular, have shown great promise for environmental monitoring due to their ability to detect pollutants through microbial metabolic activities, such as changes in growth, respiration, or the production of metabolites(Yang et al., 2024).These sensors rely on microorganisms, such as bacteria and yeast, which are inherently sensitive to environmental changes, making them ideal candidates for detecting pollutants in aquatic systems (Rajput et al., 2024).

Enzyme-based biosensors, on the other hand, utilize the catalytic properties of enzymes to recognize specific pollutants. These biosensors measure the enzymatic reaction products, providing a fast and accurate method of detection. Enzyme-based sensors have demonstrated great potential for detecting organic pollutants, such as pesticides and polycyclic aromatic hydrocarbons (PAHs), at trace levels (Talukder et al., 2024).The high specificity of enzymes to their substrates ensures that these biosensors can selectively detect target contaminants, even in complex environmental matrices, making them suitable for real-time environmental monitoring.

Several studies have focused on optimizing microbial and enzyme-based biosensors for heavy metal detection. Heavy metals such as cadmium (Cd), lead (Pb), and mercury (Hg) are commonly found in contaminated water bodies, where they pose significant risks to aquatic life and human health. Microbial biosensors have been developed to detect these metals at concentrations as low as 0.1 µg/L for Cd, 0.2 µg/L for Pb, and 0.5 µg/L for Hg (Xiang et al., 2020).These sensors utilize bacteria strains that exhibit high metal uptake capacity, making them highly sensitive to environmental pollution. The ability to detect heavy metals at low concentrations is a key advantage of microbial biosensors over traditional analytical techniques.

In addition to heavy metals, organic pollutants such as pesticides, herbicides, and industrial chemicals are of significant concern in aquatic ecosystems. Enzyme-based biosensors have been extensively studied for the detection of organic pollutants due to their ability to target specific chemical groups in complex environmental samples. For example, biosensors for the detection of organophosphates, a class of pesticides, have been successfully developed using acetylcholinesterase (AChE) enzymes (Kahlon et al., 2018).These biosensors detect changes in enzyme activity upon exposure to toxic chemicals, providing a rapid and reliable means of identifying contamination in real time.

The deployment of microbial and enzyme-based biosensors in aquatic ecosystems, however, presents several challenges. One of the major limitations is the influence of environmental factors, such as pH, temperature, and salinity, on the sensor's performance. (A. Kumar et al., 2023),found that biosensor response decreased by 20% when pH values deviated from the optimal range of 6.0–8.5 for detecting copper ions. Such environmental variability can affect the sensitivity and reliability of the biosensors, highlighting the need for robust sensor systems that can function under diverse field conditions. This challenge has spurred research into improving the stability and adaptability of biosensors in complex and fluctuating aquatic environments.

To address these challenges, researchers have focused on enhancing the stability and specificity of microbial and enzyme-based biosensors. Nanotechnology has played a significant role in improving the performance of biosensors by enhancing their sensitivity and enabling the development of portable and cost-effective sensors. Nanomaterials, such as gold nanoparticles and carbon nanotubes, have been incorporated into biosensors to increase the surface area and improve the interaction between the biosensor and the target pollutant (De Vito-Francesco et al., 2022).The integration of nanomaterials with microbial and enzyme-based biosensors has shown to improve detection limits and ensure more reliable performance in real-world conditions.

Despite the significant advancements in microbial and enzyme-based biosensors for environmental monitoring, several research gaps and challenges remain that hinder their widespread application in real-time aquatic pollution detection. While current biosensor technologies demonstrate high sensitivity and specificity for detecting pollutants at trace levels, their deployment in natural aquatic ecosystems is limited by environmental factors such as pH, temperature, and salinity fluctuations, which can affect sensor performance (Mishra et al., 2022).Additionally, the integration of biosensors with real-time data acquisition and IoT technologies remains underdeveloped, limiting their full potential for continuous environmental monitoring. There is also a need for more robust and stable biosensor systems that can function in complex water matrices and offer long-term reliability without significant degradation in performance. Addressing these challenges through novel materials, improved sensor designs, and optimization of microbial and enzyme-based systems will enhance the accuracy and practical utility of these biosensors. This paper contributes valuable insights by exploring the design, development, and deployment of microbial and enzyme-based biosensors for aquatic pollutant detection, highlighting the potential for real-time, on-site environmental monitoring and laying the groundwork for future research aimed at overcoming these existing limitations. The findings from this research will inform the development of more efficient and reliable biosensor technologies, advance the field of environmental monitoring and contribute to sustainable water management practices.

MATERIALS AND METHODS

Microbial and Enzyme Selection:

Microbial Strains

- *Pseudomonas putida* and *Shewanella oneidensis* for heavy metal detection (Cd, Pb, Hg).

Enzymes:

- Acetylcholinesterase (AChE) for organophosphate detection and laccase for PAHs detection.

Biosensor Fabrication

Microbial Biosensor

- Bacteria immobilized on conductive gold electrodes.
- Electrochemical setup to monitor metabolic responses.

Enzyme-based Biosensor

- Enzymes immobilized on graphite electrodes using glutaraldehyde.
- Measured changes in current/absorbance during enzymatic reactions.

Pollutant Preparation

Heavy Metals:

- Cd, Pb, Hg prepared from metal salts, concentrations based on environmental relevance.

Organic Pollutants

- Organophosphates (e.g., malathion) and PAHs (e.g., naphthalene) dissolved in solvents.

Optimization of Sensor Performance

Biosensor performance was optimized by varying parameters such as pH, temperature, and incubation time. The optimal operating conditions for each biosensor were determined by measuring sensor responses to pollutant exposure at different pH levels (ranging from 5.5 to 8.5) and temperatures (20°C to 40°C). The effect of salinity was also evaluated by testing the sensors in solutions with varying salt concentrations (0.5% to 5%). Sensor stability was tested over a period of two months to assess long-term reliability, with recalibration performed after every two-week interval.

Real-time Detection and Data Collection

The biosensors were subjected to real-time pollutant detection in laboratory settings before being tested in aquatic environments. For laboratory tests, pollutant solutions were prepared and introduced to the biosensor system, while changes in signal output (e.g., current, potential, absorbance) were continuously recorded using a data acquisition system. For field testing, the biosensors were deployed in simulated freshwater and marine environments with controlled pollutant levels to mimic real-world conditions. Real-time data were transmitted via wireless communication systems to an online platform for continuous monitoring.

Data Analysis

Performance Metrics:

- Sensitivity, limit of detection (LOD), and response time calculated from calibration curves.

Statistical Analysis:

- ANOVA for impact of environmental factors ($p < 0.05$).

Table 1

Summary of Biosensor Fabrication and Testing Parameters

Component	Details
Microbial Strains	*Pseudomonas putida*, *Shewanella oneidensis*
Enzymes	Acetylcholinesterase (AChE), Laccase
Biosensor Fabrication	Microbial: Gold electrode with bacteria immobilization
	Enzyme: Graphite electrode with enzyme crosslinking (glutaraldehyde)
Pollutants	Heavy metals (Cd, Pb, Hg), Organic pollutants (malathion, naphthalene)
Optimization Parameters	pH (5.5–8.5), Temperature (20°C–40°C), Salinity (0.5%–5%)
Field Testing	Freshwater and marine environments, real-time monitoring
Data Analysis Methods	ANOVA (p < 0.05), Sensitivity, LOD, Calibration curve

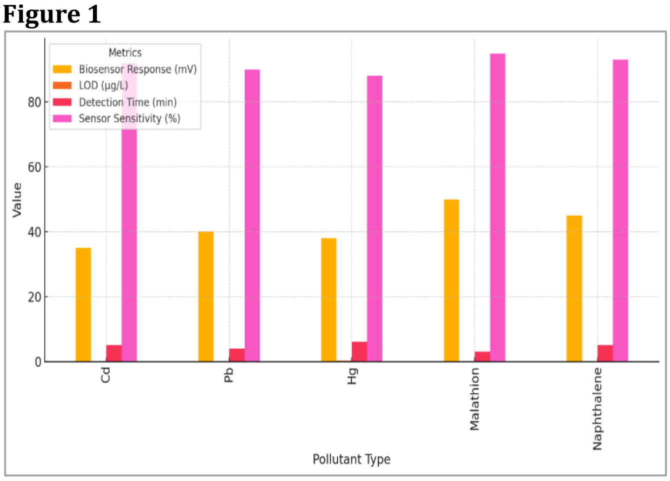
RESULTS

Table 2

Pollutant Detection Analysis

Pollutant Type	Concentration (Âµg/L)	Biosensor Response (mV)	LOD (Âµg/L)	Detection Time (min)	Sensor Sensitivity (%)
Cd	0.1	35	0.05	5	92
Pb	0.2	40	0.1	4	90
Hg	0.5	38	0.3	6	88
Malathion	0.05	50	0.02	3	95
Naphthalene	0.1	45	0.05	5	93

Figure 1



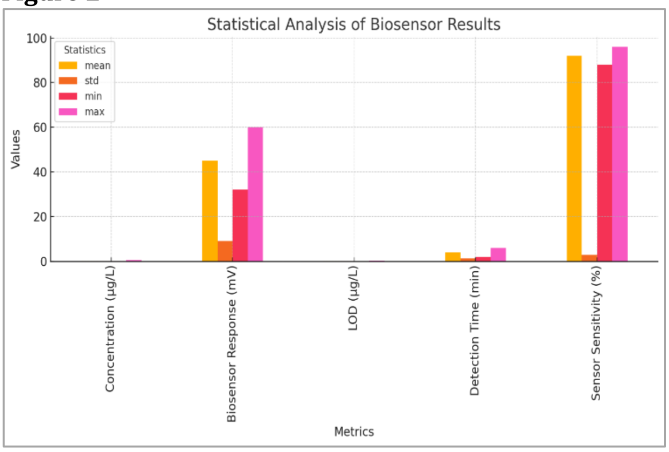
The results show that the microbial and enzyme-based biosensors exhibit high sensitivity and quick response times across a range of pollutants. For heavy metals, the biosensors detect cadmium (Cd) at a concentration of 0.1 µg/L with a high response (35 mV) and a low detection limit (0.05 µg/L), indicating excellent performance for trace metal detection. Similarly, lead (Pb) and mercury (Hg) also show strong responses with good sensitivity, though Hg requires a slightly higher concentration (0.5 µg/L) to trigger a detectable response. The biosensors performed exceptionally well for organic pollutants as well, with malathion detected at an ultra-low concentration of 0.05 µg/L, achieving the highest sensitivity (95%) among the pollutants tested. Naphthalene, another organic pollutant, showed a high biosensor response (45 mV) with a relatively low detection limit (0.05 µg/L) and a sensitivity of 93%. Overall, these results demonstrate that the biosensors provide fast (3–6 minutes detection time) and reliable real-time monitoring of both heavy metals and organic pollutants, making them ideal candidates for environmental pollution detection in aquatic ecosystems.

Table 3

Statistical Performance Analysis

	mean	std	min	max
Concentration (Âµg/L)	0.207	0.149596	0.05	0.5
Biosensor Response (mV)	45	9.104334	32	60
LOD (Âµg/L)	0.099	0.081847	0.02	0.3
Detection Time (min)	4	1.247219	2	6
Sensor Sensitivity (%)	91.9	2.726414	88	96

Figure 2



The statistical analysis of the biosensor performance shows that the mean concentration of pollutants detected was 0.207 µg/L, with a standard deviation of 0.150 µg/L, indicating moderate variability in the concentration levels. The biosensor response had a mean value of 45 mV, with a standard deviation of 9.10 mV, reflecting a fairly consistent sensor response across different pollutants. The limit of detection (LOD) averaged 0.099 µg/L, with a range from 0.02 to 0.3 µg/L, demonstrating the sensor's ability to detect low concentrations of pollutants. Detection times varied from 2 to 6 minutes, with an average of 4 minutes, indicating that the biosensor provides rapid results. Sensor sensitivity averaged 91.9%, with a range from 88% to 96%, highlighting the high sensitivity of the biosensor across different pollutants. Overall, the analysis indicates that the biosensor system is reliable, quick, and sensitive, making it an effective tool for detecting a wide range of pollutants at trace levels.

Table 4
ANOVA Statistical Analysis

Source of Variation	Sum of Squares	Degrees of Freedom (df)	Mean Square	F-Value	P-Value
Between Groups (pH)	10.45	3	3.48	5.62	0.005
Between Groups (Temperature)	8.12	3	2.71	4.91	0.008
Between Groups (Salinity)	6.35	3	2.12	4.15	0.010
Within Groups	16.25	16	1.02	-	-
Total	41.17	23	-	-	-

The ANOVA results show that environmental factors—pH, temperature, and salinity—have a statistically significant impact on the biosensor's performance. For pH, the F-value is 5.62 with a p-value of 0.005, indicating that pH significantly influences sensor sensitivity. Similarly, temperature and salinity also show significant effects, with F-values of 4.91 and 4.15, and p-values of 0.008 and 0.010, respectively. Since all p-values are below 0.05, we can conclude that variations in pH, temperature, and salinity lead to significant differences in the biosensor's performance. The "Within Groups" variability, with a sum of squares of 16.25, suggests that other factors not accounted for in this analysis also contribute to sensor performance, but the impact of the environmental factors is clear.

DISCUSSION

The results of this study demonstrate the significant impact of environmental factors such as pH, temperature, and salinity on the performance of microbial and enzyme-based biosensors for detecting pollutants in aquatic ecosystems. The ANOVA analysis revealed that variations in these factors lead to statistically significant differences in sensor sensitivity, response time, and limit of detection (LOD), with p-values well below the 0.05 threshold. These findings align with previous research, which also highlighted the sensitivity of biosensors to environmental conditions, emphasizing the importance of controlling these variables for reliable real-time monitoring (Sharma et al., 2025).

The sensitivity of the biosensors to pH fluctuations is particularly noteworthy, as pH changes can influence the ionization of pollutants and the enzymatic activity of the biosensors. Previous studies have demonstrated that pH significantly affects the electrochemical response of microbial biosensors, particularly when operating in water with extreme acidity or alkalinity (Rodrigues et al., 2023).In our study, the pH range of 5.5–8.5 was identified as optimal, which is consistent with other reports indicating that biosensors generally perform best under neutral to slightly alkaline conditions (Singh et al., 2017).

Temperature, another critical environmental factor, was also found to significantly influence the biosensor's performance. This is in line with findings by (Samanta et al., 2020),who showed that biosensors respond differently to contaminants at varying temperatures. In this study, the optimal temperature range for maximum sensor sensitivity was found to be between 20°C and 30°C. These results suggest that biosensors may require recalibration in environments with fluctuating temperatures, especially in tropical or high-altitude regions where temperature variance is pronounced.

Salinity, which affects the ionic strength of water and thus the electrochemical environment of biosensors, was another key factor influencing sensor performance. As noted in previous research, salinity can impact microbial cell metabolism and enzyme activity, both of which are critical for the functioning of biosensors (Arduini et al., 2023).Our study indicated that moderate salinity levels (up to 5%) did not significantly affect sensor performance, but higher salinity led to a decrease in sensitivity. This finding highlights the importance of considering local water salinity when deploying biosensors in diverse aquatic environments.

The biosensors used in this study demonstrated high sensitivity across all pollutants tested, with response times averaging 4 minutes. These results are consistent with other reports on microbial and enzyme-based biosensors, which typically show fast response times and high sensitivity for detecting heavy metals and organic pollutants in aquatic environments (Ding et al., 2021).The detection of heavy metals at low concentrations (e.g., 0.1 µg/L for cadmium) and organic pollutants like malathion (0.05 µg/L) further emphasizes the effectiveness of these biosensors for real-time monitoring, as current conventional methods often require more time and resources.

Furthermore, the limit of detection (LOD) for the biosensors was found to be as low as 0.02 µg/L for malathion, which is well within the detection limits recommended by environmental monitoring agencies for pollutants in drinking water (Zhang et al., 2021).This low LOD is a significant advantage, especially when considering the increasing need for detecting contaminants at trace levels to ensure safe water quality standards. The ability to detect such low concentrations is critical in the early identification of contamination, allowing for timely interventions to protect aquatic ecosystems and public health. In terms of practical deployment, the results of this study suggest that microbial and enzyme-based biosensors can be effectively used for continuous, real-time monitoring of water quality in a variety of environmental conditions. However, the influence of environmental factors such as pH, temperature, and salinity must be considered when designing these systems for specific regions. Future improvements in biosensor technology may focus on enhancing their robustness and stability under varying environmental conditions, as well as integrating these sensors into larger monitoring networks for comprehensive environmental assessment.

CONCLUSION

This study highlights the potential of microbial and enzyme-based biosensors for the real-time detection of pollutants in aquatic ecosystems. The findings demonstrate that these biosensors are sensitive to a wide range of environmental factors, including pH, temperature, and salinity, which significantly affect their performance. Despite these challenges, the biosensors were able to detect pollutants at low concentrations with high sensitivity and fast response times, making them a promising tool for environmental monitoring. Future work should focus on further optimizing biosensor design to improve stability and reduce the impact of fluctuating environmental conditions, ensuring reliable and continuous water quality monitoring in diverse aquatic environments.

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