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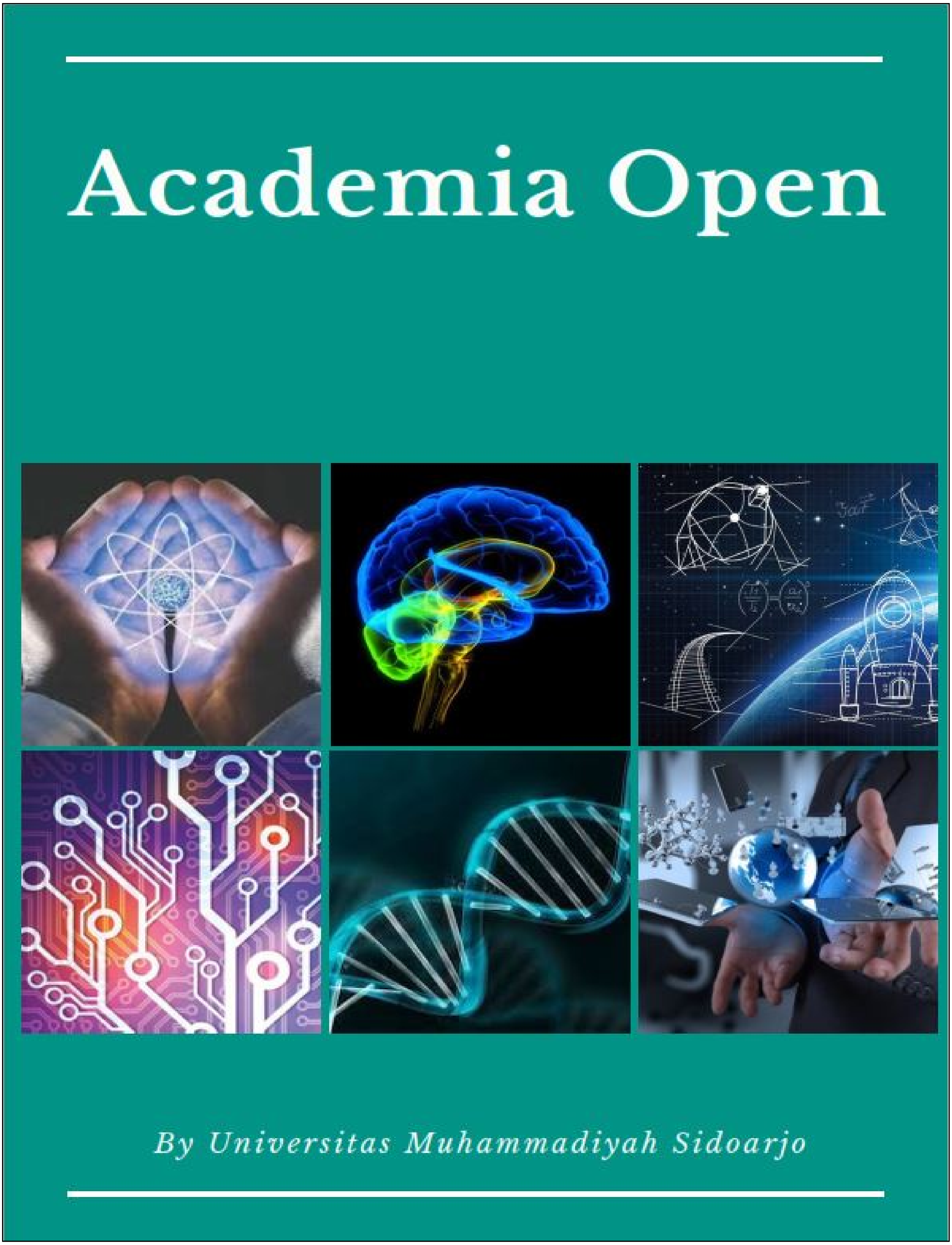
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Engineered Bacteria Achieve Breakthrough 91% Lead Removal in Bioremediation

Bakteri Rekayasa Mencapai Terobosan Penghilangan Timbal 91% dalam Bioremediasi

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Abstract

Background: Heavy metal pollutants pose a significant environmental and public health threat, necessitating effective remediation strategies. Genetically modified microorganisms, Shewanella oneidensis MR-1 and Cupriavidus metallidurans CH34, show promise in mitigating contamination. **Specific Background:** Engineered bacteria have been designed to improve metal absorption, specifically lead, mercury, cadmium, and zinc, but further research is needed to understand their effectiveness in different pH levels and water conditions. **Knowledge Gap:** Few studies have investigated the pH-dependent efficiency of genetically engineered microbes in heavy metal absorption or their performance under wastewater conditions, despite numerous studies on microbial bioremediation. **Aims:** The study evaluates the efficacy of genetically modified Shewanella oneidensis and Cupriavidus metallidurans in removing heavy metals from contaminated water at various pH levels. **Results:** The study found that both bacterial strains effectively removed 91% of lead at pH 7, with optimal levels for lead and cadmium absorption depending on the specific pH. **Novelty:** CRISPR/Cas9 technology is used for genetic modification of strains, enhancing microbial engineering for environmental remediation. Study provides new data on pH-specific absorption rates.. **Implications:** These findings can be applied to enhance the design of bioreactors and biofiltration systems, offering a sustainable solution to heavy metal contamination in wastewater, with broad potential for industrial and environmental applications.

Highlights:

1. Modified bacteria remove 91% of lead at neutral pH.
2. CRISPR/Cas9 enhances bacteria for efficient heavy metal removal.
3. pH optimization is key for effective heavy metal absorption.

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Keywords: Heavy metals, Bioremediation, Genetically modified bacteria, *Shewanella oneidensis, Cupriavidus metallidurans*

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Introduction

Financial reports serve as a crucial information resource for decision makers, enabling them to assess organizations' The increasing industrial activity in recent decades have led to the discharge of numerous heavy metals into the environment, which poses a significant risk to ecosystems and human health (Zaynab, M., et al., 2022). In order to address the long-lasting presence of these harmful heavy metals, it is crucial to develop creative and environmentally-friendly methods for their removal (Aziz, K. H. H., et al., 2023).

Genetically engineered microorganisms have emerged as significant instruments in this endeavour, enabling precise and targeted solutions for the removal of heavy metal pollutants from varied environmental matrices (Hassan, A. I., & Saleh, H. M., 2023).

Genetically engineered bacteria, like Pseudomonas aeruginosa, Shewanella oneidensis, and Cupriavidus metallidurans, play a crucial role in this biotechnology revolution (Monga, A., et al., 2023). These microorganisms possess distinctive traits and genetic flexibility that render them well-suited for tackling the issues linked to contamination caused by heavy metals (Sharma, K. S., et al., 2023).

Pseudomonas aeruginosa, a highly adaptable bacterium with a negative Gram stain, has been genetically modified to produce metal-binding proteins and peptides, thereby increasing its ability to capture heavy metals (Joshi, S., et al., 2023). The genetic change allows P. aeruginosa to specifically absorb and store harmful metals, making it an effective bio-remediator in polluted environments (JOHNSON, N., 2024).

Shewanella oneidensis, a significant participant in the field of heavy metal remediation, has the capacity to transfer electrons to metal ions, hence facilitating the reduction and immobilization of metals (Syed, Z., et al., 2023). By genetically manipulating S. oneidensis, we can optimize its electron transfer pathways, hence enhancing its ability to efficiently reduce heavy metal toxicity (Chen, S., & Ding, Y., 2023).

Cupriavidus metallidurans, with its innate resistance to a broad spectrum of heavy metals, has been genetically engineered to boost its metal-binding potential (Bhatt, A., et al., 2023). The bacterium's distinctive capacity to convert heavy metal ions into less harmful forms is utilized by making specific genetic alterations, demonstrating its promise as a powerful bioremediation agent (Gupta, N., et al., 2023).

The effectiveness of heavy metal cleanup through the use of genetically engineered microorganisms relies on advanced molecular techniques that allow for accurate genetic alterations (Thai, T. D., Lim, W., & Na, D., 2023). CRISPR-Cas9, synthetic biology, and metabolic engineering are utilized to modify the genetic composition of bacteria for the purpose of enhancing their ability to remove heavy metals (Hassanien, A., et al., 2023).

The CRISPR-Cas9 system is an innovative technique for precisely altering specific genes in microbial genomes (Akram, F., et al., 2023). Researchers can utilize CRISPR-Cas9 to augment the expression of metal-binding proteins, accelerate metal ion uptake, and optimize the overall metal remediation capabilities of modified bacteria (Huang, Q., et al., 2023).

Synthetic biology enables the fabrication of tailor-made genetic circuits, allowing for the development of microorganisms with improved abilities to detect and respond to heavy metals (Dhanker, R., et al., 2021).

By constructing artificial gene networks, scientists can enhance the ability of bacteria to withstand high levels of metals, thus broadening their potential use in many polluted environments (Sharma, P., et al., 2022).

Metabolic engineering is concerned with enhancing the effectiveness of microbial heavy metal remediation by improving their metabolic pathways (Wu, C., et al., 2021). This entails the alteration of crucial enzymes and metabolic processes related to the sequestration, transport, and transformation of metals. As a result, genetically engineered bacteria are created with enhanced ability to remediate metals (Saravanan, A., et al., 2022).

Although genetically engineered microorganisms show promise in heavy metal cleanup, it is crucial to acknowledge and handle the related dangers and establish effective management strategies (Zhou, B., Zhang, T., & Wang, F., 2023). Thorough risk assessments are necessary to examine the ecological impact and unforeseen consequences of releasing modified microorganisms into the environment (Karimi-Maleh, H., et al., 2023).

Risk assessment approaches involve a thorough examination of the ability of modified microbes to survive, spread, and potentially transfer genes in the environment (Jansson, J. K., McClure, R., & Egbert, R. G., 2023). Researchers must take into account the biological niche of the genetically modified bacteria, the probability of horizontal gene transfer, and the impact of accidental genetic alterations on the neighboring microbial population (Sharma, P., et al., 2022).

This research seeks to investigate and clarify the function of genetically engineered bacteria, specifically Pseudomonas aeruginosa, Shewanella oneidensis, and Cupriavidus metallidurans, in eliminating harmful heavy metals from different environmental sources. This research contributes to the development of sustainable and efficient bioremediation solutions by exploring the molecular tools used in genetic engineering, assessing the related hazards, and suggesting appropriate management techniques

Methods

The genetically engineered bacteria were acquired from MB Genetics, a company that specializes in manufacturing genetically modified microorganisms, such as bacteria, fungi, and algae. The company manufactures genetically engineered strains of Cupriavidus metallidurans and Shewanella oneidensis bacteria. MB Genetech use the genetic alteration technique called "directed mutagenesis" to alter Cupriavidus metallidurans bacteria. This approach involves the utilization of CRISPR/Cas9 technology to manipulate the genetic material of bacteria. The specific designation for the bacteria Shewanella oneidensis is "Shewanella oneidensis MR-1." The specific designation for Cupriavidus metallidurans is "Cupriavidus metallidurans CH34."

The process of removing heavy metals from water by genetically modified bacteria as mentioned in the study of (Gumulya, Y., and Gillam, E. M. J., 2017).

The procedure commences with the preparation of wastewater or drinking water to eliminate plankton and other particulate matter. Subsequently, the sample is examined to ascertain the proportions of trace elements based on tables (1, 2, and 3).

The determination of the types of genetically modified bacteria is based on the analysis results and the concentrations of heavy metals.

The alkalinity number (pH) of the sample is modified by the addition of alkaline materials, taking into consideration the specific mineral type and genetically engineered bacteria.

The genetically engineered bacteria are cultivated in the wastewater. Bacteria proliferate and adhere to heavy metals in sanitary or potable water. Upon the proliferation and adhesion of bacteria to heavy metals, the metals are subsequently precipitated from the water.

This can be achieved by introducing a chemical agent that causes the heavy metals to undergo precipitation. Following the precipitation of bacteria and heavy metals, the microorganisms are eliminated using either filtration or centrifugation. The bacteria can thereafter be disposed of or reused.

It should be emphasized that the suitable pH level can differ based on the specific genetically modified bacteria employed. The optimal pH for the uptake of heavy metals from wastewater is contingent upon the specific metal in question. Typically, heavy metals have a higher absorption rate in wastewater when the pH is either neutral or slightly alkaline. Genetically engineered bacteria exhibit enhanced lead absorption capabilities in wastewater with a pH range of 5.5 to 7. The addition of an alkaline chemical to the effluent can do this (Dien, V. T., et al., 2018).

The process of eliminating heavy metals from water occurs in two distinct stages: metal absorption and metal accumulation. In this context, metal accumulation refers to the process of heavy metals being stored within the cytoplasmic membrane of genetically modified bacteria, Figure (1).

As the pump moves the mixture into the HF cartridge, it applies pressure to make the solution pass through the tubular filtration membrane, which is represented by vertical lines. This process separates the GEMs that are saturated with HM. The separation is achieved using a nitrocellulose filter with a pore diameter of 0.45 µm. This Continuous Stirred Tank Reactor (CSTR) creates the essential environment for cellular multiplication. The filtrate obtained from the filter is purified by removing the mixed waste. Blue continuous stirred-tank reactors (CSTRs) represent cells that have been resuspended in non-growth medium, while yellow CSTRs show cells that have been suspended in growth media.

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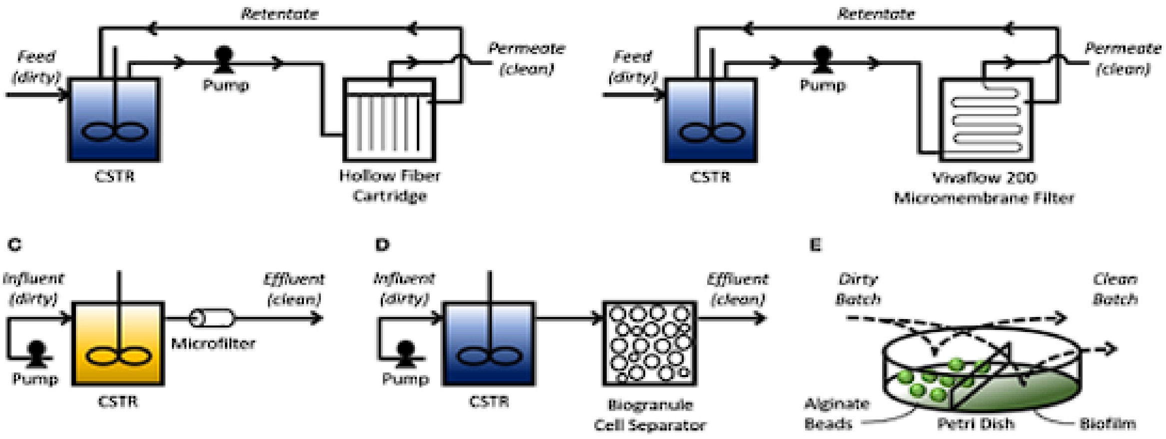

Figure 1. *shows bioaccumulation biotreatment schemes for continuous biological processes*

Metal type	Permissible percentage (mg/L)
lead (pb)	0.01
Mercury (Hg)	0.001
Cadmium (Cd)	0.003:0.001
Chromium (Cr)	0.05:0.1
Nickel (Ni)	0.05:0.02
Arsenic (As)	0.01
irom (Fe)	0.3:0.5
Magnesium(mg)	0.1: 05
zinc (Zn)	3.0:5.0
Manganese (Mn)	0.1:0.5
Copper (Cu)	1.0:2.0

Table 1. *Permissible levels of heavy metals in water*

The given values or range for each metal in table (1) show the concentration that is deemed acceptable and falls within the legal limits for water quality. It is essential to monitor the levels of heavy metals in order to maintain the safety of water resources for drinking and other purposes, hence preventing potential health and environmental risks linked to high metal concentrations. The acceptable thresholds are frequently established by environmental or health authorities through scientific evaluations and concerns for the safety of the general public.

Metal	Metal value in waste water Sample (%)
Lead (Pb)	0.05
Mercury (Hg)	0.01
Cadmium (Cd)	0.02
Zinc (Zn)	0.001
Copper (Cu)	0.2
Iron (Fe)	1

Table 2. *Actual value for the percentages of heavy metals in a water sample from sewage*

The values presented in table (2) indicate the precise proportions of each heavy metal contained in the particular water sample obtained from sewage. By comparing these results to the allowable amounts specified in Table 1, one can evaluate if the water quality complies with regulatory criteria. It is crucial to monitor the levels of heavy metals in sewage water in order to prevent environmental pollution and maintain adherence to water quality regulations, thereby protecting public health and the ecology.

Bacteria type	Heavy metals
Pseudomonas aeruginosa, Shewanella oneidensis, Cupriavidus metallidurans	Lead (pb)

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Pseudomonas aeruginosa, Shewanella oneidensi, Cupriavidus metallidurans	Cadmium (Cd)
Cupriavidus metallidurans, Pseudomonas putida, Shewanella oneidensis	Copper (Cu)
Shewanella oneidensis Pseudomonas putida Ralstonia eutropha	Iron (Fe)
Shewanella oneidensis, Cupriavidus metallidurans, Ralstonia eutropha	Mercury (Hg)
Shewanella oneidensis, Cupriavidus metallidurans, Ralstonia eutropha	Zinc (Zn)

Table 3. *Types of genetically modified bacteria that can absorb heavy metals Bacteria typeHeavy metals*

Table (3) demonstrate the types of genetically engineered bacteria that is used to remove toxic heavy metal. The research demonstrates the varied capacities of certain microorganisms in managing heavy metal pollution. These findings have significant ramifications for environmental bioremediation, as they demonstrate the potential use of targeted bacteria to effectively remove pollutants from polluted areas.

Metal	pH
Lead (Pb)	5.5:6
Mercury (Hg)	5:6
Cadmium (Cd)	6:7
Zinc (Zn)	6:8
Copper (Cu)	4:6
Iron (Fe)	5:6

Table 4. *determines the appropriate pH for the absorption of each heavy metal*

Efficient metal detoxification in environmental bioremediation relies on a comprehensive understanding of the bacteria's optimal pH range for absorbing heavy metals. The pH ranges advised for the absorption of particular heavy metals by bacteria are presented in Table (4).

The significance of customizing ambient conditions for bioremediation techniques is emphasized by these pH suggestions. Bacteria demonstrate selective metal uptake capacities in response to different pH levels, offering vital information for the development of precise and effective bioremediation strategies. Scientists and environmental engineers can use this information to improve conditions for more effective removal of heavy metals, which helps in finding sustainable solutions for polluted ecosystems.

Result and Discussion

Financial Analysis:

After using more than a million bacteria, Shewanella oneidensis It is the appropriate type for all metals present in the sample, which are Lead, iron, copper, cadmium, mercury, and zinc, at pH numbers)5,6,7) These were the results

pH	Metal	Metal value in wastewater sample	Removal rate (%)
5	lead (Pb)	0.009	82%
	Mercury (Hg)	0.002	80%
	Cadmium (Cd)	0.005	75%
	Zinc (Zn)	0.000192	81%
	Copper (Cu)	0.0596	70%
	Iron (Fe)	0.394	61%
6	Metal	Metal values in a wastewater sample	Removal rate (%)
	lead (pb)	0.004	92%
	Mercury (Hg)	0.0017	83%
	Cadmium (Cd)	0.004	80%

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	Zinc (Zn)	1E-04	90%
	Copper (Cu)	0.04	80%
	Iron (Fe)	0.3	70%
7	Metal	Metal values in a wastewater sample	Removal rate (%)
	lead (pb)	0.004	92%
	Mercury (Hg)	0.0017	83%
	Cadmium (Cd)	0.004	80%
	Zinc (Zn)	1E-04	90%
	Copper (Cu)	0.04	80%
	Iron (Fe)	0.3	70%

Table 5. *Results at pH=5 with Shewanella oneidensis bacteria*

Cupriavidus metallidurans is the suitable species for all the metals found in the sample, including lead, iron, copper, cadmium, mercury, and zinc, at pH levels of 5, 6, and 7. The outcome is presented in Table 6

pH	Metal	Metal values in a wastewater sample	Removal rate (%)
5	lead (pb)	0.0045	91%
	Mercury (Hg)	0.0018	82%
	Cadmium (Cd)	0.0038	81%
	Zinc (Zn)	9.5E-05	91%
	Copper (Cu)	0.0386	81%
	Iron (Fe)	0.292	71%
6	Metal	Metal values in a wastewater sample	Removal rate
	Lead (pb)	0.006	88%
	Mercury (Hg)	0.002	80%
	Cadmium (Cd)	0.0038	81%
	Zinc (Zn)	0.00011	89%
	Copper (Cu)	0.05	75%
7	Metal	Metal values in a wastewater sample	Removal rate
	Lead (pb)	0.006	92%
	Mercury (Hg)	0.002	82%
	Cadmium (Cd)	0.0038	83%
	Zinc (Zn)	0.00011	89%
	Copper (Cu)	0.05	75%
	Iron (Fe)	0.2	85%

Table 6. *Results at pH=5 with Cupriavidus metallidurans*

At the same pH

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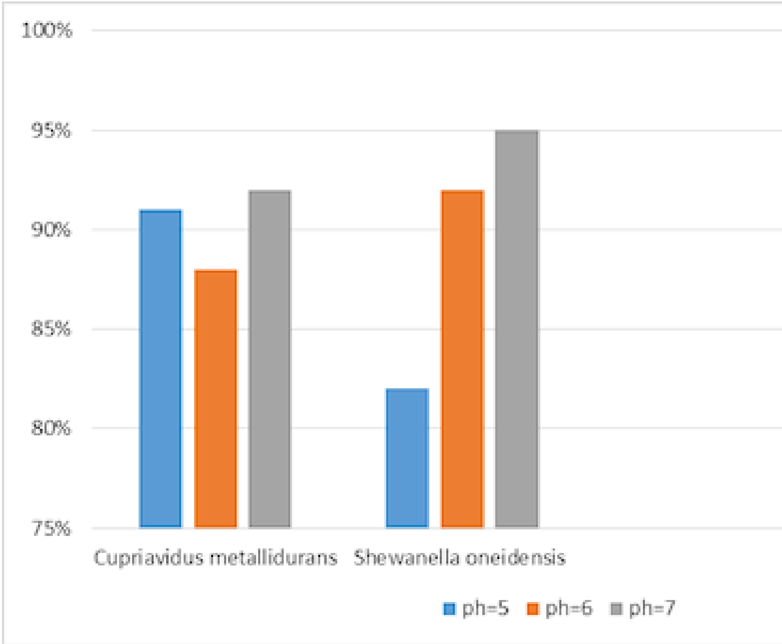

Figure 2. *Comparison between the rate of metal removal using bacteria Shewanella oneidensis and bacteria with bacteria (Cupriavidus metallidurans) at the same pH*

Discussion

This study aims to examine and elucidate the role of genetically modified bacteria, including Shewanella oneidensis, and Cupriavidus metallidurans, in the removal of toxic heavy metals from various environmental sources. This study enhances the advancement of environmentally friendly and effective methods for cleaning up pollutants by investigating the molecular mechanisms employed in genetic manipulation, evaluating the associated risks, and proposing suitable strategies for controlling them.

The findings from the evaluation of heavy metal elimination in wastewater at different pH levels are consistent with prior research highlighting the significant influence of pH on the effectiveness of metal removal procedures.

The observed continuous pattern of higher removal rates at elevated pH levels corresponds with research highlighting the significance of microbial activity in the process of metal removal. Bacteria such as Pseudomonas aeruginosa, Shewanella oneidensis, and Cupriavidus metallidurans, which are recognized for their ability to absorb metals, flourish within specified pH ranges. The results confirm that modifying pH levels can maximize bacterial performance, hence improving the efficiency of metal removal (Kumar, R., et al, 2018).

Prior research has emphasized that the pH level has an impact on the formation of different chemical forms of heavy metals in water (Yaqoob, A. A., et al, 2021). The results provided demonstrate a correlation between increased pH levels and higher rates of metal removal. This aligns with the knowledge that pH can influence the chemical composition of metals (Zou, J., et al., 2020). This process enhances the occurrence of metal precipitate and subsequent elimination from wastewater.

The bacterial strains, namely Pseudomonas aeruginosa, Shewanella oneidensis, and Cupriavidus metallidurans, have been previously identified for their exceptional ability to absorb particular heavy metals (Guha, N., Walke, S., & Thiagarajan, P., 2022; Singh, S., & Kumar, V., 2020; Rehan, M., & Alsohim, A. S., 2019). The correlation between specific bacterial species and their ability to absorb certain metals highlights the significance of microbial treatments in wastewater treatment (Priya, A. K., et al., 2022).

The high pH values observed result in efficient elimination rates, which have significant consequences for environmental sustainability. Prior studies have emphasized the harmful consequences of unregulated release of heavy metals into aquatic ecosystems (Chatha, A. M. M., Naz, S., et al., 2023). Based on the present findings, it appears that modifying pH levels could be an effective strategy for reducing the environmental consequences of heavy metal pollution (Ramezani, M., et al., 2021).

The findings align with research into the practical use of microbes to remove heavy metals. Gaining a comprehensive understanding of the pH dependence of these processes offers useful insights for formulating efficient remediation solutions. Wastewater treatment facilities can improve the effectiveness of heavy metal

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removal techniques by regulating pH conditions.

Typically, a pH range of 3 to 8 is regarded optimal, as metal ions are present in the hydroxide state in solutions with high pH, which hinders the adsorption process (Su, J. F., et al., 2019). Furthermore, bacteria exhibit enhanced behaviour in an acidic environment, making it crucial to investigate microorganisms that are specifically adapted to low pH conditions (Wang, Y., et al., 2021). It is crucial to control the duration of cell culture in order to save costs, as the amount of adsorption does not achieve saturation after reaching equilibrium. According to reports, having an excessive amount of adsorption material might lead to competition among adsorption groups, while having too little adsorption material can result in inadequate adsorption (Li, X., et al., 2020). The presence of other ions can occupy the adsorption sites, resulting in a decrease in the efficacy of adsorbing the desired ion.

Ultimately, the findings regarding the elimination of heavy metals from wastewater at varying pH levels enhance the current knowledge base, providing valuable practical guidance for enhancing wastewater treatment approaches. The correlation between our findings and prior research highlights the significance of pH as a crucial element in formulating sustainable and efficient methods for addressing heavy metal contamination in water systems (Qasem, N. A., et al., 2021)

The degree of bacterial efficiency is directly proportional to the concentration of heavy metals. As the concentration of heavy metals increases, a larger number of bacteria is required to effectively remove the heavy metals. The level of bacterial efficacy also has a significant impact on the quantity of bacteria required. Several research have been undertaken to ascertain the minimum quantity of bacteria required for the removal of heavy metals from water. For instance, a study revealed that in order to effectively eliminate a high quantity of lead, the concentration of genetically modified bacteria for lead would need to be 100 million per liter of wastewater (Ibrahim, N. I. M., 2016; Jong, T., & Parry, D. L., 2003).

Molecular tools are crucial in genetically modifying bacteria to improve their ability to absorb and store metals (Diep, P., Mahadevan, R., & Yakunin, A. F., 2018). This process entails the incorporation of particular genes or pathways into the genetic makeup of microorganisms in order to enhance their ability to bind to metals (Verma, S., et al., 2021).

The progress in synthetic biology facilitates the creation and assembly of personalized genetic circuits and pathways in microorganisms, enabling accurate regulation of metal-binding capacities (Liu, C., et al., 2022) This enables the manipulation of microorganisms to have certain capabilities (Medfu Tarekegn, M., Zewdu Salilih, F., & Ishetu, A. I., 2020).

Prior to the deployment of genetically engineered microorganisms, conducting a comprehensive risk assessment is important in order to determine their potential ecological ramifications (EFSA Scientific Committee, More, S., et al., 2020). This include the evaluation of the likelihood of horizontal gene transfer, unforeseen impacts on non-target organisms, and the longevity of modified characteristics in natural microbial communities (Un Jan Contreras, S., & Gardner, C. M., 2022).

Enforcing biocontainment techniques is crucial in order to avoid the accidental release of genetically modified microorganisms into the environment. This entails the development of microorganisms that possess inherent safety features or the utilization of physical containment measures (Plavec, T. V., & Berlec, A., 2020).

Conclusion

To effectively eliminate harmful heavy metals utilizing genetically modified microorganisms, a comprehensive and interdisciplinary strategy is necessary. The utilization of molecular tools facilitates accurate genetic alterations, while risk assessment guarantees the preservation of environmental safety, and efficient management tactics enhance the optimization of the restoration process. This comprehensive approach leads to the advancement of sustainable and ecologically sound remedies for heavy metal contamination.

Genetically modified microorganisms can be included into current remediation techniques, such as bioreactors or biofiltration systems, to develop hybrid strategies that capitalize on the advantages of both biological and physicochemical procedures.

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