Original Research Article

Isolation and screening of lead-accumulating microorganisms and their environmental applications

Hao Xia*, Changlu Chen, Wenjie Ma, Qing You
Guizhou University of Engineering Science, Bijie, Guizhou, 551700, China

Abstract: This study identified a lead-tolerant microorganism XH3 from soil near lead-zinc mine tailings in Bijie City, Guizhou Province. The strain demonstrated exceptional lead tolerance, achieving a maximum concentration of 5000 mg/L. Adsorption experiments revealed that XH3 reached a peak adsorption capacity of 219 mg/g for Pb^{2+}at 350 mg/L, with the adsorption process conforming to the Langmuir isotherm model, indicating single-layer chemical adsorption. These findings suggest the strain holds significant potential for lead-contaminated environment remediation.
Keywords: lead-fixing microorganisms; XH3 strain; lead pollution; adsorption capacity; langmuir model

1. Introduction

In recent years, with the advancement of industrialization in China, heavy metal pollution, especially lead pollution, has become increasingly severe. Lead has diverse sources, including natural ones such as volcanic eruptions and forest fires, as well as industrial activities like smelting, battery manufacturing, and mining[1,2]. Lead pollution has a significant impact on human health, particularly harming the nervous system and intellectual development of children and pregnant women. Therefore, researching methods to control lead pollution is of great significance.

2. Materials and methods

2.1. Experimental materials

2.1.1. Origin of the strain

The bacterial strains used in this experiment were sourced from soil samples collected from the tailings of a lead-zinc mine in Bijie City, Guizhou Province. The soil samples were randomly selected from different numbered soil layers, with each sample weighing approximately 200g and labeled in numbered sealed bags[3]. All samples were immediately refrigerated at-4℃ upon collection and stored until subsequent experimental use.

2.1.2. Experimental reagents

Table 1. Test agents.

Drug Name	Chemical formula/Main ingredients	Molecular weight (g/mol)	fineness (%)
lead nitrate	$Pb(NO_3)_2$	331.2	98
Pancreas yeast powder	-	2000	99
tryptone	-	2500	98
sodium chloride	NaCl	58.44	99
Lead standard solution	Pb^{2+}	-	100

2.1.3. Laboratory Equipment

Table 2. Instruments used in the experiment.

Instrument name	Instrument Model	manufacturer
electronic balance	FA2104	Shanghai Mettler Toledo Instrument Co., LTD
Vertical pressure steam sterilizer	STER-500	Shanghai Disinfection Equipment Co., LTD
pH count	PHS-3C	Beijing Jingke Instrument Co., LTD
Incubator	SHP-150	Shanghai Bosuo Instrument Co., LTD
Biochemical incubator	BSP-250B	Beijing Edward Instrument Co., LTD
Eppendorf centrifuge	H1200R	Eppendorf
Flame atomic absorption spectrometer	AAnalyst 200	PerkinElmer

2.1.4. Culture medium

The experimental media used include broth medium, lead-containing broth medium, and lead-containing agar medium. (1) The broth culture medium consists of: 0.5g pancreatic yeast powder, 1g sodium chloride, and 1g trypsin, dissolved in 100mL distilled water. Adjust the pH to 7.0 and sterilize at 121℃ for 15 minutes[4-6]. (2) The lead-containing broth culture medium is similar to the regular broth medium, with the addition of an appropriate amount of lead nitrate solution (200mg/L $^{+}$ concentration). (3) The lead-containing agar culture medium contains: 0.5g pancreatic yeast powder, 1g sodium chloride, 1g trypsin, and 2g agar. Dissolve in 100mL distilled water, adjust pH to 7.0, sterilize, then add lead nitrate solution (200mg/L $^{+}$ concentration). Pour into petri dishes to form the agar culture medium.

2.2. Experimental methods

2.2.1. Isolation and screening of lead-fixing microorganisms

(1) Strain enrichment: Add 1g of collected soil samples to LB medium and incubate at 37°C with 150rpm for 2-3 days. Preliminary screening of samples containing lead-fixing microorganisms is performed by observing color changes in the culture medium.

(2) Strain isolation and purification: Inoculate 1mL of enriched liquid culture onto LB solid medium containing lead (Pb^{2+} 200 mg/L), then incubate at 37℃ for 2-3 days. Select high-performing strains based on colony morphology on the surface, and further purify using the streak plate method[7].

(3) Slope preservation: The selected single colonies are inoculated into slope culture medium and incubated at 37℃ for 2-3 days. After colony growth, they are stored long-term in a-4℃ refrigerator.

2.2.2. Physiological and biochemical identification of bacterial strains

Based on morphological characteristics and growth traits, the isolated and purified bacterial strains were sent to Guiyang Bioengineering for gene sequencing. Identification was performed using 16S rRNA gene sequence analysis. By comparing with known bacterial sequences in the database, the phylogenetic relationship with related species was determined, followed by phylogenetic tree analysis[8].

2.2.3. Characteristics of bacterial strains

(1) MIC test: To determine the minimum inhibitory concentration (MIC) of Pb^{2+} the strain, XH3 was cultured on LB solid medium at varying concentrations (200 mg/L to 5000 mg/L) and its growth was monitored after 24 hours.

(2) Adsorption capacity experiment: Simulated lead-containing wastewater with different concentrations (Pb^{2+} concentration: 50,100,150,200,250,300,350,400,450,500mg/L) were prepared, and the bacterial strains were inoculated in these solutions. After 30 minutes of shaking culture, the supernatant was centrifuged, and the concentration in the supernatant was measured to calculate the adsorption capacity of the bacterial strains.

(3) Adsorption Isotherm Experiment: The purified bacterial strain was inoculated into 100mL LB liquid mediumin a conical flask and cultured at 37°C with 150rpm for 2-3 days. After incubation, the culture was centrifuged at 5000×g for 10 minutes, the supernatant was discarded, and the bacterial cells were washed with ultrapure water. This washing process was repeated 2-3 times to remove residual materials[9]. The resulting bacterial cells were lyophilized and stored for later use. For the adsorption experiment, 50mL of Pb^{2+} solution (50-500 mg/L) was added to 0.025g of lyophilized bacterial cells. The mixture was incubated at 25°C with 150rpm for 30 minutes. After centrifugation to separate the supernatant, the Pb^{2+} concentration was measured to calculate the bacterial adsorption capacity.

3. Results and analysis

3.1. Plate streaking and purification culture

Through enrichment culture, lead-tolerant microorganisms were isolated from soil samples collected Pb^{2+} under different conditions. Each sample was cultured in LB liquid medium containing 200 mg/L lead for 2-3 days. The medium turned black by the second day, indicating the presence of lead-tolerant microorganisms. Subsequently, the secondary enrichment culture medium was inoculated onto solid medium with 200 mg/L lead using the mixed bacterial inoculation method. Colonies were observed on plates numbered 1, 2, 4, and 5, with plate 2 showing the best bacterial growth. This strain was selected for further experiments. Purification using

the streak plate method revealed milky-white, moist colonies that turned pale yellow the next day and emitted a strong odor[10]. Genetic sequencing identified the strain as Enterobacter hormaechei subsp. XH3. Minimum inhibitory concentration (MIC) tests demonstrated that XH3 could tolerate up to 5000 mg/L of lead, exhibiting strong lead tolerance. See **Figure 1-3**.

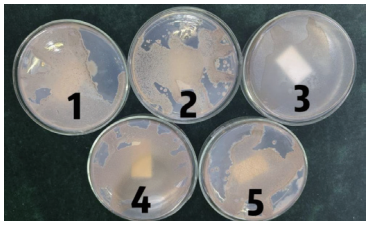

Figure 1. Plate culture map.

Figure 2. Growth profile of strain XH3 on agar plates with 5000 mg/L lead concentration.

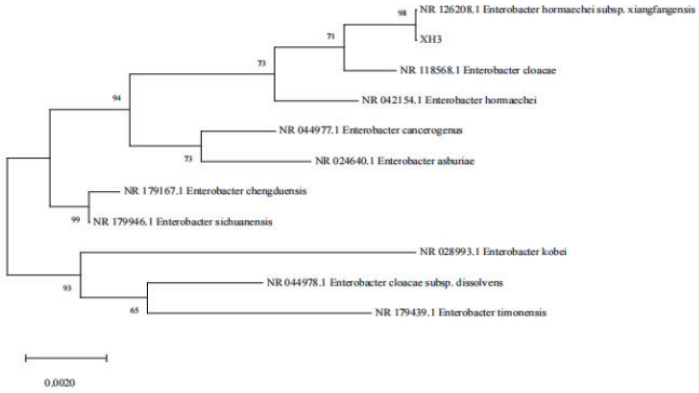

Figure 3. Phylogenetic tree of XH3-associated bacterial species.

3.2. Influence Pb^{2+} of initial concentration on XH3 adsorption

The experiment measured the adsorption capacity of strain XH3 Pb^{2+} for by setting different initial concentrations (50,100,150,200,250,300,350,400,450,500 mg/L). The results showed a certain relationship between the initial concentration of and the adsorption capacity.

(1) In the range of 50 to 200mg/L, the adsorption capacity is positively correlated with the initial concentration, and the adsorption capacity increases gradually.

(2) When the initial concentration exceeds 350mg/L, the adsorption capacity reaches the maximum value of 219mg/g.

(3) When the initial concentration is further increased to more than 350mg/L, the adsorption capacity shows a downward trend, which may be due to the repulsive force between ions, leading to the saturation of adsorption sites.

Therefore, the optimal initial concentration of 350 mg/L yields the highest adsorption capacity for strain XH3, as shown in **Figure 4**.

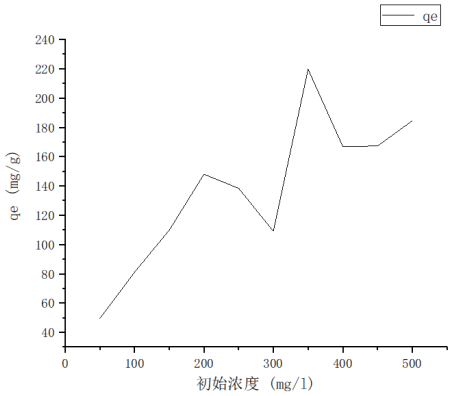

Figure 4. Effect of varying Pb^{2+} initial concentrations on XH3's adsorption capacity.

3.3. Adsorption isotherm model

To investigate the adsorption Pb^{2+} mechanism of strain XH3, three isotherm models—Freundlich, Langmuir, and Temkin—Were applied for fitting analysis.

(1) The Freundlich model showed a good fit with a correlation coefficient of 0.99014 and a P-value <0.05, confirming the adsorption process's alignment with the model. See **Figure 5**.

(2) The Langmuir model fitting results showed a correlation coefficient of 0.99645 with a P-value <0.05, indicating the adsorption process exhibited characteristics of monolayer adsorption. The calculated maximum adsorption capacity (qm) of the Langmuir model was 201.61290mg/g, which closely matched the experimental adsorption capacity (219 mg/g), confirming that the adsorption on the surface of strain XH3 was chemisorption. See Figure 6.

(3) The Temkin model fitting results show a value of 0.99591 with a P-value <0.05, indicating its suitability for describing the adsorption heat effect. See **Figure 7**.

The Langmuir model showed the best fit, indicating that the adsorption of XH3 pairs is primarily single-layer adsorption, i.e., chemical adsorption. The separation factor RL (0.50891 to 0.91199) indicated favorable adsorption for XH3 pairs. As shown in **Table 3**, the adsorption rate (RL) of XH3 for Pb2+ in this study ranges from 0.50891 to 0.91199, indicating that XH3 adsorbs Pb2+ effectively.

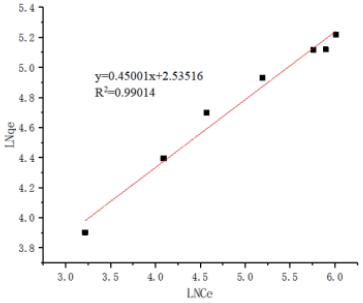

Figure 5. Freundlich isotherm.

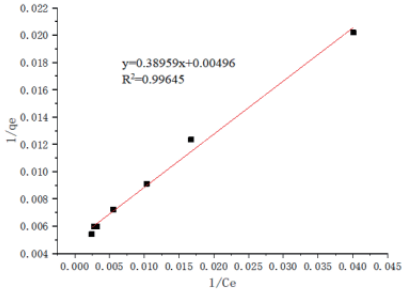

Figure 6. Langmuir isotherm.

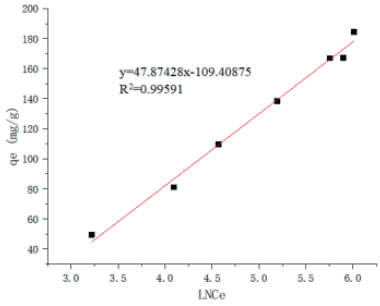

Figure 7. Temkin model.

Table 3. Adsorption isotherm parameters.

Isotherm	Parameter	
Freundlich	$k_F((mg/g)(L/mg)^{1/m})$	12.61843
	1/n	0.45001
	R^2	0.99014
	P	＜0.05
Langmuir	q_m(mg/g)	201.61290
	k_L(L/mg)	0.00193
	R_L	0.50891-0.91199
	R^2	0.99645
	P	＜0.05
Temkin	A_T(L/mg)	0.10174
	b_T(KJ/mol)	51.95134
	R^2	0.99591
	P	＜0.05

4. Conclusion

This study successfully identified a lead-resistant microorganism XH3, which demonstrates strong Pb^{2+} adsorption capacity. Experimental analysis showed that XH3 exhibited optimal growth in media containing 200 mg/L Pb^{2+}, and stable single colonies were obtained through streak purification on agar plates[11]. Minimum inhibitory concentration (MIC) experiments revealed that XH3's maximum lead tolerance reached 5000 mg/L. Adsorption results indicated that XH3 achieved peak adsorption capacity of 219 mg/g at 350 mg/L. The adsorption process followed the Langmuir isotherm model, confirming that Pb^{2+} adsorption occurs through single-layer chemical adsorption.

Overall, the XH3 demonstrates broad application potential in lead-contaminated environmental remediation.

Fundings

Project for the Growth of Young Scientific and Technological Talents in Regular Higher Education Institutions of Guizhou Province (No. Qianjiaohe KY [2022] 130); Project for the Growth of Young Scientific and Technological Talents in Regular Higher Education Institutions of Guizhou Province (No. Qianjiaohe KY [2022] 131).

About the author

Hao Xia (1991-), male, from Anqing, Anhui Province, is a teacher at the School of Ecological Engineering, Guizhou University of Engineering and Applied Technology. His main research and teaching areas include the application of environmental biotechnology in pollution control and water pollution treatment. Corresponding author of this article.E-mail:hxia@gues.edu.cn.

References

[1] Li Xiaomin. Phosphorylated zero-valent iron for lead removal from water and soil immobilization [D]. Central China Normal University, 2020.

[2] Shen Qiuyue, Cao Zhiqiang, Zhu Yuefang, Shi Weilin. Isolation and Identification of a Cadmium-tolerant Bacteria and Optimization of Adsorption Conditions [J]. Soil, 2016,48(03):615-620.

[3] Li Yinping, Zhang Zhuanyuan, Liu Xiaoyan, Che Shuai, Shi Naiwen, Chen Yiming, Yan Mingyan. Adsorption behavior and mechanism of Lead (Pb2+) by sulfate polysaccharide from Enteromorpha prolifera[J]. International Journal of Biological Macromolecules, 2022, 207.

[4] Jia-Shun Cao, Cheng Wang, Fang Fang, Jun-Xiong Lin. Removal of heavy metal Cu(II) in simulated aquaculture wastewater by modified palygorskite[J]. Environmental Pollution, 2016, 219.

[5] Wallace A B ,Varona S N ,Stiffler K A , et al. High microbial diversity, functional redundancy, and prophage enrichment support the success of the yellow pencil coral, Madracis mirabilis, in Curaçao's coral reefs.[J]. mSystems,2025,e0120825.

[6] Wu R C ,Xu Z R ,Huang L Y , et al. [Impacts of Vegetation Restoration on Soil Microbial Community Structure in Lead-Zinc Mine Tailing Ponds in the Karst Region of Southwest China].[J].Huan jing ke xue= Huanjing kexue,2025,46(10):6640-6650.

[7] Kong L ,Xue M ,Li J , et al. Effect of lead-zinc tailings on the microbial corrosion resistance of alkali-activated materials[J].Construction and Building Materials,2025,494143427-143427.

[8] Chen P ,Mei T ,He X , et al. Impacts of Lead and Nanoplastic Co-Exposure on Decomposition, Microbial Diversity, and Community Assembly Mechanisms in Karst Riverine Miscanthus Litter.[J].Microorganisms,2025,13(9):2172-2172.

[9] Zhang L ,Song Z ,Qin Q , et al. Multi-system toxicity of lead (Pb) in Procambarus clarkii: Integrated analysis of tissue damage, immune dysfunction, oxidative stress, and microbial dysbiosis via multi-omics approaches.[J].Comparative biochemistry and physiology. Toxicology & pharmacology : CBP,2025,298110331.

[10] Tao Y ,Liu D ,Shi Q , et al. Lead exposure in relation to gut homeostasis, microbiota, and metabolites.[J].Applied and environmental microbiology,2025,e0037225.

[11] Ayres R K ,Liuzzi J . Establishing a Lead Dose-Response Model to Develop Protocol for Future Microbiome Research in C. elegans Populated With Diverse Gut Microbiota[J].Current Developments in Nutrition,2025,9 (S2):107129-107129.

[12] Zafar S ,Fatima S ,Muzammil S , et al. Bioremediation of lead contaminated aquatic environment using non-pathogenic strain Klebsiella pneumoniae PbS3A2 in Labeo rohita[J].International Journal of Environmental Science and Technology,2025,22(13):1-14.