CHEMICAL ENGINEERING TRANSACTIONS
VOL. 55, 2016
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.aidic.it/cet

Guest Editors: Tichun Wang, Hongyang Zhang, Lei Tian

ISBN 978-88-95608-46-4; **ISSN** 2283-9216
DOI: 10.3303/CET1655065

Selection of Heavy Metal Zinc Adsorption Strains in Contaminated Soil

Zhuo Yang

Hebei University of Environmental Engineering, Qinghuangdao, Hebei 066000, China.
yangzhuo315566@126.com

This test focuses on the screening and identification of heavy metal zinc adsorption strains in contaminated soil. Four strains with high zinc adsorption capacity are obtained by collecting contaminated soil samples, enrichment cultivation, strain isolation, and determination of adsorption rate. The strains are named ZnA-4, ZnE-3, ZnH-2 and ZnL-2 respectively. ZnH-2 strain boasts a zinc adsorption rate of 48.30%, higher than that of any other strain, followed by ZnL-2 with an adsorption rate of 42.95%. With conventional bacteria identification method, the four strains are preliminarily indentified as *Bacillus.spp* based on the two aspects of colony morphology & characteristics and physiological & biochemical tests. The functional strains are potential remediation agents for in-situ fixation of contaminated soil.

1. Introduction

As a micronutrient necessary for the growth of animals and plants, Zinc plays an important role in regulating human immune function, maintaining the normal physiological function of the body, promoting the normal development of children, and treating of anorexia and malnutrition (Medina et al., 2005; Zhu et al., 2005; Zhou et al., 2007). With the rapid development of mining of lead, zinc and other mineral resources, metal smelting, fuel production, recent years has seen zinc and other heavy metals entering the soil environment through a variety of ways, causing serious heavy metal pollution to soil (Zhang et al., 2012). Crop failure is commonplace on zinc-contaminated soil as plants absorb zinc from the soil. To make matters worse, the zinc absorbed by the plants would pass up the food chain and ultimately endanger human health. As a result, the quality of the soil and the safety of agricultural products are of paramount importance to human health (Wang and Wei, 1995).

The traditional control methods of heavy metal pollution are chemical precipitation, ion exchange, electrolysis, and membrane separation. However, traditional methods have the disadvantages of high cost, high energy consumption, and the likelihood of secondary pollution. In contrast, microbial adsorption technology, a recent and effective method for removing heavy metals from soil, has low operating cost, and no influence on soil fertility and metabolic activity, and can prevent damages to human health and environment by pollutants transfer. Thus, the technology attracts extensive attention from researchers over the years (He 2012).

Aiming at improve bacteria resources for biotechnological treatment of zinc-contaminated soil and lay a scientific foundation for further research, this paper selects and isolates the bacterial strains with high efficiency in zinc adsorption, and identifies the species of the strains with strong adsorption capacity.

2. Materials and methods

2.1 Experiment instruments

The following instruments are used in the experiment: MLS-3020 autoclave by Sanyo; ZHWY-211B double-deck shaker by Shanghai Zhicheng Analytical Instrument Co., Ltd.; SPX-250 intelligent biochemical incubator by Ningbo Haishu Saifu Experimental Instrument Plant; electronic balance (Adventurer) by Ohaus USA; benchtop by Suzhou Antai Airtech Co., Ltd; BC/BD-325 cryogenic refrigerator by Haier; benchtop by Sanyo; optical microscope by Nikon; 4°C refrigerator (MDF-382E) by Sanyo; 752-type ultraviolet and visible spectrophotometer by Spectrum Shanghai; TGL-16M high-speed centrifuge by Hunan Saitexiang Instrument Company; Eppendorf RS-311 micropipette by Shanghai Kenqiang Instrument Co., Ltd.

Please cite this article as: Yang Z., 2016, Selection of heavy metal zinc adsorption strains in contaminated soil, Chemical Engineering Transactions, 55, 385-390 DOI:10.3303/CET1655065

2.2 The collection of soil samples
The soil samples are collected by the five-spot method. The author gathers the contaminated soil around the sewage treatment plant, and in front of the Miaogou Iron Mine, and the bottom mud at the estuary of Xiaotang River. After that, the author mixes the soil samples, shovels off the surface soil, puts the soil at the depth of 5-10cm into aseptic bags, records the sampling location and time, returns to the lab, and stores the bags in the 4°C refrigerator for future use.

2.3 Experiment methods

2.3.1 Strain enrichment and separation
The soil suspension is prepared by mixing 5g of soil sample in Zn^{2+}-containing fluid medium. After shaking culture for 3 days, it is transferred to fresh fluid medium until Zn^{2+} concentration reaches 500 mg/L. This continuous enrichment results in a highly pure, stable, enrichment culture.

The acclimated strain is then spread to a solid medium into which 500mg/L of zinc chloride has been added. Single colony is repeatedly streaked on the heavy metal-containing solid medium until the pure culture is obtained.

2.3.2 Determination of strain adsorption capacity
(1) Cell Preparation

The activated cells are picked from the slant, shaken for 20 hours, collected, and washed with deionized water and centrifuged. Repeat the washing and centrifuging process three times, and the cells are ready for use.

(2) Adsorption Test

Weigh a certain amount of dry cells, add them into 50mL of Zn^{2+}-containing aqueous solution, conduct oscillation adsorption according to different experimental requirements, and carry out centrifuging at 13000r/min for 5min.

(3) Calculation

Heavy metal adsorption rate is calculated with the following formula:

Adsorption rate = $(C_0-C_t)/C_0\times100\%$

Where, C_0 is the initial concentration of heavy metal ions (mg/L); C_t is the equilibrium concentration of heavy metal ions (mg/L).

3. Experimental results

3.1 Stain enrichment and purification
The obtained strains were numbered and streaked and purified. See Table 1 for the colony morphology.

Table 1: Description of colony morphology

Stain	Characteristic of Colony					
	Shape	Surface	Humidity	Color	Size	Edge
ZnA-1	Circular	Flat	Dry	White	Large	Regular
ZnA-2	Circular	Convex	Sticky	Ivory	Extra-large	Regular
ZnA-3	Sub-circular	Flat	Dry	Ivory	Extra-large	Regular
ZnA-4	Sub-circular	Flat	Dry	White	Large	Jagged
ZnA-5	Sub-circular	Flat	Dry	White	Large	Jagged
ZnB-1	Circular	Convex	Sticky	Red	Medium	Regular
ZnB-2	Circular	Flat	Wet	Ivory	Medium	Regular
ZnB-3	Circular	Flat	Dry	White	Medium	Regular
ZnB-4	Oval	Convex	Sticky	White	Extra-large	Regular
ZnB-5	Circular	Flat	Wet	Ivory	Large	Regular
ZnC-1	Sub-circular	Flat	Dry	White	Large	Snowflake
ZnC-2	Circular	Flat	Wet	Ivory	Large	Regular
ZnC-3	Circular	Flat	Sticky	Ivory	Large	Regular
ZnC-4	Circular	Flat	Dry	White	Large	Jagged
ZnC-5	Circular	Flat	Dry	White	Large	Irregular
ZnD-1	Circular	Flat	Dry	White	Extra-large	Regular
ZnD-2	Circular	Convex	Sticky	Ivory	Medium	Regular
ZnD-3	Circular	Convex	Wet	Ivory	Medium	Regular
ZnE-1	Irregular	Convex	Dry	White	Large	Snowflake
ZnE-2	Circular	Flat	Wet	Ivory	Large	Regular
ZnE-3	Circular	Convex	Wet	Ivory	Medium	Regular

Stain	Characteristic of Colony					
	Shape	Surface	Humidity	Color	Size	Edge
ZnF-1	Circular	Flat	Dry	Light red	Extra-large	Irregular
ZnF-2	Circular	Flat	Sticky	Light red	Large	Regular
ZnF-3	Circular	Flat	Dry	White	Large	Regular
ZnF-4	Circular	Flat	Sticky	Light red	Medium	Irregular
ZnF-5	Circular	Convex	Sticky	White	Extra-large	Jagged
ZnF-6	Irregular	Convex	Sticky	Light red	Large	Jagged
ZnF-7	Circular	Flat	Dry	Ivory	Medium	Regular
ZnF-8	Circular	Flat	Dry	White	Extra-large	Jagged
ZnH-1	Irregular	Convex	Dry	White	Large	Snowflake
ZnH-2	Sub-circular	Flat	Wet	Ivory	Large	Jagged
ZnH-3	Sub-circular	Flat	Dry	Ivory	Large	Regular
ZnH-4	Sub-circular	Flat	Dry	White	Large	Jagged
ZnH-5	Circular	Convex	Sticky	White	Small	Regular
ZnI-1	Circular	Flat	Wet	Ivory	Extra-large	Regular
ZnI-2	Circular	Flat	Sticky	White	Extra-large	Regular
ZnI-3	Circular	Flat	Sticky	White	Medium	Jagged
ZnJ-1	Circular	Flat	Relatively Wet	Ivory	Large	Regular
ZnJ-2	Sub-circular	Flat	Dry	White	Extra-large	Jagged
ZnJ-3	Sub-circular	Convex	Sticky	White	Extra-large	Jagged
ZnK-1	Circular	Convex	Sticky	White	Medium	Regular
ZnK-2	Circular	Flat	Sticky	Ivory	Medium	Regular
ZnK-3	Circular	Flat	Sticky	Ivory	Extra-large	Jagged
ZnL-1	Oval	Flat	Wet	Ivory	Medium	Regular
ZnL-2	Circular	Flat	Sticky	Ivory	Large	Regular
ZnN-1	Circular	Flat	Wet	Ivory	Extra-large	Regular
ZnN-2	Circular	Convex	Wet	Brown	Small	Regular
ZnM-1	Circular	Flat	Sticky	Ivory	Large	Regular
ZnM-2	Irregular	Convex	Sticky	Ivory	Extra-large	Irregular
ZnM-3	Circular	Flat	Sticky	White	Medium	Regular
ZnO-1	Sub-circular	Flat	Sticky	White	Extra-large	Irregular
ZnO-2	Circular	Flat	Wet	Light red	Extra-large	Regular
ZnO-3	Circular	Flat	Wet	Ivory	Large	Regular
ZnO-4	Circular	Flat	Dry	White	Extra-large	Regular
ZnP-1	Sub-circular	Flat	Sticky	White	Large	Jagged
ZnP-2	Circular	Flat	Wet	Ivory	Medium	Regular
ZnP-3	Circular	Flat	Sticky	White	Extra-large	Regular
ZnP-4	Circular	Convex	Sticky	Grey in the middle and transparent on the edge)	Small	Regular

3.2 Zinc adsorption test

3.2.1 Drawing of Zn^{2+} standard curve

(1) Preparation of the developer solution

Take a 4mL transparent tube, add 3mL of 4g/mL NaOH solution with a micropipette, and add 300μL of 5 g/L dithizone stock solution, stir evenly and allow the mixture to stand until two layers are formed. Then, take the upper layer as the developer solution. The developer solution should be prepared right before use.

(2) Absorption Spectrum Scanning and Standard Curve Drawing

Take a series of 15mL microcentrifuge tubes, add 0 μL, 10μL, 20μL, 30μL, 40μL, 50μL, 60μL, 70μL, 80μL, 90μL and 100μL of 100mg/L Zn^{2+} standard solution to the tubes respectively. Then, add deionized water into the tubes till the level reaches 700μL. Next, add 100μL of Triton X-100 solution, 100μL of developer solution, and 100μL of acetic acid-sodium acetate buffer solution into each tube, shake the tubes up to prepare a 1mL reaction system. With a UV-1700 spectrophotometer, measure the absorption spectrum of the reaction system at 520 nm with Zn^{2+} content of 0μg/mL and 1 μg/mL, respectively, and draw the standard curve. (See Figure

1) Use this method to determine content of metal ions in the solution after adsorption and calculate the adsorption rate (Yu et al., 2004; Li et al., 2007).

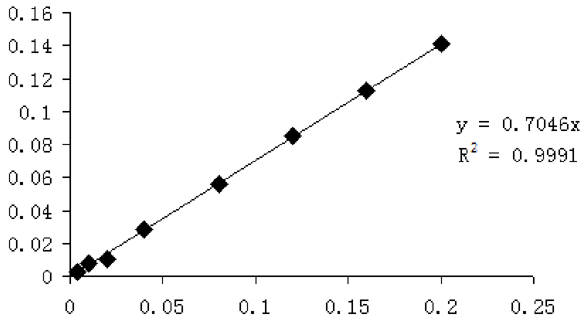


Figure 1: Zn^{2+} Standard Curve

(2) Results of Zn^{2+} Adsorption by Strains
See Table 2 for the adsorption rate of Zn^{2+} by strains.

Out of the 58 zinc resistant strains that have been screened out, 34 are capable of absorbing zinc. ZnH-2 strain boasts a zinc adsorption rate of 48.30%, higher than that of any other strain, followed by ZnL-2 with an adsorption rate of 42.95%., ZnE-3, and ZnA-4. As these four strains are more valuable for the remediation of zinc-contaminated soil, the author studies the biological characteristics of them.

Table 2: Results of Zn^{2+} Adsorption by Strains

Strain	Adsorption Rate%	Strain	Adsorption Rate%
ZnB-2	6.005	ZnB-1	3.133
ZnD-3	7.050	ZnM-3	3.303
ZnP-4	3.394	ZnK-2	1.697
ZnD-1	1.305	ZnB-5	1.567
ZnE-3	11.88	ZnL-2	42.95
ZnA-5	5.222	ZnD-2	3.916
ZnH-3	4.83	ZnH-4	1.697
ZnH-5	3.916	ZnA-4	10.84
ZnB-6	4.569	ZnF-4	6.658
ZnC-1	5.614	ZnC-3	4.439
ZnH-2	48.30	ZnL-1	3.916
ZnP-1	9.008	ZnK-1	3.133
ZnF-7	5.352	ZnO-3	4.439
ZnE-2	5.483	ZnP-3	6.658
ZnH-1	4.178	ZnF-5	3.524
ZnJ-1	5.22	ZnF-6	1.305
ZnI-1	3.394	ZnM-2	1.305

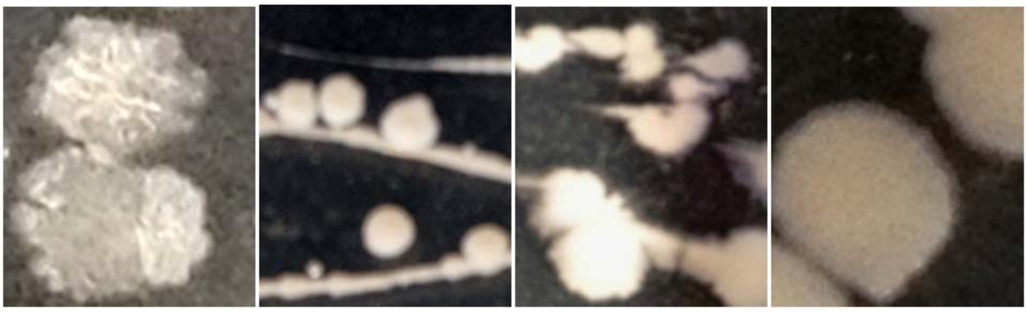
3.3 Strain identification

3.3.1 Colony characteristics

Table 3: Description of Colony Morphology

Stain	Colony Characteristics					
	Shape	Surface	Humidity	Color	Size	Edge
ZnA-4	Sub-circular	Flat	Dry	White	Large	Jagged
ZnE-3	Circular	Convex	Wet	Ivory	Medium	Regular
ZnH-2	Sub-circular	Flat	Wet	Ivory	Large	Jagged
ZnL-2	Circular	Flat	Sticky	Ivory	Large	Regular

Observing the colony morphology of the four strains of ZnA-4, Zn-3, Zn-2 and Zn-2, the author draws the preliminary conclusion that these strains are bacterial colonies which are circular/sub-circular, and white. See Table 3 and Figure 2 for detailed description.

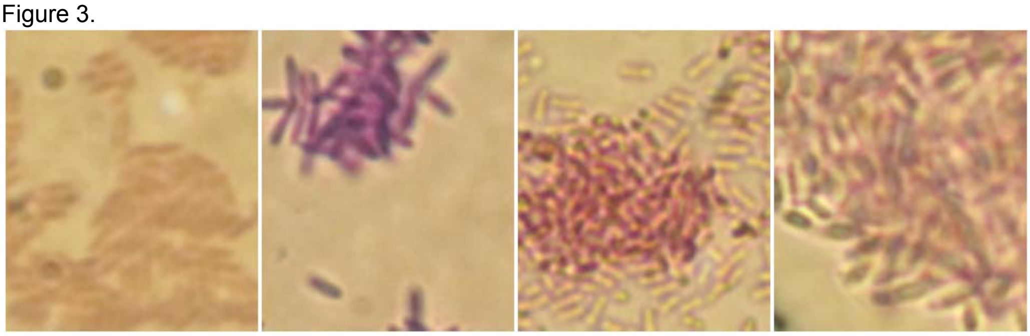


ZnA-4 ZnE-3 ZnH-2 ZnL-2

Figure 2: Colony morphology and characteristics

3.3.2 Gram staining

After Gram staining, the four strains are observed under the microscope. The author concludes that all of them arte bacillus. ZnE-3 is Gram-positive bacteria and all the other three strains are Gram-negative bacteria. See Figure 3.



ZnA-4 ZnE-3 ZnH-2 ZnL-2

Figure 3: Photos of gram stained strains

3.2 The results of physiological and biochemical experiments

See Table 4 for detailed results.

Table 4: The results of physiological and biochemical experiments

Item	Strain			
	ZnA-4	ZnE-3	ZnH-2	ZnL-2
V.P test	-	-	-	-
Nitrate reduction test	+	+	+	+
Starch hydrolysis test	+	-	+	+
Contact enzyme test	+	+	+	+
Sugar fermentation test	+	+	+	+
Citrate test	-	-	-	-
Methyl red test	+	+	+	-
Ammonia production test	+	-	+	-
Phenylalanine deaminase test	-	-	-	-
Malonate test	+	+	+	+
Denitrification test	-	+	+	+
Dextrin production test	+	+	-	+
Glucose fermentation test	+	-	+	+
	+	-	+	+
Urease test	-	+	+	-

Item	Strain			
	ZnA-4	ZnE-3	ZnH-2	ZnL-2
Lipase test	-	+	-	-
3-ketolactose determination	-	-	-	-
Production of pyocyanine	-	-	-	-
Nitrite reduction	+	+	+	+
Carbon source utilization	+	-	+	+

Note: "+"≥90% indicates that the strain is positive; "–"≥90% indicates that the strain is negative.

Comparing the above results to the Common Bacteria Identification Manual, the author draws the preliminary conclusion that: ZnA-4, ZnE-3, ZnH-2 and ZnL-2 are Bacillus spp.

4. Conclusion

In light of the adsorption capacity of heavy metal zinc in contaminated soil, this test obtains four strains with high zinc adsorption capacity, namely ZnA-4, ZnE-3, ZnH-2 and ZnL-2 respectively. ZnH-2 strain boasts a zinc adsorption rate of 48.30%, higher than that of any other strain, followed by ZnL-2 with an adsorption rate of 42.95%. As these four strains are more valuable for the remediation of zinc-contaminated soil, the author studies the biological characteristics of them, and preliminarily identifies them as *Bacillus*. In the future, researchers can make further studies on how to optimize the adsorption capacity of the strains.

Reference

He Q.X. 2012. Review of the Remediation Technology of Soils Pollution by Heavy Metals. Guangzhou Chemical Industry, 40(22): 44-46.

Li Y.P., Liu X.D., Gong H.L. 2007. Adsorption Characteristics of Soils for Lead, Copper, Zinc and Cadmium. Rock And Miner A analysis, 26(6):455-459.

Medina H., Bullón J., Ontíveros F., Chacón T., Cárdenas A., 2005, Zinc separation of aqueous solutions using emulsion liquid membranes. The pH influence, Revista de la Facultad de Ingeniería, 20, 3, 57-62.

Wang Y., Wei F.S. 1995. Soil element chemistry].Beijing: China Environmental Science Press, 42-57.

Yu C.X., Xu X.P., Shen X.X., Li H.Z. 2004. Biosorption and tolerance of Sphaerotilus Natans to Heavymetalion. Chinese Journal of Applied and Environmental Biology, 11(1):90-92.

Zhang F.L., Chen Y.H., Liang X. 2012. Research advances on screening of hyperaccumulator and tolerant plant species of Pb-Zn. Journal of Central South University of Forestry & Technology, 32(12): 92-96.

Zhou Q.X., Wei S.H., Diao C.Y. 2007. Basic Principles and Researching Progresses in Ecological Remediation of Contaminated Soils. Journal of Agro-Environment Science, 26(2): 419- 424.

Zhu Y.G., Chen B.D., Lin A.J., Ye Z.H., Wong M.H. 2005. Heavy metal contamination in Pearl River Delta-Status and research priorities. Acta Scientiae Circumstantiae, 25(12): 1575-1579