

Study on Removal of Heavy Metal Ni Pollution by Microbial Mineralization Consolidation

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Abstract. In this study, a strain of *Pichia pastoris* strain N was screened from the soil around the local electroplating plant. This carbonate mineralization bacteria produced urease during the growth and metabolism process, decomposing the substrate urea and producing ammonia ions. And carbonate ions, mineralized to consolidate the heavy metal Ni in the soil. The basic physiological and biochemical properties, growth curve, urease activity and influencing factors of strain N and mineralization analysis experiments were analyzed. The conclusions of this experiment can provide a theoretical basis for the removal of heavy metals from soil in the field and can improve the application value of this technology.

1. Introduction

Microorganisms have an irreplaceable role in removing heavy metal nickel from the soil. They have two main methods to remove heavy metals from the soil. Biomineralization has the advantages of low cost, good effect, convenient control and simple operation, which has attracted more and more people's attention and become a popular method for repairing nickel pollution in soil. The microbial enzyme-decomposing substrate mineralization and consolidation method studied in this thesis is a method for removing heavy metals in soil with great practical significance. It also provides a new research direction and good theoretical basis for repairing heavy metal pollution in soil.

2. Preparation of strain culture medium

Medium: It can provide the necessary microorganisms for the cultured microorganisms, such as carbon, nitrogen and oxygen. It usually contains all kinds of nutrients and trace elements necessary for growth.

a). **Solution preparation:** Add five grams of yeast extract, ten grams of peptone, five grams of sodium chloride in one liter of distilled water, and use a heating furnace to help dissolve. One thing to note is that there is an increase in moisture during heating. Distilled water should be added to 100 mL after the dissolution is completed. If you want to get a solid medium, add the appropriate amount of agar bar or agar powder if necessary. Prepare 10 bottles of 100 ml medium and place in the refrigerator for later use.

b). **Adjust pH:** Adjust the pH of the medium to 7.0.

c). **Sterilization:** The medium was placed in an autoclave and sterilized at 121 ° C for 20 minutes.

d). **Stand still:** Place the sterilized medium on an aseptic table until the medium is cooled to room temperature. Store at a temperature of about 4 ° C and put it in the refrigerator.



3. Determination of soil physical and chemical properties

The strain used in this experiment was taken from an electroplating factory in Yaoguan Town, Wujin District, Changzhou City. Each small bag of soil was excavated from five different locations nearby, and the five soils were mixed to select suitable strains. To this end, it is necessary to determine the physical and chemical properties of the soil, mainly to determine the pH of the soil and the heavy metal content in the soil.

- a). Take 5 parts of each of the 5 soil samples, mix until uniform, and dry in an oven for 24 hours;
- b). Drying the soil sample into a powder and storing it in a plastic bag for testing;
- c). Weigh 0.5g of soil sample into plastic steel bottle for microwave digestion;
- d). Add 6mL concentrated nitric acid, 3mL concentrated hydrochloric acid and 1mL hydrogen peroxide to the plastic cylinder, and cover the bottle cap;
- e). The digestion tank is placed in an oven and reacted at 60 ° C for 1 day;
- f). Open the plastic steel bottle, wash the digestion product in the plastic cylinder with nitric acid repeatedly into a 50mL test tube, and dilute to 50mL with distilled water;
- g). Preparing standard solutions of Ni, Cr, Cu, Zn, Cd and Pb;
- h). The soil stock solution is diluted to 10^{-1} , 10^{-2} and 10^{-3} times in a small test tube to prevent the measured value from being too large, resulting in an error;
- i). The content of heavy metals in the stock solution after digestion is measured by a flame spectrophotometer. If it is greater than the standard value, the diluted soil digestion solution is replaced for determination.

4. Drawing of urea concentration and OD430 standard curve

In order to determine the effect of different pH and temperature on the rate of urea decomposition, a standard curve of urea concentration is needed to reflect changes in urease activity.

- a). Prepare 5 bottles of urea solution at concentrations of 0.5g/L, 1 g/L, 2 g/L, 2.5 g/L and 4 g/L, respectively;
- b). Formulating the developer dimethylaminobenzaldehyde: adding 4 g of p-dimethylaminobenzaldehyde, 4 mL of 98% HCl and 96 mL of absolute ethanol, mixing well and storing in a dark bottle;
- c). 5 mL of each of the 5 concentrations of urea solution was placed in a 10 mL small test tube, 0.5 mL of the color developer was added, and after 10 minutes, the concentration was measured in an ultraviolet spectrophotometer having a wavelength of 430;
- d). The resulting data is processed and plotted as a chart.

5. Physical and chemical properties of soil

The pH of the soil was determined to be 5.27. The highest content of heavy metals in the soil was Cr and Zn, which reached 3.989 and 3.959 mg/L, indicating that the strains also had good resistance to Cr and Zn. Next is Pb, the concentration reached 2.239mg / L, and the content in the soil is also higher. This was followed by Ni, Cd and Cu at concentrations of 1.045, 0.714 and 1.267 mg/L, respectively. It can be seen that the heavy metal content in the soil of the electroplating plant is relatively high, which ensures the tolerance of bacteria and good vitality. The strains screened out were screened at different concentrations of Ni and found to grow at concentrations of 50, 100, 200, 300 and 400 mg/L. The strain N2 was grown at 50, 100 and 200 mg/L. The best is that the growth slows down at 300 mg/L, and it grows after 2 days at 400 mg/L. Therefore, this experiment used the best growing strain N1 at 100mg/L for subsequent experiments.

6. Plate coating and strain preservation of strains

The cultured bacterial liquids N1 and N2 were diluted, diluted to 10^{-6} , and plated, and some of the obtained strains are shown in Fig. 1 (N2 on the left and N1 on the right). The selected strains were streaked and placed in a refrigerator and stored at 4 °C.



Fig.1 The spread-plate of N

7. Growth tree of strain

After the sequence was tested, the obtained sequence was compared with the database to obtain a similar strain. The N and MEGA were compared with N to remove the head and tail parts, and the growth tree was drawn as shown in Fig. 5.

The strains have their own genus, and the different bacterium attributes and sequences are completely different, and the difference is very large. After sequencing, the BLAST of NCBI network can be used to find the registered strains with similar sequences, and then download similar strains. After being placed in MEGA versus DNA, the similarity of the strains can be further analyzed in detail to make a growth tree of the strain. It can be seen from Fig. 2 that strain N and *Klebsiella* have certain similarities, and the similarity rate of the most similar strain reaches nearly 100%, so it can be considered that strain N is *P. pastoris*. *Kosakonia radicincitans* strain 189 and *Klebsiella pneumoniae* strain lsy1 belong to the same genus.

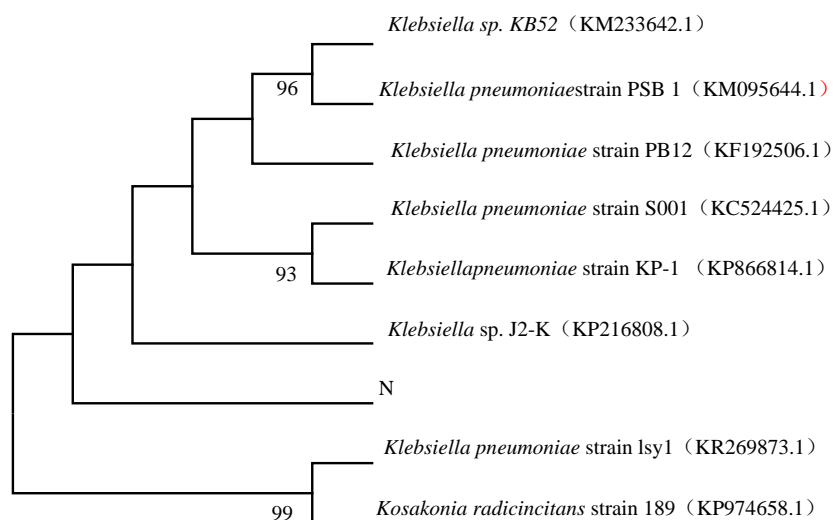


Fig.2 The growth tree of the strain N

8. Standard curve of urea concentration and OD430

The absorbance of 5 bottles of urea standard solution was measured and plotted as a standard curve as shown in Fig. 3. The standard curve of urea concentration and absorbance is the basis of the subsequent urease activity analysis. The magnitude of the error affects the determination of the effect of subsequent single factors on the urease activity of the strain. Too high will lead to the prediction of urease activity too low, while too low will lead to low Urease activity is predicted to be artificially

high, so the degree of precision must be controlled to at least 0.99. It can be seen from Fig. 3 that the error value of the urea concentration standard curve measured by this method is very low, reaching 0.9989, which provides data accuracy and method guarantee for the subsequent pH and temperature measurement.

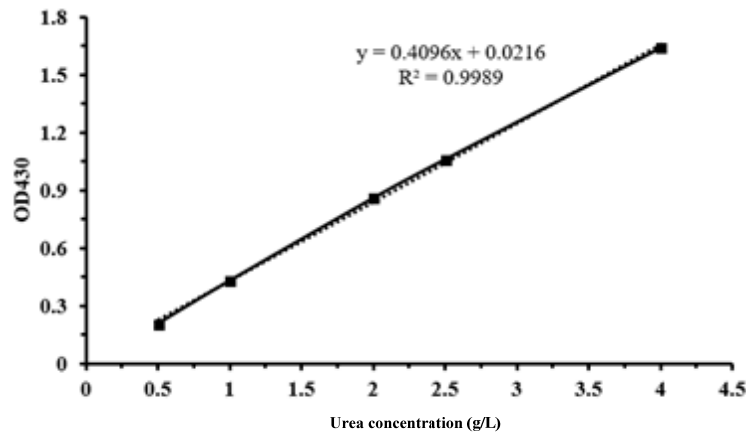


Fig.3 Standard curve of urea concentration and OD430 value

9. XRD analysis of mineralized products

The mineralized product was ground and subjected to X-ray powder diffraction, and the results are shown in Fig. 4.

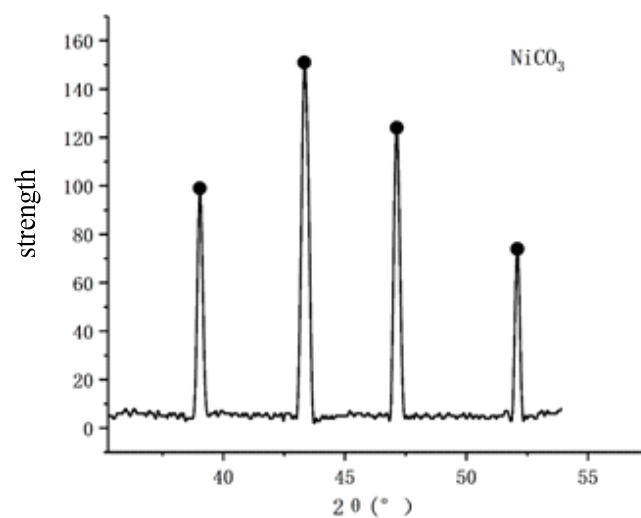


Fig.4 XRD patterns of mineralization products

The mineralized product is subjected to bottle pouring, centrifugation, washing, centrifugation and grinding to bagging, in which unpredictable contaminants are doped, which leads to impure product, so it must be carried out before testing. Washing can improve the accuracy of the results. XRD is used here. XRD is the abbreviation of X-ray diffraction. The use of X-rays, neutrons or electrons to analyze the microstructure and composition of powder samples is a scientific technique for analyzing the composition of materials. It can be seen from Fig. 4 that the four peaks of NiCO_3 in the XRD pattern of the mineralized product are very obvious. After analysis in software, it is found that the FDM value

of NiCO_3 in the mineralized product is 0.8 (the smaller the content is, the higher the content is), accounting for 1%. It has reached 98%, and the peak shape is sharp, and the half width is small, indicating that the crystallization is better. Therefore, the main product in the mineralization product is NiCO_3 , and the mineralization effect is very remarkable, which further proves that the mineralization effect of the strain N is good.

10. FT-IR analysis of mineralized products

The mineralized product was ground into a powder and sent to Fourier transform infrared analysis, and the results are shown in Fig. 5. Fourier infrared spectroscopy can help analyze the functional group components of a substance and is the most commonly used analytical tool for chemical analysis. The main functional group of NiCO_3 is carbonate, the main bond is C-O bond, and the absorption frequency in the infrared region is 1850~1350. With this, it can be judged whether or not carbonate is produced in the FT-IR. As can be seen from Figure 5, there are three strong absorption peaks, the first is located at 1060.77 cm^{-1} , which is the characteristic absorption peak of primary alcohol, probably due to the organic acid residue produced by strain N; the second is in At 1631.12 cm^{-1} , the standard infrared spectrum of $\text{CO}_3^{2-}(\text{CO})$ has a stretching vibration of 1850~1600 cm^{-1} . The strong absorption at this stage is very characteristic, indicating that a large amount of CO_3^{2-} is produced in the mineralized product; The third is at 3435.53 cm^{-1} , which is a strong absorption peak of -OH, and the sample may absorb moisture to cause a water peak. In general, the results of Fourier transform infrared analysis show that the carbonate is more stable than the ammonia ion, and can act as a site and a trace metal to adsorb on the surface of the strain to form a mineral precipitate.

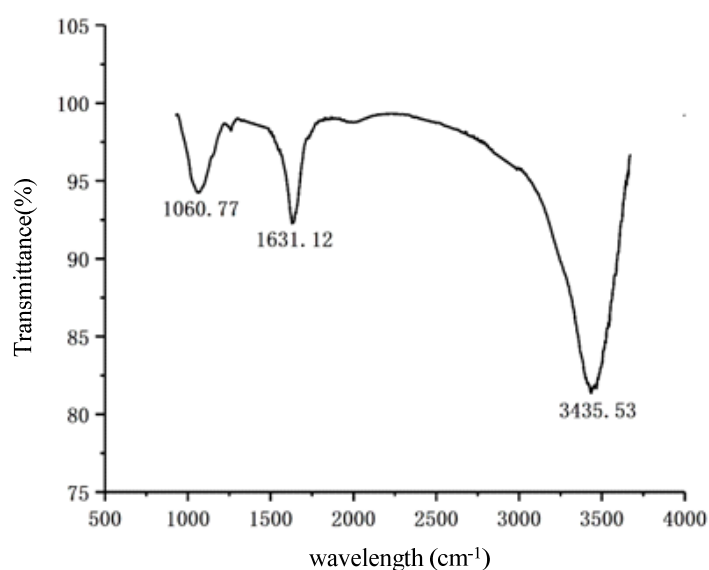


Fig.5 FT-IR patterns of mineralization products

11. Summary

In this experiment, strains N screened by electroplating plants were used for Gram staining, growth factors, growth curves, urease activities, urease activity factors and mineralization analysis experiments. In addition, soil physical and chemical properties experiments were carried out. The experimental results show that the heavy metal Ni in the microbial mineralization consolidation soil is a very feasible method, but it is necessary to work hard on the source and stability of the strain and the scale of the application, to get closer to the application in the field. Higher application value contributes to the removal of soil heavy metal Ni.

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