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A new strain for recovering precious metals from waste printed circuit boards

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ABSTRACT

A new strain, *Pseudomonas Chlororaphis* (*PC*), was found for dissolving gold, silver, and copper from the metallic particles of crushed waste printed circuit boards (PCBs). The optimized conditions that greatly improved the ability of producing CN^- (for dissolving metals) were obtained. Dissolving experiments of pure gold, silver, and copper showed that the metals could be changed into Au^+, Ag^+, and Cu^{2+}. *PC* cells and their secreta would adsorb metallic ions. Meanwhile, metallic ions destroyed the growth of *PC*. Dissolving experiments of metallic particles from crushed waste PCBs were performed by *PC*. The results indicated that 8.2% of the gold, 12.1% silver, and 52.3% copper were dissolved into solution. This paper contributed significance information to recovering precious metals from waste PCBs by bioleaching.

1. Introduction

China has been one of the largest e-waste dumping grounds in the world. About 2.5 million tons of e-waste (both self-generated and imported from developed countries) appeared in Chinese mainland (Ongondo et al., 2011) every year. Among which, waste printed circuit boards (PCBs), the core component of electronics products, were also produced. Waste PCB contains nearly 28% metals and the purities of metals are more than 10 times higher than that of rich-content minerals (Li et al., 2007.). Therefore, recovering waste PCBs has now become increasingly important, which is mostly done by small backyards workshop using crude technologies (such as acid-washing or open incineration). However, serious pollution has been generated in the recovering process such as exposure of polybrominated diphenyl ethers, polychlorinated dibenzo-*p*dioxins and dibenzofurans (Duan et al., 2011), and heavy metals (Leung and Wong, 2008; Ilgin and Gupta, 2010). Therefore, environment-friendly technology is a pressing demand for treating waste PCBs.

Physical methods are preferred for recovering waste PCBs (Ruan and Xu, 2012a,2012b; Ruan et al., 2013; Veit and Bernardes, 2006). However, physical treatments are insufficient in recovering precious metals. Abundant precious metals (such as gold and silver) were contained in waste PCBs (Li et al., 2012). Small proportion and inertial characteristics of precious metals cause difficulties in separation by physical methods (Li and Xu, 2010). Precious metals were lost in physical recovering process.

Therefore, developing new technology for recovering precious metals is urgently demanded for improving added value of waste PCBs recovery.

Bioleaching is widely used in mineral processing (Watling, 2006). It has the advantages of environment-friendly and low-cost. However, its application for recovering waste PCBs is still in its infant. Currently, Xiang et al. (2010) reported the application of bioleaching for recovering copper from waste PCBs. *Chromobacterium Violaceum* (*CV*) was the most used microbe in the bioleaching process for recovering precious metals from waste PCBs (Pant et al., 2012). Precious metals were dissolved into solution by CN^- emitted from *CV*. Chemical equation of the dissolution process could be presented as:

$$4Au + 8CN^- + O_2 + 2H_2O = 4Au(CN)_2^- + 4OH^- \quad (1)$$

Although *CV* has great ability to produce CN^-, it is not convenient to be employed in industrial application. *CV* mainly appears in tropical and subtropical regions. The special demand on living condition restrains the scope of its industrial application in recovering precious metals from waste PCBs. Therefore, it will be of great significance to search suitable strain for the industrial bioleaching process of recovering precious metals from waste PCBs.

Pseudomonas strains also have the ability to produce CN^- though the ability is not as strong as *CV*. However, *pseudomonas* strains have an important advantage of being employed in the industrial application for recovering precious metals from waste

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PCBs. They are easy to be found in rhizosphere microbial communities, and have strong ability of acclimation.

In this study, a new strain of *pseudomonas* was found, which could produce CN^- to recover precious metals from waste PCBs. Its category was distinguished by the method of DNA phylogenetic tree. Factors influencing the new strain for producing CN^- (PH, temperature, additive, rotation) were investigated. Then, bioleaching process of copper, silver, and gold from crushed waste PCBs by the new strain was observed. This strain can be employed for recovering precious metals from waste PCBs. The goal of this paper is to search a suitable strain for the industrial bioleaching process of recovering precious metals from waste PCBs and will contribute a lot to the development of new technology for recovering precious metals and a value-added e-waste recovery.

2. Materials and methods

2.1. Screening of pseudomonas

Soil for screening *pseudomonas* was obtained from reed root at mining region. Then, 0.2 ml soil extract (1 g soil dissolved in 10 ml sterile water) was inoculated to NB medium (comprised of beef extract, peptone, NaCl, and agar), which contained sterile penicillin, novobiocin, and cycloheximide. After 72 h of cultivation, various strains of Pseudomonas were selected and purified.

2.2. Monitoring of CN^--producing ability of pseudomonas

Color reaction was adopted to identify which strains could produce CN^-. The strain which produced CN^- would turn the yellow test paper (dipped in the solution of 0.5% picric acid and 2% sodium carbonate) to red. Turning time and color grade showed the CN^--producing ability.

For investigating the ability of producing CN^-, 1 ml *Pseudomonas* solution was cultured for 6 h, 12 h, 24 h, 36 h, 72 h, 120 h, and 196 h in nutrient solution (pH = 7; 25 °C) respectively. Concentrations of CN^- produced by the strain in nutrient solutions were measured by titration of silver nitrate (National standard of China, 2009). $[Ag(CN)_2]^-$ will be generated when CN^- meets $AgNO_3$. The excess Ag^+ will gather indicator and turn the solution from yellow to orange red. The concentrations of CN^- can be computed by:

$$\rho = \frac{c(V_a - V_0) \times 52.04 \times V_1 \times 1000}{V} \quad (2)$$

where c is the concentrations of silver nitrate, mol/L; V_a, the volume of silver nitrate solution, ml; V_0, the volume of silver nitrate solution for blank control, ml; V, the total volume of sample, ml; and V_1, the volume of the tested sample, ml.

2.3. 16S rDNA sequence determination of the pseudomonas

DNAs of *Pseudomonas* strains were extracted by the method of freeze–thaw. Then, DNAs were amplified under the primers of 27F(5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R(5′-GGTTACCTTGTTACGACTT-3′), and the sequences of DNAs were detected. According to the gene sequences, homology analyses of the strains were performed by comparing to GenBank data via software of DNAMAN (McMahan et al., 2012). Then, phylogenetic tree of the strains were constructed by MEGA 5.

2.4. Investigation of precious-metal-leaching ability

Leaching ability of the strains to precious metals can be indicated by the concentrations of dissolved metallic ions in nutrient solution. Metallic particles were fed into 750 ml nutrient solution (in 1 L culture bottle). Then, 3 ml strain was inoculated into nutrient solution. After cultivation, concentrations of metals in the supernatant of nutrient solution were monitored. Concentration of Au^+ in nutrient solution was detected by the method of Inductively Coupled Plasma Optical Emission Spectrometer (ICP–OES). Concentrations of Ag^+ and Cu^{2+} were monitored by the method of Atomic Absorption Spectrometry (AAS).

2.5. Percentage analysis of Au, Ag, and Cu in the mixed metals from crushed waste PCBs and the bioleaching process

Mixed metallic particles were obtained from corona-electrostatic separation of crushed waste PCBs. The process of gaining the mixed metallic particles was given in Fig. 1. Waste PCBs of mobile phone were crushed into mixed particles (size 0.6 mm) of metals and nonmetals. Then the mixed particles were sorted into metallic particles and nonmetallic particles by corona-electrostatic separation. Separation rate of corona-electrostatic separation was greater than 95%. The mixed metallic particles mainly contained copper and zinc as well as some precious metals of gold and silver. The nonmetallic particles mainly contained epoxy and fiberglass. 4 g of mixed metallic particles (contained little nonmetallic particles) were dissolved by aqua regia and diluted to 100 ml by distilled water in a 100 ml volumetric flask. Then the concentration of Au^+ in the volumetric flask was detected by the method of ICP–OES and the concentrations of Ag^+ and Cu^{2+} were monitored by the methods of AAS.

Mass percentage of Au, Ag, and Cu in the mixed metallic particles could be calculated by Eq. (3):

$$\eta = \frac{c \times 0.1}{4} \times 100\% \quad (3)$$

where η is the mass percentage of metal in the mixed metallic particles, c is the concentration of metal in volumetric flask (g/L).

Bioleaching experiments of mixed metallic particles were performed in lab. 4 g mixed metallic particles collected from crushed waste PCBs were fed into 250 ml of nutrient solution and the strain was inoculated into the nutrient solution. Under the optimized conditions of producing CN^-, ten times of bioleaching were performed to dissolve the 4 g of mixed metallic particles and each time lasted 72 h in 250 ml of nutrient solution. Then, the concentrations of Au^+, Ag^+, and Cu^{2+} in the supernatant fluid of nutrient solution were detected and the mass of dissolved Au^+, Ag^+, and Cu^{2+} could be computed by multiplying metal concentration to the volume of nutrient solution.

3. Results and discussion

3.1. Screening of pseudomonas strains and the phylogenetic tree

According to color reaction, eleven strains (marked as $1^{\#}$, $2^{\#}$, $3^{\#}$, $4^{\#}$, $5^{\#}$, $6^{\#}$, $7^{\#}$, $8^{\#}$, $9^{\#}$, $10^{\#}$, and $11^{\#}$ respectively), which were found to have the ability of producing CN^-, were selected from the soil extract and presented as Fig. 2. The Figure showed that strain $6^{\#}$ had the greatest ability to produce CN^-.

After DNA extraction, PCR amplification, and sequences determination, phylogenetic tree of the strains were constructed by comparing the gene sequence to GenBank and presented as shown in Fig. S1. According to phylogenetic tree, the genetic relationship of the eleven strains was determined as shown in Table 1.

The threshold of homology judgment of strains by 16SrDNA sequences is 98%. That is, if the similarity is less than they are not the same species. Thus, $1^{\#}$, $4^{\#}$, and $6^{\#}$ strains belonged to *Pseudomonas Mosselii*, *Pseudomonas Aeruginosa*, and *Pseudomonas Chlororaphis* respectively (sequences of strain $6^{\#}$ were placed in supporting

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