American Journal of Environmental Science and Engineering
2018; 2(4): 72-78
http://www.sciencepublishinggroup.com/j/ajese
doi: 10.11648/j.ajese.20180204.14
ISSN: 2578-7985 (Print); ISSN: 2578-7993 (Online)

Preliminary Bioleaching of Heavy Metals from Contaminated Soil Applying *Aspergillus niger* F2

Deng Xinhui[1], Chen Runhua[2, *], Shi Yan[3, *], Zhuo Shengnan[3, *]

[1]College of Life Science and Chemistry, Hunan University of Technology, Zhuzhou, China

[2]College of Environmental Science and Engineering, Central South University of Forestry and Technology, Changsha, China

[3]School of Metallurgy and Environment, Central South University, Changsha, China

Email address:

xhdeng2007@126.com (Deng Xinhui), chen12@csu.edu.cn (Chen Runhua), 851610184@qq.com (Zhuo Shengnan)

[*]Corresponding author

To cite this article:

Deng Xinhui, Chen Runhua, Shi Yan, Zhuo Shengnan. Preliminary Bioleaching of Heavy Metals from Contaminated Soil Applying *Aspergillus niger* F2. *American Journal of Environmental Science and Engineering*. Vol. 2, No. 4, 2018, pp. 72-78.
doi: 10.11648/j.ajese.20180204.14

Received: November 23, 2018; **Accepted**: December 8, 2018; **Published**: January 22, 2019

Abstract: A new strategy of heavy metal biobleaching was proposed based a fungal strain identified as *Aspergillus niger* and named F2. F2 displayed great ability of heavy metal resistance and organic acid production. The temperature, pH, carbon source, and nitrogen source have great influences on the heavy metal bioleaching from contaminated soil by F2. The optimum temperature and pH for biobleaching were 30°C and 5.0, respectively. The total heavy metal bioleached by F2 with sucrose, glucose, maltose, lactose and starch as carbon source were 69.86%, 66.57%, 64.59%, 0.92%, and 69.01%, respectively, while the total heavy metal bioleached by F2 with $NaNO_3$, NH_4NO_3, peptone, and yeast extract as nitrogen source were 64.10%, 64.05%, 65.87% and 66.27% individually. Our finding provided a new perspective for the treatment of heavy metal contaminated soil.

Keywords: *Aspergillus niger*, Bioleaching, Soil, Organic Acid, Copper, Lead, Zinc, Cadmium

1. Introduction

To achieve green and sustainable development of the globe strongly demand an economically feasible and eco-friendly sustainable process for heavy metal contaminated soil remediation. A variety of technologies for the remediation of heavy metal contamination soil have arisen, such as immobilization/fixation, oxidation/reduction, and flushing/leaching [1-3]. In the processing, chemical reagents such as surfactant, phosphate, H_2O_2, H_2SO_4, and limestone are used to immobilize heavy metal in soil [4-6]. Plants and microorganisms are used to extract heavy metal from soil. All the technologies of remediation on heavy metal contaminated soil come down to two principles: One principle is to decrease the toxicity but not remove heavy metal from the contaminated soil [7-8], the other principle is to directly extract heavy metal from the contaminated soil. There are advantages and shortcomings for each technology. The former has potential hazards of secondary pollution, the later has the problems of long extraction time and low extraction quantity. To address these challenges, it is necessary to develop a new technology for remedying the contaminated soil in an environment friendly and time saving manner.

There are many plants and microorganisms used to extract heavy metal from the polluted soil. Phytoextraction was developed early in America in 1998, Oat, Barley, and Indian mustard were used to extract zinc from pollution soil [9]. Alyssum species was used to extract nickel and cobalt from nickel-contaminated soil in England [10]. The antimony and arsenic contaminated soil were treated with vetiveria zizanioides by Yang [11]. *Acidithiobacillus thiooxidans* and *Brettanomyces* B65 were used to co-bioleaching heavy metal from tannery sludge [12]. *Hymeniacidon heliophile* and *Bacillus sp*. isolated from the sponge cells were used to leach electronic waste [13]. *Acidithiobacillus thiooxidans*, *Alicyclobacillus sp*., *Acidithiobacillus ferrooxidans*, *Sulfobacillus sp*. *Penicillium chrysogenum*, and *Penicillium simplicissimum* are all used in the technology of bioleaching [14]. The period of microbiological repair techniques is much shorter than phytoextration, therefore, many researchers focus on them.

The smelting activity has a serious impact on the environment. Especially, the discharge of smelting waste water caused severe contamination of soil with heavy metals (such as Cu, Zn, Cd, and Pb) [15-16]. Obviously, the technology of immobilization/fixation and oxidation/reduction are unsuitable for the compound heavy metal pollution remediation. The micro-bioleaching technology is environmentally friendly, fast, and cheap, meanwhile, the microbiology used for heavy metal extraction must have the ability of heavy metals resistance and organic acid production. In this paper, a new fungal strain was isolated and identified. The potential of this strain for heavy metal contaminated soil remediation was investigated.

2. Material and Method

2.1. Sample Collection and Chemical Analysis

The contaminated soil used in this study was collected from the top soil (0-20cm) of the sites around (1000 m) a Smelting Plant located in City Zhuzhou, Hunan Province, Central South of China. The collected soil was air dried, crushed and sieved through a 2-mm sieve to remove sundries. Then the clean sample was mechanically mixed to ensure homogeneity and stored in a plastic container for subsequent experiments. The prepared samples were sent to Wuhan Chujiang Environmental Protection Technology Co. LTD to identify the heavy metal's (copper (Cu), lead (Pb), zinc (Zn), and cadmiun (Cd)) content. The pH of the soil was determined via a pH-meter (pHS-3B) according to the method of potentiometric determination of solution pH value. The ratio of soil and water was 1:2.5. All experiments were conducted in triplicate. The soil's physico-chemical characteristics are presented in Table 1. Zn, Cd, Pb and Cu were confirmed in the contaminated soil. It worth to not that the contents of Zn and Cd are great higher than the national standard and pose a significant threat to human health and the environment.

Table 1. *Heavy metal content and pH value of 0-20cm soil layer around smelting plant.*

Item	Pb (mg/kg^{-1})	Zn (mg/kg^{-1})	Cd (mg/kg^{-1})	Cu (mg/kg^{-1})	pH
Value	486	6.75*103	319	486	7.09

2.2. Fungal Isolating, Purification and Acclimation

For isolating some indigenous strains to treat the contaminated soil, 0.1g of contaminated soil was added to 9.9 mL of sterile deionized-water, and shake well for several times, then remained static for a while. The supernatant was withdrawn and gradually diluted (1mL supernatant was added to double-deionized water) into a series of concentrations. 0.1mL of 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} diluents were spread on the agar plates contained Pb 1500 mg/L^{-1}, Cd 80 mg/L^{-1}, Cu 2000 mg/L^{-1} and Zn 2000 mg/L^{-1}, respectively. The agar plate was prepared by adding 30g of sucrose, 1g of K_2HPO_4, 3g of $NaNO_3$, 0.5g of $MgSO_4$, 0.5g of KCl, 0.01g of $FeSO_4$, and 20g of agar in 1L of deionized water. The spread plates were incubated in Electro-thermal incubator at 30°C for 7 days. The strains presented on the plates were isolated and purified, and then inoculated into 50 ml of liquid medium, which containing Glucose (90g/L^{-1}), KH_2PO_4 (0.05g/L^{-1}), $NaNO_3$ (3g/L^{-1}), $MgSO_4$ (0.5g/L^{-1}), KCl (0.5g/L^{-1}), and $FeSO_4$ (0.01g/L^{-1}). The pH in the medium was adjusted to 6.7 using 0.1mol/L^{-1} HCl. From the third day, the pH value of the liquid medium repreasented the organic acids production by F2 was monitored [14].

2.3. Identification of the Strain

A fungal strain with high ability of heavy metal resistance and organic acid production was sent to AXY Biotechnology Co Ltd to perform the identification the strain by 18SrDNA and ITS sequencing. The gene primers were:

ZJ-NS1: 5'- GTAGTCATATGCTTGTCTC -3'
ZJ-NS8: 5'- TCCGCAGGTTCACCTACGGA -3'
ZJ-ITS4: 5'- TCCTCCGCTTATTGATATGC -3'
ZJ-ITS5: 5'- GGAAGTAAAAGTCGTAACAAGG -3'

The sequences were initially analyzed at NCBI server (http://www.ncbi.nlm.nih.gov/) using BLAST tools. A phylogenetic tree was constructed using the neighbor-joining method with MEGA 7 software, and the morphology was observed with optical microscope (LJ-CLP03).

2.4. Heavy Metal Resistance and Organic Acid Production of F2

The spores were inoculated on the agar plates containing heavy metal using inoculating stick. The contents of heavy metal in agar plate are as follows: Cu were 1800 mg/L^{-1}, 2000 mg/L^{-1}, and 2500 mg/L^{-1}; Zn were 4000 mg/L^{-1}, 5000 mg/L^{-1}, 6000 mg/L^{-1}, and 7000 mg/L^{-1}; 6000 mg/L^{-1}, 7000 mg/L^{-1}, and 8000 mg/L^{-1} for Pb; 1800 mg/L^{-1}, 2000 mg/L^{-1}, 2200 mg/L^{-1}, and 2400 mg/L^{-1} for Cd. After inoculation, the growth of the colonies was determined and recorded. On the other hand, the spores were inoculated in the above liquid medium with sucrose as sole carbon source and $NaNO_3$ as nitrogen source. pH was determined using a pH-meter (pHS-3B) every day.

2.5. Study on Different Factors Affect Heavy Metal Bioleaching by F2

The addition of different carbon sources and nitrogen sources were optimized to reach the maximum heavy metal bioleaching efficiency. Glucose, sucrose, maltose, lactose and soluble starch were used as carbon source, while $NaNO_3$, NH_4NO_3, peptone, and yeast extract were used as nitrogen sources. The bioleaching medium was composed of 90 g L^{-1} of carbon source and 3 g of nitrogen source. Other components of medium are as follows: KH_2PO_4 (0.05g/L^{-1}), $MgSO_4$ (0.5g/L^{-1}), KCl (0.5g/L^{-1}), and $FeSO_4$ (0.01g/L^{-1}) [17]. When different carbon sources were studied, $NaNO_3$ was used as nitrogen source, while glucose was used as carbon source

when different nitrogen sources were investigated. 49 mL of liquid medium was put in a 250 mL of autoclaved conical flasks. 1 mL of spore suspension containing about 10^7 spores was aseptically added to each conical flask, and then put in a rotary shaker with the speed of 120 rpm at 30°C for 7 days. Then 2.5g of sterilized soil was added to each flask and continued to incubate for another 7 days. Moreover, bioleaching temperature and pH were also studied. Three temperature levels including 25°C, 30°C, and 35°C and three pH levels including 5.0, 7.0, and 9.0 were selected to investigate the effect on the heavy metal bioleaching efficiency. The sterile deionized water was added every other day to keep the weight balance of each conical flask. The number of spores was counted using a haemocytometer and standardized to approximately 7.5×10^8 spores per milliliter. The bioleaching amount of different heavy metals with different carbon/nitrogen source after 14 days were compared by determining the concentration of Pb, Zn, Cd, and Cu in the filtrates. Sterile experimental conditions were achieved by autoclave. Each flask contained liquid medium was autoclaved at 115°C for 30min prior to inoculation. The contaminated soil was autoclaved separately. Each sample was conducted in triplicate. The bioleaching percentage was calculated according to the following formula: bioleaching percentage (%) = heavy metal in liquid medium / heavy metal in soil (2.5g) *100%.

3. Result and Discussion

3.1. Isolation, Acclimation and Identification of Aspergillus Niger

The strain with the ability of heavy metal resistance and organic acid production was selected for identification based on the analysis of the 18S rDNA gene sequence and ITS gene sequence. The 18S rDNA sequence has 1671 base pairs (bp), while ITS4-5 sequence has 625 bp. The blast result showed that the gene sequence of both 18S rDNA and ITS4-5 has 99% similarity with those of *Aspergillus niger*. The morphology of the strain was shown in Figure 1, the phialides arise circumferentially and that black conidia obscure the vesicle, which is the typical characteristics of *Aspergillus niger*. A phylogenetic tree was then constructed using the neighbor-joining method with MEGA 7 software (Figure 2). The phylogenetic tree obtained from the comparisons of ITS4-5 sequences showed the stain tightly clustered with *A. niger*. Thus, according to the gene sequence, the morphology, and phylogenetic tree, the strain was identified as *Aspergillus niger*, and named F2. *Aspergillus niger* F2 was sent to be preserved in institute of microbiology in Guangdong, China, the preservation number is GDMCC No. 60213.

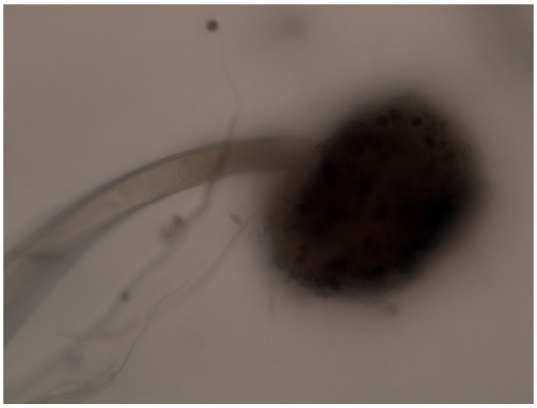


Figure 1. *Mycelium and spore morphology of Aspergillus niger F2.*

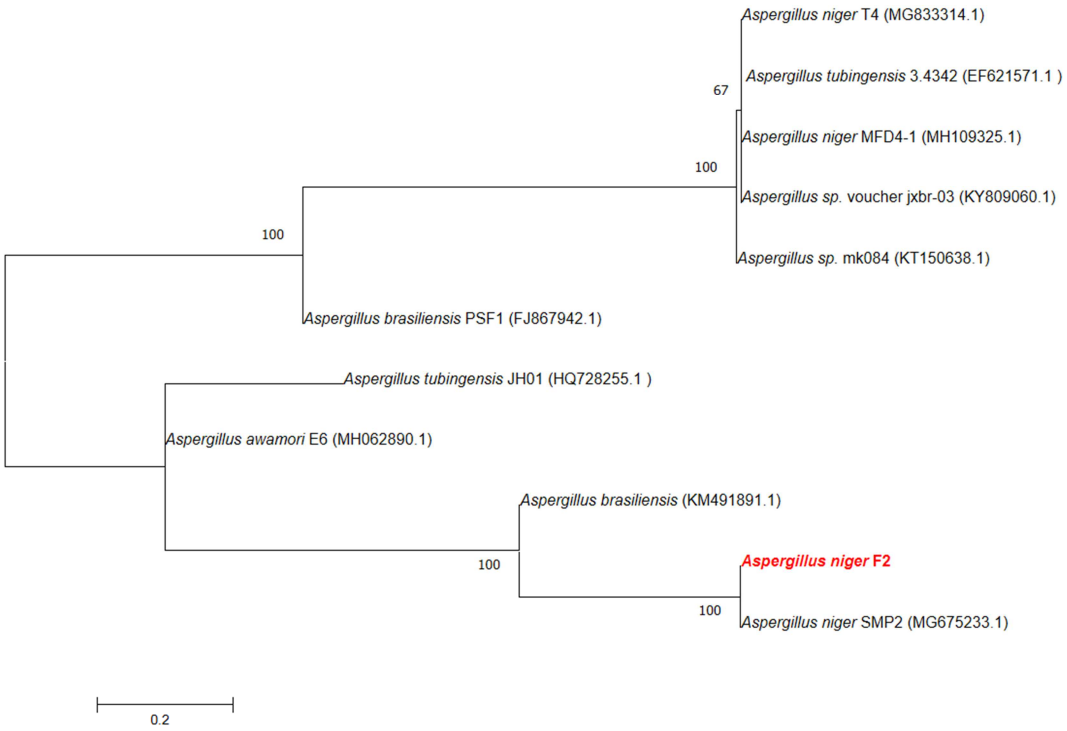


Figure 2. *Molecular Phylogenetic analysis by Maximum Likelihood method.*

3.2. The Resistance of F2 to Heavy Metals

The growth of F2 under Cu stress is shown in Figure 3a. The F2 colony with the diameter of 0.9 cm was inoculated on the plate contained Cu with the concentration of 1800 mg/L^{-1}. Then, the colony diameter was 2.07cm after 7 days culture. In other words, the colony diameter of F2 was increased of 1.17 cm in seven days. Under 2000 mg/L^{-1} of Cu stress, the colony diameter was 1.20 cm after 7 days culture and only the 0.30 cm of colony diameter was increased. Under 2500 mg/L^{-1} of Cu stress, the colony diameter was only 1.07 cm and 0.17 cm of the colony diameter was increased. The results showed that F2 grew well on 2000 mg/L^{-1} Cu stress, and the maximum copper concentration for strain F2 to tolerate was 2000 mg/L^{-1}.

The growth of F2 under Zn stress is shown in Figure 3b. On the concentration of 4000 mg/L^{-1}, the colony diameter was increased from 0.9 cm to 1.03 cm after one day growth. The colony diameter was increased to1.10 cm under the concentration of 4000 mg/L^{-1} stress, while it was increased to 0.93 cm under 5000 mg/L^{-1} stress after two days growth. Moreover, F2 grew very slow and the colony diameter was only increased of 0.93cm under both 6000 mg/L^{-1} and 7000 mg/L^{-1} Zn stress. After six days growth, the colony diameter of strain F2 was increased to 1.90cm, 1.60 cm, 1.10 cm, and 1.00 cm under 4000 mg/L^{-1}, 5000 mg/L^{-1}, 6000 mg/L^{-1}, and 7000 mg/L^{-1}, respectively. The result showed that the growth of F2 was influenced by Zn. When the concentration of Zn exceeded 5000 mg/L^{-1}, F2 grew slow or even stopped growing.

F2 grew under Pb stress is shown in Figure 3c. under the concentration of 7000 mg/L^{-1} stress, F2 grew slow at the beginning and then rapid. After two days growth, the colony diameter increased from 0.9 cm to 1.07 cm, then increased to 1.77 cm after three days growth and to 4.90 cm after seven days growth. Under the concentration of 8000 mg/L^{-1} stress, the growth rhythm was similar to that under 7000 mg/L^{-1} stress, but the growth rate under 8000 mg/L^{-1} stress was slower than that under 7000 mg/L^{-1} stress. Under 8500 mg/L^{-1} stress, strain F2 began to grow on fifth day and the colony diameter increased to 1.40 cm after seven days growth. Under 9000 mg/L^{-1} stress, no growth was observed even after 14 days culture. There was little influence on the growth of F2 under 7000 mg/L^{-1} stress and large influence under 9000 mg/L^{-1} stress.

The growth of F2 under Cd stress is shown in Figure 3d. F2 grow well under 1800 mg/L^{-1} Cd stress. When Cd concentration was 2000 mg/L^{-1}, F2 began to grow after three days culture, and colony diameter increased from 0.90 cm to 1.90 cm after six days culture. Under 2200 mg/L^{-1} Cd stress, F2 grow slow during the first three days, and colony diameter increased from 0.90 cm to 1.30 cm. Under 2400 mg/L^{-1} Cd stress, there was no growth for F2. Therefore, the upper limit concentration of Cd for F2 to tolerate was 2200 mg/L^{-1}.

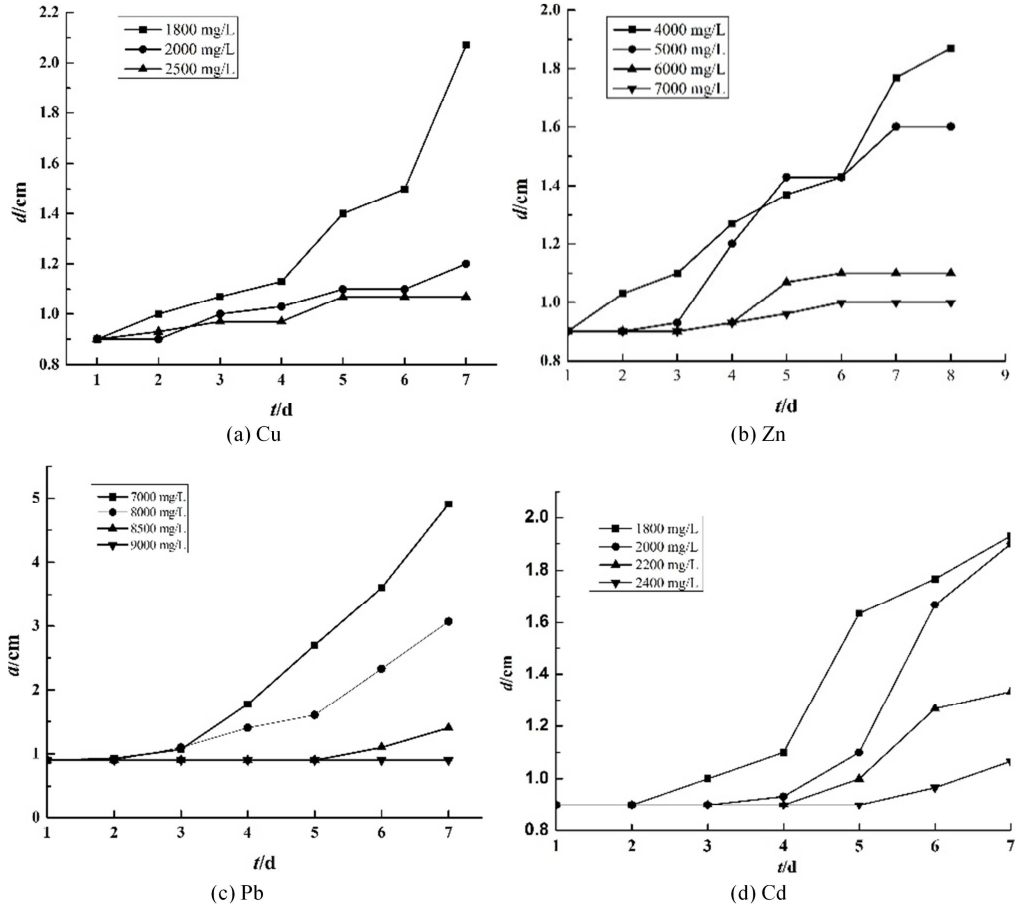
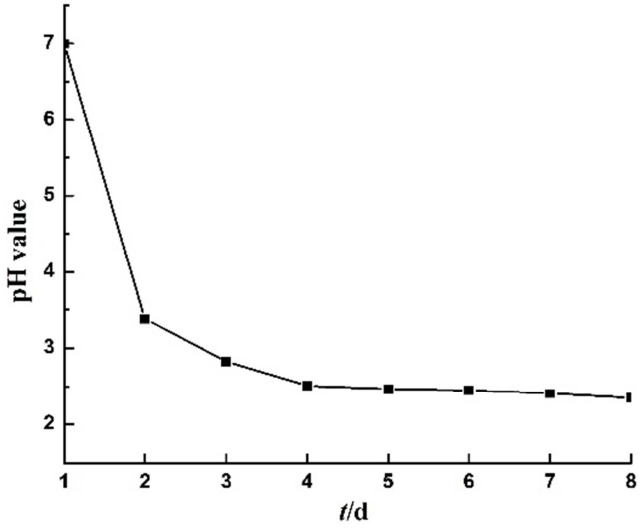


Figure 3. *The resistance strain of Cu, Zn, Pb and Cd.*

F2 shows different resistance to different heavy metals, and the order of the maximum heavy metal tolerance concentration is Zn > Pb > Cu > Cd. This order may be related to the toxicity of heavy metal itself [18-19]. Under low heavy metal concentration stress, F2 grows well. Under higher heavy metal concentration stress, F2 firstly adapts to the higher heavy metal environment for a while and then begains to grow. Under the highest heavy metal stress, strain F2 stops growing or grows very slow. Resistance to mixed heavy metals by strain F2 would be studied in the next.



(n=3)

Figure 4. *Medium pH value of the strain.*

3.3. Acid-producing Capacity of Strain F2

The pH of the liquid medium was monitored every day (Figure 4). The pH of the liquid medium rapidly dropped from 7.0 to 3.39 after one day culture, while the pH of the liquid medium dropped slowly to 2.4 in the following five days. The reduction of pH means the carbon source in liquid medium was metabolized and some organic acids were produced [17, 20]. The kinds of organic acids and the metabolism of F2 will be studied in the next study.

3.4. The Effect of Carbon Source on Heavy Metal Bioleaching by F2

When the sucrose was used as carbon source (Table 2), bioleaching percentages of Cd, Cu, Pb, and Zn are 35.89%, 48.19%, 100%, and 70.86%, respectively. The bioleaching percentage of Cd, Cu, Pb, and Zn are 27.38%, 46.09%, 100%, and 67.49% when glucose as carbon source. The bioleaching percentage of Cd, Cu, Pb, and Zn are 25.53%, 46.87%, 100%, and 65.16% when maltose as carbon source. The bioleaching percentage of Cd, Cu, Pb, and Zn are 0.443%, 0.705%, 3.81%, and 0.77% lactose as carbon source. The bioleaching percentage of Cd, Cu, Pb, and Zn are 32.11%, 46.46%, 100%, and 70.15% when the starch as carbon source. The total heavy metal bioleaching of sucrose, glucose, maltose, lactose, and starch were 69.86%, 66.57%, 64.59%, 0.92%, and 69.01%, respectively. For Cd, Cu and Zn, the bioleaching percentage is the highest when using sucrose as carbon source. For Pb, all the bioleaching percentages are 100% except for using lactose as carbon source. The heavy metal bioleaching percentage of lactose is the lowest than any other carbon sources, while the heavy metal bioleaching percentage of sucrose is the highest than any other carbon sources. The effect of carbon source on bioleaching was very significant due to organic acids produced by F2 based on the oxidation of carbon sources.

Table 2. *Heavy metal bioleaching efficiency of strain F2 applying different carbon source.*

Carbon source	Cd		Cu		Pb		Zn	
	Average (%)	Variance (%)	Average (%)	Variance (%)	Average (%)	Variance (%)	Average (%)	Variance (%)
sucrose	35.89	1.0	48.19	1.3	100	0.0	70.86	1.0
glucose	27.38	1.2	46.09	1.0	100	0.0	67.49	1.0
maltose	25.53	2.0	46.87	1.5	100	0.0	65.16	2.5
lactose	0.443	3.0	0.705	1.7	3.81	1.0	0.77	1.0
starch	32.11	1.5	46.46	1.9	100	1.5	70.15	1.0
control	2.13	0.12	1.14	0.32	1.21	0.21	3.14	0.11

3.5. The Effect of Nitrogen Source on Heavy Metal Bioleaching by F2

The effect of single nitrogen source on the heavy metals bioleaching was shown in Table 3. When the $NaNO_3$ was used as the nitrogen source, bioleaching percentages of Cd, Cu, Pb, and Zn were 36.61%, 48.23%, 100%, and 71.54%, respectively. When the NH_4NO_3 was used as the nitrogen source, bioleaching percentages of Cd, Cu, Pb, and Zn were 31.95%, 52.76%, 100%, and 71.47%, respectively. When the peptone was used as the nitrogen source, bioleaching percentages of Cd, Cu, Pb, and Zn were 35.98%, 53.81%, 100%, and 73.70%, respectively. When the yeast extract was used as nitrogen source, bioleaching percentages of Cd, Cu, Pb, and Zn were 38.47%, 51.84%, 100%, and 74.77%, respectively. The total heavy metal bioleaching percentages of $NaNO_3$, NH_4NO_3, peptone, and yeast extract were 64.10%, 64.05%, 65.87%, and 66.27%, respectively. The result showed that the total heavy metal bioleaching percentage of organic nitrogen sources is slightly higher than that of inorganic nitrogen source. When using yeast extract as the nitrogen source, the total heavy metal bioleaching percentage is a little highest than any other nitrogen. However, the effect of nitrogen source for F2 on bioleaching of heavy metals is not significant.

Table 3. *Heavy metal bioleaching efficiency of strain F2 applying different nitrogen source.*

Item	Cd		Cu		Pb		Zn	
	Average (%)	Variance (%)	Average (%)	Variance (%)	Average (%)	Variance (%)	Average (%)	Variance (%)
$NaNO_3$	36.61	1.02	48.23	2.02	100	0.00	71.54	1.86
NH_4NO_3	31.95	3.17	52.76	2.23	100	0.00	71.47	1.99
peptone	35.98	1.33	53.81	2.03	100	0.00	73.70	3.01
yeast extract	38.47	1.82	51.84	2.85	100	0.00	74.77	1.26
control	2.13	0.12	1.14	0.32	1.21	0.21	3.14	0.11

3.6. The Effect of Temperature on Heavy Metal Bioleaching by F2

The effects of temperature on the heavy metal bioleaching were shown in Figure 5. The bioleaching percentages of Cd, Cu, Pb, and Zn were 35.89%, 48.19%, 100%, and 70.86%, respectively, at 25°C. The bioleaching percentages of Cd, Cu, Pb, and Zn were 41.89%, 47.45%, 100%, and 79.56%, respectively, at 30°C. The bioleaching percentages of Cd, Cu, Pb, and Zn were 36.97%, 47.42%, 100%, and 74.60%, respectively, at 35°C. The total bioleaching percentage was 69.86% at 25°C, 77.41% at 30°C, and 73.03% at 35°C. The highest bioleaching efficiency was obtained at 30°C. This may be due to that 30°C is the optimal temperature for the metabolism of *Aspergillus niger* [21], more organic acids were produced under this conditions. The highest bioleaching percentage of Cu was obtained at 25°C. The highest bioleaching percentage of both Cd and Zn was obtained at 30°C. However, the bioleaching percentage of Pb was not influenced by temperature, which may be related to the content and the special form of Pb in soil [22].

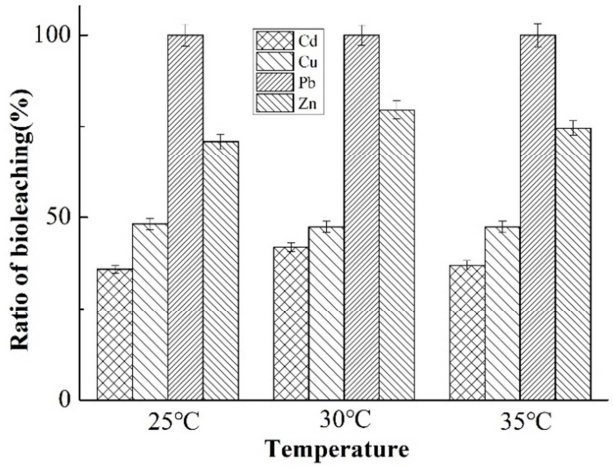


Figure 5. *Heavy metal bioleaching efficiency of strain F2 at different temperature.*

3.7. The Effect of pH on Heavy Metal Bioleaching by F2

The effects of heavy metal bioleaching under different pH were shown in Figure 6. The bioleaching percentages of Cd, Cu, Pb, and Zn were 38.16%, 45.41%, 100%, and 75.99%, respectively at pH 5. The bioleaching percentages of Cd, Cu, Pb, and Zn were 35.89%, 48.19%, 100%, and 70.86%, respectively, at pH 7. The bioleaching percentages of Cd, Cu, Pb, and Zn were 34.94%, 47.19%, 100%, and 74.08%, respectively, at pH 9. The total heavy metal bioleaching percentage was 74.09% at pH 5.0, 69.86% at pH 7.0, and 72.47% at pH 9.0. The bioleaching percentages of Cd and Zn at pH 5.0 was higher than those of other pH. The bioleaching percentage of Cu at pH 7.0 was higher than that at pH 5.0 and 9.0. However, the bioleaching percentage of Pb was not influenced by pH. The highest total bioleaching percentage was obtained at pH 5.0, while the lowest total bioleaching percentage was obtained at pH 7.0. In other words, the total bioleaching percentage in neutral medium was lower than that in both acid medium and alkaline medium. The production of organic acids at different pH by F2 will be studied in the further study.

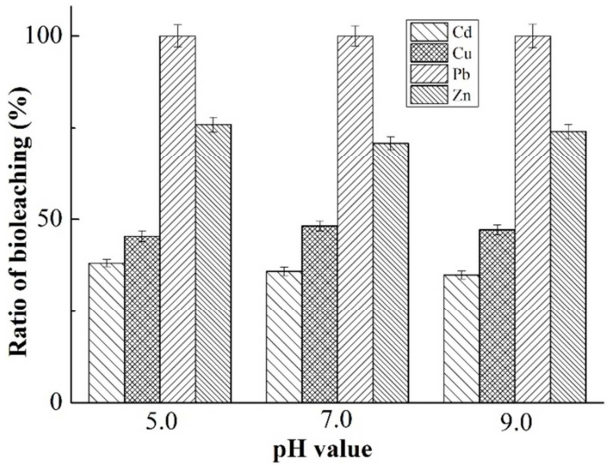


Figure 6. *Heavy metal bioleaching efficiency of strain F2 at different pH.*

4. Conclusion

A. niger F2 isolated from the contaminated soil displayed great ability of heavy metal resistance and organic acids production. F2 grew well is under 2500 mg/L^{-1} of Cu, 5000 mg/L^{-1} of Pb, 8500 mg/L^{-1} of Zn, and 2200 mg/L^{-1} of Cd stress. pH of the liquid medium decreased during the process of bioleaching by F2. The sugars from plants is more suitable for F2 as carbon source than those from animals, while the monosaccharides are more suitable carbon source for F2 than disaccharides and polysaccharides. Organic nitrogen source is more beneficial to strain F2 for bioleaching than inorganic nitrogen source. In general, the effect of carbon source on bioleaching is more significant than nitrogen source. The bioleaching percentage of F2 is also influenced by temperature and pH. From above, strain F2 has great potential for bioleaching heavy metal from contaminated soil. Further study is needed to develop the techniques for the extraction of

heavy metals from contaminated soil using F2. Thus, the mechanism of bioleaching heavy metals from contaminated soil using F2 involved of organic acids production and of heavy metal change before and after bioleaching will be elucidated.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (51474102, 51804353), the Hunan Natural Science Foundation (2015JJ3059, 2018JJ3885).

References

[1] H. Mubarak, L. Y. Chai, N. Mirza, Z. H. Yang, A. Pervez, M. Tariq, S. Shaheen, and Q. Mahmood (2015) Antimony (Sb)-pollution and removal techniques-critical assessment of technologies. Toxicological & Environmental Chemistry 0277-2248, 1029-0486.

[2] Z. H. Yang, Y. Liu, Y. P. Liao, L. Y. Chai, Q. Z. Li, and Q. Liao (2016) Isolation and identification of two novel alkaligenous arsenic (III)-oxidizing bacteria from a realgar mine, China. Clean-Soil, Air, Water 44, 1-7.

[3] M. A. Tobor-Kaplon, J. Bloem, P. F. A. M. Ro¨ Mkens, and P. C. De Ruiter (2006) Functional stability of microbial communities in contaminated soils near a zinc smelter (Budel, The Netherlands). Ecotoxicology 15, 187-197.

[4] S. Das, and Y. P. Ting (2017) Improving gold (Bio) leaching efficiency through pretreatment using hydrogen peroxide assisted sulfuric acid. Clean–Soil Air Water 6, 8-12.

[5] S. R. Burckhard, A. P. Schwab, and M. K. Banks (1995) The effects of organic acids on the leaching of heavy metals from mine tailings. Journal of Hazardous Materials 41, 135-145.

[6] S. W. Zhou, and M. G. Xu (2007) The progress in phosphate remediation of heavy metal contaminated soils. Acta Ecologica Sinica 7, 3043-3049.

[7] Z. H. Yang, Z. Zhang, L. Y. Chai, Y. Wang, Y. Liu and R. Y. Xiao (2016) Bioleaching remediation of heavy metal contaminated soils using *Burkholderia sp*. Z-90. Journal of Hazardous Materials 301, 145-152.

[8] P. Rasoulnia, and S. M. Mousavi (2016) Maximization of organic acids production by *Aspergillus niger* in a bubble column bioreactor for V and Ni recovery enhancement from power plant residual ash in spent-medium bioleaching experiments. Bioresource Technology 216, 729-736.

[9] E. Stephend, and L. Kochian (1998) Phytoextraction of zinc by oat (Avena sativa), barley (Hordeum vulgare), and Indian mustard (Brassica juncea). Environment Science &Technology 32, 802-806.

[10] Y. M. Li, R. E. Brewer, and J. S. Jaynelkin (2003) Phytoextraction of nickel and cobalt by hyperaccumulator alyssum species grown on nickel-contaminated soils. Environment Science & Technology 37, 1463-1468.

[11] M. Nosheen, M. Hussani, L. Y. Chai, Y. Wang, K. M. Jamil, K. Q. Ullah, H. M. Zaffar, F. Umar, S. Rizwana, and Z. H. Yang (2017) The potential use of vetiveria zizanioides for the phytoremediation of antimony, arsenic and their Co-contamination. Bulletin of Environmental Contamination and Toxicology 4, 511-517.

[12] D. Fang, and L. X. Zhou (2007) Enhanced Cr bioleaching efficiency from tannery sludge with coinoculation of *Acidithiobacillus thiooxidans* TS6 and *Brettanomyces* B65 in an air-lift reactor. Chemosphere 69, 303-310.

[13] E. R. Enrique, A. M. Maria, A. O. N. Claudio, C. R. E. Denise, O. Renato, O. Guilherme, and R. C. Marcio (2017) Bioleaching of electronic waste using bacteria isolated from themarine sponge hymeniacidon heliophila (Porifera). Journal of Hazardous Materials 329, 120-130.

[14] X. H. Deng, L. Y. Chai, Z. H. Yang, C. J. Tang, H. X. Tong, and P. F. Yuan (2012) Bioleaching of heavy metals from a contaminated soil using indigenous *Penicillium chrysogenum* strain F1. Journal of Hazardous Materials 233-234, 25-32.

[15] Y. Liu, C. J. Wang, H. Liu, H. Z. Ma, Q. H. Zhang, T. Feng, and D. L. Sun (2015) Spatial distribution and ecological risk assessment of heavy metals in soil around a lead and zinc smelter. Chinese Journal of Environment Engineering 1, 477-484.

[16] Z. J. Xu, C. H. Wu, F. Liu, and X. Y. Qiu (2007) Study on combined pollution character of heavy metals in soils around a typical lead-zinc smeltery. Journal of Hunan University Science Technology (Natural Science Edition) 1, 111-114.

[17] H. N. Bahaloo, S. M. Mousavi, and S. A. Shojaosadati (2016) Bioleaching of valuable metals from spent lithium-ion mobile phone batteries using *Aspergillus niger*. Journal of Power Sources 320, 257-266.

[18] L. Chen, Y. F. Song, W. Zhang, X. Y. Li, L. Wang, P. H. Ji, and X. X. Yang (2008) Assessment of toxicity effects for cadmium contamination in soils by means of multi-indexes. Journal of Environmental Science 9, 2606-2612.

[19] P. A. Wani, and M. S. Khan, Heavy metal toxicity and their remediation by soil microbes. 2011, LAP Lambert Academic Publishing, pp. 2-5.

[20] X. F. Zeng, S. H. Wei, L. N. Sun, D. A. Jacques, and J. X. Tang (2015) Bioleaching of heavy metals from contaminated sediments by the *Aspergillus niger* Strain SY. Journal of Soils Sediments. 4, 1029-1038.

[21] A. Sharma, V. Vivekanand, and R. P. Singh (2008) Solid state fermentation for gluconic acid production from sugarcane molasses by *Aspergillus niger* ARNU employing tea waste as the novel Solid support. Bioresource Technology 9, 3444-3450.

[22] Y. X. Chen, Y. M. Hua, S. H. Zhang, and G. M. Tian (2005) Transformation of heavy metal forms during sewage sludge bioleaching. Journal of Hazardous Materials 1-3, 196-199.