Responses of bacterial community to potential heap construction methods of fine-grained copper tailings in column bioleaching system

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Abstract: The column bioleaching of copper flotation tailings was comparatively investigated using layered heap construction method (LM), agglomerate heap construction method (AM), and pellets-sintering heap construction method (PM). The bacterial communities of free, attached, weakly-attached, and strongly-attached microbes in the later bioleaching stage were investigated. In AM group, the addition of lump sulphide ore resulted in the low leachate pH, high ferric iron concentration, and rapid microbial adsorption, which obtained the maximum copper extraction (60.1%) compared with LM (54.6%) and PM (43.9%) groups. The relative abundance of dominant genera and microbial communities of different microbiota underwent changes in three heap construction methods. The alpha-diversity indexes of attached, weakly-attached, and strongly-attached microbes were different, while no significant change was observed in free bacteria. The variation of whole bacterial community was significantly associated with solution pH, total iron, and ferric iron concentrations. Pearson correlation analysis and partial least square path model both indicated that attached bacteria made larger contribution to the copper extraction of tailings.

Key words: fine-grained tailings; column bioleaching; heap construction method; free and attached bacteria; microbial diversity

1 Introduction

Flotation is the most widely used extraction technology in copper extractive fields worldwide and can produce 80% of the copper production of this commodity [1]. Meanwhile, flotation process can create numerous tailings annually. In large copper mining, one ton of copper concentrate generated produces 151 t of tailings [2]. The accumulation and storage of tailings in open-air condition can cause potential environmental and societal problems. The physicochemical and biological reactions that occur in the stock heap improve the trace metals mobility of exposed tailings. As a result of the oxidation of sulfur minerals, the generation of acid drainage (low pH and high heavy metals) poses a great danger to farmland and underground water [3−5]. Tailings can also be considered as a secondary source because they contain a large number of valuable metals such as Cu, Co and Ni, which can mitigate the inconsistency between the decline in high-grade minerals and the rise in the demand for metal resources [6,7]. Hence, the recycling processing of tailings and the recovery of valuable metals from this waste are necessary to increase the utilization values of tailings [8].

Bioleaching is a low-cost and eco-friendly extraction technology for the recovery of trace metals from tailings and low grade ores [9,10]. However, tailings with high level of fines can reduce the permeability of leachate, restrict the transfer of gases (e.g., oxygen and carbon dioxide), and delay the microbial adsorption especially in dump bioleaching operation, and thereby decrease the dissolution efficiency of tailings [11]. Several efforts have been made to increase the permeability of stock heap and improve the extraction yields. Thin-layer conveying and dumping performance accompanied by other technological improvements is used to enhance the heap permeability and copper extraction from low-grade copper oxides of Yangla Copper Mine located in Yunnan Province of China [12]. The need for agglomeration method of ores with binders such as polymer materials is recommended in heap leaching operation to gain high metal leaching rates if the ores contain lots of fines [13]. These strategies have promoted the industrial practice of highly fine-grained tailings heap bioleaching [14]. However, comparative study on the effects of different potential heap constructions on tailings dissolution is very limited, and there are also few records on the correlations between bacterial community and copper extraction of tailings using diverse heap construction methods.

The bioleaching process involves multiple biological, chemical and physical reactions [15,16]. Microbial interaction is considered as the main driving factor to dissolve minerals via various biochemical pathways [17]. Microbial ecology studies relevant to the essential leaching parameters of leachate in the bioleaching method can approximate the phylogenetic microbiota interaction [18]. Bacterial communities of free and attached cells, growing in different bioleaching subsystems, are different and play specific roles of elemental sulfur and ferrous iron oxidization in promoting the bioleaching efficiency through direct or indirect mechanisms [19,20]. However, the attached microbial community on the tailings surface, accounting for most of total biomass, is still not well understood, especially concerning the linkage between attached community structure and copper extraction. It is necessary to monitor the community compositions of free and attached bacteria to obtain complete comprehension in the contributions of distinct microbiota to tailings bioleaching.

In this work, we constructed the column bioleaching of flotation tailings using three different heap construction methods. The bacterial communities in three groups were analyzed by 16S rRNA MiSeq high-throughput sequencing. The major objective of this study was to address the following questions: do different heap construction methods alter the bioleaching behaviors and microbial diversities of free and attached bacteria? and how these changes affect the tailings bioleaching efficiency?

2 Experimental

2.1 Ore samples

Copper flotation tailings used in this study were collected from Lualo Tailing Dam of Zambia. Particle size distribution of tailings was ~75 μm (48%), 75−150 μm (43%) and 150−270 μm (9%), respectively. X-ray diffraction analysis showed that SiO_2 (60.7%), Fe_xO (25.6%) and MgO (3.2%) were the main components of tailings. Copper phase analysis showed that tailings contained 0.06% free copper oxide, 0.01% combined copper oxide, 0.06% secondary copper sulfide and 0.07% primary copper sulfide. The main element contents of tailings were 0.2% Cu, 19.7% Fe and 2.3% S. Another kind of lump sulphide ores sized at 2.5−5 mm, which served as the core of agglomerate ores adhering the flotation tailings, were obtained from Yulong Copper Mine in Changdu city of China. The main element composition was 2.8% Cu, 16.6% Fe and 17.4% S.

2.2 Preparation of mixed mesophilic acidophiles

The enrichment of the mixed mesophilic acidophiles for subsequent column bioleaching is as described by our previous study [21]. Briefly, acid mine drainage and leaching residue samples were obtained from a mine located in Jiangxi Province of China. Two samples were combined and then inoculated into culture medium (pH 2.0) at 30 °C to enrich the acidophilic consortium [6]. Cultures were supplemented with 4.5% $FeSO_4 \cdot 7H_2O$, 1.0% S^0 and 2.0% tailings as energy sources. Finally, mixed mesophilic acidophiles were obtained when the microbial density exceeded 1×10^8 cells/mL. The top four dominant genera in enriched acidophilic consortium were *Acidithiobacillus*, *Leptospirillum*,

Sulfobacillus and *Ferroplasma*.

2.3 Heap construction method and column bioleaching

Three kinds of potential heap construction methods including the layered heap construction method (LM), the agglomerate heap construction method (AM), and the pellets-sintering heap construction method (PM) were carried out (Fig. 1). LM was performed via the alternant loading of tailings and quartz sands sized at 2.5−5 mm. As a result, four layers of 5 cm-thick quartz sands and 5 cm-thick tailings were placed into the column, respectively. AM was processed via the blend of 10% H_2SO_4 solution and mixed ores (tailings mixed with lump ores). Then, agglomerate ores were put into the column. For PM operation, tailings added with 1.5% bentonite were granulated and sintered into agglomerate pellets (800 °C, 10 min), and subsequently loaded into the column. The column bioreactor was 50 cm high and the internal diameter was 7 cm. In three bioleaching groups, tailings with equivalent mass (1.3 kg) were loaded into each column. Column bioleaching experiments were operated at ambient temperature for 83 d including acid leaching process (10 d) using a 1.6 L culture medium of pH 1.5.
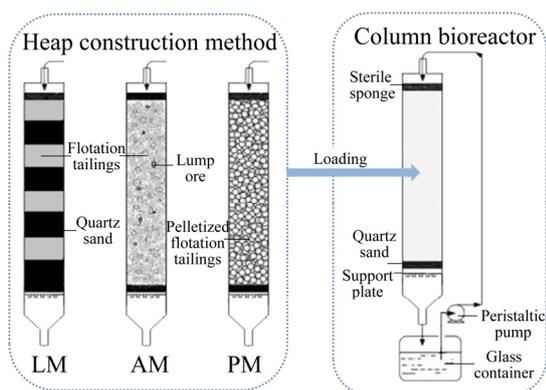

Fig. 1 Design of potential heap construction methods and configuration of column bioreactor (LM−Layered heap construction method; AM−Agglomerate heap construction method; PM−Pellets-sintering heap construction method)

2.4 Chemical and microbiological analysis

Column bioleaching tests were monitored regularly for the measurements of pH (pHS−3C, Leici, China), redox potential (ORP, BPH−220, Bell, China), cell density (BX41, Olympus, Japan) and total iron concentration (inductively coupled plasma-atomic emission spectrometry, Optima 5300 DV, Baird, China) in leachate solution. Ferrous iron concentration was measured by phenanthroline colorimetry method, and ferric iron concentration was equal to the difference of total iron and ferrous iron concentrations. Copper extraction was calculated according to

$$\eta=(W_1-W_2)/W_1\times100\%$$

where W_1 represents the Cu content of tailings before bioleaching, and W_2 represents the Cu content after bioleaching.

The genomic DNA of mixed mesophilic acidophiles (inoculum) and free microbes in leachate after bioleaching tests were extracted using a TIANamp Bacteria DNA kit (Tiangen Biotech., China). The genomic DNA of attached microbes, weakly-attached and strongly-attached microbes in three heap construction methods after bioleaching was extracted by the E.Z.N.A. Soil DNA kit (Omega Bio-Tek Inc., USA). Procedures for amplification, sequencing of 16S rRNA gene and raw data preprocessing are as described in our previous study [21].

2.5 Statistic analysis

One-way analyses of variance (ANOVA) followed by the least significant difference (LSD) test were carried out using IBM SPSS Statistics 21.0 software to determine the difference in leachate properties, relative abundance of dominant genera and alpha-diversities after bioleaching experiments in three heap construction methods. Dissimilarity tests (Adonis) was used to evaluate the significant differences in microbial community composition among three bioleaching groups. Non-metric multidimensional scaling analysis (NMDS) was carried out to compare the bacterial community structure using R 'vegan' package. Mantel test analysis was conducted to determine the relationship between leachate variables and bacterial community. Pearson correlation test was used to determine the correlation among copper extraction, leachate factors and dominant genera. Partial least squares path modeling (PLSPM) was performed using 'amap', 'shape', 'diagram' and 'plspm' packages to show the association of copper extraction with leachate pH, ferric iron and microbial communities within three heap construction methods.

3 Results and discussion

3.1 Bioleaching of copper tailings by mixed mesophilic acidophiles

Different heap construction methods clearly impacted the bioleaching behaviors of three experimental groups. Leachate pH values increased rapidly in initial bioleaching process, which corresponded to the consuming proton stage of ferrous iron oxidation [11]. The pH values decreased gradually from Day 25 in LM and AM groups, and finally, the pH value of AM (1.3) was significantly ($P<0.05$) lower than that of LM (1.6) and PM (2.4) groups (Table 1). Among three groups, the highest solution cell density was observed in LM group during the whole bioleaching performance. Meanwhile, the time of bacterial numbers increase in LM solution (on Day 32) was later than AM group (on Day 17). The lowest permeability of LM group led to the longer time of microbial adsorption onto tailings surface. The lump sulphide ore was used in AM bioleaching system, and sufficient energy sources (16.6% Fe and 17.4% S) accelerated the microbial growth in early experimental stage. However, the only decline phase among three groups was also found in AM group from Day 62, which can be attributed to the energy source consumption, increase in metal ions, and organic substances in last phage [22]. Non-obvious change of free cell density was presented in PM group. This was attributed to the sulfur element loss in the sintering treatment of tailings and the persistent higher solution pH, which severely inhibited the growth of acidophiles [23].

In acid leaching stage, ferrous iron was released from the tailings dissolution and was the main component of total iron in leachate. With the addition of mixed mesophilic acidophiles on Day 10, ferric iron resulted from the ferrous iron oxidation by the iron-oxidizers almost accounted for all of the total iron concentrations. However, ferric iron and total iron concentrations presented different change trends in three groups. Compared with LM and PM groups, the ferric iron concentration was higher and increased steadily in AM bioleaching process. The LM operation caused a lower permeability, but the ferric iron concentration was higher than that of PM group. In addition, the ferric iron concentration began to decline in bioleaching stage of PM group, which might infer the formation of jarosite due to the higher leachate pH values. After the bioleaching tests, the ferric iron concentration of LM group (2.6 g/L) was significantly ($P<0.05$) lower than that of AM (19.7 g/L) group, but higher than that of PM (0.11 g/L) group (Table 1). The iron ion concentration changes did not alter the variation tendency of leachate ORP values in three groups. In acid leaching operation, the ORP values decreased to about 350 mV, but were sharply enhanced on Day 5 with the increase of ferric iron concentration and then remained stable in subsequent bioleaching process.

Copper extraction of tailings based on copper contents was 60.1% in AM group, which was significantly ($P<0.05$) higher than that of AM (54.6%) and PM (43.9%) groups (Table 1). Pearson correlation analysis showed a significantly negative correlation between leachate pH and copper extraction ($P<0.001$), but a significantly positive correlation between copper extraction and total and ferric iron concentrations ($P=0.006$). In AM group, more sulfur element and ferrous iron were dissolved from the lump sulphide ores and supported the growth of mesophilic acidophiles. Elemental sulfur

Table 1 Leachate properties after column bioleaching experiments and Pearson correlation analysis between leachate properties and copper extractions of flotation tailings using three heap construction methods

Group	pH [a]	ORP/ mV	Cell density/ (10^6 cells·mL^{-1})	Total iron concentration/ (g·L^{-1})	Ferric iron concentration/(g·L^{-1})	Copper extraction/%
LM	1.6±0.1	674.5±0.5	18.8±0.4	2.7±0.1	2.6±0.1	54.6±0.9
AM	1.3±0.1	597.0±58.0	3.6±0.4	20.0±1.3	19.7±1.2	60.1±1.0
PM	2.4±0.1	617.0±2.0	3.6±0.3	0.15±0.1	0.11±0.1	43.9±0.9
Copper extraction [b]	−0.985 (<0.001)	−0.075	0.185	0.826 (0.006)	0.827 (0.006)	−

[a] Means with different small letters in the same column are significantly different according to LSD test ($P<0.05$); [b] Significant differences ($P<0.05$) are indicated in parentheses according to Pearson correlation analysis

was oxidized into H_2SO_4 by sulfur-oxidizers, leading to the lower solution pH. Ferrous iron was oxidized into ferric iron serving as the major oxidizing reagent, which accelerated the tailings dissolution and Cu recovery of AM group [24]. In three heap construction methods, the solution permeability order was PM>AM>LM. Low solution permeability hindered the microbial colonization of mixed acidophiles and restricted oxygen and carbon dioxide transfer in ore body of LM group. These negative factors reduced the activity of microorganisms and slowed down the kinetics of tailings dissolution compared with AM group [25]. For PM group, high solution pH promoted the formation of jarosite inferred by the ferric ion concentration reduction [26]. The jarosite particles could wrap the tailings surface and block the interaction between tailings and acidophilic microbes, resulting in a lower copper extraction.

3.2 Bacterial community structure and diversity

Analysis of 16S rRNA gene sequences showed that the bacterial community compositions of free and attached microbes including weakly-attached and strongly-attached bacteria in three heap construction methods were different at the genus level (Fig. 2(a)). In the initial inoculum, the genera *Acidithiobacillus* (67.8%) and *Sulfobacillus* (24.6%) were the preponderant microorganisms, and the proportions of *Leptospirillum* and *Ferroplasma* were less than 9%. After the bioleaching of the tailings, the most abundant genera were *Acidithiobacillus*, *Leptospirillum*, *Ferroplasma*, and *Acidiphilium*. Comparisons of the differences in these bacterial genera among free, attached, weakly-attached, and strongly-attached microbes are shown in Figs. 2(b₁−b₄). Heap construction method significantly affected the distributional difference of the same dominant genus in three bioleaching groups. For the free bacterial composition, by comparing AM and PM groups, *Acidithiobacillus* and *Leptospirillum* were significantly ($P<0.05$) more abundant, and *Acidiphilium* were significantly ($P<0.05$) less abundant in LM (Fig. 2(b₁)). The most abundant genus in AM was *Ferroplasma*, while *Acidiphilium* accounted for the largest proportion in PM. The relative abundance of genera *Acidithiobacillus*, *Sulfobacillus* and *Acidiphilium* presented similar variation trends in attached and weakly-attached

microbes among three bioleaching groups (Figs. 2(b₂, b₃)). In addition, the abundance of *Leptospirillum* and *Ferroplasma* ranked first in PM and AM groups, respectively. In strongly-attached microbial community, genera *Acidithiobacillus* and *Acidiphilium* accounted for the largest proportions in PM group (Fig. 2(b₄)). The genera *Ferroplasma* and *Sulfobacillus* had the highest abundance in LM group, but the abundance of *Leptospirillum* was significantly ($P<0.05$) lower than that in the other two groups.

The alpha diversity estimated by Shannon index of free bacterial community showed no significant ($P>0.05$) changes among three bioleaching groups, but significant ($P<0.05$) changes were observed in attached, weakly-attached, and strongly-attached microbial communities (Fig. 3). Shannon indexes of weakly-attached and strongly-attached communities in AM group were significantly ($P<0.05$) lower than those of LM and PM groups. The microbial growth of AM group entered into the decline phase in last bioleaching stage. Therefore, the deterioration of bioleaching environments and the consumption of energy sources might reduce the alpha diversity. Furthermore, Shannon index of PM group was lower than that of the LM group in attached cell community. The inadequate iron, sulfur energy sources, and higher solution pH in PM bioleaching system could result in the decrease in alpha diversity.

Non-metric multidimensional scaling (NMDS) analysis based on Bray−Curtis distance was utilized to analyze the overall structural variations of bacterial microbiota after bioleaching in three potential heap construction methods (Fig. 4). The results of NMDS analysis showed that bacterial communities were clearly separated among LM, AM and PM groups. Dissimilarity test using Adonis further confirmed significantly ($P<0.05$) different structures of microbial communities in the four groups including the inoculum. Besides, it was noted that attached bacterial community was clustered together with weakly-attached microbes of three groups, and distinguished from the free and strongly-attached microbial communities, which might be explained by the similar variation trends in the relative abundance of the dominant genera in attached and weakly-attached microbiota.
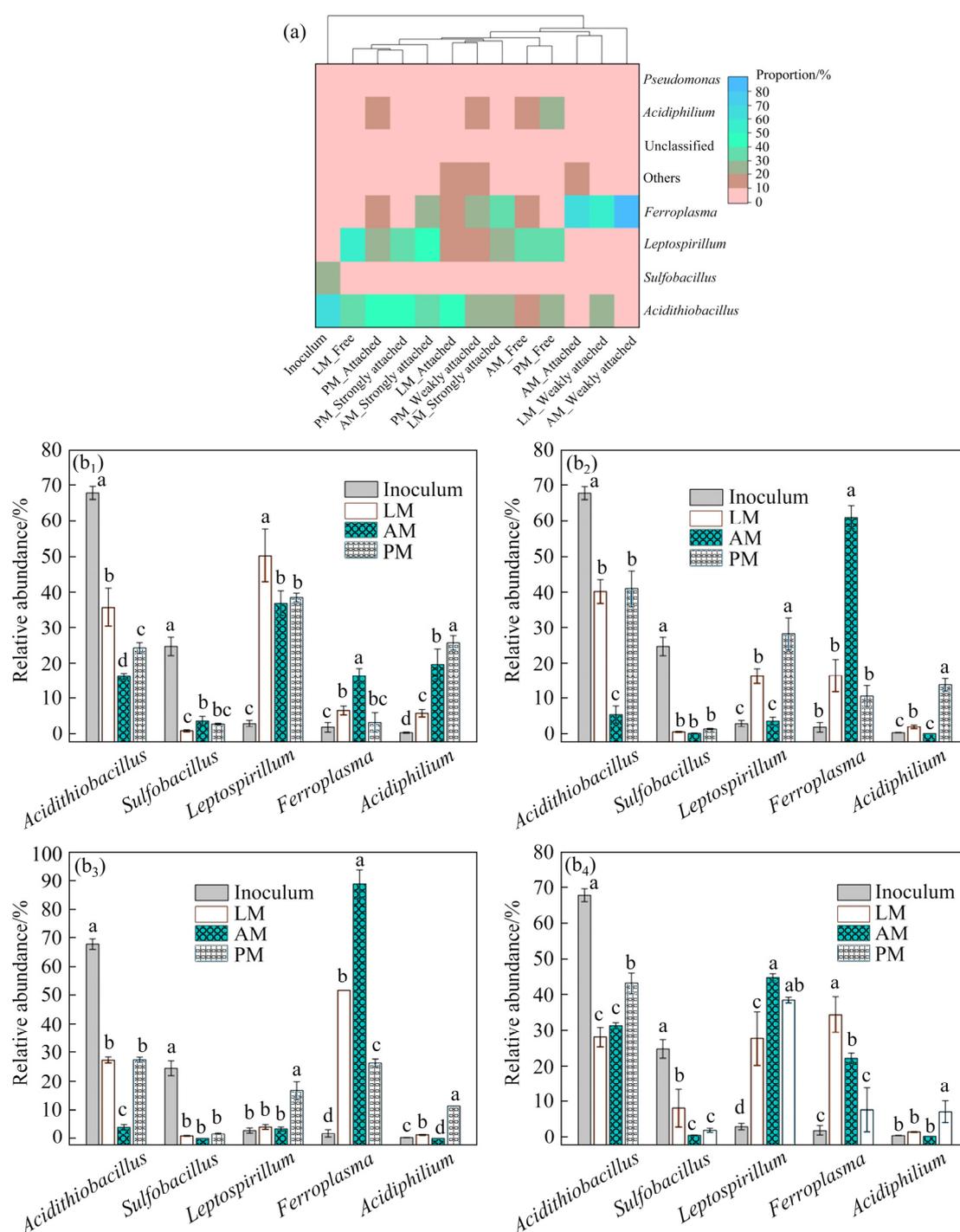
Fig. 2 Microbial compositions with different heap construction methods (a), and relative abundances of dominant genera in free (b$_1$), attached (b$_2$), weakly-attached (b$_3$) and strongly-attached microbes (b$_4$) (Different small letters above error bars indicate the significant difference according to LSD test ($P<0.05$))

3.3 Links among bacterial community, leachate property and copper extraction

Mantel test analysis was applied to exploring the associations between leachate properties and bacterial community structures in three groups (Table 2). The results showed that solution pH ($P=0.024$), total iron ($P=0.007$), and ferric iron ($P=0.007$) concentrations were significantly associated with the whole bacterial community. Leachate factors listed in Table 2 had a significant correlation with dominant genus *Acidithiobacillus* ($P<0.01$), but no significant associations were found between leachate factors and *Sulfobacillus*. In addition, total iron and ferric iron concentrations
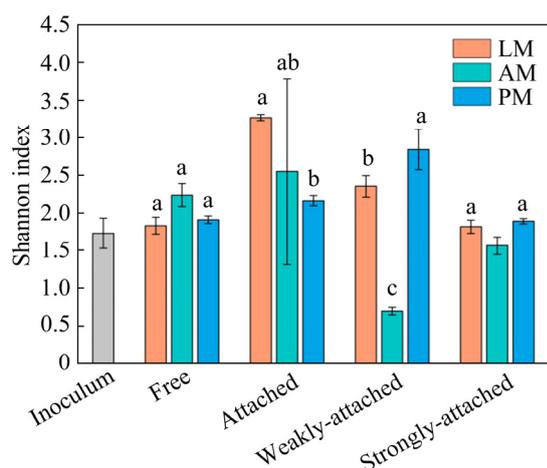
Fig. 3 Alpha diversity (Shannon index) with different heap construction methods (Different small letters above error bars indicate significant difference according to LSD test ($P<0.05$))

Fig. 4 Non-metric multidimensional scaling analysis results of bacterial community based on Bray−Curtis distance indices and dissimilarity test (Adonis) showing differentiation in microbial structures of different heap construction methods (F, A, W and S represent free, attached, weakly-attached and strongly-attached microbial communities, respectively)

had significant associations with the relative abundance of *Leptospirillum*, *Ferroplasma* and *Acidiphilium*, and pH was also associated with the changes of *Acidiphilium*.

Correlations between copper extractions of tailings and dominant genera in free, attached, weakly-attached, and strongly-attached microbial communities are given in Table 3. There was only one genus *Ferroplasma* in free microbial community
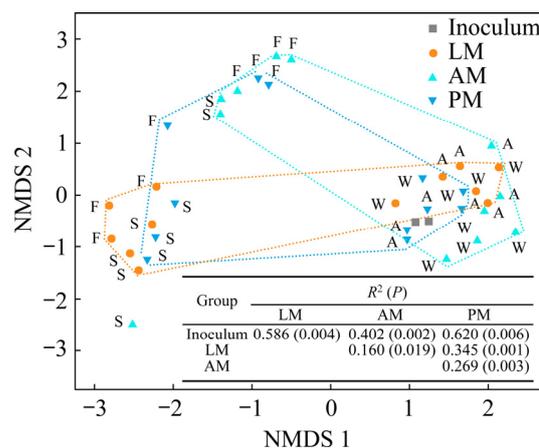
closely correlated to copper extraction ($P=0.005$). However, in attached and weakly-attached microbial communities, five dominant genera all had significant correlations ($P<0.05$) with copper extractions, and *Acidithiobacillus*, *Ferroplasma*, and *Acidiphilium* were significantly ($P<0.05$) correlated to copper extractions in strongly-attached

Table 2 Mantel test analysis results of relationship between leachate properties and bacterial community

Bacterial community	pH	ORP	Cell density	Total iron	Ferric iron
Whole	0.126 (0.024)	0.067	0.016	0.232 (0.007)	0.232 (0.007)
Acidithiobacillus	0.331 (0.001)	0.299 (0.001)	0.476 (0.001)	0.243 (0.002)	0.244 (0.001)
Sulfobacillus	−0.037	−0.008	0.105	0.002	0.002
Leptospirillum	−0.003	0.061	0.023	0.106 (0.033)	0.106 (0.031)
Ferroplasma	0.085	0.001	−0.055	0.203 (0.009)	0.203 (0.009)
Acidiphilium	0.400 (0.001)	0.101	0.006	0.121 (0.045)	0.122 (0.046)

Significant differences ($P<0.05$) are indicated in parentheses

Table 3 Pearson correlation analysis results between dominant genera and copper extractions of flotation tailings in three heap construction methods

Dominant genera	Free	Attached	Weakly-attached	Strongly-attached
Acidithiobacillus	−0.223	−0.771 (0.015)	−0.756 (0.018)	−0.829 (0.006)
Sulfobacillus	0.099	−0.96 (<0.001)	−0.973 (<0.001)	0.015
Leptospirillum	0.078	−0.937 (<0.001)	−0.951 (0.002)	0.152
Ferroplasma	0.838 (0.005)	0.705 (0.034)	0.950 (<0.001)	0.668 (0.049)
Acidiphilium	0.45	0.972 (<0.001)	0.966 (<0.001)	0.916 (0.001)

Significant differences ($P<0.05$) are indicated in parentheses

microbial community. These results indicated that, compared with free microbes, attached microbes played more important role in affecting copper bioleaching efficiencies of tailings [27]. It is shown that there were substantial negative correlations between *Acidithiobacillus*, *Sulfobacillus*, and *Leptospirillum*, but *Ferroplasma* and *Acidiphilium* had significant positive correlations with copper extractions. The genera *Ferroplasma* and *Acidiphilium* were able to adapt to the adverse environments in last bioleaching stage. Organic matter originated from exudates and cell lysates of microorganisms could inhibit the growth of autotrophs [28]. The growth of *Ferroplasma* and *Acidiphilium* with organic matter as an energy source reduced the toxicity to autotrophs and therefore made positive contributions to the Cu recovery of tailings [29,30].

In addition to correlation analysis, the relative importance of the associations of copper extractions with leachate pH, ferric iron, and microbial properties was analyzed using the partial least square path model (PLSPM) (Fig. 5). Leachate pH had significant negative effects on copper extraction ($P<0.05$) and ferric iron ($P<0.05$), but a significant positive effect was shown between ferric iron and copper extraction ($P<0.01$). Leachate pH had no significant effect both positively or negatively ($P>0.05$) on free and attached community structures, but there were significant positive effects ($P<0.001$) on weakly-attached and strongly-attached microbial communities. Ferric iron concentration changes had significant positive correlations ($P<0.01$) with free and strongly-attached microbial communities and significant negative correlations ($P<0.05$) with attached and weakly-attached microbial communities, indicating that ferric iron was a more important factor affecting microbial community composition and structure in the later bioleaching stage. Free bacterial community had no impacts on copper extraction of tailings. However, attached bacterial community was positively correlated with copper extraction ($P<0.01$), and weakly-attached and strongly-attached microbial communities showed a negative correlation with copper extractions ($P<0.01$). These findings were consistent with the Pearson correlation analysis results (Table 3) demonstrating the significance of attached microbes to the copper recovery of tailings. A previous study

also found that the contribution of attached cells in sulphide ore bioleaching was high at 50.5% [31].
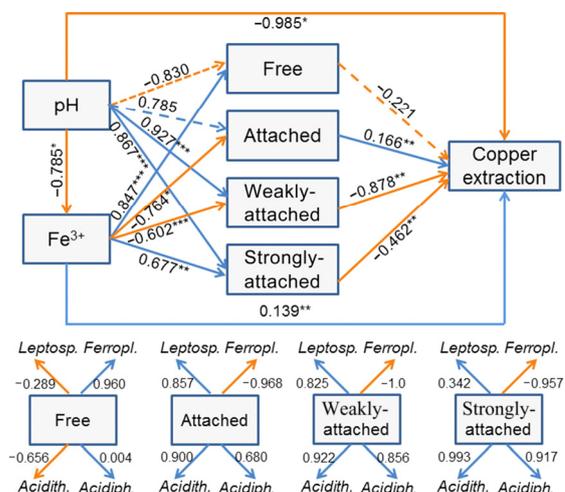

Fig. 5 Partial least square path modeling showing association of copper extractions with leachate pH, ferric iron and microbial communities of free, attached, weakly-attached and strongly-attached microbes with different heap construction methods (Goodness of fit of the modeling is 0.7648; Blue and red arrows indicate positive and negative path coefficients, and solid and dashed lines indicate significant ($P<0.05$) and insignificant path coefficients ($P>0.05$), respectively; *, ** and *** indicate significances at the levels of $P<0.05$, 0.01 and 0.001, respectively)

4 Conclusions

(1) Different heap construction methods clearly impacted the bioleaching behaviors and copper extractions of the tailings of three experimental groups.

(2) The alpha diversity (except free microbiota) and beta diversity in three groups were significantly changed. Solution pH and ferric iron significantly influenced the whole microbial community and the most dominant genera.

(3) Pearson correlation and PLSPM analysis both indicated that the attached microbes played a positive contribution to the copper recovery of tailings compared with free microbes.

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细粒铜尾矿生物柱浸体系下细菌群落对潜在的铺矿形式的响应

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摘 要：比较研究分层铺筑(LM)、聚团铺筑(AM)和烧结球团铺筑(PM)三种铺矿形式对铜浮选尾矿生物柱浸的影响。解析尾矿浸出后期三组浸矿体系下游离、吸附、弱吸附和强吸附微生物的群落结构。在 AM 浸出组，用于尾矿聚团的块状硫化矿的添加使浸出溶液 pH 值降低，三价铁离子浓度升高和微生物快速吸附。相比于 LM(54.6%)和 PM(43.9%)浸出组，AM 浸出组得到最高的铜浸出效率(60.1%)。在不同尾矿铺筑形式下，不同微生物群的优势属的相对丰度和群落结构均发生明显变化。尾矿铺筑形式改变吸附、弱吸附和强吸附微生物群落的 α-多样性指数，但未改变游离微生物群落的 α-多样性。溶液 pH 值、总铁和三价铁浓度与整体细菌群落的变化呈现显著相关性。皮尔逊相关分析和偏最小二乘路径模型均表明吸附微生物对尾矿中铜浸出的贡献更大。

关键词：细粒尾矿；生物柱浸；铺矿形式；游离和吸附细菌；微生物多样性

(Edited by Bing YANG)