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Carbon Material with High Specific Surface Area Improves Complex Copper Ores' Bioleaching Efficiency by Mixed Moderate Thermophiles

Xiaodong Hao ^{1,2}, Xueduan Liu ^{1,2}, Ping Zhu ^{1,2}, Aijia Chen ^{1,2}, Hongwei Liu ^{1,2}, Huaqun Yin ^{1,2}, Guanzhou Qiu ^{1,2} and Yili Liang ^{1,2,*}

¹ School of Minerals Processing and Bioengineering, Central South University, Changsha 410083, China; haoxiaodongxyz@163.com (X.H.); xueduanliu@yahoo.com (X.L.); zhuping199508@163.com (P.Z.); 165611029@CSU.com (A.C.); hongweiliu@csu.edu.cn (H.L.); yinhuaqun_cs@sina.com (H.Y.); qgzhoucsu@163.com (G.Q.)

² Key Laboratory of Biometallurgy of Ministry of Education, Changsha 410083, China

* Correspondence: liangyili@hotmail.com; Tel.: +86-731-8883-0546

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Abstract: The catalysis of carbon materials with different specific surface areas (SSA) (2, 400, 800 and 1200 m²/g) on complex copper ores bioleaching by moderately mixed thermophiles was investigated. The copper extractions increased with the rise in SSA of carbon materials. A recovery of 98.8% copper in the presence of 1200 m²/g activated carbon was achieved, and improved by 30.7% and 76.4% compared with biotic control and chemical leaching. Moreover, the addition of 1200 m²/g activated carbon adsorbed large amount of bacteria, accelerated the oxidation rate of ferrous iron and maintained the solution redox potential at relatively low values, and significantly increased the dissolution of primary copper sulfide (62.7%) compared to biotic control (6.0%). Microbial community succession revealed that activated carbon changed the microbial community composition dramatically. The *S. thermosulfidooxidans* ST strain gained a competitive advantage and dominated the microbial community through the whole bioleaching process. The promoting effect of carbon material with higher SSA on copper extraction was mainly attributed to better galvanic interaction, biofilm formation, direct contact and lower redox potential.

Keywords: complex copper ores; specific surface area; primary copper sulfide; low redox potential; microbial community succession

1. Introduction

Due to the continuous decrease of valuable copper minerals, the beneficiation and utilization of complex and low-grade copper ores that used to be considered as wastes needs a relook. Bioleaching has become a preponderant technology for copper extraction from complex copper ores on account of its advantages in high processing capacity, low-cost production and eco-friendly for nature. Moreover, primary copper sulfide (chalcopyrite) including chalcopyrite-bearing mineral is refractory to be dissolved due to the formation of a passivation layer, stable structural configuration and higher lattice energy [1–3]. Therefore, increasing the bioleaching efficiency and ultimate copper extractions from complex copper sources is the most urgent problem affecting the economy of copper mining.

For bioleaching technology, the addition of modifier is beneficial to increasing the copper recovery rate. It has been documented that some catalytic agents (e.g., Ag⁺, polyethylene glycol and Cl[−]) were used to improve the copper sulfide ores dissolution [4,5]. Ag⁺ catalysis is due to the formation of a complex porous layer on sulfide mineral particles instead of the deposition of a tenacious sulfur layer. Ag₂S–Ag film modifies the structure of the S⁰ layer, improves its electrical conductivity, and accelerates

the electronic transfer through the Fe^{3+} oxidant [6]. Polyethylene glycol can increase the bacterial attachment of *A. ferrooxidans* and promote the sulfur oxidation accumulated on mineral surfaces in the leaching process, finally enhancing the bioleaching efficiency [7]. Cl^- is another important factor for chalcopyrite bioleaching through improving dissolution kinetics by reducing the activation energy. However, Cl^- would inhibit microbial activities and growth even at low concentration (0.1%, wt/vol) [8]. Therefore, the catalysts listed above are hardly available because of expensive price or deleterious effects to bioleaching bacteria.

Carbon materials are produced and obtained relatively easily. Their specific structures offer them exceptional properties, which make these materials suitable for use in various biotechnological applications. Hydrophilic carbon-based materials with high surface area and electrical conductivity are commonly used as electrode materials in the field of microbial fuel cells (MFCs) [9]. A high surface area allows high extent of bacterial attachment and increases electron-exchanged frequency between bacteria and the electrode [10,11]. High electrical conductivity can reduce the ohmic resistance of the electron flow in MFCs. Carbon nanotubes (CNT) and activated carbons possess uniform structural units but are prone to forming bundles of randomly tangled agglomerates caused by the strong Van der Waals forces [12]. These properties render exceptional adsorption ability and are used for removal of heavy metals, organics and biological impurities [13]. In addition, carbon materials are also applied in the biometallurgy field. The formation of chalcopyrite/carbon aggregates change the electrical conductivity of the reaction product layer, and further increase the chalcopyrite dissolution rate by ferric sulfate [14]. Subsequent studies speculate that the enhanced copper efficiency of chalcopyrite by adding activated carbon is owing to the galvanic interaction between activated carbon and chalcopyrite [15]. Zhang et al. [16] indicate that the solution with the concentration of 3.0 g/L activated carbon is most beneficial to the dissolution of low-grade primary copper sulfide ores using mixture of *A. ferrooxidans* and *A. thiooxidans*. Liang et al. [17] show the optimum concentration of activated carbon enhancing chalcopyrite dissolution is 2 g/L with extreme thermophile *Acidianus manzaensis*. However, previous studies just focused on the effects of carbon material load on chalcopyrite bioleaching. The relationship between the properties of carbon materials and the complex copper ores dissolution process remains unknown. Meanwhile, many reports show that moderate thermophiles, such as *S. thermosulfidooxidans*, *L. ferriphilum*, *A. caldus* and *F. thermophilum*, can greatly improve the reaction kinetics and avoid excessive passivation, thus improving sulfide ore extractions [18]. Therefore, it is necessary to explore the role of the moderate thermophiles in complex copper ores' bioleaching with the assistance of carbon material.

Therefore, complex copper ores bioleaching using carbon materials with different specific surface area by mixed moderate thermophiles is investigated. The objectives of this study are to determine the copper bioleaching efficiency of complex copper ores using carbon materials with different specific surface area; to identify the effects of carbon material on bioleaching process, biosorption behavior and community succession of mixed moderately thermophiles; to investigate the mechanism of copper bioleaching coupled with carbon materials with high specific surface area. We expect that this study will shed light on carbon material-aided bioleaching technology.

2. Materials and Methods

2.1. Complex Copper Ores and Carbon Materials

The complex copper ore used in this study were obtained from Luanshya copper mine in the copperbelt of Zambia. It was air-dried, ground and passed through a 75 μm plastic sieve before leaching experiments. The ore samples were digested by an acid mixture of HF , HNO_3 and HClO_4 (Yongfa chemical manufacturing Co. Ltd., Wuhan, China) on an electric heating plate (XJS20-42, Labotery Instruments Co. Ltd., Tianjin, China). Elemental contents were measured with an inductively coupled plasma-optical emission spectrometer (ICP-OES, Optima 5300 DV, Baird Instruments Co. Ltd., Shanghai, China). The results were as follows (wt/wt): 2.3% Cu, 3.8% Fe, 1.3% S, 7.9% K, 7.8% Al,

3.1% Mg, 1.9% Ca, 0.4% Na and 0.3% Mn. The copper phase analysis displayed that complex copper ores contained 1.1% primary copper sulfide, 0.7% secondary copper sulfide, 0.5% free copper oxide and 0.1% combined copper oxide. X-ray diffraction (XRD, Model DX-2700, Shanghai instruments Co. Ltd., Shanghai, China) analysis presented feldspar (47.5%) and mica (31.2%) as the major components, and quartz (7.6%), montmorillonite (6.3%), fluorite (4.8%) and amphibole (2.6%) were the minor ones.

Carbon materials used in this study were purchased from Xingbang activated carbon Co., Ltd. in Guangdong Province of China, and the particle sizes were under 75 μm . The properties of the carbon materials are listed in Table 1.

Table 1. Properties of carbon materials used in the present study.

Carbon Materials	Specific Surface Area (m^2/g)	Electrical Conductivity (S/m)	pH	S (%)	Fe (%)
Graphite (C2)	2	0.6	5.2	0.9	0.9
Activated carbon I (C400)	400	0.1	6.2	0.4	0.8
Activated carbon II (C800)	800	0.1	6.9	0.5	0.3
Activated carbon III (C1200)	1200	0.1	5.9	0.2	0.3

2.2. Preparation of the Mixed Moderate Thermophiles

Four moderately thermophilic strains (*Leptospirillum ferriphilum* DX, *Acidithiobacillus caldus* DX, *Sulfobacillus thermosulfidooxidans* ST and *Ferroplasma thermophilum* L1) used in this study were isolated from an acid mine drainage in China [19–21]. Each strain grew in basal salt media at 45 °C except that *L. ferriphilum* DX subcultured at 40 °C. *L. ferriphilum* DX grew at an initial pH of 1.6 and supplemented with $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (44.7 g/L); *A. caldus* DX was maintained with an initial pH of 2.0 and added to S^0 (10 g/L); *S. thermosulfidooxidans* ST was at an initial pH of 1.6 and supplemented with $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (44.7 g/L) and 0.02% (wt/vol) yeast extract; and *F. thermophilum* L1 grew in basal salt medium at an initial pH of 1.0 and added with $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (44.7 g/L) and 0.01% (wt/vol) yeast extract. All strains were subcultured every 15 days and maintained as active cultures in basal salt medium at our laboratory. The basal salt medium contained the following ingredients (g/L): $(\text{NH}_4)_2\text{SO}_4$ (3), K_2HPO_4 (0.5), KCl (0.1), Ca $(\text{NO}_3)_2$ (0.01) and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.5). Four strains were collected by centrifuging at $10,000 \times g$ for 15 min when the bacterial density of a culture reached $1\text{--}2 \times 10^8$ cells/mL. Finally, equal bacterial density of each of the aforementioned four strains were pooled, resulting in mixed moderate thermophiles in subsequent experiments.

2.3. Bioleaching Experiments and Scanning Electron Microscope (SEM) Analysis

Bioleaching experiments were carried out in 250 mL of shake flasks containing 100 mL sterilized basal salt medium. Each of four carbon materials was mixed with complex copper ore samples (10%, wt/wt) in a porcelain mortar, respectively, and pestle for 5 min before added into each bioleaching systems. The pulp densities of complex copper ores and carbon materials were 2% and 0.2% (wt/vol), respectively. The initial solution pH was 1.8 adjusted by sulfuric acid solution (1:1, vol/vol). The initial bacterial density was 4.4×10^7 cells/mL. Shake flasks were incubated in a rotary shaker at 175 rpm and constant temperature of 45 °C. Biotic control experiment (Ore + B, B represented moderate thermophiles) was performed in parallel without the addition of carbon materials. The chemical leaching (abiotic control) experiments (Ore, Ore + C2, Ore + C400, Ore + C800 and Ore + C1200) were also conducted without adding bacteria and/or carbon materials. The water evaporation was compensated for with sterilized distilled water every two days. The losses due to samples collected for the physicochemical analysis of solution at regular intervals were compensated for with sterilized fresh basal salt medium. The experiments were run in triplicate.

Scanning electron microscope (SEM) analysis (Quanta-200, FEI Instruments Co., Eindhoven, The Netherlands) was performed on the 5th day of bioleaching experiments to explore the adsorbing capacity of carbon materials on microbes. Samples (5 mL, including the bioleached residues and

solution) from the bioleaching systems were collected. The bioleached residues were separated via the centrifugation at $3000 \times g$ for 1 min, then vacuum freeze-dried and used for the SEM analysis.

2.4. Physicochemical Analysis

The total iron and copper concentrations in solution during complex copper ores bioleaching were determined by ICP-OES. The ferrous iron concentration was measured with the phenanthroline spectrophotometry method, while ferric iron concentration marked the difference between the concentrations of total iron and ferrous iron. The solution pH values were determined with a digital pH meter (PHS-3C, Leici Analytical Instruments Co., Ltd., Shanghai, China), and redox potentials (ORP) were measured by a platinum electrode with an Ag/AgCl electrode as reference (BPH-221, Bell Analytical Instruments Co., Ltd., Dalian, China). The bacterial density of the solution was monitored by direct counting using a hemocytometer with an optical microscope (BX41, Olympus Instruments, Tokyo, Japan). Residue samples were collected and dried at regular intervals as the bioleaching process continued for copper phase analysis.

2.5. Microbial Community Dynamic Analysis

2.5.1. Total Genomic DNA Extraction

Bioleaching experiments were shut down after 15 days. The microorganisms from the biotic control and $1200 \text{ m}^2/\text{g}$ activated carbon added groups on days 4, 8, 12 and 15 were withdrawn. Samples (50 mL, containing residue and solution) in two bioleaching groups were harvested by centrifugation at $10,000 \times g$ for 15 min. The total genomic DNA was extracted using the E.Z.N.A. Soil DNA kit (Omega Bio-Tek Inc., Newark, NJ, USA). In addition, genomic DNA of the initial mixed moderate thermophiles was extracted using the TIANamp Bacteria DNA kit (Tiangen Biotech Co., Ltd., Beijing, China) in accordance with the manufacturer's instructions. The crude genomic DNA was purified by 1.0% (wt/vol) agarose gel and stored at -20°C until it was used. The experiments were run in triplicate.

2.5.2. Polymerase Chain Reaction (PCR) Amplification and Sequence Analysis

Two primers of F515 (5'-GTGCCAGCMGCCGCGGTAA-3') and R806 (5'-GGACTACHVGGG TWTCTAAT-3') were used to amplify the V4 hypervariable region of the bacterial and archaeal 16S rRNA gene fragments. Polymerase chain reaction (PCR) systems and procedures were as described by Hao et al. [22]. Sequencing process was carried out on Illumina MiSeq sequencing platform. The MiSeq 500 cycles kit was applied for 2×250 bp paired-ends sequencing on MiSeq machine. Raw sequences resulted from the Illumina MiSeq sequencing run were processed according to the galaxy pipeline on our local server [23]. The detailed process of Illumina sequencing data analysis was as referred to by [24].

3. Results and Discussion

3.1. Effect of Carbon Materials with Different Specific Surface Area (SSA) on Copper Extractions

The leaching rates of Cu with time from complex copper ores in the presence of different carbon materials were shown in Figure 1. In this study, the electrical conductivity of graphite (0.6 S/m) was much higher than the other three activated carbons (0.1 S/m) (Table 1), but the copper extractions were significantly enhanced by activated carbons instead of graphite, which suggested that SSA was the key factor affecting Cu leaching efficiency.

The copper extractions improved with the increase in SSA of carbon materials both in chemical leaching and bioleaching experiments. In abiotic control experiments, without the addition of the mixed moderate thermophiles, the final copper extractions were 22.4% (Ore), 32.5% (Ore + C2), 37.9% (Ore + C400), 40.9% (Ore + C800) and 49.5% (Ore + C1200) on day 15, respectively. However,

in bioleaching experiments, the copper extractions reached up to 68.1% (Ore + B), 77.2% (Ore + B + C2), 85.7% (Ore + B + C400), 95.2% (Ore + B + C800) and 98.8% (Ore + B + C1200) within 15 days of the bioleaching operation. The copper yields in Ore + C1200 and Ore + B + C1200 groups increased 27.1% and 30.7% compared to Ore and Ore + B, respectively. The results showed that the presence of C1200 clearly enhanced copper extractions, which was in accordance with previous studies [15–17]. They indicated that the enhanced dissolution rates of copper ores could mainly be attributed to the galvanic interaction between activated carbon and sulfide ores. On the other hand, the copper yields in Ore + B and Ore + B + C1200 groups increased 45.7% and 49.4% compared to Ore and Ore + C1200, demonstrating that the presence of bioleaching bacteria with the assistance of C1200 played an important role in the Cu recovery [25].

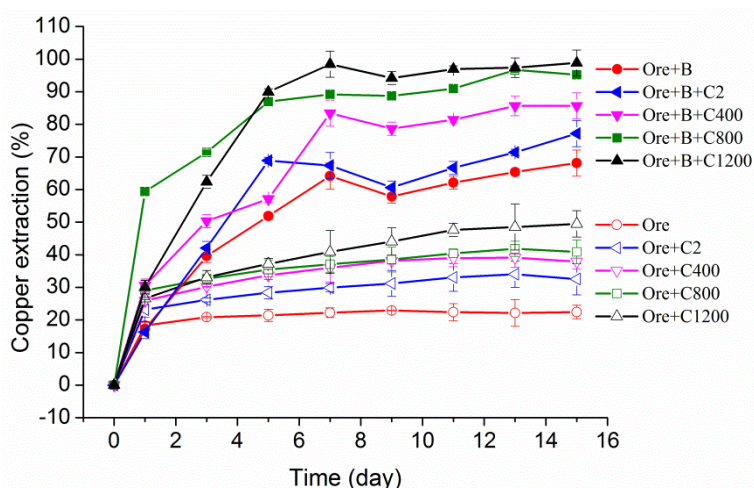


Figure 1. Variation of copper extractions during complex copper ores bioleaching process in the presence of carbon materials with different specific surface area by mixed moderate thermophiles. B represents the mixed moderate thermophiles.

3.2. Effect of Carbon Materials with Different SSA on Leaching Parameters

The changes in pH, ORP values, ferric and ferrous iron ratios and bacterial density in solution with time in the presence of different carbon materials were presented in Figure 2. In the bioleaching run, the solution pH values were lower than those of chemical leaching most of the time (Figure 2a). In the bioleaching groups having inoculum, the mixed moderately thermophiles (*A. caldus* DX and *S. thermosulfidooxidans* ST) were able to oxidize S^0 and reduced inorganic sulfur compounds (RISCs) derived from complex copper ores resulting in the decrease in pH even in the presence of carbon materials. Interestingly, the solution pH increased with the increase in SSA of carbon materials in chemical and bioleaching experiments. This was because the pH values were integrated results of acid consumption and acid generation during the leaching process. Carbon materials with high SSA promoted the dissolution of complex copper ores, and more alkaline substances (e.g., alkaline oxide and acid oxide) were released and neutralized by protons leading to the high solution pH.

As shown in Figure 2b, the carbon materials with different SSA induced different solution ORP values. In the bioleaching operation, the redox potentials increased sharply in 5 days then were stable in the subsequent process. The redox potentials were higher in Ore + B, Ore + C2 + B and Ore + C400 + B groups maintaining at around 610–650 mV than approximately 510 mV in Ore + C800 + B and Ore + C1200 + B groups. The ORP values of the abiotic groups were lower than those of corresponding bioleaching runs except the Ore + C800 group. The iron-oxidizing microbes (*L. ferriphilum* DX, *S. thermosulfidooxidans* ST and *F. thermophilum* L1) in mixed moderate thermophiles could oxidise ferrous iron into ferric iron resulting in high solution ORP values, and facilitated Cu bioleaching. In the chemical leaching operation, the ORP values were low in Ore, Ore + C2 and

Ore + C1200 groups maintaining around at 350 mV. Notably, the Ore + C400 and Ore + C800 groups stayed at the similar ORP values of about 500–550 mV with Ore + B + C800 and Ore + B + C1200 on day 15. However, the extremely high copper extractions were only obtained in Ore + B + C800 and Ore + B + C1200. It was indicated that the high SSA of activated carbon-aided bioleaching could accelerate the dissolution of complex copper ores at the appropriate ORP values. The redox potential of leaching solution was a dominating factor influencing the dissolution of sulfide, especially for chalcopyrite. Former research had proved that sulfide minerals dissolution could be intensified by controlling redox potential at relatively low values [26,27]. Third et al. designed a computer-controlled reactor by arresting the air supply to the reactor when the solution ORP was greater than a designated set point. They found that leaching at a low redox potential of 380 mV–420 mV (vs. Ag/AgCl) achieved high copper recoveries. Zhao et al. also showed that bornite could decrease the solution ORP and maintained it at an appropriate range (380–480 mV vs. Ag/AgCl) to promote chalcopyrite dissolution. In this study, C800 and C1200 caused relatively low solution ORP (500 mV) compared with the other three bioleaching groups (600–650 mV) facilitating the increase of copper extractions.

The ferric and ferrous iron concentration ratios in the bioleaching run were identified in Figure 2c. This shows the ratios increased with the rise in SSA of carbon materials indicating high SSA improved the oxidation rate of ferrous iron. Ferric ion acted as an efficient oxidant and was found to be beneficial to dissolve sulfide ore in both chemical leaching and bioleaching [28]. A high ferric and ferrous iron concentration ratio in Ore + B + C1200 resulted in high copper extraction but not the highest solution ORP, which suggested the addition of C1200 could influence the trend of redox potentials.

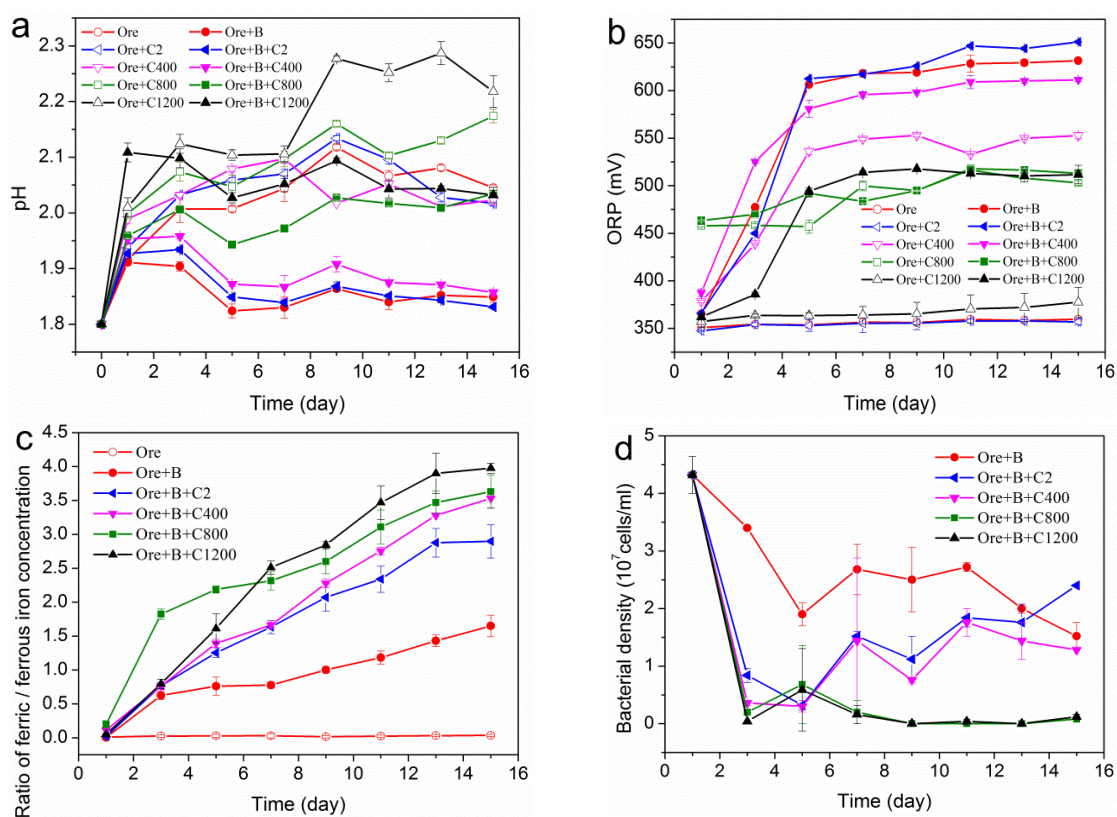


Figure 2. Variations of (a) pH, (b) redox potential, (c) ratio of ferric and ferrous iron and (d) bacterial density in solution during complex copper ores bioleaching in presence of carbon materials with different specific surface area by mixed moderate thermophiles. B represented the mixed moderate thermophiles.

The variation of bacterial density in solution was shown in Figure 2d. The bacterial density declined sharply in the first 3 days. It descended with increasing SSA of carbon materials. The bacterial density dropped from 4.4×10^7 to 1.9×10^7 (Ore + B), 3.2×10^6 (Ore + B + C2), 3.0×10^6 (Ore + B + C400), 6.8×10^6 (Ore + B + C800) and 5.9×10^6 cells/mL (Ore + B + C1200) on 5th day of the bioleaching run. It showed a slight growth from the 5th to 7th days and then kept relatively stable in solution of Ore + B, Ore + B + C2 and Ore + B + C400. However, there was no obvious growth of microbes in the solution with 800 m²/g or 1200 m²/g activated carbon, and bacterial density was near zero since the bioleaching process began. The results might indicate that activated carbon with high SSA had a strong ability to adsorb bioleaching microbes, and the decrease of bacterial density in solution could not decrease the final copper extractions.

3.3. Adsorption Behaviors of Bioleaching Microbes onto Complex Copper Ores and Carbon Materials

SSA played key roles in bacterial attachment and influenced the reaction between the bacteria and the ores. The adsorption feature of mixed moderate thermophiles onto the matrix (complex copper ores and carbon materials) surfaces on the 5th day of bioleaching was performed by SEM (Figure 3a–e). Compared with Ore + B, the amount of bacteria adsorbed onto the carbon materials improved with the rise in SSA resulting in low bacterial density in solution (Figure 2d) suggesting the high SSA could not inhibit the bacterial growth.

Microbial colonization and subsequent biofilm formation on the solid surface were prerequisite and important in sulfide minerals bioleaching. The amount of attached bacteria and the adsorption rates were significantly related to the corresponding metal dissolution rates [29]. Dong et al. indicated that the bacterial adsorption quantity (*A.t ferrooxidans* LD-1) onto the five copper sulfide minerals had the same order as that of copper extractions (djurleite > bornite > pyritic chalcocopyrite > covellite > porphyry chalcocopyrite). Biosorption depended on both the biochemical properties of the bacteria and the interfacial properties of the solids [30]. High surface area, a microporous structure and high degree of surface reactivity, make activated carbons versatile adsorbents for both microbe and low copper ores [31]. Attached bacteria produced extracellular polymeric substances (EPS) and formed a biofilm on the ore interface. The biofilm created many discrete and protective microenvironments against a possibly infertile bulk-solution composition or other dynamic forces such as solution flows or abrasion, and facilitated the dissolution of minerals mediated by acidophiles [32].

Former research demonstrated the adsorption by activated carbon would probably decrease the attachment of microbes onto the ore surface [33]. It was also generally accepted that the adsorption quantity of cells onto the mineral surface was significant for the sulfide ores dissolution in the initial leaching process [34]. However, such a negative effect was inessential because the catalytic effect of activated carbon played a dominant role. Figure 1 showed that the copper extractions of bioleaching in 800 m²/g and 1200 m²/g activated carbon added groups were the highest although low bacterial density in solution was observed. The presence of bioleaching bacteria in biofilm enhanced the electron transfer and galvanic interactions between the activated carbon of high SSA and the ores, and further improved copper extraction efficiency [35].

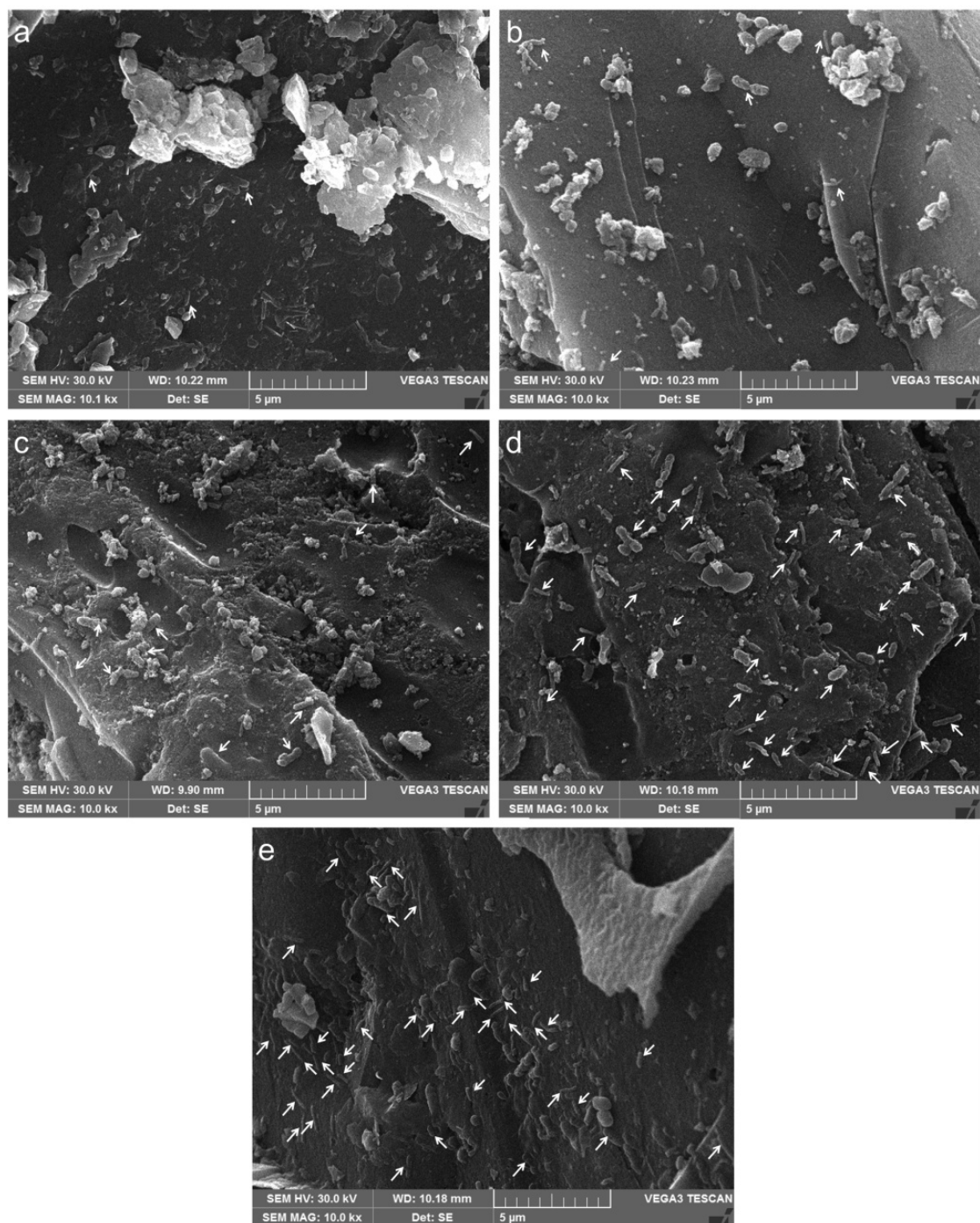


Figure 3. Scanning electron microscope (SEM) images of microbial adsorption onto solid surfaces of (a) complex copper ores, carbon materials with specific surface area of (b) $2 \text{ m}^2/\text{g}$, (c) $400 \text{ m}^2/\text{g}$, (d) $800 \text{ m}^2/\text{g}$ and (e) $1200 \text{ m}^2/\text{g}$ on the 5th day of bioleaching experiments. The white arrows represented the bioleaching microbes.

3.4. The Copper Phase Analysis of Bioleached Residues

The copper phase analysis of raw ores and bioleached residues collected on days 4, 8, 12 and 15 during the bioleaching process in Ore + B and Ore + B + C1200 groups was performed (Figure 4). In Ore + B group, primary copper sulfide (PCS) could hardly be dissolved, and its copper recovery rate was only 6.0% in 15 days. However, the PCS bioleaching rates in Ore + B + C1200 group were 15.8%,

37.8%, 44.8% and 62.7% on days 4, 8, 12 and 15, respectively. The results indicated that the addition of activated carbon with high SSA significantly facilitated the dissolution of PCS. In addition, free copper oxide (FCO) and secondary copper sulfide (SCS) obtained higher extraction rates, which reached up to 97.1% and 97.7% in Ore + B group, and 98.3% and 98.1% in Ore + B + C1200 group on the 15th day. It showed that activated carbon made no difference on FCO and SCS bioleaching in this study. However, lower quantities of combined copper oxide (CCO) were dissolved, and the leaching rates were just 10% and 22.5% in the two experimental groups.

Copper phase analysis showed that, in Ore + B + C1200 group, the activated carbon dramatically enhanced the PCS (chalcopyrite) recovery rate compared with Ore + B. As shown in Figure 2b, the ORP values in Ore + B + C1200 were significantly lower than Ore + B from the 5th day of bioleaching. Previous studies reported that low redox potential was in favor of the chalcopyrite bioleaching with the assistance of bioleaching bacteria [36,37]. The passivation layer hindering bioleaching performance, which was considered to mainly consist of disulfide (S_2^{2-}), polysulfide (S_n^{2-}), elemental sulfur (S^0) and jarosite, rarely formed at low redox potential value [38–40]. In addition, high redox potentials also caused the rapid deterioration of ion diffusion performance of EPS and readily formed the passivation layer [41,42]. Therefore, many efforts have been made to control the redox potential at an appropriate range to improve chalcopyrite bioleaching efficiency [43]. Hiroyoshi et al. [44] proposed the two-step dissolution model of chalcopyrite in acid solution. Chalcopyrite was firstly reduced to the secondary mineral chalcocite (Cu_2S) by ferrous iron at low potential, then chalcocite was oxidized by ferric iron and/or dissolved oxygen to copper ion and elemental sulfur. The presence of activated carbon could change the transition path of electrons through galvanic interaction at a low redox potential, and the functional group (e.g., carboxyl) that existed on activated carbon surfaces could improve the hydrophilicity of ores and electron transfer from electrodes to bacteria, finally enhancing the copper dissolution [35].

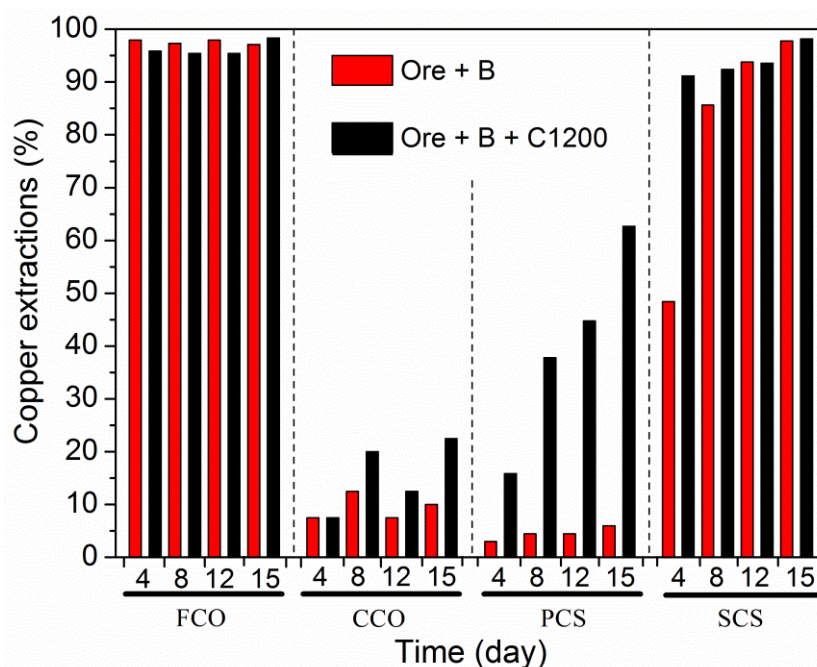


Figure 4. Variation in copper extractions of free copper oxide (FCO), combined copper oxide (CCO), primary copper sulfide (PCS) and secondary copper sulfide (SCS) in bioleached residues compared with raw ores during bioleaching process in Ore + B and Ore + B + C1200 groups by mixed moderately thermophiles.

3.5. Microbial Community Dynamics Analysis

The microbial community dynamics change in the bioleaching system was important for improving the dissolution of sulfide minerals. Therefore, microbial community succession on days 4, 8, 12 and 15 during bioleaching in Ore + B and Ore + B + C1200 groups was characterized. As shown in Figure 5, *L. ferriphilum* DX, *A. caldus* DX, *S. thermosulfidooxidans* ST and *F. thermophilum* L1 were 24.3%, 26.1%, 26.5% and 23.2% in initial inoculum, respectively. Compared with the relative percentages of each species in the two bioleaching systems, the addition of activated carbon obviously affected the microbial community composition.

In Ore + B group, *L. ferriphilum* DX accounted for 18.8% and 16.5% of the cultures on days 4 and 8, then increased to 21.6% and 20.2% on the 12th and 15th days. The proportion of *A. caldus* DX decreased gradually as the bioleaching process continued, representing just 10.7% on day 15. The percentage of *S. thermosulfidooxidans* ST increased and became the dominant species through the whole bioleaching stages, accounting for 56.2% (day 4), 64.6% (day 8), 61.1% (day 12) and 69.0% (day 15) of the cultures, respectively. However, *S. thermosulfidooxidans* ST were the predominant species and accounted for at least 98% in the bioleaching process of Ore + B + C1200 group. Activated carbon might reduce the numbers of *ferriphilum* DX and *A. caldus* DX in bioleaching systems. The percentage of the two species accounted for just under 2%, and *F. thermophilum* L1 was not detected in two bioleaching systems.

The addition of activated carbon (1200 m²/g) had great effects on microbial succession and mineral dissolution during complex copper ores bioleaching. *S. thermosulfidooxidans* ST could adapt to the variant leaching environments including the addition of activated carbon much more than the other three microbes. One possible reason was the growth energy. Compared with the sulfur-oxidizer *A. caldus* DX and the iron-oxidizer *L. ferriphilum* DX, both ferrous iron and elemental sulfur could be utilized by *S. thermosulfidooxidans* ST. Therefore, *S. thermosulfidooxidans* ST gained a competitive advantage and dominated the microbial community in two bioleaching groups. Another possible reason was *Sulfobacillus* spp. could adapt to adverse environments attributed to their ability to survive as endospores and grew with organic matter as an energy source [45]. However, organic matter that originated from exudates and cell lysates of microorganisms were absorbed by activated carbon, which had toxicity to autotrophs and inhibited the growth of them [46]. Thus, the versatility and resilience of *S. thermosulfidooxidans* ST made them function independent of *A. caldus* DX and *L. ferriphilum* DX and played an important role in the bioleaching system with the addition of activated carbon. Although *F. thermophilum* L1 also could use ferrous iron and organic matters as energy sources, its growth may be restrained by high solution pH in the two bioleaching groups.

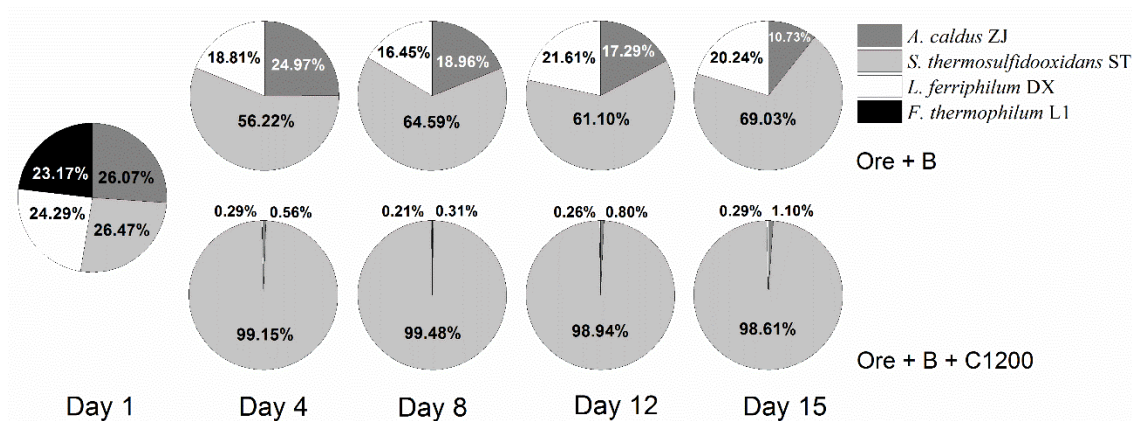


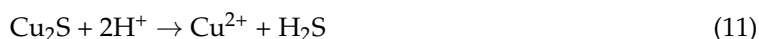
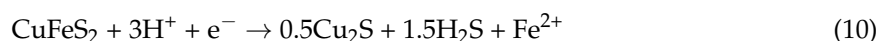
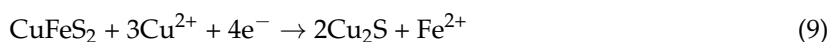
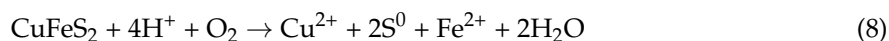
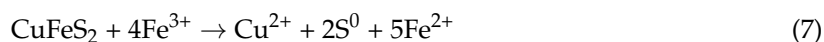
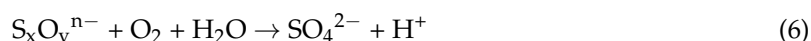
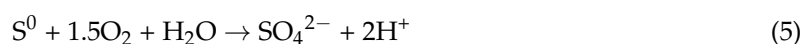
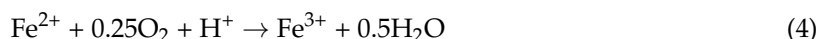
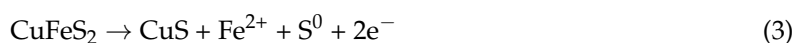
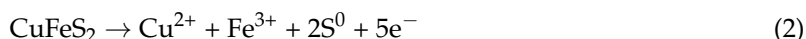
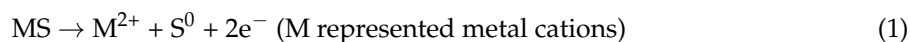
Figure 5. Microbial community dynamics change of the mixed moderate thermophiles during complex copper ores bioleaching process in Ore + B and Ore + B + C1200 groups.

In Ore + B group, *A. caldus* DX, which could use reduced inorganic sulfur compounds (RISCs) as energy sources, grew better than ferrous iron oxidizer *L. ferriphilum* DX before the 8th day. Elemental

sulfur and ferrous iron generated from the dissolution of copper ores provided energy sources and nutrients for the growth of microbes. Oxidation of elemental sulfur yielded considerably more energy than that of ferrous iron [47]. Furthermore, the highest pH values above 2.2 were out of optimal range for the growth of *L. ferriphilum* DX, but it benefited the growth of *A. caldus* DX [48]. All these factors resulted in a relatively high proportion of *A. caldus* DX in the initial bioleaching stage of Ore + B. Afterwards, the proportion of *A. caldus* DX showed a downward trend as bioleaching progressed, but the percentage of *L. ferriphilum* DX increased, which was in accordance with the rise of ferric and ferrous iron ratios (Figure 1b).

3.6. A Model for the Effects of Activated Carbon with High SSA on Complex Copper Ores Bioleaching

To interpret the effects of activated carbon with high SSA on complex copper ores' bioleaching using the mixed moderate thermophiles, a mechanism model was established (Figure 6). I: the addition of activated carbon constituted the galvanic interaction with complex copper ores, intensified the anodic oxidation of SCS [Equation (1)] and PCS [CuFeS₂, Equations (2) and (3)], resulting in more S⁰ and Fe²⁺ dissolved from copper ores; II: microorganisms with better adsorbed abilities, such as *S. thermosulfidooxidans* ST, adhered to the activated carbon surface and accompanied by the production of EPS formed biofilm creating favorable bioleaching microenvironments which were different from the situation in solution and could facilitate the sulfide ores' bioleaching process. During this period, released Fe²⁺ and S⁰ could be subsequently oxidized by iron oxidizer and sulfur oxidizer to generate ferric sulfate [Equation (4)] and sulfuric acid [Equations (5) and (6)]; III: PCS in ores was probably dissolved by ferric ion and proton attack in the presence of bacteria [Equations (7) and (8)], which was generally accepted as indirect mechanism [49]; IV: the addition of activated carbon with high SSA controlled the redox potential at an appropriate range for a longer period of time where PCS was supposed to be reduced to Cu₂S [Equations (9) and (10)], and further oxidized rapidly to Cu²⁺ as presented in Equation (11) [27,50], thus resulting in extremely high copper extraction of more than 98% in Ore + B + C1200.



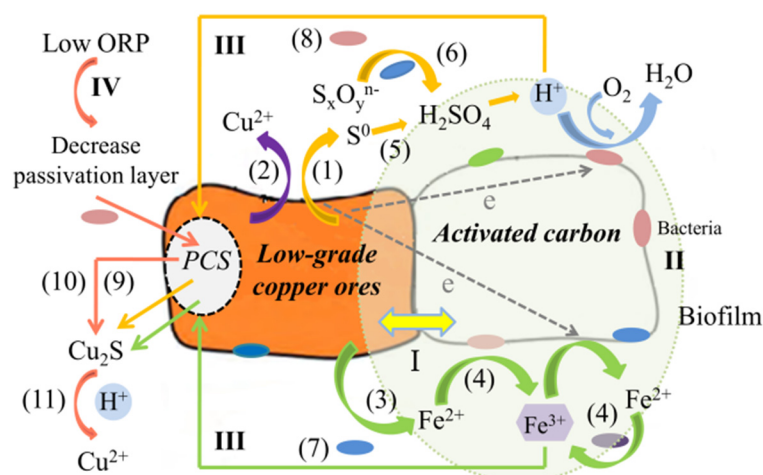


Figure 6. The model for complex copper ores' bioleaching coupled with activated carbon with high specific surface area in the presence of mixed moderate thermophiles. The numbers I–IV represent the beneficial or potential bioleaching mechanisms, and (1)–(11) represent the Equations (1)–(11).

4. Conclusions

Copper bioleaching efficiency from complex copper ores increased with the rise in SSA of carbon materials by mixed moderate thermophiles. The copper extraction was 98.8% in Ore + B + C1200, and improved by 30.7% and 76.4% compared with Ore + B and Ore groups. The addition of activated carbon (1200 m²/g) adsorbed large amount of bacteria, accelerated the oxidation rate of ferrous iron and maintained the solution ORP at relatively low values, and significantly improved PCS (chalcopyrite) dissolution (62.7%) compared to Ore + B group (6.0%). The microbial community dynamics changed greatly in response to the activated carbon (1200 m²/g) addition in the bioleaching system. *S. thermosulfidooxidans* ST gained a competitive advantage and overwhelming superiority. *S. thermosulfidooxidans* ST was absorbed by carbon material with high SSA and contributed to better galvanic interaction, biofilm formation, direct contact and lower potential values resulting in an extremely high copper extraction of complex copper ores. In the industrial bio-heap leaching operation, the carbon materials with high SSA and crushed ores can be premixed in appropriate proportions before heap construction. Moreover, the cheaper and easily-obtained carbon materials with high SSA should be searched for or produced, and utilized on an industrial scale.

Author Contributions: X.H., X.L., G.Q. and Y.L. conceived and designed the experiments; X.H. performed the experiments; X.H., H.L. and H.Y. analyzed the data; P.Z., and A.C. contributed reagents/materials/analysis tools; and X.H. wrote this paper.

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