Explore the recycling of bioleaching functional bacteria and sulfur substrate using the sulfur-covered biochar particles

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Research

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Abstract

Background Bioleaching has been attracting attention recent years as an emerging sediment heavy metal pollution remediation technology. However, the use of sulfur powder as sulfur substrate causes the problem of “post-acidification”, and the free bioleaching functional bacteria which are susceptible to environmental impact during reactor operation cannot be used efficiently for multiple rounds. These problems can be solved if the sulfur substrate and the bioleaching functional bacteria can be recycled simultaneously after bioleaching. A new kind of sulfur substrate, the laboratory-made sulfur-covered biochar particles, were used in the bioleaching experiment, compared with sulfur powder and sulfur powder mixed with the surfactant rhamnolipid. Results The sulfur-covered biochar particles exhibited superior bioleaching performance, including faster acidification rate, SO_4^{2-} production rate and heavy metal bioleaching rate, and higher heavy metal solubilization percentage (Ni 33.76%; Cu 66.16%; Zn 65.494%), which was resulted from the acceleration of bioleaching reaction by the bioleaching functional bacteria immobilized on the biochar surface. Otherwise, the sulfur-covered biochar particles could be reused in the second round, and the heavy metal solubilization percentage (Ni 32.84%, Cu 69.93%, Zn 67.17%) was comparable with that of the first round. Nevertheless, the sulfur content became the main limiting factor causing poor bioleaching performance during the third round. The sulfur mixed with the surfactant rhamnolipid did not show significant effect in promoting acidification and heavy metal solubilization due to high levels of organic matter and the impact of the low pH value. Conclusion The research indicated the laboratory-made sulfur-covered biochar particles could realize the dual immobilization of the bioleaching functional bacteria and the sulfur substrate to support their recycling and reuse in the second bioleaching round. In the future research, the way to maintain the reuse of the sulfur-covered biochar particle for more rounds will be explored.

Background

In order to ensure the normal traffic of waterways and the safety of water quality, regular dredging work is inevitable. However, the heavy metal content of the dredged sediment often exceeds the environmental quality standard due to human activities. The heavy metal pollution remediation of the sediment is urgent. Compared to chemical methods, such as the direct use of mineral acids or chelating agents [1-3], bioleaching as an emerging sediment heavy metal pollution remediation technology has the advantages of environmentally friendly, energy-saving and low cost [4]. Bioleaching is a biological remediation technology utilizing acidophilus autotrophic bacteria including *At.ferrooxidans*, *At.thiooxidans*, *Thiobacillus thioparas*, *Leptospirillum ferrooxidans*, *Thiobacillus thioparas* etc. to perform bioleaching reaction [5]. These bioleaching functional bacteria can oxidize sulfur, reduced sulfur compound or/and ferrous ions to produce sulfuric acid and ferric ions, which have strong leaching ability [6] and can solubilize metals in their reduced forms and those associated with acid-soluble ores [7].

However, there are some problems in bioleaching technology that hinder its development in engineering applications.

On the one hand, the most common used sulfur substrate in the bioleaching is sulfur powder, because it has a large specific surface area and can facilitate the adsorption and the growth of bacteria on the substrate to enhance the oxidation rate of sulfur [8]. However, the sulfur powder has high hydrophobicity and it is difficult to disperse in the liquid phase. Only 40-60% of the sulfur powder can be utilized in the bioleaching process in general [9]. The sulfur powder remained in the sediment increases the operational cost and can cause the problem of “post-acidification”, which complicates the subsequent disposal of the sediment. The general solution is to add supplementary surfactant or to replace the sulfur powder with bio-sulfur [10-12]. Nevertheless, the addition of the industrial chemical surfactant might influence the growth of the bacteria and can cause secondary pollution. The bio-surfactant costs high and is not suitable for large-scale engineering applications; while the bio-sulfur has the disadvantages of limited sources, easy formation of colloidal solution.

On the other hand, in the microbial engineering application, the free bacteria in the reactor often underperform due to not being well colonized and being susceptible to environmental fluctuations [13]. This problem can be solved utilizing microorganisms immobilization technology. The technology immobilized specific microorganisms on a carrier, restricting or positioning them in a certain area, so the microorganisms can maintain a high density and high biological activity, and can proliferate quickly [14]. This technology has the advantages of less microorganisms loss, strong toxicity resistance, and reusable microorganisms [15, 16]. The choice of the carrier material is crucial to this technology. A proper carrier can not only increase the number and the activity of the microorganisms, but also achieve efficient recycle of the microorganisms.

These two problems can be solved at the same time if a proper carrier can be utilized to immobilize sulfur substrate and bioleaching functional bacteria, because the residual sulfur substrate and the active bioleaching bacteria can be reused for multiple times by recycling the carrier.

In order to overcome the “post-acidification” problem and simplify the steps of the repetitive addition of bioleaching inoculum during the operation of the bioleaching sequencing batch reactor, the research selected bamboo biochar as the carrier to produce the recyclable sulfur-covered biochar particles by solidifying melted elemental sulfur on the surface of the bamboo biochar. The research investigated the bioleaching effect of the sulfur-covered biochar particles compared with the sulfur powder and the sulfur powder mixed with the surfactant rhamnolipid. The research also explored the potential and the mechanism of the immobilization of the bioleaching functional bacteria on the sulfur-covered biochar particles and their recycling use. The research also explored the change in the structure of microflora during the bioleaching process using the integrated high-throughput absolute abundance quantification (iHAAQ) technology [17].

Materials And Methods

Properties of sediment

The sediment sample selected in this study was from Puti Lake, Jiaxing, Zhejiang Province, China (E 120.73°, N 30.95°). The grab dredger was used to collect the sediment sample from the bottom layer within 0-30 cm. The coarse suspended matter was removed using a 20 mesh screen. The physical and chemical properties of the sediment were measured as follows: pH 7.29, Eh -159.83 mv, total nitrogen 3.04 g/kg, total carbon 31.11 g/kg, acid volatile sulfur (AVS) 2.20 mg/kg and total solid 22.10%. The concentrations of various heavy metals were as follows: Ni 84 mg/kg, Cu 284 mg/kg, Zn 394 mg/kg. The heavy metal concentrations of the sediment were analyzed using the method mentioned in Analysis of heavy metal contents after the acid digestion of the sediment.

Properties of sulfur substrate

The sulfur substrate used in the research included sulfur-covered biochar particles, sulfur powder and sulfur powder mixed with surfactant rhamnolipid (see Table 1 for details). The sulfur-covered biochar particles were prepared by the laboratory: The bamboo biochar was purchased from Lin'an Yaoshi Biochar Industry Co., Ltd. Bamboo sawdust was anaerobic-burned at 500 °C for 8h, and the bamboo biochar particles were sieved with a mortar mill to obtain the particle size required for the research. After that, the bamboo biochar particles were washed three times with distilled water, and dried at 105 °C for 6h. The surface area of the prepared bamboo biochar particles was 332.10 m²·g⁻¹. The main components included C (56.05 %), H (1.32 %), N (0.23 %), O (2.62 %), ash (39.78 %), P (0.29 %) and Na (0.01 %).

And the sulfur-covered biochar particles were produced by solidifying melted elemental sulfur on the surface of the bamboo biochar at 120 °C. After cooling down, the weight of the attached melted sulfur on the bamboo biochar particles was measured in order to control the same sulfur content in each experiment group.

Table 1 The properties of the different sulfur substrates

Preparation of the sludge-enriched inoculum

The inoculum used in this experiment was obtained from the acclimation of the sludge indigenous bacterium in Hangzhou Qige Wastewater Treatment Plant. And the preparation methods were consistent with the previous research [18].

Bioleaching experiment

Three experiment groups were set up according to the sulfur substrate added: sulfur powder group (experimental code: S-A), sulfur-covered biochar particles group (experimental code: SC-A), sulfur powder mixed with rhamnolipid (experimental code: R-A). The bioleaching experiments were conducted in a 250 mL conical flask containing 2.5 g dry weight of sediment, 150 mL of distilled water (the sediment concentration was decided by pre-experiment), 3 g/L of sulfur substrate (calculated by sulfur content) and 3 mL of sludge-enriched inoculum (-A represented the addition of the inoculum). Control groups

Code	Sulfur substrate	Sulfur content	Particle size	Source	Note
S	sulfur powder	≥ 99.5%	200-300 μm	Shijiazhuang Jiyanzhengnong corporation	/
SC	sulfur-covered biochar particles	70%	irregular particles with size of about 5 mm	prepared by the laboratory	prepared by solidifying melted elemental sulfur on the surface of the bamboo biochar
R	sulfur powder mixed with rhamnolipid	≥ 99.5%	200-300 μm	VICTEX corporation	rhamnolipid surfactant is secreted by <i>Pseudomonas</i> . The added concentration in the research was 0.3 g/L [10]
C	none	/	/	/	control check group, no sulfur substrate added
N	none	/	/	/	sterilization treatment group, added 200 mg/L NaN ₃

without sulfur substrate and sterilization groups without sulfur substrate but with 200 mg/L NaN₃ were set at the same time (the experimental code: C/N).

The conical flasks were placed in a shaking incubator at 28 °C and 180 r/min. Each treatment consisted of nine parallel groups, three of which were used to measure pH, concentration of SO₄²⁻ and heavy metal on a daily basis. Distilled water was added daily to compensate for the loss of vaporization. The supernatant was withdrawn daily and the concentration of SO₄²⁻ and heavy metals was analyzed. The other six parallel groups were tested for microbiological analysis on Day 4 and Day 9, respectively.

When it came to Day 9, the Tessier sequential extraction method was employed to determine the content of different heavy metal forms in the solid phase after bioleaching [19]. The forms of heavy metals were represented as follows in Fig. 2: Res=residual state; Org=organic state; Fe-Mn=iron-manganese oxidation state; Car=carbonate-bound state; Exc=exchangeable state.

After the first round, the sulfur-covered biochar particles of the SC-A group were recovered by filtration and washed with sterile physiological saline 3 times before being used for the second round of bioleaching. The experimental conditions of the second round were the same as those of the first round, but no bioleaching functional bacteria or sulfur substrate were added in all experimental groups. The third round of bioleaching experiment was carried out by the same experimental method. The pH value,

concentration of SO_4^{2-} and heavy metals of the samples were measured every day to investigate the bioleaching effect of the sulfur-covered biochar particles in the multiple recycling rounds.

Analysis method

The pH value was measured using pH meter (PB-10) using the National Standard Method HJ 962-2018. The concentration of SO_4^{2-} was detected by ion chromatography (ICS-1100); total nitrogen, total carbon, and total phosphorus of the sediment sample were determined using an elemental analyzer (Elementar vario MAX CNS). The specific surface area of the biochar samples were measured using the American Tristar III3020 automatic specific surface area. The BET (Brunauer–Emmerr–Teller) equation was used to calculate the surface area of the bamboo biochar particles [20]. The EA110 elemental analyzer was utilized to determine the percentages of elements C, H, and N in the biochar samples [21]. Field emission scanning electron microscope (FEI SIRION-100) was utilized to observe the surface structure and the colonization of the microorganisms on the sulfur-covered biochar particles.

Analysis of heavy metal content

The heavy metal concentration of the sample was detected by an inductively coupled plasma mass spectrometer (ICP-MS) (PQMS 10-5000S-AR091). The limit of detection (LOD) of the ICP-MS is 1ppb, the accuracy was < 5%, and the relative standard deviation (RSD) was < 5%. To control the analytical quality of the analytical procedure, a certified reference material (GBW-07405) was applied and analyzed following the same procedure. The recoveries of Ni, Cu and Zn were in the range of 74.3%~113.3% (n=3). The recoveries of heavy metals during the sequential extraction process were in the ranges of 79.08~117.92%, 76.16~107.94% and 74.93~113.62%, respectively. The method to calculate the recoveries of heavy metal during the sequential extraction process was written in the Supplementary Material. These results indicated that our methods were reliable and precise enough for the purposes of this study.

Microbiological analysis

Using the iHAAQ methodology proposed by Lou et al., the qPCR analyses were performed to quantify the specific genes of the extracted DNA with three replicates using a StepOnePlus™ Real-Time PCR System instrument (Applied Biosystems, Foster City, CA, USA). The high-throughput sequencing was performed by using Illumina Miseq platform following standard protocols. The quality control of raw sequencing reads was performed using QIIME software (version 1.7.0). The absolute abundance of each genus level of the top 30 most abundant microorganisms in the sediment was calculated by multiplying the total abundance of bacteria (the copy number of the 16S rRNA gene in the V4 region measured by qPCR) and the corresponding relative abundance obtained by high-throughput sequencing [17].

Data analysis

The mathematical calculations involved in the study were done using Matlab 2017 software. The chart making and function curve fitting involved in the research were completed using Origin 8.0 software. The correlation analysis was performed using SPSS V22.0 software.

Heavy metal bioleaching curves were fitted using logistic equations [22]: (see Equation 1 in the Supplementary Files)

In the formula, M is the concentration of heavy metal (mg/kg) in the liquid phase; M_{limit} is the upper limit bioleaching concentration of heavy metal (mg/kg); t is the bioleaching time; x , B , and p are constants.

We assumed that when $M = 95\%M_{\text{limit}}$, the bioleaching is finished. Then based on equation (1), $T_{95\%}$ could be calculated using equation (2): (see Equation 2 in the Supplementary Files)

Using equation (1) to further determine the derivative of the time t and the bioleaching time $T_{V_{\text{max}}}$ (day), at which the maximum bioleaching rate V_{max} ($\text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$) is reached, can be obtained.

Results

Bioleaching performance of sulfur-covered biochar particles

There was no significant difference in acidification rate within the experiment groups after the addition of the sludge-enriched inoculum. The SC-A group achieved the fastest acidification rate (pH reached 2.35 on the Day 5) and the lowest pH value (pH reached 1.80 on the Day 9) (Fig. 1). Although the specific surface area of the sulfur powder was much larger than that of the sulfur-covered biochar particles, the acidification rate of the S-A group was slightly slower than that in the SC-A group (pH reached 2.44 on the Day 6). In the R-A group, rhamnolipid was added as a surfactant to make the sulfur powder more easily dispersed in the liquid phase, but its acidification rate was the slowest (pH reached 2.36 on the Day 8). In order to investigate the acidification performance of the indigenous microorganisms in the sediment, the C group and the N group were set. When no sulfur substrate was added (the C group), the presence of indigenous microorganisms in the sediment also kept the system acidic (pH was about 5.5), while the pH of the N group remained at around 7.

The shorter the time bioleaching finished, the faster the total bioleaching rate. The SC-A group with the fastest acidification rate and the shortest the bioleaching time had the fastest total bioleaching rate (Table 2), and it also reached the highest heavy metal solubilization (Ni 33.76%; Cu 66.16%; Zn 65.49%). While the total bioleaching rate of the R-A group was slower than those of the S-A group and the SC-A group, these experiment groups with inoculum added all reached almost the same heavy metal solubilization at the end of the bioleaching experiment (Fig.2).

Table 2 Parameters of heavy metal curve fitting when different sulfur substrate used

	Code	M _{limit}	x	p	B	T _{Vmax} (day)	V _{max} (mg·kg ⁻¹ ·day ⁻¹)	T _{95%} [day]	R ²
Ni	S-A	30.06	3.073	2.501	32.14	2.188	7.708	10.25	0.9618
	SC-A	29.87	2.939	2.772	31.41	2.238	8.461	8.675	0.9755
	R-A	39.62	5.592	1.897	41.45	3.197	4.698	26.99	0.9809
Cu	S-A	193.5	3.850	4.890	197.2	3.537	65.31	7.06	0.9939
	SC-A	195.2	3.627	4.351	200.4	3.271	73.87	6.27	0.9882
	R-A	214.5	6.020	5.485	215.0	5.692	50.59	10.30	0.9974
Zn	S-A	237.8	2.952	4.117	245.3	2.616	90.78	6.08	0.9800
	SC-A	247.2	2.833	4.455	252.3	2.555	104.35	5.50	0.9882
	R-A	298.6	5.088	3.185	303.7	4.147	52.53	12.92	0.9829

For the three heavy metals studied, the heavy metal solubilization percentage was in decreasing order: Cu>Zn>Ni (Fig. S1), Zn and Cu were likely to be leached while Ni was least likely to be leached, which was similar to previous studies [23, 24].

The logistic formula (1) can fit the heavy metal bioleaching curve very well (see Table 2 for detail parameters of curve fitting). Formula (2) can be used to calculate the time required for each sulfur substrate to reach the bioleaching end point. Formula (1) can be used to obtain V_{max} (mg·kg⁻¹·day⁻¹) and the time it appears T_{Vmax} (day).

According to Table 2, the V_{max} was related to the heavy metal leached. The V_{max} of the three heavy metals in the increasing order was Zn>Cu>Ni. Chen et al. (2004) found that the higher the initial heavy metals contend in the sediment, the faster the V_{max}, which was consistent with our result.

The SC-A group had the fastest acidification rate, SO₄²⁻ production rate, total bioleaching rate, the largest V_{max} and the corresponding shortest T_{Vmax}, and it also reached the highest heavy metal solubilization. In addition, the sulfur substrate could be reused when the sulfur-covered biochar particles were recycled. All of these indicated that sulfur-covered biochar particles had superior bioleaching ability and the potential for recycling.

Bioleaching functional bacteria immobilization effect of sulfur-covered biochar particles

In order to test the bioleaching functional bacteria immobilization effect of sulfur-covered biochar particles, samples were collected for microbiological analysis in the middle of the experiment (Day 4) and the end of the experiment (Day 9). The integrated high-throughput absolute abundance quantification (iHAAQ) technology were used to investigate the changes in the flora structure and abundance during bioleaching (Fig.3). The result showed that the absolute abundance of Top 30 microorganisms in the SC-A group was less than the S-A group in the middle of the experiment. Among the dominant microorganisms associated with bioleaching, only *Acidithiobacillus* and *Sulfuritalea* were more abundant in the SC-A group. However, the situation reversed at the end of the experiment, the sulfur-covered biochar

particles of the SC-A group enriched higher abundance of the Top 30 microorganisms, regardless of whether they were related to bioleaching.

At the same time, we performed scanning electron microscope observations of the sulfur-covered biochar particles recycled from the first round of bioleaching. The observation showed that large amount of rod shaped microorganisms was immobilized on the surface of the sulfur-covered biochar particles, and the sulfur layer on the surface became rough and complicated due to metabolism of the bioleaching bacteria(Fig.4).

Reuse of recoverable sulfur-covered biochar particles in bioleaching

In order to investigate the feasibility of reusing the sulfur-covered biochar particles in multiple bioleaching rounds, the sulfur-covered biochar particles used in the first bioleaching round were recycled to be reused in the second and the third round of bioleaching, meanwhile no more sulfur substrate and sludge-enriched inoculum were added. The results showed that both the first round and the second round could reach pH 2.5 when it came to Day 9, but the pH value of the third bioleaching round only reached 3.5 when the experiment came to an end. Although the final pH values of the first round and the second round were very close, the SO_4^{2-} production of the second round was much lower than the first round, and the SO_4^{2-} production rate of the third round stayed flat. At the end of the second bioleaching round, the heavy metals solubilization was Ni 32.84%, Cu 69.93% and Zn 67.17%, while only Ni 20.09%, Cu 10.28% and Zn 37.20% in the third round. Therefore, the recycled sulfur-covered biochar particles could support the second round of bioleaching, and the heavy metals solubilization was satisfied. When it came to an end of the third bioleaching round, only 20.25% elemental sulfur still remained on the biochar particles.

Discussion

Acidification and oxidation of sulfur

It is generally accepted that the solubilization of the acid-soluble metal sulphides is caused by the attack of the proton (the “polysulfide pathway”) [25, 26], while the acid-insoluble metal sulphides are dissolved by the combination of oxidative attack and proton attack (the “thiosulfate pathway”) [27]. Therefore, we can investigate the acidification rate and the production of SO_4^{2-} to judge the bioleaching of heavy metals from the side, and the result showed that the faster the pH declined, the faster the SO_4^{2-} was produced, indicating that the acidification of the system was caused by the sulfur oxidation to produce H_2SO_4 .

Sulfur powder is highly hydrophobic and difficult to disperse in the liquid phase, but it has a large specific surface area, so it is more prone to adsorb microorganisms and promote their growth, and its production rate of SO_4^{2-} tends to be fast [9]. However, the acidification rate and SO_4^{2-} production rate of the S-A group were slower than the SC-A group. Many studies have reported that the biochar is redox-active due to its quinone group and aromatic structure [28-30]. We performed Fourier transform infrared

spectroscopy on the biochar particles used in the experiment. The results showed that the infrared absorption peak appeared at 1610 cm^{-1} , indicating the existence of quinone structure (Fig S2). The electron transfer in the process of microbial oxidation of elemental sulfur could be enhanced, resulting from the transformation of the oxidation and reduction states of quinone structure. Therefore, the comparable acidification rate of sulfur-covered biochar particles and sulfur powder may result from the acceleration of sulfur oxidation rate, especially when microbial oxidation was happening at the same time.

Rhamnolipid is a biological surfactant commonly produced by strains of the genus *Pseudomonas*. The release of this biological surfactant promotes emulsification of the hydrocarbon phase, rendering such lipophilic molecules available to the metabolic pathways of microorganisms [31]. However, the R-A group did not show a significant effect in promoting the bioleaching acidification. This could be caused by the organic matters the rhamnolipid contained, which inhibit the autotrophic metabolism of indigenous sulfur-oxidizing bacteria. The growth of bioleaching functional strains could be inhibited by organic compounds such as pyruvic acid, citric acid, oxaloacetic acid, and glucose [32-35].

Heavy metal bioleaching performance

A large proportion of Cu in the sediment sample existed in the organic state (49.14%), and Zn mostly existed in exchangeable, carbonate-bound, and iron-manganese oxidation states (57.30%). In this case, Zn and Cu had high bioleaching levels in the experiment. The research found that the residual state was the most difficult form to be leached [36, 37], this is because that heavy metals in the residual form bound to a resistant crystal structure rarely contact sulfuric acid [38]. Therefore, the amount of the residual form determined the upper limit of the bioleaching of heavy metals and Ni mostly existing in the residual form was the most difficult metal to be leached.

The kind of sulfur substrate affected the acidification rate, which in turn affected the total bioleaching rate, and the distribution of the initial form of the heavy metal

determined the level of heavy metal solubilization in bioleaching. This explained why three experiment groups had different total bioleaching rate, but they all reached similar heavy metal solubilization and the distribution of heavy metal form in the sediment after bioleaching.

Extraction of metals by anionic surfactant rhamnolipid through solubilization has been known and previously applied with effective results [39-41]. However, the R-A group did not show significant effect in promoting heavy metal solubilization in the research. This may be because that as the pH in the bioleaching system declined, the water solubility, surface tension and the number of heavy metal binding sites of the rhamnolipid continued to decrease [40, 42]. Otherwise, the complicated competitive ions and ligands in the sediment system were important factors affecting the adsorption and solubilization of heavy metals by rhamnolipid.

Immobilization function of biochar

The microbiological analysis and the scanning electron microscope result proved that the sulfur-covered biochar particles could enrich and immobilize bioleaching functional bacteria from the sediment.

The reason why sulfur-covered biochar particles could immobilize large amount of bacteria may be related to its carrier bamboo biochar. Biochar contains a series of nutrients (such as K^+ , Mg^{2+} , Na^+ , N, P, etc.), and because of its negative surface charge, it can also absorb salt ions in the surrounding environment to provide nutrients for microorganisms [43]. The adsorption of heavy metals leached during the bioleaching process on the biochar could reduce heavy metals bioavailability, which alleviated their toxicity to the microorganisms [44, 45]. Besides, the large specific surface area and the high pore volume of the biochar can provide safe and suitable microenvironment for microorganisms to grow [46]. Otherwise, the sulfur melted on the surface of the bamboo biochar particles provided the elemental sulfur as sulfur substrate while also expanding the reaction area to a certain extent. Therefore, compared with sulfur powder, the addition of sulfur-covered biochar particles had a stronger promotion effect on the growth of sulfur-oxidizing autotrophs.

Potential reuse of sulfur-covered biochar particles

The recycled sulfur-covered biochar particles had satisfied bioleaching performance in the second round of bioleaching, but their sulfur content became the main limiting factor in the third or more rounds. Similar conclusion could also be obtained by combining the distribution figure of the elemental sulfur in different rounds (Fig. 5). In addition, we found that there was still much elemental sulfur that was not converted to SO_4^{2-} but was wasted or converted to other sulfur compounds remained in the sediment, which indicated the complexity of the sulfur oxidizing bacteria metabolism during bioleaching process in the sediment.

The sulfur residue remained on the biochar particles was different from sulfur powder, it was immobilized on the biochar particles and could be recycled efficiently, which avoided the problem of “post-acidification” and secondary pollution. No inoculum addition during the second bioleaching round indicated that the use of the sulfur-covered biochar particles could also simplify the operational step of the inoculum addition. Nevertheless, the sulfur-covered biochar particles could only maintain two rounds of bioleaching, and the third and more rounds of bioleaching were limited due to insufficient sulfur substrate. In the future research, it is considered to supplement a small amount of sulfur powder or new sulfur-covered biochar particles to provide an additional sulfur source in the third or more rounds of bioleaching, so as to maintain the reuse of the sulfur-covered biochar particles. In the current chemical fertilizer industrial production, the production operation of sulfur-covered urea particles is realized, which manifested that the industrial production of the sulfur-covered biochar particles is also practicable.

Conclusion

The result showed that the sulfur-covered biochar particles had the fastest acidification rate, SO_4^{2-} production rate and heavy metal bioleaching rate, and the highest heavy metal solubilization, which was

resulted from the acceleration of bioleaching reaction by the bioleaching functional bacteria immobilized on the biochar surface. Meanwhile, the dual immobilization of the bioleaching functional bacteria and the sulfur layer on the sulfur-covered biochar particles realized their recycling and their reuse in the second bioleaching round. In the future research, a small amount of sulfur powder or new sulfur-covered biochar particles will be supplemented to achieve more rounds of recycling of the sulfur-covered biochar particles, so as to maintain the reuse of the sulfur-covered biochar particles, so as to overcome the “post-acidification” problem and simplify the steps of the repetitive addition of bioleaching inoculum during the operation of the bioleaching sequencing batch reactor.

List Of Abbreviations

S-A: the sulfur powder groups with inoculum added; SC-A: the sulfur-covered biochar particles groups with inoculum added; R-A: the sulfur powder mixed with rhamnolipid groups with inoculum added; C: the control groups; C-A: the control groups with inoculum added; N: the NaN_3 groups

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

CCW performed all experiments, analyzed the data and was a major contributor to writing the manuscript. LPL was major contributor to supervision, guided the laboratory experiments and contributed to writing the manuscript. MYJ guided the bioleaching experiment and contributed to writing the manuscript. LCH,

YCC, YTS helped with the experiment. HZW, QL, CFS, and BLH contributed to supervision and writing the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

Acidification (solid line) and SO₄²⁻-production (dotted line) during bioleaching.

Figure 2

Solubilizations of Ni, Cu, Zn, and distribution of heavy metal forms before and after bioleaching. H represented for sediment without bioleaching treatment. The triangle represents the solubilization of each group.

Figure 3

The fold change of the absolute abundance of the SC-A group vs. the S-A group. The number 4 and 9 represented the sample of the Day 4 and the Day 9.

Figure 4

The scanning electron microscope result of the SC-A group on the Day 9. The magnifications of (a) and (b) were (1000×) and (5000×), respectively.

Figure 5

Acidification, SO₄²⁻ production and the distribution of the elemental sulfur in the three bioleaching rounds. (Acidification curve: solid line; SO₄²⁻ concentration: dotted line)

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