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Mechanism underlying the bioleaching process of $LiCoO_2$ by sulfur-oxidizing and iron-oxidizing bacteria

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Benefiting from lower operational costs and energy requirements than do hydrometallurgical and pyrometallurgical processes in metal recovery, the bioleaching of $LiCoO_2$ through the use of sulfur-oxidizing and iron-oxidizing bacteria has drawn increasing attention. However, the bioleaching mechanism of $LiCoO_2$ has not been clearly elaborated. In the present study, the effects of the energy source of bacteria, such as Fe^{2+}, pyrite and S^0, and the products of bacterial oxidation, such as Fe^{3+} and sulfuric acid, on the chemical leaching of $LiCoO_2$ were studied. The results indicated that lithium was dissolved by acid, and cobalt was released by the reduction of Fe^{2+} and acid dissolution. The recovery of Li^+ and Co^{2+} could be significantly improved by pH adjustment. Finally, optimal recoveries of Li^+ and Co^{2+} were observed in the pyrite group, reaching 91.4% and 94.2%, respectively. By using pyrite as the energy source, the role of bacteria in bioleaching of $LiCoO_2$ was investigated. The results showed that bacteria could produce sulfuric acid by oxidizing pyrite to promote the mobilization of Li^+ and Co^{2+}. The recovery of lithium and cobalt could be increased to 100.0% and 99.3% by bacteria. Moreover, extracellular polymeric substances secreted by bacteria were found to be a factor for the improvement of Li^+ and Co^{2+} recovery.

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[**Key words:** Bioleaching; $LiCoO_2$; Pyrite; Sulfur-oxidizing and iron-oxidizing bacteria; Extracellular polymeric substances]

Lithium-ion batteries are widely used as power sources for electric vehicles because of their excellent electrochemical properties (such as high density, long life, small size, and lightweight, among others) (1,2). However, when lithium-ion batteries reach their service life, the treatment of the discarded batteries becomes a serious issue for many countries (3,4). It has been estimated that more than 0.5 million tons of discarded lithium-ion batteries will be produced in China in 2020 (5). Due to the presence of toxic elements and compounds in discarded lithium-ion batteries, their improper disposal poses a serious threat to the environment and human health (6). Additionally, various valuable metals (such as lithium and cobalt) are present in discarded lithium-ion batteries, and the recycling of these metals generates remarkable economic and social benefits (7).

The components of lithium-ion batteries are a cathode, an anode, a separator, electrolytes, collectors and a metal protective shell (8). The cathode is made of lithium mixed metal oxide, such as $LiCoO_2$, $LiMn_2O_4$, $LiNiO_2$, $LiFePO_4$ and $LiNi_xCo_yMn_zO_2$ (9). As the cathode is the most valuable component in lithium-ion batteries (10), many researchers have focused on the recovery of metals from the cathode (7,11).

Hydrometallurgical and pyrometallurgical processes are the most widely used methods to recover lithium and cobalt from discarded lithium-ion batteries (9,12–14). However, their shortcomings include strict equipment requirements, high energy consumption and potential safety risks, forcing people to find alternative methods (15). Bioleaching, which can overcome these shortcomings, is considered a promising alternative for the recovery of discarded lithium-ion batteries (16,17).

Bioleaching is a promising technology, which utilizes microorganisms for metal recovery from low grade ore (18,19), waste printed circuit boards (20), sewage (21), spent catalyst (22) and discarded lithium-ion batteries (8). Acidophilic sulfur-oxidizing and iron-oxidizing bacteria are commonly used for metal extraction, such as *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, *Leptospirillum ferrooxidans*, and *Sulfobacillus thermosulfidooxidans*, among others (23). These bacteria use inorganic compounds (Fe^{2+}, FeS_2 and reduced S) as an energy source and utilize their ability to facilitate metal dissolution through a series of biooxidation and bioleaching reactions (24).

Although bioleaching has some advantages in metal recovery, some shortcomings persist in the bioleaching of electronic wastes, such as a long leaching time and a low pulp density, limiting its commercial application. Therefore, an in-depth understanding of the mechanism of bioleaching of metals could provide important theoretical support for the optimization and development of processes. Different mechanisms have been proposed according to different research purposes and bacteria. In the bioleaching of metal sulfides using sulfur-oxidizing and iron-oxidizing bacteria, the indirect mechanism is the most recognized mechanism (25). In the direct mechanism, the bacteria contribute to generating the oxidizing agent, ferric iron. The metal ions and reduced sulfur from metal sulfides are then released through the attack of Fe^{3+}. Finally, the reduced sulfur is biooxidized to sulfuric acid through the

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thiosulfate or polysulfide pathway (25). During the bioleaching of waste printed circuit boards (PCBs), the metals in PCBs are dissolved by the Fe^{3+} generated by bacterial oxidation (26). During the bioleaching of Ni–Cd batteries, Ni and Cd are mobilized by the sulfuric acid produced by bacteria (27). Some researchers (15–17,28–30) have studied the bioleaching of lithium-ion batteries, but there is no consistent conclusion regarding the bioleaching mechanism of lithium-ion batteries. Zeng et al. (30) indicated that during the bioleaching of $LiCoO_2$, $LiCoO_2$ is dissolved by the reaction (Eq. 1), and the presence of copper ions can accelerate the reaction rate. However, Xin et al. (31) compared the effects of different energy sources on the bioleaching of $LiCoO_2$ and found that Li^+ was released due to acid dissolution and Co^{3+} in $LiCoO_2$ was mobilized by the combined effects of Fe^{2+} reduction and acid dissolution. The reaction is shown in Eq. 2. Therefore, to better understand the bioleaching of lithium-ion batteries, more effort is required to reveal the bioleaching mechanism of lithium-ion batteries.

$$4LiCoO_2 + 12H^+ \rightarrow 4Li^+ + 4Co^{2+} + 6H_2O + O_2\uparrow \tag{1}$$

$$2FeSO_4 + 2LiCoO_2 + 4H_2SO_4 \rightarrow Fe_2(SO_4)_3 + 2CoSO_4 + Li_2SO_4 + 4H_2O \tag{2}$$

Moreover, the effect of different energy sources (Fe^{2+}, pyrite, S and their combination) on the bioleaching of lithium-ion batteries has been investigated by several researchers (16,17,31). However, few studies have addressed whether these energy sources themselves might react with lithium-ion batteries. The study of the chemical leaching of lithium-ion batteries through the use of these energy sources could contribute to elucidating the role of bacteria in the bioleaching of lithium-ion batteries, thus revealing the bioleaching mechanism of lithium-ion batteries.

In the present study, $LiCoO_2$ was selected as the research objective. The bioleaching mechanism of $LiCoO_2$ was investigated using sulfur-oxidizing and iron-oxidizing bacteria. The effects of the energy source of bacteria, such as Fe^{2+}, pyrite and S^0, and the products of bacterial oxidation, such as Fe^{3+} and sulfuric acid, on the chemical leaching of $LiCoO_2$ were studied. Using pyrite as an energy source of bacteria, the role of bacteria in bioleaching of $LiCoO_2$ was studied and modeled by comparing the results under biotic and abiotic conditions. Moreover, two hypothetical bioleaching mechanism of $LiCoO_2$ were proposed and validated.

MATERIALS AND METHODS

Materials and bacteria Lithium cobalt oxide was purchased from Titan, with a content of 99.8%. The pyrite samples were kindly supplied by one Gold Mine Co. Ltd., P.R. China, containing 30 ± 0.5% Fe, 28 ± 0.3% S and 3 ± 0.1% As. The X-ray diffraction (XRD) results showed that the main compositions of the sample were pyrite and quartz. The sample was sieved to obtain a particle size distribution between 63 μm and 90 μm. All chemicals were of analytical grade reagents, and all aqueous solutions were prepared using distilled water.

The acidophilic microorganisms were isolated from mineral drainage and domesticated for a long time in our laboratory to achieve a high bioleaching activity of pyrite. The organisms were studied by analyzing the V4 region of 16S rDNA using a high-throughput DNA sequencing technique. The predominant organisms were *Leptospirillum ferriphilum* sp. and *S. thermosulfidooxidans* spp. The 9K medium was used for the bacterial culture, and the contents of the media were as follow (g/L): 3.0 $(NH_4)_2SO_4$, 0.1 KCl, 0.5 $MgSO_4 \cdot 7H_2O$, 0.5 K_2HPO_4 and 0.01 $Ca(NO_3)_2$. Pyrite with a mass percentage of 10% was used as an energy source for the bacteria. All the data presented in this study were obtained from triplicate experiments with a p-value < 0.05.

Chemical leaching of $LiCoO_2$ without pH adjustment Deionized water, 30 g/L Fe^{2+}, 30 g/L Fe^{3+}, 100 g/L pyrite and 28 g/L S^0 were used to leach $LiCoO_2$. The contents of the iron and S^0 in pyrite were 30% and 28%, respectively, and therefore 30 g/L Fe^{2+}, 30 g/L Fe^{3+} and 28 g/L S^0 were selected in these experiments.

Specific contents of $FeSO_4 \cdot 7H_2O$, $Fe_2(SO_4)_3$, pyrite and S^0 were added into different 250-ml Erlenmeyer flasks containing 100 ml of 9K medium. The initial pH was adjusted to 1.20 with 5 mol/L sulfuric acid solution. Then, 15 g/L $LiCoO_2$ was added to the solution. Samples were collected at 1, 2, 3, 5 and 7 h to analyze the pH and concentrations of Li^+ and Co^{2+}.

Chemical leaching of $LiCoO_2$ with pH adjustment The experiment was designed similar to the previous description. The difference was that the pH during the leaching process was maintained at 1.20 using 5 mol/L sulfuric acid solution.

Bioleaching of $LiCoO_2$ The design of the bioleaching experiments is shown in Table 1. Ninety milliliters of 9 K medium and 10 ml stock culture were prepared in groups A, B and F. One hundred milliliters of 9 K medium was prepared in groups C, D and E. Ten grams of pyrite were added in groups A–F, and the initial pH was adjusted to 1.20 with 5 mol/L sulfuric acid solution. Group F was sterilized by autoclaving. Then, 15 g/L $LiCoO_2$ was added to groups A–F. All groups were cultivated in a rotary shaker at 42°C at 180 rpm. The pH of groups A, C and F was maintained at 1.20 with 5 mol/L sulfuric acid solution after the addition of $LiCoO_2$. The same amount of acid solution as in group A was added to group D. Groups B and E were evaluated without pH adjustment. Samples were collected at regular intervals to analyze their pH, oxidation-reduction potential (ORP) and concentrations of Li^+, Co^{2+}, Fe^{2+} and Fe^{3+}.

Effect of the electron donor on bioleaching of $LiCoO_2$ The bioleaching experiments were carried out with 90 ml 9K medium, 10 g pyrite, 10 ml stock culture and 1.5 g $LiCoO_2$. After 24 h of culture, different amounts of $FeSO_4 \cdot 7H_2O$ (0, 1, 3, 5 g/L Fe^{2+}) were added to the leachate. The samples were collected at intervals to analyze the concentrations of Li^+, Co^{2+}, Fe^{2+} and Fe^{3+}.

Effect of electron transfer on the bioleaching of $LiCoO_2$ The experiments were carried out in a 250-ml Erlenmeyer flask with 90 ml 9 K medium, 10 g pyrite, 10 ml stock culture and 1.5 g $LiCoO_2$. The initial pH was adjusted to 1.20. Then, 0 g/L and 0.01 g/L riboflavin were added to the medium. The samples were collected at intervals to analyze ORP and the concentrations of Li^+, Co^{2+} and Fe^{3+}.

Extraction and analysis of the biochemical characterization of extracellular polymeric substances A culture with 90 ml 9 K medium, 10 g pyrite and 10 ml stock culture was carried out for 7 d. Then, extracellular polymeric substances were extracted from the culture using the EDTA method (32). The protein was measured using the Bradford assay (33). Uronic acids and polysaccharides were analyzed according to Govender and Gericke (34). The protein and polysaccharide in extracellular polymeric substances were extracted according to Vimalnath and Subramanian (35).

Effect of extracellular polymeric substances on the chemical leaching of $LiCoO_2$ Different content of extracellular polymeric substance extraction (0, 10, 30, and 50 ml) were added to a 250-ml Erlenmeyer flask. The extracellular polymeric substance extraction was then supplemented to 100 ml with 9K medium. Subsequently, 10 g pyrite and 1.5 g $LiCoO_2$ were added to the solution. The samples were collected at intervals to analyze the concentrations of Li^+ and Co^{2+}.

Effect of protein and polysaccharide extracted from extracellular polymeric substances on the chemical leaching of $LiCoO_2$ The protein and polysaccharide extracted from 100 ml extracellular polymeric substances extraction were added to the different solutions with 100 ml 9 K medium and 10 g pyrite, respectively. Next, 1.5 g $LiCoO_2$ was added to the solution to start the reaction. The samples were collected at intervals to analyze the concentrations of Li^+ and Co^{2+}.

Effects of glucose, uronic acid and cysteine on the chemical leaching of $LiCoO_2$ The simulated composition of the extracellular polymeric substances, including glucose, cysteine and uronic acid, were used to experimentally investigate its effect on the chemical leaching of lithium cobaltate. Thus, 1 g/L glucose, 1 g/L uronic acid and 1 g/L cysteine were added to the different solutions with 100 ml 9 K medium and 10 g pyrite, respectively. Next, 1.5 g $LiCoO_2$ was added to the solution to start the reaction. The samples were collected at intervals to analyze the concentrations of Li^+ and Co^{2+}.

Culture conditions and analytical methods All experiments in this paper were conducted in a 250-ml Erlenmeyer flask with an initial pH of 1.20, 42°C and 180 rpm. The pH and ORP were measured using a pH meter and Eh meter (Mettler model FE20, Mettler Toledo, Shanghai, China). The concentrations of Li^+ and Co^{2+} were determined with an atomic absorption spectrometer (iCE 3000, Thermo Fisher, Cambridge, UK). The concentration of Fe^{2+} was determined by titration with potassium dichromate in the presence of the indicator *N*-phenylanthranilic acid. The concentration of Fe^{3+} was determined by titration of EDTA at pH 2 with sulfosalicylic acid as an indicator.

TABLE 1. The variables designed with different groups.

Group	9K medium (ml)	Inoculum (ml)	Sterilization	$LiCoO_2$ (g/L)	pH adjustment
A	90	10	–	15	Adjust pH = 1.20
B	90	10	–	15	–
C	100	–	–	15	Adjust pH = 1.20
D	100	–	–	15	The same amount of acid with group A
E	100	–	–	15	–
F	90	10	Sterilization	15	Adjust pH = 1.20

RESULTS AND DISCUSSION

Chemical leaching of $LiCoO_2$ without pH adjustment During the bioleaching of lithium-ion batteries, pyrite, S^0, Fe^{2+} or their combination were commonly used as an energy source for bacteria. The Fe^{3+} and H_2SO_4 were the products of the bacterial oxidation of these energy sources. The effects of pyrite, S^0, Fe^{2+}, Fe^{3+}, and H_2SO_4 on the chemical leaching of $LiCoO_2$ were investigated. The results are shown in Fig. 1.

As indicated in Fig. 1A, the addition of $LiCoO_2$ could lead to a rise in pH. The most obvious increase in pH occurred in the pyrite group with a pH increase from 1.20 to 3.97 within 7 h. A rise in pH in the water and S^0 groups ensued. The pH in the Fe^{2+} group rose rapidly at first and then remained at approximately 2.40. It was inferred from the brown precipitate at the bottom of flask that ferric hydroxide precipitate was produced in the Fe^{2+} group. However, in the Fe^{3+} group, the pH only increased from 1.20 to 1.47.

As shown in Fig. 1B, the final recovery of Li^+ in the other groups was almost the same as that in the water group. It was indicated that no other substance except H^+ contributed to the dissolution of Li^+. These results suggested that the lithium in $LiCoO_2$ was dissolved by acid. Although the final recovery of Li^+ was similar in all groups, the dissolution rate of Li^+ in the Fe^{3+} group was slower than in the other groups.

As shown in Fig. 1C, the recovery of Co^{2+} in the pyrite and Fe^{2+} groups was significantly higher than in the other three groups. The optimal recovery of Co^{2+} was found in the pyrite group and reached 54.0% in 7 h. The Fe^{2+} group followed with a cobalt recovery of 51.2%. The final recoveries of Co^{2+} in the remaining groups were all approximately 26.0%. However, there were some difference in the dissolution rate of Co^{2+}. The dissolution rate of Co^{2+} in the Fe^{3+} group was the slowest. The dissolution rate of Co^{2+} in the S^0 group was higher than that in the water group during the first 3 h. The recovery of Co^{2+} in the S^0 group reached 27.3% in 2 h, while the water group required 3 h to achieve a Co^{2+} recovery of 26.5%. The rate in the Fe^{3+} group was slowest, and the cobalt recovery was only 25.2% up to 7 h.

There are two forms of cobalt in $LiCoO_2$ (31). Most of them are in the form of Co^{3+}, which is difficult to be dissolved directly with acid. The remaining cobalt is in the form of Co^{2+}, which can be easily mobilized by acid. For convenience of recovery, Co^{3+} was required to be reduced to Co^{2+}. As there were no reducing agents in the groups of the water, S^0 and Fe^{3+} leaching system, only Co^{2+} could be dissolved into the leachate. Since the mass ratio of Co^{2+} and Co^{3+} in $LiCoO_2$ is basically fixed, the final recoveries of Co^{2+} in these three groups were all approximately 26.0%. However, the recoveries of cobalt in the pyrite and Fe^{2+} groups were significantly higher than those in these three groups. The results indicated that the two groups not only mobilized Co^{2+} in $LiCoO_2$ but also extracted part of the Co^{3+}. This phenomenon was due to the redox reaction between Fe^{2+} and Co^{3+} as follows:

$$Fe^{2+} + Co^{3+} \rightarrow Fe^{3+} + Co^{2+}(CoO) \tag{3}$$

The reaction could be confirmed by the change in Fe^{2+} and Fe^{3+} in the groups of Fe^{2+} and pyrite, as shown in Fig. 1D. The Fe^{3+} in the group of pyrite first increased and then decreased. The reason for the increase in Fe^{3+} is indicated by Eq. 3. Because of the high pH (above 3.0), the formation of ferric hydroxide led to a decrease in Fe^{3+}.

In these experiments, the final recoveries of Li^+ and Co^{2+} in the Fe^{3+} group were similar to those in the other groups, but the dissolution rates of Li^+ and Co^{2+} were slower than those in the

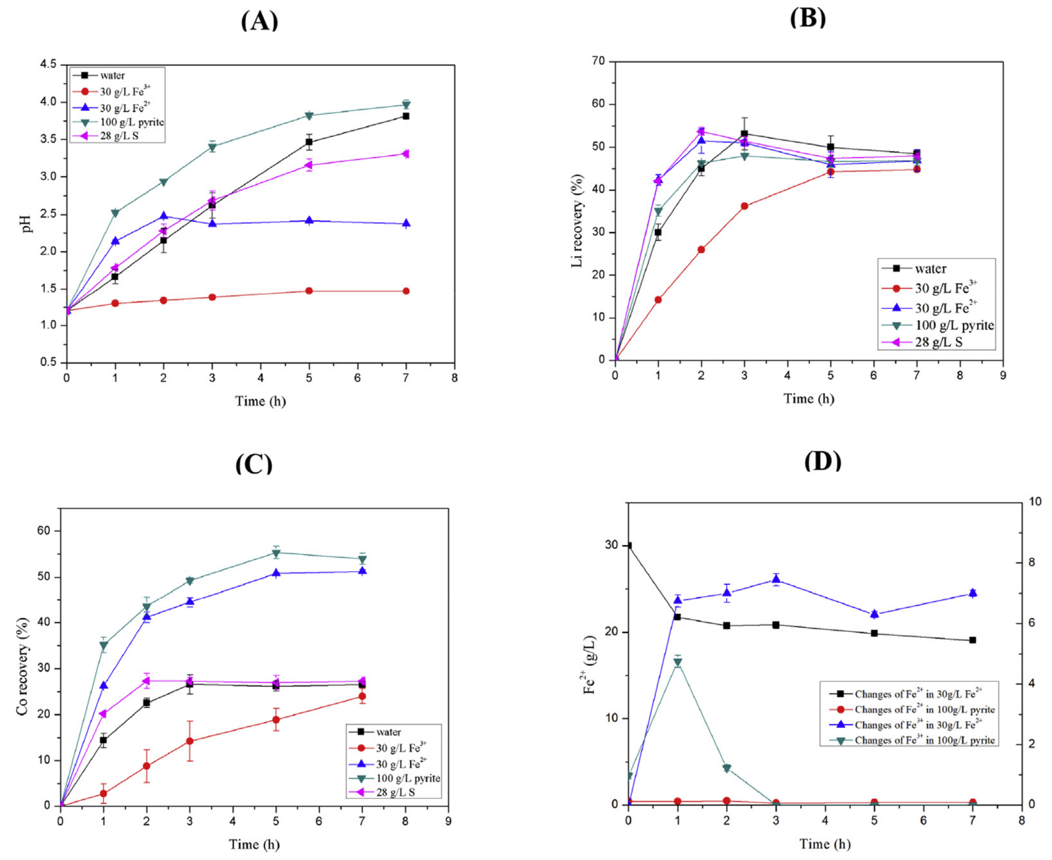

FIG. 1. Changes in the pH (A), Li recovery (B), Co recovery (C), and concentrations of Fe^{2+} and Fe^{3+} (D) with time during chemical leaching without pH adjustment.

other groups. This finding might be the result of the hydrolysis of Fe^{3+}. The hydrolysis of Fe^{3+} was very complex, and the first step of the hydrolysis could be explained as follows (17):

$$xFe^{3+} + yH_2O \leftrightarrow Fe_x(OH)_y^{(3x-y)+} + yH^+ \quad (4)$$

In Eq. 4, x and y represent the number of iron ions and the hydroxyl number.

As shown in Eq. 4, H^+ was produced because of the hydrolysis of Fe^{3+}, which might explain why the increase in pH in the group of Fe^{3+} was much slower than that in the other groups. In addition to Eq. 4, there were some polyreactions in the hydrolysis of Fe^{3+} (36,37). First, the hydrolysis product of Fe^{3+} aggregated into highly positively charged polymers by olation (37). Then, these polymers further accumulated to form larger polymers and reduced the electric charges by oxolation (36). The formation of these polymers might retard the contact of H^+ and $LiCoO_2$, which could result in the reduction of dissolution rates of Li^+ and Co^{2+}.

Chemical leaching with pH adjustment The dissolution of Li^+ and Co^{2+} are acid-consuming processes in the leaching of $LiCoO_2$, such that the H^+ in the solution might not satisfy the mobilization of Li^+ and Co^{2+} when H^+ cannot be generated in a timely and rapid manner. Therefore, in this section, the pH of the solution was adjusted to 1.20 with 5 mol/L sulfuric acid solution, and the results are shown in Fig. 2. As illustrated in Fig. 2A, the greatest consumption of H^+, which reached 540.5 mmol/L, occurred in the Fe^{2+} group. The H^+ consumed in the pyrite group was 462.5 mmol/L. Moreover, the amounts of consumption of H^+ in the water and S^0 groups were almost the same and reached 245.0 and 243.0 mmol/L, respectively. They were less than those in the Fe^{2+} and pyrite groups. The Fe^{3+} group only consumed 131.5 mmol/L of H^+.

TABLE 2. Final Li and Co recovery in chemical leaching of $LiCoO_2$ with and without pH adjustment at 7 h.

Group	Li recovery (%)		Co recovery (%)	
	Without adjusting pH	Adjusting pH	Without adjusting pH	Adjusting pH
Water	48.4	65.9	26.4	33.7
30 g/L Fe^{3+}	44.7	56.4	24.0	29.8
30 g/L Fe^{2+}	46.8	85.1	51.2	90.1
100 g/L pyrite	46.9	91.4	54.0	94.2
28 g/L S	47.9	68.2	28.6	35.9

The final Li^+ and Co^{2+} recoveries of $LiCoO_2$ at 7 h with and without pH adjustment are shown in Table 2. The results indicated that the recoveries of Li^+ and Co^{2+} were significantly increased by pH adjustment. The results were similar to those of Xin et al. (17).

The variation of Li^+ recovery with time during chemical leaching of $LiCoO_2$ with pH adjustment is shown in Fig. 2B. The results showed that the recoveries of Li^+ in the Fe^{2+} and pyrite groups were significantly higher than in the other groups. The dissolution rate of Li^+ in the S^0 group was faster than in the water group, but the final recovery of Li^+ in these two groups was similar. The slowest dissolution rate of Li^+ and the lowest final recovery of Li^+ was observed in the Fe^{3+} group.

As illustrated in Fig. 2C, with the pH adjustment, the recovery of Co^{2+} in the Fe^{2+} and pyrite groups increased significantly from 51.2% to 54.0%–90.1% and 94.2%. In these two groups, the pH adjustment played a significant role in the recovery of Co^{2+} from $LiCoO_2$. However, the recovery of Co^{2+} in the other three groups

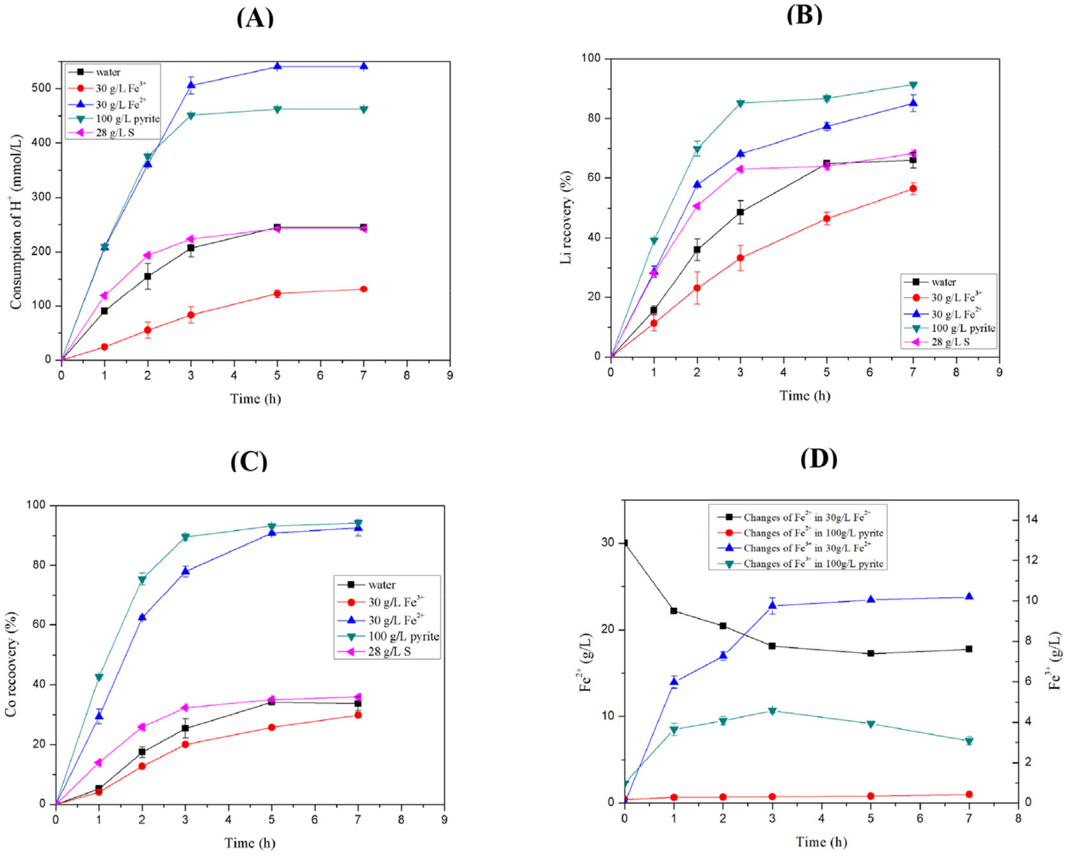

FIG. 2. Variation in the consumption of H^+ (A), Li recovery (B), Co recovery (C), and concentrations of Fe^{2+} and Fe^{3+} (D) with time during chemical leaching with pH adjustment.

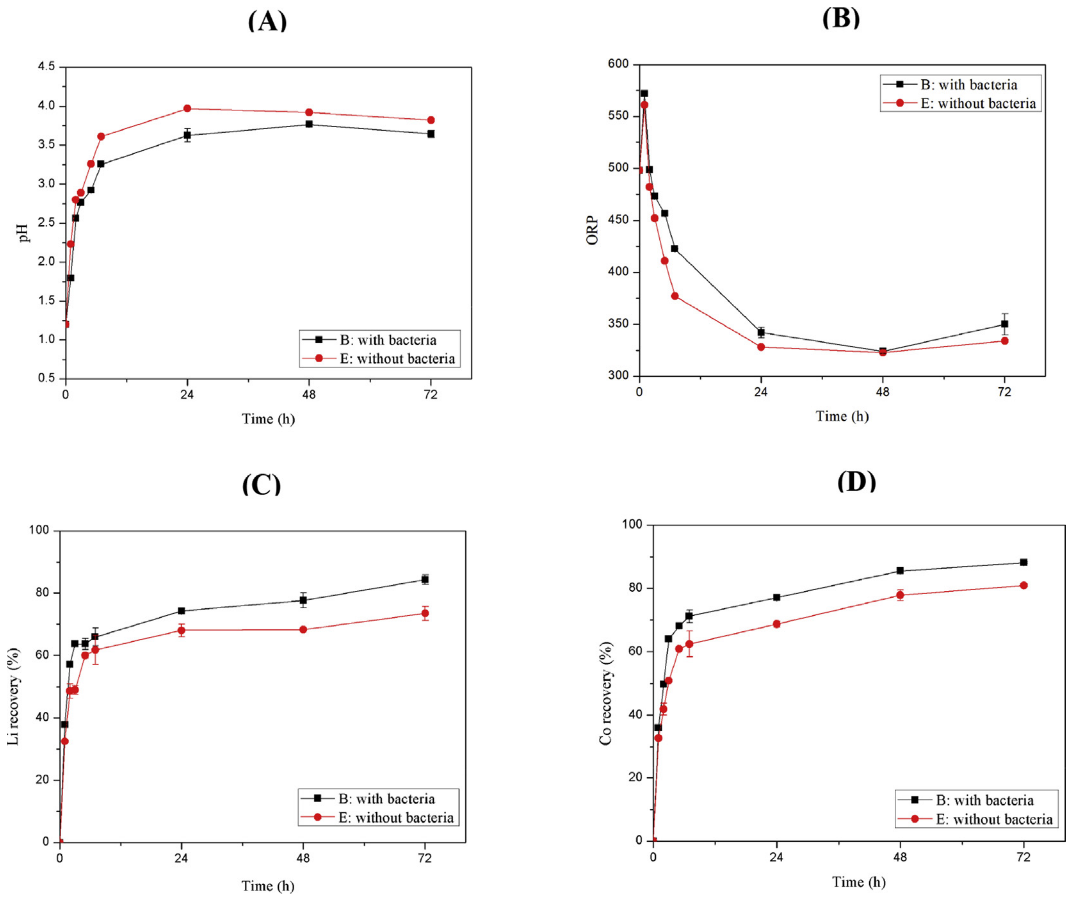

FIG. 3. Changes in the pH (A), ORP (B), Li recovery (C) and Co recovery (D) with time during leaching of $LiCoO_2$ without pH adjustment.

was increased slightly by pH adjustment. It was confirmed that only Co^{2+} but not Co^{3+} in $LiCoO_2$ could be dissolved by acid. In these three groups, Co^{2+} recovery in the S^0 group was the highest and that in the Fe^{3+} group the lowest.

The recoveries of Li^+ and Co^{2+} were almost consistent with the consumption of acid. The more acid consumed, the more Li^+ and Co^{2+} were recovered. It was indicated that acid played a vital role in the leaching of $LiCoO_2$. However, there was an exception. The pyrite group consumed less acid than the Fe^{2+} group, but it had a higher recovery of Li^+ and Co^{2+}, potentially because the sulfur in the pyrite was oxidized to sulfuric acid by Fe^{3+} to compensate for the consumption of acid (31). As mentioned above, the Li^+ in the $LiCoO_2$ was dissolved by acid. However, the recoveries of Li^+ in the water, S^0 and Fe^{3+} groups were still much lower than in the Fe^{2+} and pyrite groups after pH adjustment. This result might be due to the inclusion of lithium in Co^{3+}. Before Co^{3+} was mobilized, the lithium trapped in the Co^{3+} could not be dissolved by acid. The dissolution rate and finial recovery of Li^+ and Co^{2+} in the Fe^{3+} group was the lowest. The formation of hydrolytic polymer of Fe^{3+} might be the reason for the low recovery of Li^+ and Co^{2+}. Moreover, the recoveries of Li^+ and Co^{2+} in the Fe^{2+} group (85.1% and 90.1%, respectively) were lower than those in the pyrite group (91.4% and 94.2%, respectively). As shown in Fig. 2D, more Fe^{3+} was formed in the Fe^{2+} than the pyrite group. The reduced recovery of Li^+ and Co^{2+} might have been caused by the presence of more Fe^{3+} in the Fe^{2+} group.

The role of bacteria in the bioleaching of $LiCoO_2$ During the chemical leaching of $LiCoO_2$, lithium was released by acid dissolution, and cobalt was mobilized by the combined work of Fe^{2+} reduction and acid dissolution. The optimal recoveries of Li^+ and Co^{2+} were in the pyrite group, so pyrite was selected as the bacterial energy source for bioleaching of $LiCoO_2$. A series of experiments were designed to verify the role of bacteria in the bioleaching of $LiCoO_2$. In the bioleaching of metal sulfides, waste printed circuit boards or spent catalyst using sulfur-oxidizing and iron-oxidizing bacteria, the role of bacteria was to oxidize the Fe^{2+}, S^0 or pyrite to provide Fe^{3+} and H^+. Fe^{3+} and H^+ were the attacking agents, which were used to dissolve metal sulfides or metals in the waste printed circuit boards and spent catalyst. As mentioned in the above section, the dissolution of Li^+ and Co^{2+} could be accelerated by H^+ but retarded by Fe^{3+} during the chemical leaching of $LiCoO_2$. The experimental design used to verify the role of bacteria in the bioleaching of $LiCoO_2$ is shown in Table 1, and the results are shown in Figs. 3 and 4.

Without pH adjustment, the leaching of $LiCoO_2$ in biotic (group B) and abiotic conditions (group E) are conducted. As illustrated in Fig. 3C and D, a higher recovery of Li^+ and Co^{2+} was achieved under biotic (group B) compared with abiotic conditions (group E). The results indicated that the presence of bacteria was beneficial for the leaching of $LiCoO_2$. The formation of sulfuric acid from pyrite by bacteria might be the main reason for the improvement of Li^+ and Co^{2+} recovery. It could be confirmed by the results shown in Fig. 3A, which demonstrated that the rise in pH was reduced under biotic (group B) compared with abiotic conditions (group E).

The leaching of $LiCoO_2$ under biotic (group A) and abiotic conditions (group C) with pH adjustment are conducted in this part. As shown in Fig. 4A, the consumption of H^+ under biotic (group A) and abiotic conditions (group C) was 515 mmol/L and 546.5 mmol/L, respectively. The results indicated that the amount of acid required to adjust the solution pH to 1.20 was less under biotic (group A)

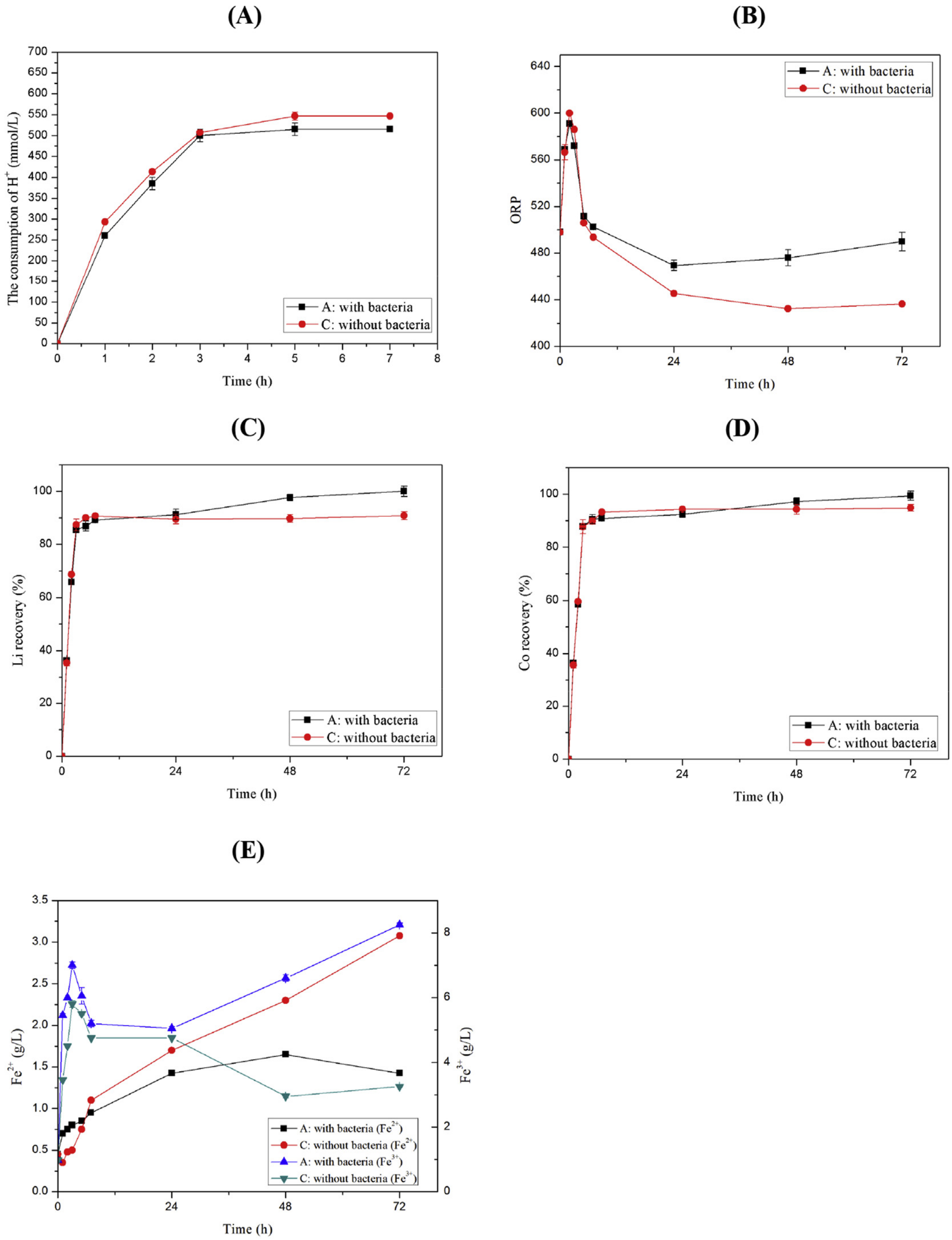

FIG. 4. Variation in the consumption of H^+ (A), ORP (B), Li recovery (C), Co recovery (D), and concentrations of Fe^{2+} and Fe^{3+} (E) with time during leaching $LiCoO_2$ with pH adjustment.

than abiotic conditions (group C). This result confirmed that the bacteria could oxidize pyrite to produce sulfuric acid. The recovery of Li^+ and Co^{2+} under biotic (group A) and abiotic conditions (group C) with pH adjustment are shown in Fig. 4C and D. The results illustrated that the recovery of Li^+ and Co^{2+} was slightly higher under abiotic (group C) than biotic conditions (group A) during the first 24 h. These findings indicated that the presence of bacteria in the leaching of $LiCoO_2$ was not beneficial for the dissolution of Li^+ and Co^{2+} at this time. Two reasons for this phenomenon were speculated. (i) Competition for Fe^{2+} might occur between iron-oxidizing bacteria and Co^{3+}. The iron-oxidizing bacteria needed to gain energy by oxidizing Fe^{2+}, and Co^{3+} was required to be reduced to Co^{2+} by Fe^{2+}. (ii) The presence of Fe^{3+} was not conductive to the dissolution of Li^+ and Co^{2+} from $LiCoO_2$. Therefore, the Fe^{3+} produced by iron-oxidizing bacteria might be the reason for the reduction of Li^+ and Co^{2+} recovery during the bioleaching of $LiCoO_2$, as confirmed by the results shown in Fig. 4E. The Fe^{3+} concentration was higher under biotic (group A) than abiotic conditions (group C).

As shown in Fig. 4C and D, the recovery of Li^+ and Co^{2+} under biotic and abiotic conditions reached approximately 90.0% at 24 h. The results indicated that most of the Li^+ and Co^{2+} in the $LiCoO_2$

was dissolved during this phase. The main role of bacteria in the bioleaching of $LiCoO_2$ was to produce sulfuric acid by oxidation of sulfur from pyrite. To clearly describe the bioleaching of $LiCoO_2$, a model was constructed as shown in Fig. 5. In this model, pyrite is attacked by Fe^{3+} to initially provide Fe^{2+} and S^0. Then, the Co^{3+} is reduced to Co^{2+} by Fe^{2+} through a chemical reaction, and Fe^{3+} is again formed. Moreover, the S^0 is oxidized to sulfuric acid by bacteria. The Li^+ and Co^{2+} are dissolved by sulfuric acid.

The results of this study are consistent with those reported by Xin et al. (31), who compared the effect of different energy sources (Fe leaching system, S leaching system, Fe+S leaching system) on the bioleaching of $LiCoO_2$ and found that Li^+ was mobilized due to acid dissolution and Co^{2+} was released by acid dissolution after insoluble Co^{3+} was reduced to soluble Co^{2+} by Fe^{2+} in both FeS_2 and $FeS_2 + S$ systems.

As shown in Fig. 4C and D, the recovery of Li^+ and Co^{2+} remained almost unchanged under abiotic conditions (group C) and reached 90.8% and 94.8% at 72 h. However, the recovery of Li^+ and Co^{2+} under biotic conditions (group A) reached 100.0% and 99.3% at 72 h. In addition to the production of acid and ferrous ions, bacteria have other effects on the bioleaching of $LiCoO_2$. As shown in Fig. 4E, the Fe^{2+} concentration was higher under abiotic (group C) than biotic conditions (group A) after 24 h. The results indicated that the remaining $LiCoO_2$ in the solution was not dissolved by the chemical reduction of Fe^{2+}. Moreover, the Fe^{3+} concentration in abiotic (group C) was lower than that in biotic (group A) after 24 h. The result showed that the remaining $LiCoO_2$ that could not be dissolved under abiotic conditions (group C) was not due to the restriction of Fe^{3+}.

Two hypothetical bioleaching mechanisms were proposed to explain these roles of bacteria in the bioleaching of $LiCoO_2$, and they are shown in Fig. 6. Mechanism 1 is as follows: In the process of the biooxidation of Fe^{2+} to Fe^{3+}, the electrons from Fe^{2+} were transferred to oxygen by a series of electron transfer chains. In the bioleaching of $LiCoO_2$, these electrons from Fe^{2+} might be partly transferred to Co^{3+}. As a result, the recovery of Co^{2+} was increased. Mechanism 2 is described as follows: During the bioleaching of $LiCoO_2$, bacteria might secret extracellular polymeric substances on pyrite and $LiCoO_2$. Some chemicals inside the extracellular polymeric substances might be beneficial to the recovery of Li^+ and Co^{2+} from $LiCoO_2$.

In addition, the recovery of Li^+ and Co^{2+} under abiotic conditions (group D) with the same amount of acid as the biotic conditions (group A) was lower than under abiotic condition (group C) during the first 7 h but then similar to the conditions shown in group C (Supplementary material Fig. S1). The recoveries of Li^+ and Co^{2+} were improved under sterile (group F) than abiotic conditions (group C) but lower than under biotic conditions (group A) (Supplementary material Fig. S1).

Confirmation of mechanism 1 Mechanism 1 supposed that electron transfer played an important role in the bioleaching of $LiCoO_2$. To verify this hypothesis, two experiments were designed.

Effect of the electron donor on the bioleaching of $LiCoO_2$ If the hypothesis was correct, there were two directions for these electrons from Fe^{2+}: one was transferred to oxygen, and the other was transferred to Co^{3+}. It was assumed that the proportion of these electrons was constant in the two directions. As a result, the increase in the electron donor concentration was able to transfer more electrons to Co^{3+} by bacteria. As a result, more Co^{3+} could be dissolved. The most direct way to increase the electron donor concentration was to increase the concentration of Fe^{2+}.

During the bioleaching of $LiCoO_2$ with pH adjustment, the effect of bacteria appeared after 24 h. Thus, different amounts of Fe^{2+} were added to the culture at 24 h to increase the amount of electron donors. The recoveries of Li^+ and Co^{2+} were used to verify the effect

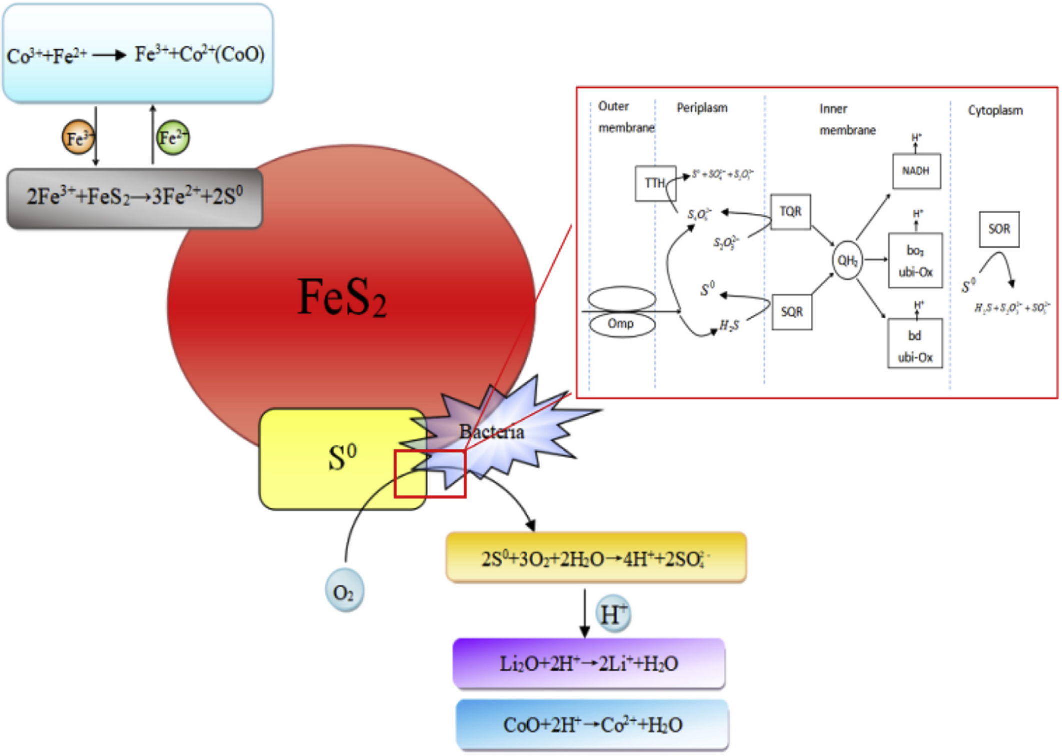

FIG. 5. The leaching mechanism of $LiCoO_2$. The red box indicates the membrane model of S metabolism. Electrons obtained by SQR, TQR from inorganic sulfur compounds are transferred to the electron transfer chain by quinone and then used by NADH complex I to generate reducing power or by terminal oxidases bd or bo3 to form H^+. TTH, tetrathionate hydrolase; SQR, sulfide quinone reductase; TQR, thiosulfate quinone oxidoreductase; SOR, sulfur oxygenase reductase; Omp, outer membrane protein.

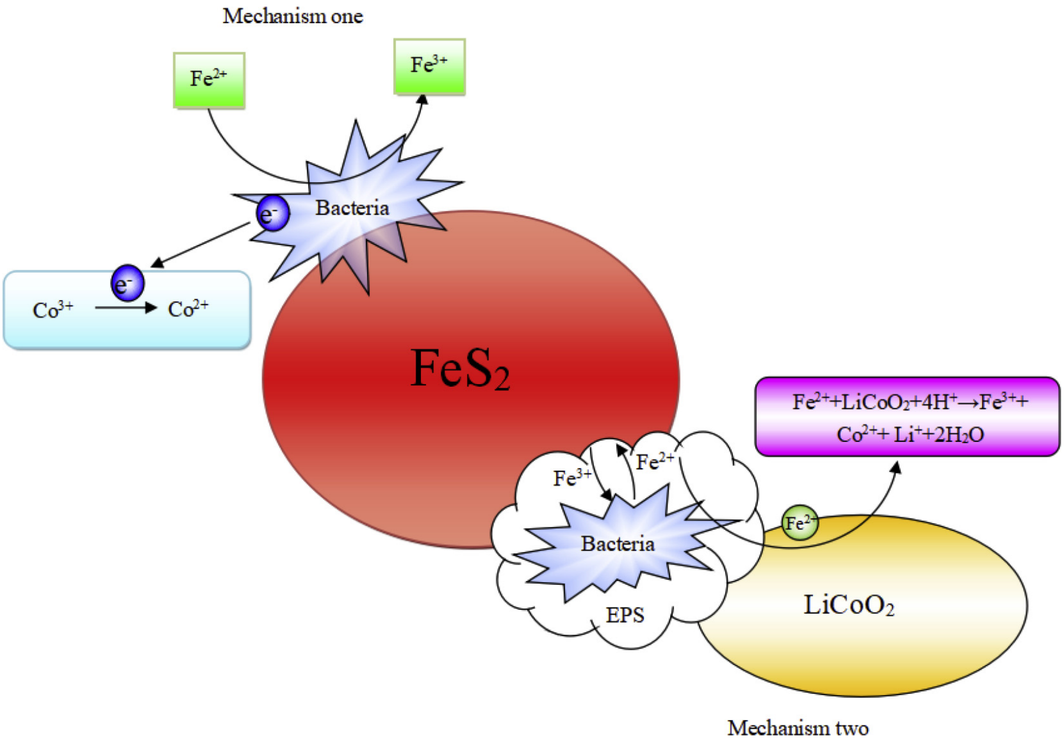

FIG. 6. The hypothetical bioleaching mechanisms of $LiCoO_2$.

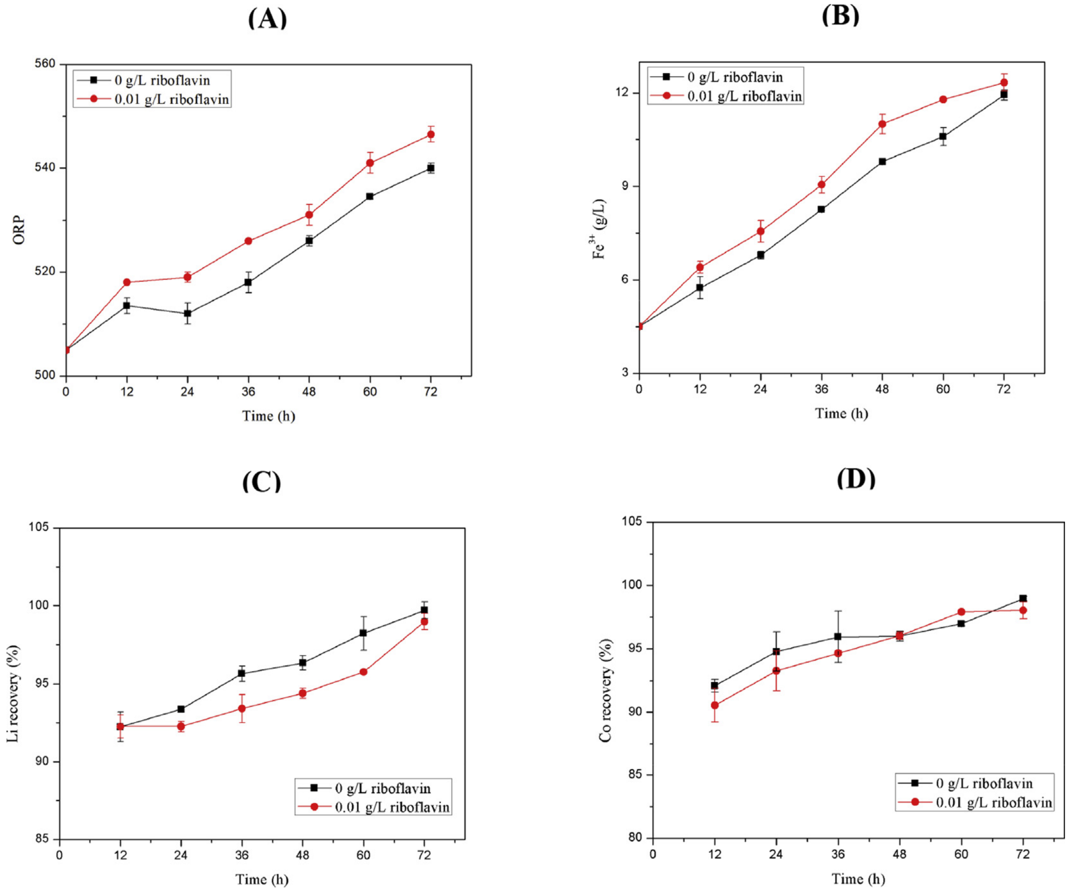

FIG. 7. Effect of acceleration of the electron transfer rate on the bioleaching of $LiCoO_2$. ORP (A), concentrations of Fe^{3+} (B), Li recovery (C) and Co recovery (D).

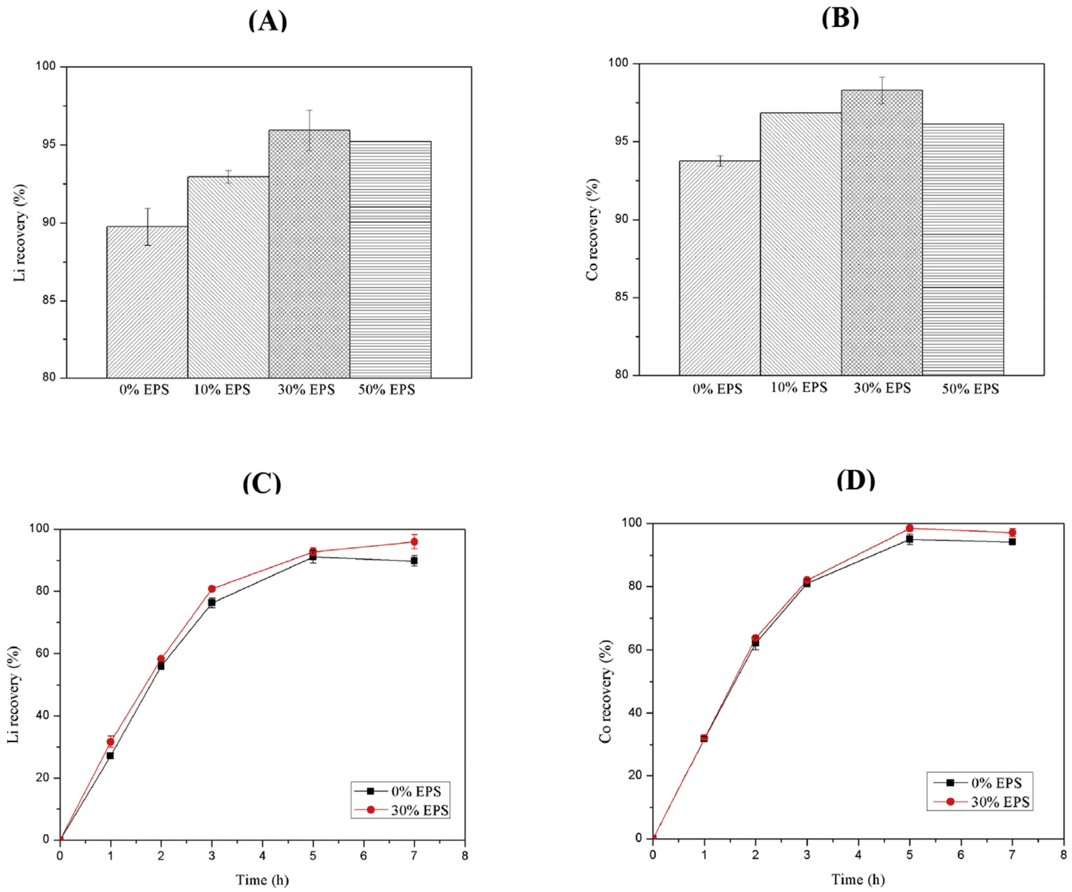

FIG. 8. Changes in Li (A) and Co (B) recovery with time during chemical leaching of $LiCoO_2$ by addition of extracellular polymeric substances (EPS). Li (C) and Co (D) recovery with the addition of different amounts of extracellular polymeric substances during chemical leaching of $LiCoO_2$ at 7 h.

of the increasing electron donor on the bioleaching of $LiCoO_2$. The results are shown in Supplementary material Fig. S2.

As shown in Figs. S2A and B, the added Fe^{2+} was basically oxidized completely by the bacteria at 42 h, which indicated that more electrons were accepted by electron acceptors through the electron transport chain pathway. However, the recovery of Li^+ and Co^{2+} did not increase with the increment of the Fe^{2+} concentration, as shown in Figs. S2C and D. These results implied that increasing the amount of the electron donor could not pass more electrons to Co^{3+}.

Effect of acceleration of the extracellular electron transfer rate on the bioleaching of $LiCoO_2$ During the bioleaching of $LiCoO_2$, there were two electronic flow directions from Fe^{2+}. The distribution proportion of the two electronic might be not fixed under different conditions. Thus, by increasing the electron donor concentration, more electrons might be transferred only to oxygen rather than to Co^{3+}. To transfer more electrons to Co^{3+}, an electron transfer mediator was used to increase the extracellular electron transfer rate. Riboflavin is a commonly used electron transfer mediator (38). In this section, riboflavin was added to the bioleaching of $LiCoO_2$ to accelerate the extracellular electron transfer rate. As shown in Fig. 7A and B, the rate of the biooxidation of Fe^{2+} to Fe^{3+} was accelerated by the addition of riboflavin. It was suggested that the electron transfer rate of bacteria could indeed be accelerated by riboflavin. However, as shown in Fig. 7C and D, the recoveries of Li^+ and Co^{2+} were not increased by the addition of riboflavin. These results indicated that the amount of electrons transferred to Co^{3+} could not be increased by accelerating the extracellular electron transfer rate.

In summary, mechanism 1 might be incorrect.

Confirmation of mechanism 2 Extracellular polymeric substances are complex mixtures of polymers excreted by bacteria through instinct or in response to environmental conditions (39). They play an important role in almost all cell interface activities, such as the aggregation of bacterial cells, adherence to surfaces, flocculation and biofilm formation, cell–cell recognition and sorption of exogenous organic compounds and inorganic ions (40). During the bioleaching of sulfide ore, the dissolution of sulfide ore can be significantly accelerated by extracellular polymeric substances (25). During the bioleaching of $LiCoO_2$, extracellular polymeric substances might have similar effects. Therefore, the extracellular polymeric substances of bacteria, which were extracted by the EDTA method, were utilized for the chemical leaching of $LiCoO_2$ in this study, and the results are shown in Fig. 8. The EDTA used to extract extracellular polymeric substances had little effect on the recovery of Li^+ and Co^{2+} (data not shown). By measuring the contents, the main components in the extracellular polymeric substances were 2.7 μg/ml protein, 110.1 μg/ml polysaccharide and 140.1 μg/ml uronic acid.

As shown in Fig. 8A and B, the addition of extracellular polymeric substances was beneficial to the dissolution of Li^+ and Co^{2+}. As shown in Fig. 8C and D, optimal recovery of Li^+ and Co^{2+} was observed with 30% extracellular polymeric substances.

The composition of extracellular polymeric substances was very complex, and the extracellular polymeric substances were mainly composed of proteins, carbohydrates, uronic acid, and lipids. To verify which component of the extracellular polymeric substances had a promoting effect on the leaching of $LiCoO_2$, protein,

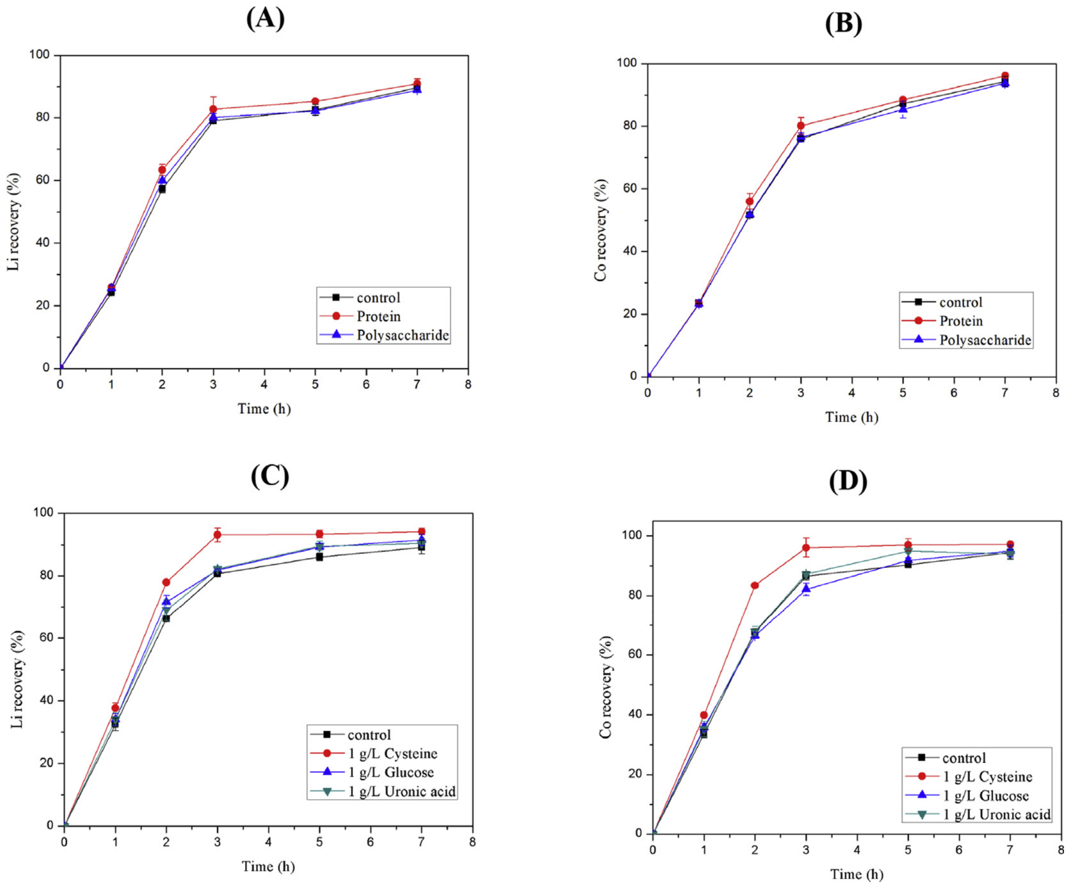

FIG. 9. (A, B) Effects of protein and polysaccharide extracted from extracellular polymeric substances on the chemical leaching of $LiCoO_2$. (A) Li and (B) Co recovery. (C, D) Effects of cysteine, glucose and uronic acid on the chemical leaching of $LiCoO_2$. (C) Li and (D) Co recovery.

polysaccharide and uronic acid were selected for analysis. As shown in Fig. 9A and B, the addition of protein extracted from extracellular polymeric substances was beneficial to the dissolution of Li^+ and Co^{2+}, but the addition of polysaccharide wasn't. Moreover, cysteine and glucose were selected as the representative monomers of protein and polysaccharide for the chemical leaching of $LiCoO_2$, and the results are shown in Fig. 9C and D. The leaching of Li^+ and Co^{2+} could be promoted by cysteine but not by glucose and uronic acid. This result might be due to the reducibility of the sulfhydryl groups in cysteine. The Co^{3+} might be reduced by these sulfhydryl groups. Wang et al. (41) validated that cysteine could clearly accelerate the oxidation rate of pyrite. The results indicated that the proteins in extracellular polymeric substances were beneficial to the leaching of $LiCoO_2$.

In the present study, the bioleaching mechanism of $LiCoO_2$ was investigated. During the bioleaching of $LiCoO_2$, Li^+ was dissolved by acid and Co^{2+} was released by the reduction of Fe^{2+} and acid dissolution. As a result, 91.4% Li^+ and 94.2% Co^{2+} were extracted from $LiCoO_2$ via the chemical leaching of pyrite with pH adjustment. The bacteria could produce sulfuric acid by oxidizing pyrite to promote the mobilization of Li^+ and Co^{2+}. With pH adjustment, the recovery of Li^+ and Co^{2+} could be increased to 100.0% and 99.3% by bacteria. The extracellular polymeric substances secreted by bacteria were confirmed to be a factor for the improvement of Li^+ and Co^{2+} recovery. The findings of the present study could provide theoretical support for the development and commercial application of the bioleaching of lithium-ion batteries.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jbiosc.2019.03.007.

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