A novel microbial-plant coupled reactor for remediating acid mine drainage and optimization of carbon sources

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Abstract

Acid mine drainage (AMD) is recognized as one of the most serious contamination sources in the nonferrous metal mining industry. In this study, aerobic strains VCZ02 and VCZ09, which were identified as *Leclercia adecarboxylata* and *Klebsiella aerogenes,* were screened from 11 strains of copper-zinc-resistant bacteria in the soil of the Dexing copper mine with Cu^{2+}/Zn^{2+} removal rates of 46.32% / 41.03% and 57.96% / 67.05%, respectively. The composition of extracellular polymers plays an important role in the removal of heavy metals by these two strains. A mixed community consisting of VCZ02 and VCZ09 was coupled with *Sagittaria sagittifolia L.* to construct a microbial-plant coupled reactor to remediate AMD. Under the optimal condition of sodium acetate as carbon source, the pH of AMD increased from less than 5 to above 6.5, showing Cu^{2+}/Zn^{2+} removal rates of 70-80% and above 30%, respectively. SEM–EDS results showed that VZC02 and VZC09 in the coupled reactor also helped with resisting the toxicity of heavy metals to plants by forming biofilms on the root surface and increasing the content of heavy metals on the surface of roots, thus improving the treatment effect of plants. This study provides a theoretical basis for the bioremediation of AMD and its application.

1 Introduction

Currently, acid mine drainage (AMD) is considered to be the most serious environmental problem associated with the metal and coal industry (Zhang, 2011). During the mining process, metallic minerals are exposed to microorganisms, oxygen and water, resulting in oxidation of associated sulfur elements and leaching of heavy metals (Hughes et al., 2013). After being carried by rainfall, AMD with SO_4^{2-}, H^+ and various heavy metals as the main ions is formed. AMD carrying heavy metals flows into the surrounding environment, causing not only environmental acidification and heavy metal contamination but also threatening human health through the food chain (He and Chen, 2014). Therefore, the removal of heavy metals from AMD has been a major problem in recent years.

At present, there are many treatment methods for AMD, including adsorption (Dlamini et al, 2019), membrane filtration (Ricci et al, 2015), advanced oxidation processes (Munyengabe et al., 2020), and chemical reactions (Kefeni et al., 2017). Nevertheless, these methods are extremely resource intensive with high costs or defective in treating AMD with high concentration of ions, which limits their large-scale application (Shi et al., 2019). Moreover, some improved physicochemical methods aiming at reducing the cost have made progress, while secondary toxic chemical sludge generated by these technologies may be the culprit leading to further environmental problems (Iqbal et al., 2009; Rawat et al., 2014; Masindi et al., 2018).

Consequently, as a low-cost and environmentally friendly technology, biological methods, including plant technology and microbial technology, are extremely promising (Shi et al., 2020). Compared with traditional methods, biological methods also show an advantage in remediating heavy metal pollution in large water basins with concentrations of heavy metals below 100 mg/L (Aryal and Liakopoulou-Kyriakides, 2015). In addition, some microorganisms in the contaminated soil have strong reduction and fixation of heavy metals as well as exhibit tolerance (Fei et al., 2022), which has promising applications for heavy metal remediation in water. For microbial remediation, extracellular polymeric substances (EPS) secreted by species-rich biofilms can adsorb heavy metals and reduce toxic effects, while the porous structure of biofilms can also promote heavy metal absorption (Liu et al., 2018). Emerging technologies such as microbial fuel cell (MFC) have been applied to treat AMD, in which anaerobic microorganisms separate metals from liquids by oxidizing metal ions and promoting their formation as precipitates (Cheng et al., 2007). MFC can not only remove heavy metals but also use microorganisms to generate electricity, which has broad development potential in clean production. However, MFC is more suitable for the removal of heavy metals in small volume (generally less than 1L) of soil and water bodies. There are a lot of restrictions when applied to magnified water bodies such as the reactor structure, membrane as well as buffering capacity (Pandit et al., 2020). AMD treatment is also often achieved by constructing a continuous alkalinity production (SAPS) reactor, which, in addition to the carbon source layer required for microbial growth, needs to be filled with an alkalinity layer to raise the pH of the reactor effluent (Jung et al., 2014). In fact, anaerobic bacteria play a key role in both MFCs and SAPS in terms of treating AMD due to their purification effect on multiple heavy metals (Lu et al., 2011; Le Pape et al., 2017; Yan et al., 2018). However, as an anaerobic species, SRB grow under harsh conditions, which is not practical in industrial applications. Microorganisms growing under aerobic conditions grow faster than anaerobic microorganisms. Consequently, if biosorption is the main removal route, aerobic microorganisms have greater advantages for more tolerant growth conditions and faster growth rates.

Indigenous bacteria generally showed higher resistance to pollutants for their adapted enzymatic reactions (Ighalo et al., 2022), therefore, bioremediation strains are easier to obtain in areas that have been contaminated for a long time (Wen et al., 2018; Gallardo-Rodríguez et al., 2019). Mixed microorganism culture has many advantages over single culture, such as increasing the number of substrates available and enhancing heavy metal resistance (Cui et al., 2017; Kang et al., 2016; Liu et al., 2018). Wen et al. used three screened copper-resistant bacteria to construct a flora and inoculated it into sludge to enhance the removal of heavy metals. In this system, bacteria formed a stable, balanced, and drug-resistant flora, and the copper removal rate reached 98.0 ± 0.3% (Hughes et al., 2013). It can be seen that constructing flora is beneficial for microorganisms in practical applications. Moreover, heavy metals are likely to be more toxic to free individual microorganisms, so it is a common method to attach microorganisms to the carrier, which can also prevent the loss of microorganisms and ensure their retention in the reactor before adapting to the environment (Wen et al., 2018).

In this study, a microbial-plant coupled reactor was constructed to remediate AMD based on strains with purification ability for Cu^{2+} and Zn^{2+} which were screened from soil contaminated by AMD. In addition, the mechanisms of four carbon sources affecting the treatment effect of the reactor and the reinforcement between microorganisms and plants were further investigated, aiming at providing an economical and suitable treatment method for field application in mitigating heavy metal contamination of mining areas.

2 Materials And Methods

2.1 Strain isolation and identification

Five grams (fresh weight) of soil from the Dexing Copper Mine was suspended in 100 mL of sterile water and shaken at 150 rpm for 30 minutes. The suspension phase was oscillated in LB medium for 24 h before the nutrient solution was serially diluted and spread on LB agar medium (pH 5, Cu^{2+} 25 mg/L, Zn^{2+} 20 mg/L) and then incubated at 28°C for 2–4 d to screen the copper-zinc resistant bacteria.

Colonies with different morphologies were selected and streaked. After repeating 4 times, pure strains were obtained. They were cultured in LB medium (pH = 5) containing 25 mg/L Cu^{2+} and 20 mg/L Zn^{2+}. After 3 days, the remaining heavy metal concentration and pH were measured to obtain strains with Cu^{2+} and Zn^{2+} remediation abilities. 16S rDNA analysis of screened strains was conducted by Shanghai Majorbio Biopharm Technology Co. Ltd.

2.2 Reactor parameters

After reviewing the literature, emergent vegetations such as *Pontederia cordata L.*, *Sagittaria trifolia L.*, *Acorus calamus L.*, *Canna indica L.* and *Cyperus alternifolius L.* were selected for pre-experiments. Taking into account the biomass, heavy metal enrichment effect and translocation capacity, *Sagittaria trifolia L.* was selected as the coupling plant in the reactor to make it more effective and sustainable. Screened bacterial strains were coupled with *Sagittaria sagittifolia L.* to construct a microbial-plant reactor, and its structure is shown in Fig. 1. The *Sagittaria trifolia L.* used in the experiment came from seedlings purchased through online channels, and plants of good growth and similar size were selected for the experiment after one week of incubation in the laboratory. Elastic filler from Shanghai TBR Braid Co. Ltd. was placed in the reactor to offer attachment for biofilms grown from strains VCZ02 and VCZ09 (1:1). Emerging plants Sagittaria sagittifolia L. were planted in the upper part of the reactor, which was beneficial for the remediation of Cu and Zn pollution as well as adjusting the pH of the water. The wastewater was pumped from the water inlet at the bottom of the reactor and flowed out from the water outlet at the top of the reactor.

The inlet water was prepared according to the AMD component of the Dexing Copper Mine: COD 200 mg/L, TP 0.4 mg/L, TN 2 mg/L, and nitrogen was provided by $(NH4)_2SO_4$ while phosphorus was provided by $K_2H(PO_4)_3$. The reactor flow was 3.91 ml/min with a hydraulic retention time of 8 h, and the air-water ratio was 5:1. Water samples were collected daily to determine the pH and Cu^{2+} and Zn^{2+} concentrations in inlet and outlet water.

2.3 Carbon source experiment

To explore the effect of different carbon sources on treatment, the reactor was supplied with carbon sources commonly used in sewage treatment plants, including sucrose, sodium acetate, glucose and methanol. The concentration of the carbon source in the influent water was 3 g/L, which has been proven to be the optimal level for bacterial growth in previous experiments. The carbon source in the influent water was changed every 5 days, and the whole experiment lasted for 48 days (including 4 weeks for flora to form biofilms). The inlet and outlet water of the reactor during the running period were analyzed for pH and heavy metal concentration. Before the carbon source was changed, the EPS of the biofilm on the filler in every period was analyzed.

2.4 Measurement of EPS

First, 2 mL of a 2 mol/L NaOH solution was added to 1 mL of biofilm and brought up to 10 mL with deionized water. The mixture was shaken at a speed of 150 r/min for 2.5 h and then centrifuged at 10,000 r/min for 20 min. The supernatant was passed through the membrane to obtain extracellular polymer substance (EPS) (Zeng et al., 2020), and the sum of protein and polysaccharide was the total amount of EPS. Furthermore, the polysaccharide content was measured by using the phenol-sulfuric acid method with a glucose standard (Jain et al., 2017). Protein was determined according to the modified Coomassie blue staining method (Cui et al., 2020) with bovine serum albumin as the standard. Then, 0.05 ml of EPS solution or standard sample in 3 ml of Coomassie brilliant blue developer (Nanjing Jiancheng Technology Co., Ltd.) in a centrifuge tube. After standing for 10 minutes, the absorbance was measured at 595 nm, and the protein content was calculated according to the formula.

2.5 Metabolic diversity in the reactor

The Ecolog of Biolog system was used to study the microbial metabolism in the reactor. Biolog EcoPlate™ consisted of 96 wells divided into three groups containing 31 of the most common carbon sources, specifically for microbial metabolism research and community structure analysis. Each well contains a redox dye indicator to reflect the nicotinamide adenine dinucleotide (NADH) produced by cellular respiration.

The dilutions were seeded into Eco plates at 150 μL/well. The plate was cultured under dark conditions at 28°C for 10 days and analyzed by a microplate reader every 12 hours (dual-wavelength data: OD_{590}-OD_{750}). The absorbance of each well directly reflected the strength of the carbon source utilization ability and was measured by the average well color development (AWCD).

2.6 Elemental analysis

The experimental sample solution was filtered through a 0.45 μm filter. An inductively coupled plasma emission spectrometer (ICP–OES) (Thermo Scientific ICAP 6000 series, Thermo Fisher Scientific Inc., USA) was used to accurately determine the Cu^{2+} and Zn^{2+} concentrations in the filtrate. The roots of Sagittaria were freeze-dried for SEM–EDS (JSE-6330F) analysis to obtain the surface morphology and relative content of Cu and Zn on the surface.

3 Results And Discussion

3.1 Isolation and identification of Cu-Zn-remediation strains

The strains isolated from the soil samples of the mining area were able to thrive on plates containing Cu and Zn. The bacterial colonies are all round, while their size, edge shape, color, and surface state are different. According to the morphological characteristics of the colonies, 11 strains of bacteria were isolated, all of which have the potential to remediate copper and zinc pollution.

After being cultured in LB medium containing heavy metals for 3 days, the pH generally rose from 5 to more than 8, and the removal rate of Cu/Zn as well as the final pH values are shown in Fig. 2. All 11 strains can remove Cu^{2+} and Zn^{2+} from the prepared AMD, while VCZ02 and VCZ09 showed the best comprehensive removal effect of these two heavy metals. Among these strains, VCZ09 has the highest removal rate of Zn^{2+}, which can reach 67.05%; VCZ02 has a greater advantage in removing Cu^{2+}, which can reach 46.32%.

Compared with single strain cultures, the mixed flora demonstrated greater resistance and efficiency for the remediation of heavy metals (Cui et al., 2017; Kang et al., 2016). To obtain a more stable and more abundant bacterial population for application, VCZ02 and VCZ09 were selected with elevated pH and removal rates of Cu^{2+} and Zn^{2+} as comprehensive indicators. Plate confrontation experiments showed that there was no antagonism. Therefore, a flora was constructed by these two strains for subsequent experiments.

VCZ02 and VCZ09 showed higher Cu^{2+} and Zn^{2+} removal abilities than other strains, and their 16S rDNA sequences were determined to identify strains. Genetic results of 16S rDNA showed that these two strains were highly similar to *Leclercia* and *Klebsiella* (Table 1). VCZ02 belongs to *Leclercia adecarboxylata*, while VCZ09 belongs to *Klebsiella aerogenes*. The phylogenetic tree was constructed as shown in Fig. 3.

Table 1
The result of the alignment with 16S rDNA sequences of organisms in the EzTaxon database

Strains	Species	Gene identity	Access no.
VCZ02	*Leclercia adecarboxylata NBRC 102595*	99.57%	BCNP01000062
VCZ09	*Klebsiella aerogenes KCTC 2190*	99.57%	AB004754

Leclercia adecarboxylata is a phosphate-solubilizing microorganism that has been isolated from the plant rhizosphere, plant body (Teng et al., 2019; Danish et al., 2019) and mining soil (Han et al., 2019), and has been found to have a variety of heavy metal resistances. As a plant-associated bacterium, it can produce various biomass-promoting substances to improve the growth of plants (Kumawat et al., 2019). Secreted EPS and groups on its cell surface can absorb heavy metal ions and have been shown to successfully passivate Pb by forming a stable crystal structure (Teng et al., 2019; Teng et al., 2020). Therefore, it is expected to be applied in remediating heavy metal contamination.

Klebsiella aerogenes was isolated from pesticide-stressed soil. It can effectively promote plant growth and enhance plant degradation of pollutants (Rani et al., 2019). In addition, organic matter such as motor oil can also be degraded by it (Alshebani, 2012). This kind of bacteria has not been studied much. Early scholars have proven that it can accumulate heavy metals in cells during rapid proliferation. However, metal absorption will be significantly reduced at lower pH values (Rudd et al., 1983). Currently, *Klebsiella aerogenes* is rarely used in the actual processing of heavy metals.

Both *Leclercia adecarboxylata* and *Klebsiella aerogenes* are gram-negative bacteria whose cell membranes contain a large number of lipopolysaccharides, peptidoglycans and phospholipids (Nikaido and Nakae, 1980). Most of these components are accompanied by carbonyl, hydroxyl, carbon/oxygen single bonds and other functional groups. It is generally believed that the oxygen and nitrogen atoms in the oxygen-containing groups tend to take electrons from other atoms due to the unsaturated outer electron layer, thus making the oxygen negatively charged. Consequently, the positively charged Cu^{2+} and Zn^{2+} combine with the oxygen negative ions to form a chemical bond or even a more stable coordination structure, thus realizing the adsorption of heavy metal ions (Saranya et al, 2018).

3.2 Effect of carbon sources on the microbial-plant reactor

3.2.1 pH of reactor effluent

Both plants and microorganisms can regulate the pH of acidic water that flows through (Asad et al., 2019). As shown in Fig. 4, under the coupled effect of plants and microorganisms, the effluent pH was increased compared to the influent.

Among the four carbon sources, sodium acetate showed the greatest advantage in increasing the pH value of the effluent with a stable pH of 6.3 ~ 6.7, while methanol showed a weaker advantage with a pH value of 5.9 ~ 6.2. Following the above two carbon sources, glucose and sucrose performed the worst, with a pH of 5.6 ~ 6.0. When the experiment of each group was carried out to the 5th day, the pH of the effluent was unstable, indicating that the reactor performance decreased with time and adding bacteria are essential to ensure stable operation (Wen et al., 2018).

3.2.2 Heavy metal concentration in reactor effluent

Sagittaria has been reported to have the ability to enrich Cu and Zn (Shirinpur-Valadi et al., 2019; Xu et al., 2012). In addition, bacteria have been identified as one of the major biosorbents for metal detoxification because their cell walls, cell membranes, and secreted EPS are composed of a variety of organic functional groups that are highly efficient at chelating metals (Saranya et al., 2018). In addition, the high porosity of biofilms can also facilitate the entry of heavy metal ions into the cell interior (Liu et al., 2018).

As shown in Fig. 5(a), the Cu concentration in the effluent was reduced under all four carbon sources, while there were significant differences between the various groups. Concentrations of Cu in effluent of the sucrose group, sodium acetate group, glucose group and methanol group were 6.75–8.82 mg/L, 2.92–4.85 mg/L, 7.79–9.26 mg/L and 8.43–9.16 mg/L, respectively. Apparently, sodium acetate has a better advantage on Cu^{2+} removal and facilitated the microbial-plant combination reactor reach a removal rate of 70.23 ~ 81.66%. It can be inferred that sodium acetate is more conducive to microbial growth on the biofilm. As shown in Fig. 5(b), in addition to sodium acetate, methanol also helped improve the removal rate of Zn^{2+} to approximately 32%. Considering the effect of the carbon source on the removal of the two heavy metals by microorganisms, sodium acetate was the best carbon source.

In existing static pot experiments, the plants' removal rate of Cu and Zn can reach approximately 30–40% (Sricoth et al., 2018). However, the removal rate will be greatly reduced if it becomes a continuous dynamic inflow, and campanula can only remove 5.18% Cu and 24.2% Zn in water (Soda et al., 2012). In addition, existing studies were conducted under neutral conditions, and turning the feed water acidic will not only impair biological growth but also reduce the precipitation of heavy metals. As a consequence, it will exhibit a lower heavy metal removal rate than 20%.

Nevertheless, under the acid influent conditions in this study, the removal rate of Cu was still higher than previous results under neutral conditions, suggesting that the presence of microorganisms enhances the plant's remediation effect on heavy metals (Asad et al., 2019). According to the solubility product of $Zn(OH)_2$ and $Cu(OH)_2$, the pH range of Zn^{2+} precipitation is 5.4 ~ 8.0, and the pH range of Cu^{2+} precipitation is 4.4 ~ 6.4. Referring to Fig. 4, the effluent pH values of the sucrose, glucose, and methanol groups were all around 6 while the sodium acetate group showed a higher pH value above 6.5 of effluent, which partially explained the poor removal effect of Zn^{2+} and Cu^{2+} in the first three groups.

The temperature of the laboratory was low during the experimental period, which had a negative impact on the growth of microorganisms and plants, resulting in inactive biological metabolism and slow biofilm renewal. During the process of this experiment, biofilms adsorbed heavy metals in water, and the adsorption sites were gradually occupied. In this situation, no new sites were generated, and the adsorbed heavy metals may also have desorbed. Consequently, the concentration of heavy metals in the effluent showed an upward trend in the later period of the second stage, as shown in Fig. 5.

3.2.3 Impact of carbon source on EPS

In the adsorption of heavy metals, EPS is a key channel for bacteria to remove heavy metals (Gallardo-Rodríguez et al., 2019). Table 2shows the amounts of EPS secreted by biofilms under different carbon sources.

Table 2
EPS of biofilms under different carbon sources

Carbon source type	Polysaccharide	Protein	EPS
Sucrose	0.043	0.102	0.145
Sodium acetate	0.044	0.168	0.212
Glucose	0.062	0.148	0.210
Methanol	0.071	0.134	0.205

The protein content of EPS secreted by fresh biofilms is slightly higher than that of polysaccharides, indicating that the EPS are mainly proteins. Studies have shown that proteins can protect cells from harmful elements (Yin et al., 2011) and that bacteria can regulate their own EPS production, resulting in more functional groups capable of binding heavy metals (Naik et al., 2012).

With the pumping of acidic heavy metal wastewater, the protein concentration gradually increased, which also confirms the microbial self-regulatory mechanisms. Under the continuous supply of four carbon sources, the amounts of EPS were not equal as shown in Table 2. Glucose and methanol are beneficial for the secretion of polysaccharides, while sodium acetate can promote the secretion of proteins. The cations in the water chelated with one or more kinds of polysaccharides or proteins and combined with the EPS through surface complexation, eventually reducing the concentration of heavy metals in the water (Yang et al., 2016).

In the process of continuous inflow, sodium acetate can induce the biofilm to secrete the maximum amounts of EPS. Figure 4 also confirms that the removal rate of heavy metals is the highest under the action of sodium acetate, which shows that EPS does play an important role in removing heavy metals. It has also been proven that biofilms composed of VCZ02 and VCZ09 have meaningful prospects in the remediation of heavy metal pollution.

3.3 Changes in microbial metabolic diversity

Average well color development (AWCD) is often used to evaluate the overall ability of a microbial community to degrade multiple carbon sources at a certain point of time, reflecting the difference in activity between multiple different microbial communities (Rutgers et al., 2016). The change rate (slope) of AWCD value and the ultimate AWCD value in variation curve reflect the ability of soil microorganisms to utilize carbon source. The higher the AWCD value is, the better the carbon metabolism capacity of this community (Zeng et al., 2018). The determined AWCD values of the biofilm with a carbon source of sodium acetate before and after the start-up of the reactor are shown in Fig. 6.

The AWCD value reflects the metabolic rate of the biological community during the growth process and the final degree. During the 12–84 h of preculture, the AWCD value of the reactor before the start-up (filming was completed and the reactor was not pumped with acidic leaching water) rose rapidly, followed by a slower rise between 84–132 h. The AWCD changes were not very obvious after 132 h, indicating that the carbon metabolism capacity of the community was nearly stable at this time.

As the reactor started to run, the Cu^{2+}, Zn^{2+} and low pH in the prepared AMD exerted a negative impact on the growth of biofilms. It can be clearly seen from Fig. 6 that the biofilm metabolism in the reactor was significantly slower than that in Before, and the carbon metabolism capacity stabilized earlier because the AWCD value began to level off at approximately 80 h.

After the reactor was started, the AWCD value of the upper biofilm was the highest, while the metabolic rates of the middle and lower microorganisms were similar in the first 75 hours. However, the final AWCD value after 10 days showed that the upper layer > middle layer > lower layer, which means that the upper layer microorganisms have a stronger carbon source utilization capacity. The upper layer of the biofilm in the reactor is in contact with the plant roots, and the small molecular organic matter secreted by the plant roots will provide additional nutrients for the growth of microorganisms. The two promote each other and result in the vigorous growth of microorganisms in this part.

3.4 Effects of microorganisms on plant root surface morphology

To study the effect of added microorganisms on Sagittaria, the roots of plants in three groups were compared: deionized water without microorganisms (CK), acid-leached water without microorganisms (CK+) and acid-leached water with microorganisms (BA). The surface morphology and elemental content of roots in the three groups were determined using scanning electron microscopy and energy dispersive spectrometry (SEM–EDS), and the results are shown in Fig. 7 and Table 3.

The roots in the CK group showed a smooth surface and robust growth conditions with more regularly arranged vertical fibers with clear contours. Compared with the smooth surface of roots in the CK group, Fig. 6 (c) indicates that the acidic leachate water in the CK + group increased the furrows and surface roughness of the Sagittaria roots, suggesting that heavy metal ions and H^+ could damage the roots. However, with the presence of the added flora of VCZ02 and VCZ09, roots in the BA group showed a smoother surface than those in CK+, which revealed that the presence of microorganisms could alleviate the stress of Cu^{2+}, Zn^{2+} and H^+ and thus protect the roots from harsh environments.

Greater magnification helped to observe more microscopic root surfaces, and Fig. 7(b) (d) (f) shows the root surface conditions of CK, CK + and BA under 5000×, respectively. The root surface of CK was very smooth with no obvious bumps, grooves, or fibers, whereas the root surface in Fig. 7 (d) and Fig. 7 (f) was significantly rougher with debris and particles. In addition, a large number of gathered short rod-shaped bacteria can be observed on the root surface in Fig. 7 (f). This evidence suggests that microorganisms growing on the filler also contact the plant roots through water flow and attach to the plant root surface, forming biofilms to mitigate the damage of heavy metal ions and H^+.

EDS showed that the main elements on the surface of Sagittaria were C, O, P, S, K, Ca, Cu and Zn (Table 3). A large amount of Cu was adsorbed on the surface of roots in the CK + group, and the weight percentage increased from 0 in CK to 6.44% in CK+. The results of the CK + and BA groups indicated that the combination of plants and microorganisms could adsorb more heavy metals on the root surface of Sagittaria. The content of Cu increased from 6.44% in the CK + group to 12.52% in the BA group, while the content of Zn increased from 1.60–3.41%, which showed a twofold increase in the adsorption ability of roots.

Table 3
Element contents on the root surface of Sagittaria in different groups

element	CK		CK+		BA	
	Wt%	At%	Wt%	At%	Wt%	At%
C	25.95	36.65	35.42	46.62	26.95	38.82
O	48.81	51.75	47.56	47.00	47.96	51.85
P	5.23	2.86	4.50	2.30	5.32	2.97
S	3.14	1.66	2.69	1.33	2.15	1.16
K	13.61	5.91	0.36	0.15	1.06	0.47
Ca	1.97	0.83	1.78	0.70	1.55	0.67
Cu	0.00	0.00	6.44	1.60	12.52	3.41
Zn	1.29	0.34	1.23	0.30	2.50	0.66

Wt %: weight percentage; At %: atomic percentage

4 Conclusion

In this study, bacteria in local soil were screened to construct a microbial-plant reactor with Sagittaria to remediate acidic mine drainage in a nonferrous metal mining area. A total of 11 strains of bacteria resistant to the heavy metals copper and zinc were screened from the local soil, and VCZ02 and VCZ09 were the most effective strains in the total simultaneous removal of Cu and Zn, with Cu removal rates of 46.32% and 41.03% and Zn removal rates of 57.96% and 67.05%, respectively. The 16S rDNA results showed that they were *Leclercia adecarboxylata* and *Klebsiella aerogenes*. Flora consisting of VCZ02 and VCZ09 were coupled with Sagittaria to construct a reactor treating acid mine drainage with a pH value lower than 5. With the best carbon source of sodium acetate, the pH of the effluent was maintained at 6.3 ~ 6.7 with a Cu^{2+} removal rate of 70–80% and a Zn^{2+} removal rate above 30%. Flora consisted of VCZ02 and VCZ09, which formed biofilms and increased the protein content in the secreted EPS to absorb more Cu^{2+} and $Zn^{2+,}$ therefore protecting plant roots and improving the heavy metal removal effect. The results of this study provide a theoretical basis and technical parameters for the biological purification of AMD.

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

Availability of data and materials: Not applicable

Competing interests: Not applicable

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Authors' contributions:

Conceptualization: Hai Lin; methodology: Hai Lin; formal analysis and investigation: Hai Lin; writing original draft preparation: Yalu Tang; writing, review and editing: Yalu Tang; Funding acquisition: Yingbo Dong; resources: Yingbo Dong; supervision: Yingbo Dong.

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Figures

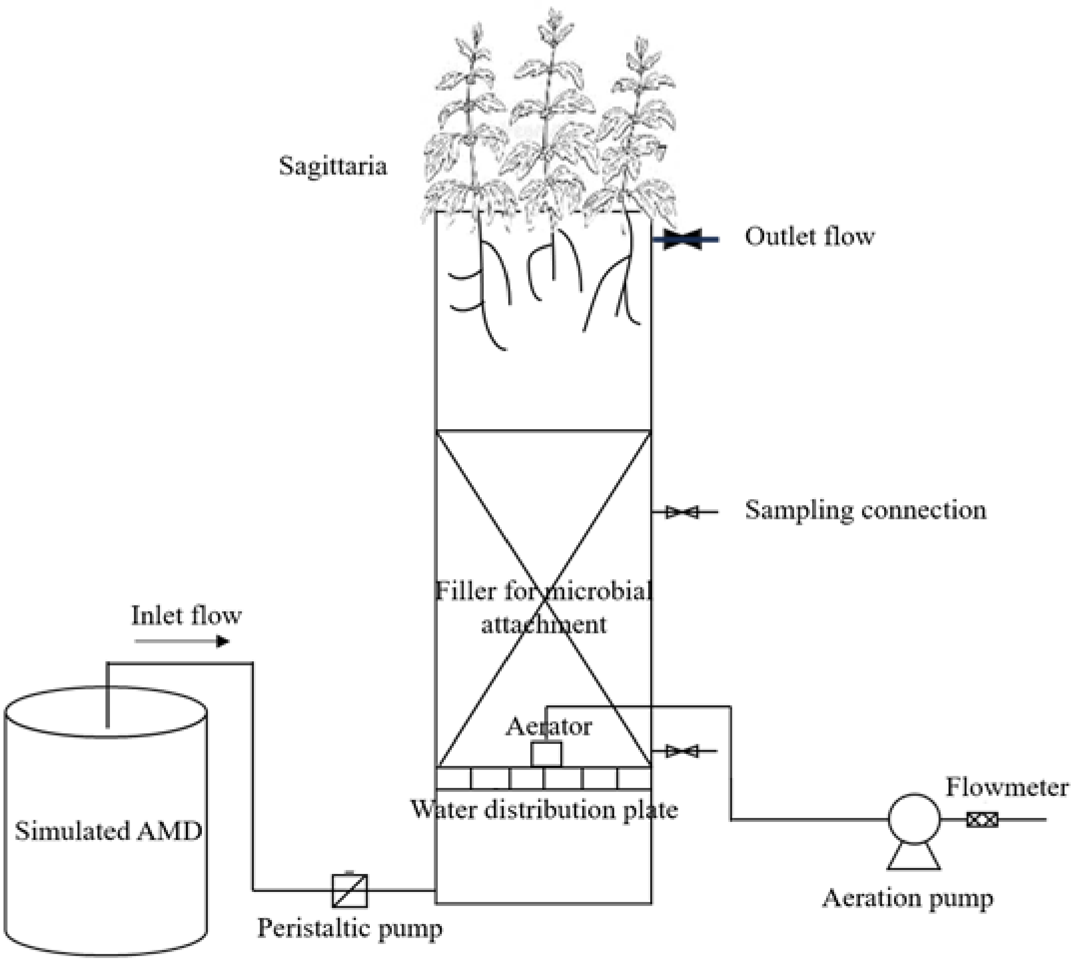

Figure 1

Schematic diagram of microbial-plant remediation of AMD

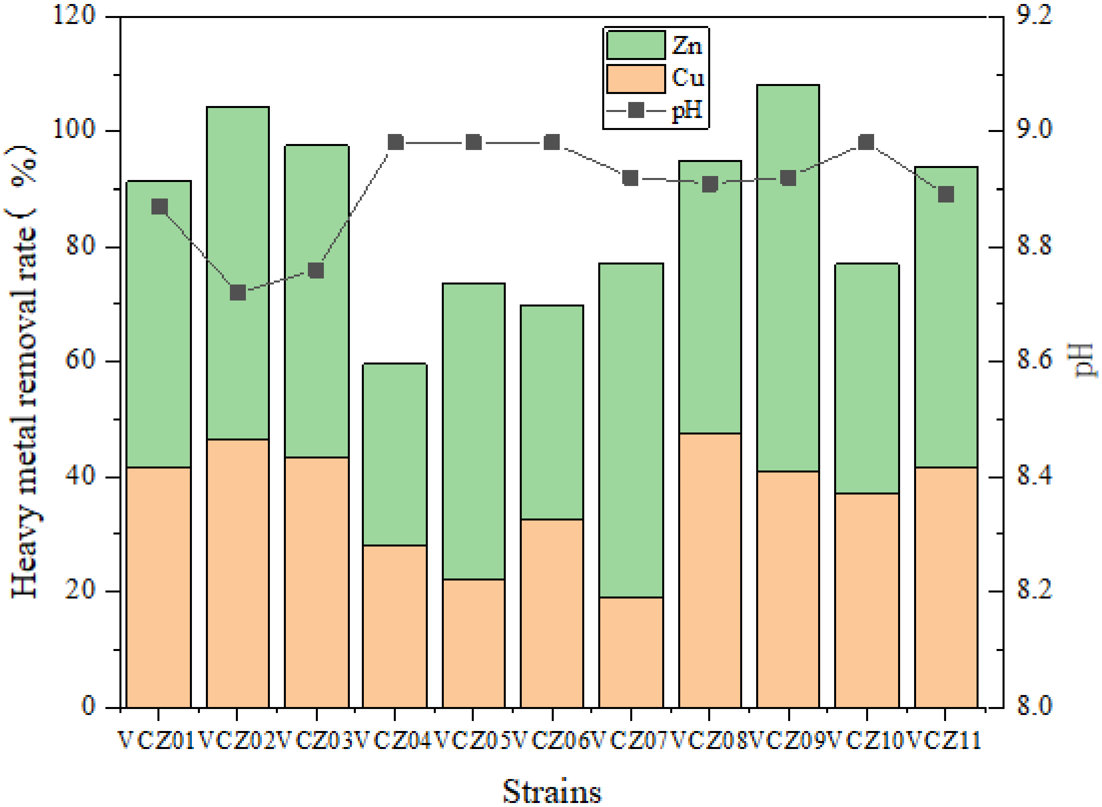

Figure 2

Effects of bacterial metabolism on heavy metal concentration and pH

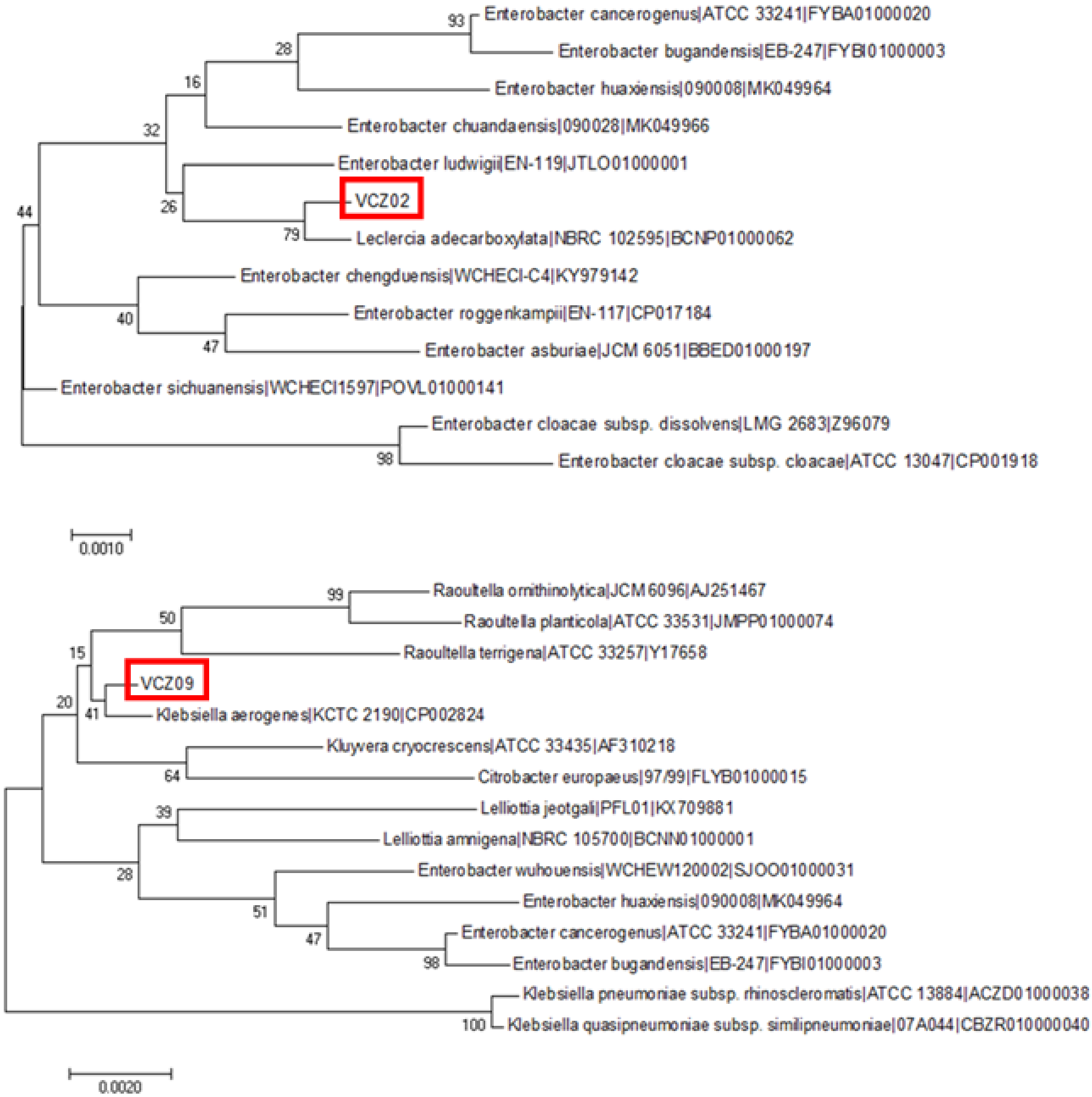

Figure 3

Phylogenetic tree of VCZ02 and VCZ09

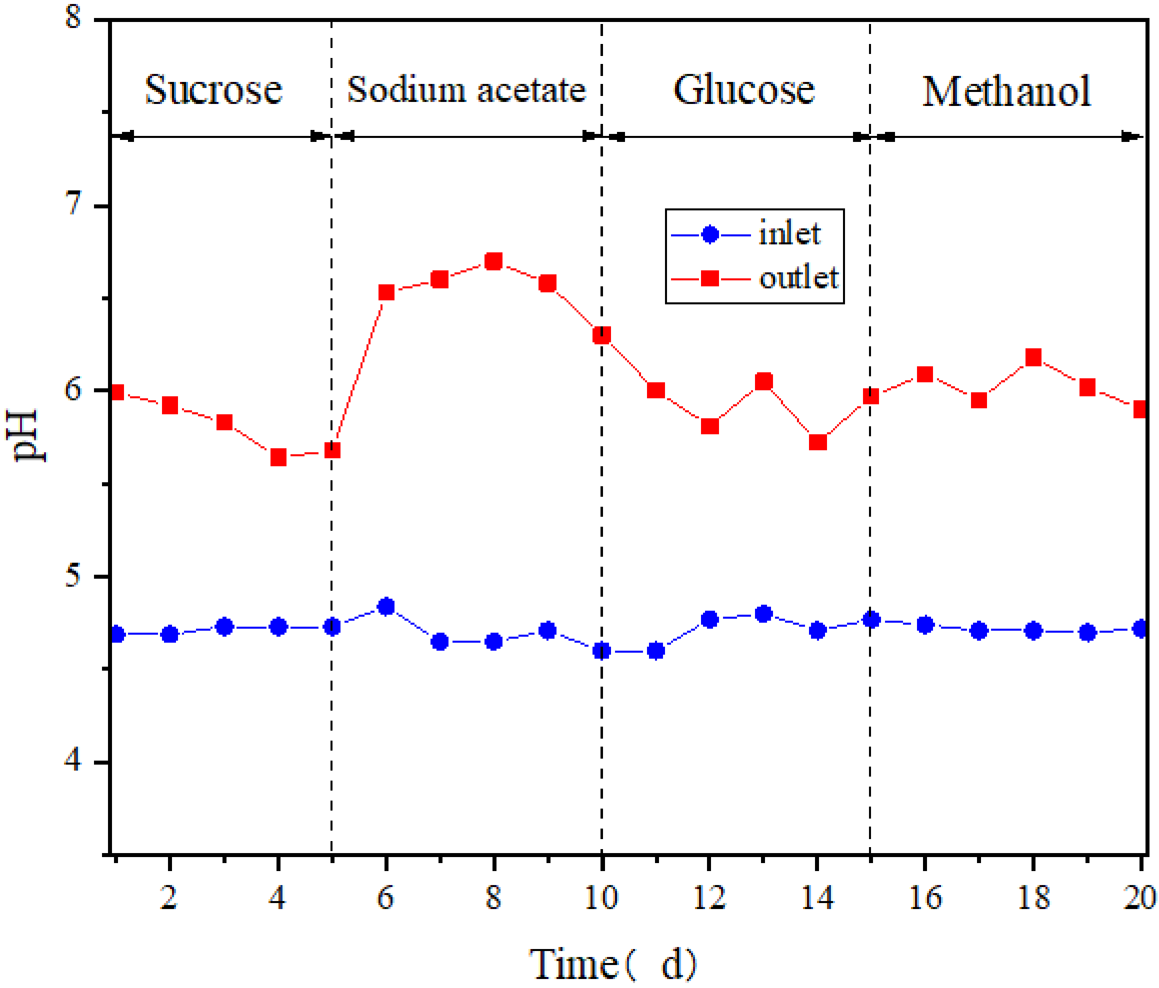

Figure 4

Effect of carbon sources on improving the pH of AMD

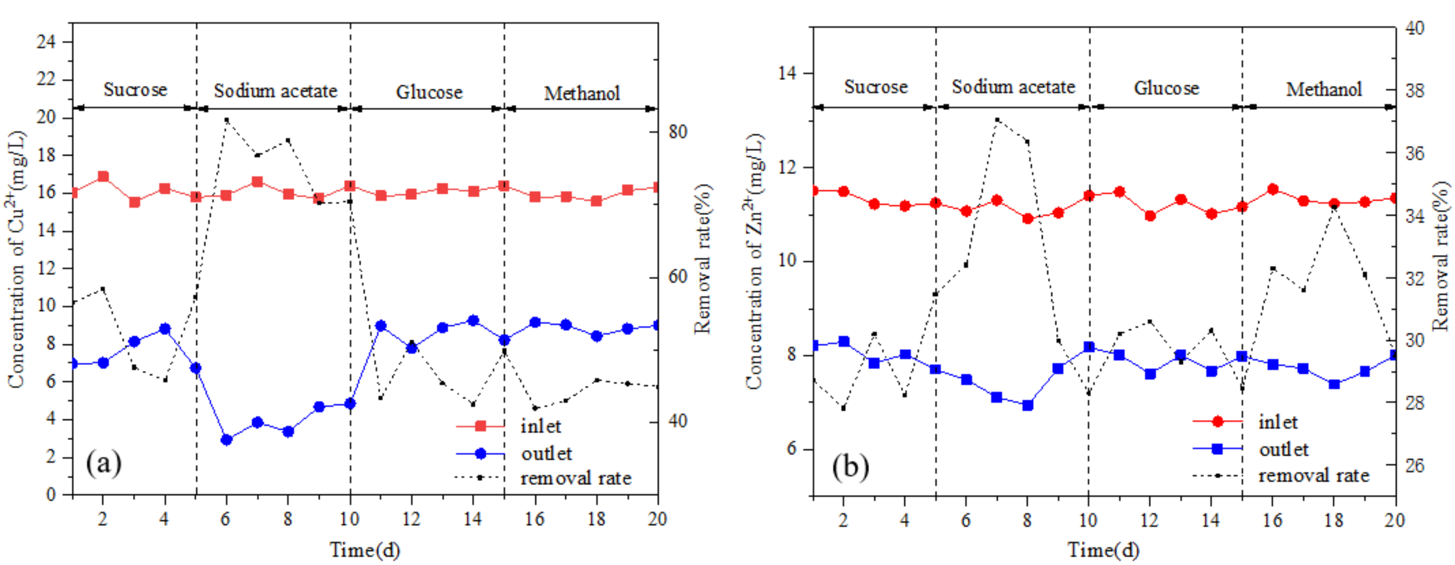

Figure 5

Removal effect of Cu^{2+} (a) and Zn^{2+} (b) in the reactor under different carbon sources

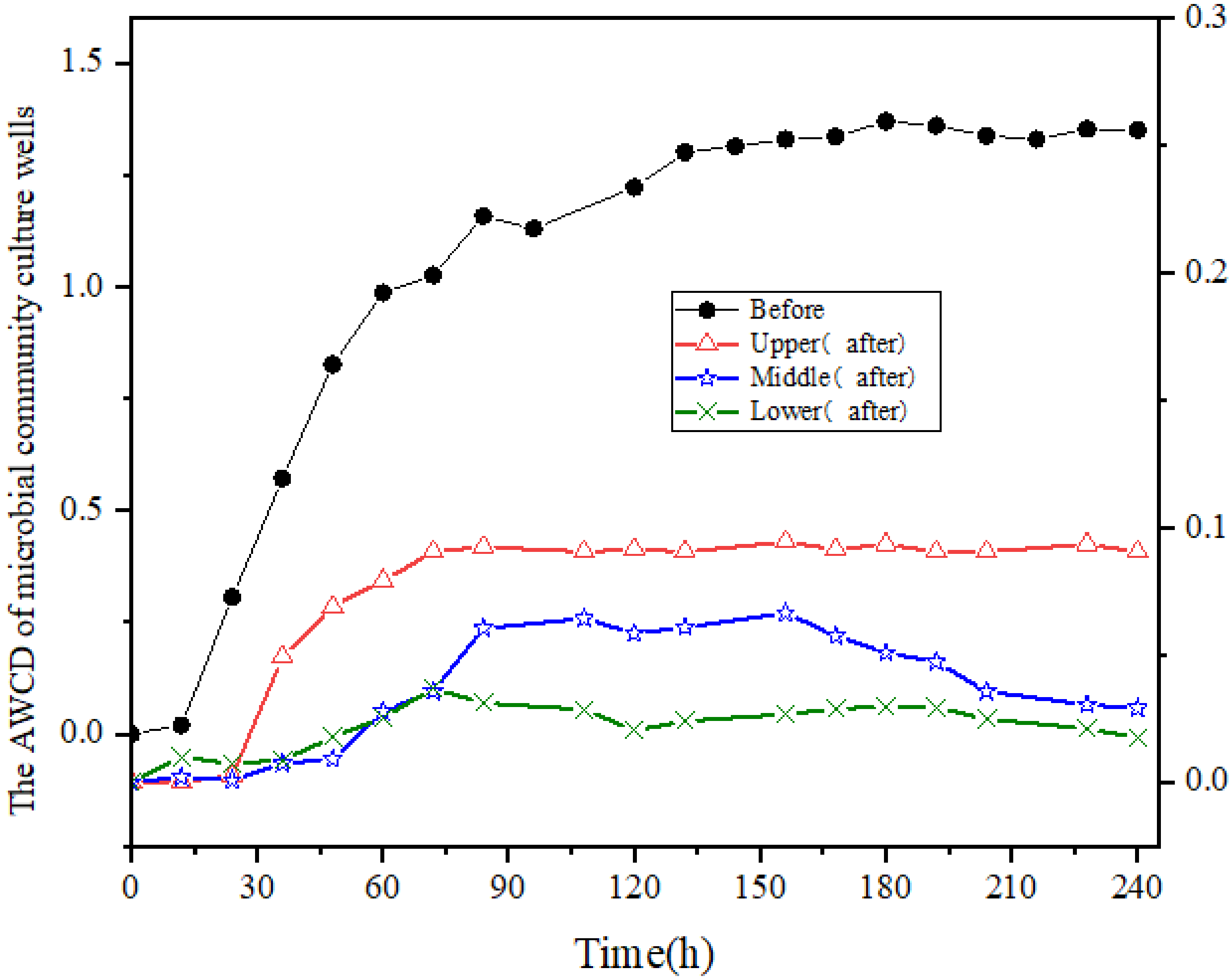

Figure 6

Average well color development (AWCD) of biofilms. The broken lines of the upper, middle and lower AWCD values after the reactor is started are drawn on the right ordinate axis

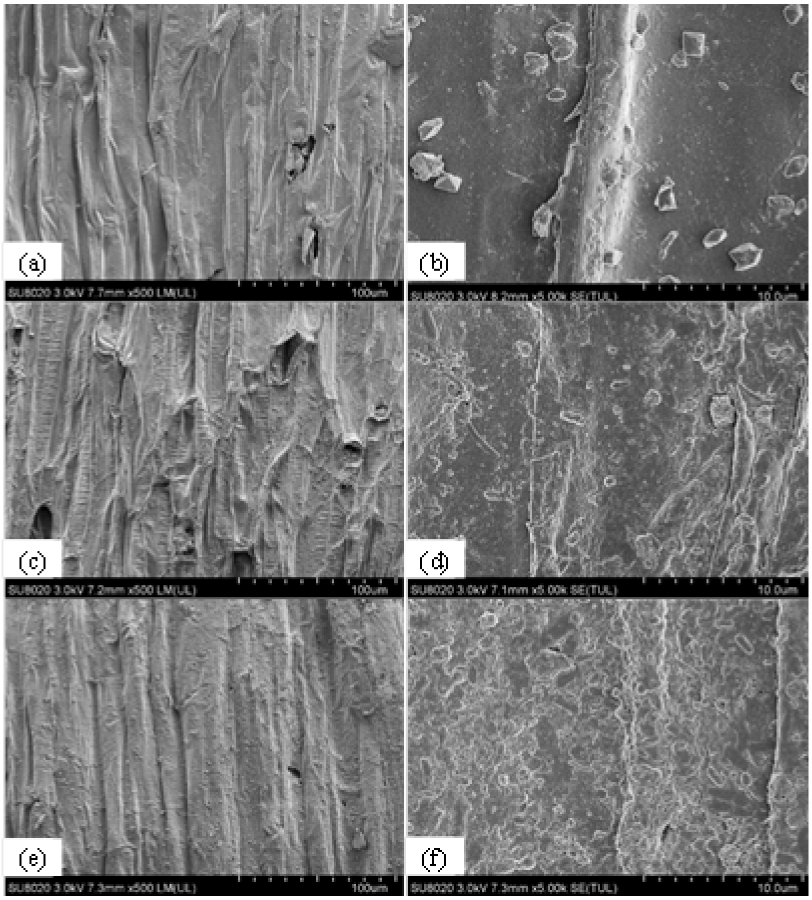

Figure 7

Surface morphology of Sagittaria roots at 500× (a, c, e) and 5000× (b, d, f) magnifications; (a) (b), (c) (d), and (e) (f) are the CK, CK+ and BA groups, respectively