Biopolymer-assisted enzyme-induced carbonate precipitation for Cu-contaminated loess remediation

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Abstract

Wastewater, discharged in copper (Cu) mining and smelting, usually contains a large amount of Cu\(^{2+}\). Immobilizing Cu\(^{2+}\) in aqueous solution and soils is deemed crucial in preventing its migration into surrounding environments. In recent years, the enzyme-induced carbonate precipitation (EICP) has been widely applied to Cu immobilization. However, the effect of Cu\(^{2+}\) toxicity denatures and even inactivates the urease. In the present work, the biopolymer-assisted EICP technology was proposed. The inherent mechanism affecting Cu immobilization was explored through a series of test tube experiments and soil column tests. Results indicated that 4 g/L chitosan may not correspond to a higher immobilization efficiency because it depends as well on surrounding pH conditions. The use of Ca\(^{2+}\) not only prevented the nucleation of the urease with Cu\(^{2+}\) by the electrostatic repulsion but reduced the potential for Cu\(^{2+}\) to migrate into nearby environments when malachite and azurite minerals are wrapped by calcite minerals. The species of carbonate precipitation that are recognized in the numerical simulation supported the above claim. On the other hand, UC1 (urease and chitosan colloid) and UC2 (urea and calcium source) grouting reduced the effect of Cu\(^{2+}\) toxicity by transforming the exchangeable state-Cu into the carbonate combination state-Cu. The side effect, induced by 4 g/L chitosan, promoted the copper-ammonia complex formation in the shallow ground, while the acidic environments in the deep ground prevented Cu\(^{2+}\) from coordinating with soil minerals. These badly degraded the immobilization efficiency. The Raman spectroscopy and XRD test results tallied with the above results. The findings shed light on the potential of applying the biopolymer-assisted EICP technology to Cu-contaminated water bodies and sites remediation.

1. Introduction

Copper is an essential trace element for human health and an important component of protein and various metal enzymes in a human body (Nyvltova et al. 2022; Vitaliti et al. 2022; Xia et al. 2022). However, unbound (free) copper ions are toxic, leading to irreversible denaturation and inactivation of cellular tissues and protein structures (Li et al. 2022; Maiti and Moura 2021; Dahiri et al. 2023). In the past few decades, copper mining and smelting, metal processing, electroplating, and electronic industries discharge an excessive amount of copper into surrounding environments (Kalinovic et al. 2016; Moran et al. 2018; Yoon and Yoon 2022). This could accumulate in aquatic plants and threatens human health through food chains (Wu et al. 2012; Garcia-Carmona et al. 2019; Zhao et al. 2021). Immobilizing Cu is deemed crucial in securing human health and the safety of nearby environments (Chen et al. 2013; Schwertfeger and Hendershot 2013; How et al. 2023). To this end, many remediation technologies, such as chemical precipitation, adsorption, electrochemical treatment, and membrane filtration, have been extensively applied to the remediation of Cu-contaminated water bodies and sites (Xu et al. 2019; Dong et al. 2020; Meng et al. 2022). However, they are usually criticized for high cost, long treatment time, and poor maneuverability (Yang et al. 2016; Zhao et al. 2019; Zhu et al. 2019). In recent years, the microbial-induced carbonate precipitation (MICP) technology draws increasing attention and introduces bacteria as the nucleation site to form carbonate precipitation with the provision of CO\(_3^{2-}\) for preventing Cu's
migration (Xie et al. 2022; Xue et al. 2022a-c). Despite that, microorganisms require to live in an appropriate environment with sufficient nutrients. Further, the micrometer size of bacteria prevents them from penetrating into deeper ground, narrowing their horizon of application. In contrast, the enzyme-induced carbonate precipitation (EICP) technology utilizes plant-extracted urease to catalyze urea hydrolysis (Chen and Achal 2019; Moghal et al. 2020; Peng et al. 2020; Zeng et al. 2021; Wang et al. 2022a, b). Despite that, the effect of Cu\(^{2+}\) toxicity however denatures and inactivates the urease. Bacteria depress the effect of Cu\(^{2+}\) toxicity through the cell membrane and extracellular polymer secretion and other protection and regulatory methods. This is not applicable to the urease as it straightforwardly exposes to surrounding environments (Wang et al. 2014; Kang and So 2016; Mota et al. 2016; Jiang et al. 2019; Chung et al. 2020; Duarte-Nass et al. 2020). Chitosan is considered a highly biocompatible biopolymer obtained by the deacetylation of chitin isolated from shrimp and crab shells. The use of such a biopolymer promotes the formation of colloids and prevents direct exposure of the urease to the effect of Cu\(^{2+}\) toxicity. Apart from that, its use leads to tighter carbonate crystals, retards reaction time, and elevates precipitation mass (Badr et al. 2014; Hamdan et al. 2016; Zhao et al. 2016; Du, et al. 2021; Barroso-Martin 2022).

\[
\text{CO(NH}_2\text{)}_2 + \text{H}_2\text{O} \rightarrow 2\text{NH}_3 + \text{CO}_2 \quad (1)
\]

\[
2\text{NH}_3 + 2\text{H}_2\text{O} \leftrightarrow 2\text{NH}_4^+ + 2\text{OH}^- \quad (2)
\]

\[
\text{CO}_3^{2-} + \text{H}_2\text{O} \leftrightarrow \text{HCO}_3^- + \text{OH}^- \leftrightarrow \text{CO}_2 + 2\text{OH}^- \quad (3)
\]

\[
\text{M}^{2+} + \text{CO}_3^{2-} \rightarrow \text{MCO}_3 (s) \quad (4)
\]

Despite that, the role of chitosan colloid in conjunction with the EICP technology (referred to also as the biopolymer-assisted EICP technology) in preventing Cu\(^{2+}\) migration has not been fully explored yet. Loess is aeolian sediment widespread in Northwest China and is featured a variety of carbonates and minerals. Its interplay with the loess is still not clear. The above reveals several research gaps and shortcomings that remain to be addressed in the future. The main objectives of this study are to: (1) reveal the underlying mechanism affecting Cu immobilization using the biopolymer-assisted EICP technology; and (2) explore the potential of applying the biopolymer-assisted EICP technology to Cu-contaminated water bodies and sites remediation.

2. Materials and Methods

2.1 Urease extraction and preparation of chitosan colloid

Urease, a nickel-containing metal enzyme widely distributed in plant seeds, was extracted from Jack bean with the activity being measured 342.7 U/g. The urease extraction procedure was conducted in accordance with the following three steps: (1) Jack bean was crushed in a grinder, sieved, and mixed with
ethanol alcohol, (2) the mixture was centrifuged using a benchtop centrifuge for 30 minutes, extracting the supernatant and storing it at 4°C for 4 hours, and (3) the sediment was obtained after re-centrifugation as the urease. Then the urease was stored at -20°C to maintain its activity for future use (Fig. S1). Apart from that, the procedure applied to prepare chitosan colloid is detailed as follows: a given amount of chitosan powder (degree of deacetylation: ≥ 95%, viscosity: 100–200 mPa·s) was dissolved in 1% (m/m) acetic acid solution (CH₃COOH) for preparing chitosan solution. The chitosan solution was stirred using a magnetic stirrer. 1 M sodium hydroxide solution (NaOH) was applied to adjust its pH, deriving the chitosan colloid.

2.2 Immobilizing of copper in aqueous solution

Test tube experiments aimed to reproduce urea hydrolysis by urease and Cu-related precipitation formation applied to prevent Cu²⁺ migration using the biopolymer-assisted EICP technology. Two groups were designed, including the test group (TG) and control group (CG), in which the latter corresponded to the EICP process proceeded in the absence of chitosan colloid for the sake of comparison. The urease and chitosan colloid was mixed as a remediation solution and added to 5–50 mM Cu-contaminated solution containing 500 mM urea and 250 mM CaCl₂ where two chitosan concentrations were considered in the present work, namely, 2 g/L and 4 g/L, respectively. Apart from that, NH₄⁺ concentration was measured at 48 h after the commencement of the test tube experiment, according to Nessler’s reagent method. While Cu²⁺ concentration was measured using an atomic spectrophotometer (TAS-990; Beijing Purkinje General Instrument, China). The immobilization efficiency can be calculated as follows:

\[
\text{Immobilization efficiency} = \frac{C_0 - C_1}{C_0} \times 100\%
\]

where \(C_0\) and \(C_1\) correspond to the initial Cu²⁺ concentration and Cu²⁺ concentration after catalyzing urea hydrolysis, respectively. Fig. S1 presents more details about the test tube experiments applied to Cu immobilization using the biopolymer-assisted EICP technology, while their experimental scheme is summarized in Table S1. Considering the change in Cu species was not yet explored in the test tube experiments, it was simulated using the Visual MINTEQ software package. The extractions of NH₄⁺ and CO₃²⁻ concentrations from the test tube experiments and two chitosan concentrations (2 g/L and 4 g/L) were applied to the simulation as the input parameters. These results help explore the change of Cu species against different Cu²⁺ concentrations and improve our understanding of the relationship between the change in Cu species and the immobilization efficiency. Further, under a chitosan concentration of 4 g/L, the carbonate precipitation, when subjected to 30 mM Cu²⁺, was analyzed via FTIR (Fourier transform infrared spectroscopy) and Raman spectroscopy, respectively. The key role of functional groups on the surface of bacteria in preventing Cu²⁺ migration was explored through a Fourier transform infrared spectroscopy (Nicolet iS50; Thermo Scientific, USA). Moreover, soil minerals were recognized using a Raman spectrometer (LabRAM HR Evolution; Horiba Scientific, Japan).
2.3 Immobilizing of copper in loess

The loess was taken from 3–4 m deep ground in Jingyang County, Xi’an City, Shaanxi Province, China. The particle-size distribution curve and physical and mechanical properties of the loess are shown in Fig. S2 and Table S2, respectively. Prior to preparing the cylindrical samples, a dilute nitric acid solution was applied to wash carbonates that contain in the loess away to mimic a scenario in which the loess was exposed to the effect of long-term leaching. The loess was then dried at 105°C for 24 h, crushed, and sieved using a 2-mm opening sieve. It was mixed with 500–4000 mg/kg Cu-contaminated solution to get the Cu-contaminated soil at a given water content of 18%, followed by its still for 6 hours. Then the Cu-contaminated soil was loaded through three equal batches into a soil compartment of 180 mm in height and 61.8 mm in diameter and compacted to a given density of 1.4 g/cm$^3$. There were two grouting phases upon completing the Cu-contaminated soil preparation where a 50 mL remediation solution containing the urease and chitosan colloid was grouted into the soil in the first place (referred to also as UC1 grouting) at a slow rate of 5 mL/min using a peristaltic pump, and the other 50 mL remediation solution containing the urea and calcium source (CaCl$_2$) was grouted (referred to also as UC2 grouting) at the same rate following a 1 h curing (Fig. S1). The two grouting phases proceeded for 24 h. More details concerning the experimental scheme are supplemented in Table S3.

2.4 Sequential extractions

Given that Cu species could vary notably with increasing the depth and affect the immobilization efficiency, it was analyzed via Tessier sequential extraction procedure (Tessier et al. 1979). There were five Cu species, namely, Exchangeable state-Cu (S1), Carbonate combination state-Cu (S2), Fe-Mn oxides state-Cu (S3), Organic state-Cu (S4), and Residue state-Cu (S5). As S1 is considered to be the highest ecotoxicity species among the five species, transforming S1 into the other four species was crucial in securing the safety of surrounding environments and human health (Zhang et al. 2019). The solution obtained by Tessier sequential extraction procedure mainly aimed to measure Cu$^{2+}$ concentration toward calculating the immobilization efficiency.

2.5 Soil sample characterization

Three soil samples were taken from the shallow, mid-depth, and deep grounds of the cylinder specimen. The XRD patterns were taken in a 5–90° range with a step width of 85°. The mineral types were recognized using an X-ray diffractometer (D8 Advance; Bruker, Germany). Furthermore, the intramolecular bonds and functional groups were identified via a Raman spectrometer where a 532 nm laser excitation was introduced. The Raman spectra were taken from a 50-4000 cm$^{-1}$ range. All of the microscopic tests were conducted in triplicate, ensuring repeatability.

3. Results and discussion

3.1 Test tube experiments
The relationships of immobilization efficiency and NH$_4^+$ concentration versus Cu$^{2+}$ concentration under no calcium source are depicted in Fig. 1a. As can be seen from Fig. 1a, 5 mM Cu$^{2+}$ (the lowest in the present work) makes the urease lose its activity, leading to an inability of secreting urease for catalyzing urea hydrolysis. Cu$^{2+}$ can interact with the sulfhydryl group of the urease to change the spatial structure of its active center, causing its denaturation and inactivation (Kang and So 2016). When the chitosan intervenes in Cu immobilization, the immobilization efficiency increases with the increase in Cu$^{2+}$ concentration, while NH$_4^+$ concentration decreases with increasing Cu$^{2+}$ concentration. It is worth noting that the immobilization efficiency is generally higher under 2 g/L chitosan than under 4 g/L chitosan. This is most likely due to the fact that more NH$_4^+$ and OH$^-$ are discharged under 4 g/L chitosan toward turning surrounding environments into alkaline conditions. Such alkaline conditions promote the formation of Cu-complex substances, thus reducing the immobilization efficiency. In contrast, such alkaline conditions cannot be attained under 2 g/L chitosan toward preventing the formation of Cu-complex substances and achieving a higher immobilization efficiency. That is to say, a higher chitosan concentration does not necessarily mean a higher immobilization efficiency, and the immobilization efficiency also depends on, for example, surrounding pH conditions. In light of the above, the immobilization efficiency of approximately 95% (the highest under no calcium source) is attained under 2 g/L chitosan when subjected to 30 mM Cu$^{2+}$.

The relationships of immobilization efficiency and NH$_4^+$ concentration versus Cu$^{2+}$ concentration under CaCl$_2$ are shown in Fig. 1b. NH$_4^+$ concentration is way higher under CaCl$_2$ than that under no calcium source. Unlike no calcium source, the immobilization efficiency is higher under 4 g/L chitosan than under 2 g/L chitosan. Ca$^{2+}$ plays a leading role in preventing Cu-complex materials formation. It changes the surface charge of the urease and therefore, prevents its nucleation with Cu$^{2+}$ by the electrostatic repulsion, securing the urease activity (Fang et al. 2021). As a result, the immobilization efficiency of 100% (the highest under CaCl$_2$) is attained under 4 g/L chitosan when subjected to 30 mM Cu$^{2+}$.

Fig. 1 Relationships of immobilization efficiency and NH$_4^+$ concentration versus Cu$^{2+}$ concentration: a No calcium source; b Under CaCl$_2$

### 3.2 Numerical simulation

Since the species of carbonate precipitation has not yet been explored in the test tube experiments, a numerical simulation tends to investigate how the species of carbonate precipitation affects the immobilization efficiency. Further, preventing its formation is considered crucial in improving the immobilization efficiency because Cu-complex substances could largely elevate the potential for Cu$^{2+}$ to migrate into surrounding environments. The numerical simulation helps to gain insights into the mentioned phenomenon. The relationships of the species distribution of carbonate precipitation versus Cu$^{2+}$ concentration under 2 g/L and 4 g/L chitosan, respectively, are depicted in Fig. 2. Two species of carbonate precipitation are present under 2 g/L chitosan when subjected to Cu$^{2+}$ concentration < 30 mM,
including malachite and copper-ammonia complex ([Cu(NH$_3$)$_x$]$^{2+}$) (Fig. 2a). A transformation of the species of carbonate precipitation from malachite and copper-ammonia complex to azurite occurs when subjected to Cu$^{2+}$ concentration ≥ 30 mM. The immobilization efficiency under 4 g/L chitosan would have been higher than that under 2 g/L chitosan if the Cu-complex substances had not been formed (Fig. 1b). Cu$^{2+}$ concentration < 30 mM and 2 g/L chitosan allow the degree of urea hydrolysis to remain relatively high and turn surrounding environments into strong alkaline conditions. NH$_3$ in ammonia nitrogen appears under strong alkaline environments and can coordinate with Cu$^{2+}$ to form the copper-ammonia complex (Williamson et al. 2021). The copper-ammonia complex formation raises the potential for Cu$^{2+}$ to migrate into nearby environments toward degrading the immobilization efficiency. However, this is not the case when subjected to Cu$^{2+}$ concentration ≥ 30 mM. Cu$^{2+}$ concentration ≥ 30 mM depresses the development of strong alkaline conditions despite the presence of chitosan, impeding the copper-ammonia complex formation.

On the other hand, one single species of carbonate precipitation is present under 4 g/L chitosan when subjected to Cu$^{2+}$ concentration < 30 mM, namely, copper-ammonia complex (Fig. 2b). This gives testimony supporting the immobilization efficiency higher under 2 g/L chitosan than under 4 g/L chitosan (Fig. 1b). When subjected to Cu$^{2+}$ concentration ≥ 30 mM, one more species of carbonate precipitation is present, namely, malachite. Despite the presence of the copper-ammonia complex, 4 g/L chitosan secures more CO$_3^{2-}$ to be involved in reducing the potential for Cu$^{2+}$ to migrate into nearby environments compared to 2 g/L chitosan. This is considered the root cause leading to the immobilization efficiency higher under 4 g/L chitosan than under 2 g/L chitosan when subjected to Cu$^{2+}$ concentration ≥ 30 mM (Fig. 1b).

Fig. 2 Relationships of species distribution of carbonate precipitation versus Cu$^{2+}$ concentration: a Under 2 g/L chitosan; b Under 4 g/L chitosan

### 3.3 Precipitation microscopic analysis

The species of carbonate precipitation is also interpreted via FTIR and Raman spectroscopy, respectively. Two adsorption bands at 3415 cm$^{-1}$ and 3442 cm$^{-1}$ are recorded by FTIR test, corresponding to the N-H stretching (Fan et al. 2021) (Fig. 3a). Further, two more adsorption bands at 1670 cm$^{-1}$ and 1658 cm$^{-1}$ are formed as a result of the O-H bending vibration (Xing et al. 2021). The above results show that the amino (-NH$_2$) and hydroxyl groups (-OH) that are carried by the chitosan play parts in the coordination with Cu$^{2+}$, reducing the potential for Cu$^{2+}$ to migrate into surrounding environments. The higher the chitosan concentration, the more the surface functional groups (i.e. -NH$_2$ and -OH) available for the coordination with Cu$^{2+}$, and the higher the immobilization efficiency. On the other hand, two bands are present at 1053 cm$^{-1}$ and 1063 cm$^{-1}$ and in fact, are mainly attributed to the C-O stretching vibration (Kong et al. 2020). The C-O stretching vibration is considered to be the footprint of the presence of malachite and azurite minerals.
Under CaCl$_2$, the Raman peaks at 1010 cm$^{-1}$ and 1377 cm$^{-1}$ are linked to the presence of malachite mineral, while the Raman peak at 1382 cm$^{-1}$ gives testimony supporting the presence of azurite mineral (Fig. 3b). Apart from that, the Raman peaks at 2907 cm$^{-1}$ and 3356 cm$^{-1}$ are formed due to the presence of calcite mineral and the O-H stretching vibration, respectively (Chukanov and Vigasina 2020). In short, malachite and azurite minerals that are present in the numerical simulation are also recognized by FTIR and Raman spectroscopy. In addition to malachite and azurite minerals, the provision of Ca$^{2+}$ is accompanied by calcite minerals formation. In other words, the provision of Ca$^{2+}$ not only improves the resistance of bacteria against the effect of Cu$^{2+}$ toxicity but reduces the potential for Cu$^{2+}$ to migrate into surrounding environments when malachite and azurite minerals are wrapped by calcite minerals.

![Fig. 3](image1.png)

Microscopic analysis of precipitation considering 4 g/L chitosan concentration: a FTIR; b Raman spectroscopy

### 3.4 Soil column tests

This part mainly discusses the soil column test results. The relationships of Cu species distribution versus Cu$^{2+}$ concentration are depicted in Fig. 4 where CG, TG2, and TG4 denote no chitosan, 2 g/L chitosan, and 4 g/L chitosan, respectively. S1 is higher in CG than in TG2 and TG4. In TG2, the reduction in S1 reaches 55.2%, 59.6%, and 58.1% when subjected to 500, 2000, and 4000 mg/kg Cu$^{2+}$, respectively. Also, S1 is higher in TG4 than in TG2, meaning that 2 g/L chitosan performs better than 4 g/L chitosan in terms of transforming S1 into the other four Cu species. In TG2, the increase in S2 reaches 22.4%, 46.9%, and 51.4% when subjected to 500, 2000, and 4000 mg/kg Cu$^{2+}$, respectively. These results appear to contradict our consensus that the higher the chitosan concentration, the higher the degree of urea hydrolysis, and the higher the immobilization efficiency. Results also show that UC1 and UC2 grouting has a minimal effect on S3, S4, and S5.

Considering S1 has the highest ecotoxicity among the five species, its migration with increasing depth is worthy of investigation. The distributions of S1 against shallow, mid-depth, and deep grounds when subjected to 500, 2000, and 4000 mg/kg Cu$^{2+}$, respectively, are shown in Fig. 5. S1 is higher in CG than in TG2 and TG4 when subjected to 500 mg/kg Cu$^{2+}$, meaning that UC1 and UC2 grouting encourages the species’ transformation from S1 to S2. In TG2, S1, however, shows a small change with increasing depth, while in TG4, the maximum and minimum reduction in S1 is present in the mid-depth ground and shallow ground, respectively (Fig. 5a). When subjected to 2000 and 4000 mg/kg Cu$^{2+}$, the reduction in S1 in TG2 also presents a small change with increasing depth. The reduction in S1 in TG4 behaves similarly to TG2 (Figs. 5b and 5c).

![Fig. 4](image2.png)

Relationships of Cu species distribution versus Cu$^{2+}$ concentration (CG: under no chitosan colloid, TG2: 2 g/L chitosan colloid, and TG4: 4 g/L chitosan colloid)

![Fig. 5](image3.png)

Variations of S1 against Shallow ground, mid-depth ground, and deep ground when subjected to a 500 mg/kg Cu$^{2+}$; b 2000 mg/kg Cu$^{2+}$; c 4000 mg/kg Cu$^{2+}$
3.5 Soil sample microscopic analysis

TG2 has been neglected here in order to stand out the role of chitosan colloid in improving the immobilization efficiency. The XRD and Raman spectroscopy results of the samples taken from CG and from three different depths of TG4 are shown in Fig. 6. Results show that Cu immobilization is attained by co-precipitating with lavendulan (NaCaCu₅(AsO₄)₄Cl·5H₂O) mineral at 9.8590 Å and calcium manganese oxide hydrate (Ca₂M₅S₁₄O₂₇·xH₂O) mineral at 17.7280 Å (Figs. 6a and 6c). Results also show that Cu immobilization is also attained by coordination adsorption with soil minerals (i.e., quartz, feldspar, albite, kaolinite, and montmorillonite). As coordination adsorptions take place, their surface hydrolysis is accompanied by forming a variety of complexation products, including ≡SOCu⁺, ≡SOCu(OH), and ≡SOCuOS≡, in which S stands for soil mineral composition (Xie et al. 2022). Cu-quartz corresponds to the diffraction peak at 3.3454 Å, while Cu-kaolinite links to the diffraction peak at 3.1900 Å.

On the other hand, the Raman peak at about 460 cm⁻¹ is attributed from the presence of quartz minerals (Figs. 6b and 6d). Further, coordination adsorptions with soil minerals other than quartz and albite allow more hydroxyl groups to play parts in preventing Cu²⁺ migration, which tallies with the presence of the Raman peaks at around 3000 cm⁻¹, induced by O-H stretching vibrations (Colomban 2005; Chukanov and Vigasina 2020).

3.6 Cu surface coordination adsorption

The below content aims to compare the spatial distribution of urease activity at 2 g/L chitosan to that at 4 g/L chitosan. Then the implications of Cu²⁺ concentration on the immobilization efficiency would be discussed as well. 4 g/L chitosan allows more NH₄⁺ and OH⁻ to be distributed in the shallow ground toward turning surrounding environments into strongly alkaline conditions. Such strongly alkaline conditions are not ideal for Cu²⁺ to coordinate with soil minerals and promote the copper-ammonia complex formation. The copper-ammonia complex formation elevates the potential for Cu²⁺ to migrate into nearby environments, indicating the lowest immobilization efficiency (Figs. 5 and 7). In contrast, the alkalinity in the mid-depth ground is not as strong as in the shallow ground, and their coordination with minerals can proceed with no difficulty toward achieving the highest immobilization efficiency. Since the colloidal nature of the chitosan prevents the grouts from penetrating into the deep ground, the deep ground remains in acidic conditions, thereby impeding coordination adsorption. Compared to the mid-depth ground, a reduction in the immobilization efficiency is present in the deep ground. In light of the above, the mid-depth ground performs the best, while the shallow ground performs the worst. On the
other hand, the difference in chitosan concentration would have been affected the variation of pH ranges in the shallow ground, mid-depth ground, and deep ground if a higher Cu\(^{2+}\) concentration (i.e. 4000 mg/kg Cu\(^{2+}\)) had not been applied to the soil column tests. The variation of pH ranges in the shallow, mid-depth, and deep grounds is narrowed when subjected to 4000 mg/kg Cu\(^{2+}\), and therefore, their difference in immobilization efficiency becomes smaller compared to 500 mg/kg Cu\(^{2+}\) (Fig. 5).

2 g/L chitosan is likely to penetrate into the ground more uniformly compared to 4 g/L chitosan (Figs. 5 and 7). This makes the variation of the urease activity with increasing depth becomes smaller. In addition, the alkalinity in the shallow ground is not as strong as under 4 g/L chitosan toward preventing the copper-ammonia complex formation and providing ideal conditions for Cu\(^{2+}\) to coordinate with soil minerals. In light of the above, the shallow, mid-depth, and deep grounds perform similarly to each other in terms of immobilization efficiency. When subjected to 4000 mg/kg Cu\(^{2+}\), such alkaline environments are turned into conditions more appropriate for Cu\(^{2+}\) to coordinate with soil minerals, securing the immobilization efficiency (Fig. 5).

Results from the microscopic analysis are interpreted in more detail as follows. The intensity of quartz varies more significantly with increasing depth when subjected to 500 mg/kg Cu\(^{2+}\) (Fig. 6a). The consumption of quartz minerals is less in both the shallow and deep grounds due to the formations of the copper-ammonia complex and acidic environments, respectively. In contrast, the mid-depth ground consumes much more quartz minerals for them to coordinate with Cu\(^{2+}\). Further, the intensity of the Raman peak at 460 cm\(^{-1}\) is higher in the shallow and deep grounds than in the mid-depth ground, meaning that the copper-ammonia complex in the shallow ground and acidic environments in the deep ground retard quartz minerals’ consumption (Fig. 6b). The coordination adsorption in the mid-depth ground leads to the surface hydrolysis of minerals, forming O-H and intermolecular hydrogen bonds. The above is the root cause leading to the Raman shift to move to 2974 cm\(^{-1}\) and 1608 cm\(^{-1}\), respectively (Colomban 2005; Chukanov and Vigasina 2020). The results of the Raman spectrum analysis tally with the XRD test results.

Although the variation of the quartz intensity with increasing depth, when subjected to 4000 mg/kg Cu\(^{2+}\), behaves similarly compared to 500 mg/kg Cu\(^{2+}\), it becomes smaller (Fig. 6c). Further, 4000 mg/kg Cu\(^{2+}\) consumes quartz minerals much more than 500 mg/kg Cu\(^{2+}\). Moreover, the shallow ground starts consuming quartz minerals when no copper-ammonia complex is present. On the other hand, 4000 mg/kg Cu\(^{2+}\) encourages more coordination adsorptions to occur. The more the coordination adsorption, the higher the intermolecular bond strength. The higher hydrogen bond strength causes the Raman shift to move to 1548 cm\(^{-1}\) and 2948 cm\(^{-1}\), respectively (Fig. 6d).

In short, the chitosan prevents the urease from losing its activity while catalyzing urea hydrolysis. Despite that, a higher chitosan concentration (e.g. 4 g/L chitosan) does not necessarily tie to a higher immobilization efficiency. The immobilization efficiency also depends on surrounding pH conditions. The use of Ca\(^{2+}\) not only prevents the nucleation of the urease with Cu\(^{2+}\) by the electrostatic repulsion but
reduces the potential for Cu\textsuperscript{2+} to migrate into nearby environments when malachite and azurite minerals are wrapped by calcite minerals. These help elevate the immobilization efficiency to about 100%. The species of carbonate precipitation that are recognized in the numerical simulation support the above claim. On the other hand, S1 (Cu in the exchangeable state) reduces notably from CG (control group) to TG2 (2 g/L chitosan). UC1 (urease and chitosan colloid) and UC2 (urea and calcium source) grouting encourages the transformation from S1 to S2 (Cu in the carbonate combined state). Soil minerals in the loess soil, in fact, play parts in preventing Cu\textsuperscript{2+} migration. In spite that the use of chitosan helps secure the urease activity while catalyzing urea hydrolysis, 4 g/L chitosan also leads to the side effect (i.e. more NH\textsubscript{4}\textsuperscript{+} and OH\textsuperscript{−} to be discharged). The side effect in the shallow ground promotes the copper-ammonia complex formation, while the acidic environments in the deep ground prevent Cu\textsuperscript{2+} from coordinating with minerals, degrading the immobilization efficiency. In contrast, the alkalinity in the mid-depth ground is not as strong as in the shallow ground and therefore, the mid-depth ground provides ideal conditions for Cu\textsuperscript{2+} to coordinate with minerals, achieving the highest immobilization efficiency. A higher Cu\textsuperscript{2+} concentration (i.e. 4000 mg/kg Cu\textsuperscript{2+}) causes the shallow, mid-depth, and deep grounds to perform similarly but their difference in the immobilization efficiency becomes smaller. Compared to 4 g/L chitosan, 2 g/L chitosan can penetrate into the ground more uniformly and turns the shallow, mid-depth, and deep grounds into alkaline environments favorable for Cu\textsuperscript{2+} coordination with minerals. 4000 mg/kg Cu\textsuperscript{2+} further narrows the variation of pH ranges in the shallow, mid-depth, and deep grounds. The findings explore the potential of applying the biopolymer-assisted EICP technology to Cu-contaminated water bodies and sites remediation.

Fig. 7 Cu coordination adsorption under UC1 and UC2 grouting considering the chitosan concentration of a 2 g/L; b 4 g/L

4. Conclusions

This paper applied the biopolymer-assisted EICP technology to the immobilization of Cu\textsuperscript{2+} in aqueous solution and the loess. Based on the results and discussion, some main conclusions can be drawn as follows:

(a) Although the use of chitosan can prevent the urease from losing its activity while catalyzing urea hydrolysis, a higher chitosan concentration does not necessarily mean an improvement in the immobilization efficiency. More NH\textsubscript{4}\textsuperscript{+} and OH\textsuperscript{−} to be discharged in the shallow ground turns surrounding environments into strongly alkaline conditions and promotes the copper-ammonia complex formation. The copper-ammonia complex formation raises the potential for Cu\textsuperscript{2+} to migrate into surrounding environments, degrading the immobilization efficiency.

(b) The use of Ca\textsuperscript{2+} not only prevents the nucleation of the urease with Cu\textsuperscript{2+} but reduces the potential for Cu\textsuperscript{2+} to migrate into nearby environments when malachite and azurite minerals are wrapped by calcite
minerals. This helps elevate the immobilization efficiency to about 100%. The species of carbonate precipitation that are identified in the numerical simulation support the above claim.

(c) UC1 and UC2 grouting encourages the transformation from the exchangeable state-Cu into the carbonate combination state-Cu. Compared to 2 g/L chitosan, 4 g/L chitosan leads to the side effect. The side effect present in the shallow ground promotes the copper-ammonia complex formation, while the acidic environments in the deep ground prevents Cu$^{2+}$ from coordinating with soil minerals. Both cases badly degrade the immobilization efficiency. When subjected to 4000 mg/kg Cu$^{2+}$, the variation of pH ranges in the shallow, mid-depth, and deep grounds is narrowed, and due to this reason, their difference in immobilization efficiency becomes smaller compared to 500 mg/kg Cu$^{2+}$.

**Declarations**

**Author contributions** Yi-Xin Xie: Data curation, Formal analysis, Software, Writing - original draft. Wen-Chieh Cheng: Conceptualisation, Methodology, Writing - review & editing, Supervision, Funding acquisition. Lin Wang: Data curation, Formal analysis, Validation, Software, Writing - original draft. Zhong-Fei Xue: Data curation, Formal analysis, Validation, Software, Writing - original draft. Yin-Long Xu: Data curation, Software, Writing - original draft.

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**Competing interests** The authors declare that they have no competing interests.

**References**


Figure 1

Relationships of immobilization efficiency and NH$_4^+$ concentration versus Cu$^{2+}$ concentration: (a) No calcium source; (b) Under CaCl$_2$
Figure 2

Relationships of species distribution of carbonate precipitation versus Cu$^{2+}$ concentration: a Under 2 g/L chitosan; b Under 4 g/L chitosan
Figure 3

Microscopic analysis of precipitation considering 4 g/L chitosan concentration: a FTIR; b Raman spectroscopy
Figure 4

Relationships of Cu species distribution versus Cu$^{2+}$ concentration (CG: under no chitosan colloid, TG2: 2 g/L chitosan colloid, and TG4: 4 g/L chitosan colloid)
Figure 5

Variations of S1 against Shallow ground, mid-depth ground, and deep ground when subjected to a 500 mg/kg Cu$^{2+}$; b 2000 mg/kg Cu$^{2+}$; c 4000 mg/kg Cu$^{2+}$
Figure 6

Microscopic analysis:  

- **a** XRD test results for CG and TG4 when subjected to 500 mg/kg Cu$^{2+}$;  
- **b** Raman spectroscopy for CG and TG4 when subjected to 500 mg/kg Cu$^{2+}$;  
- **c** XRD test results for CG and TG4 when subjected to 4000 mg/kg Cu$^{2+}$;  
- **d** Raman spectroscopy for CG and TG4 when subjected to 4000 mg/kg Cu$^{2+}$
Figure 7

Cu coordination adsorption under UC1 and UC2 grouting considering the chitosan concentration of a 2 g/L; b 4 g/L

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