

Original Research

Repairing Compound-Contaminated Soil Using EK-Laccase Combination Repairing Technology

Zhaobo Wang^{1,2}, Dajun Ren^{1,2*}, Xinyue Dai^{1,2}, Chen Kang^{1,2}, Shuqin Zhang^{1,2},
Xiaoqin Zhang^{1,2}, Zhiquan Deng^{1,2}, Chaofan Huang^{1,2}, Huiwen Guo^{1,2}

¹College of Resource and Environmental Engineering, Wuhan University of Science and Technology, Wuhan, China

²Hubei Key Laboratory for Efficient Utilization and Agglomeration of metallurgic Mineral Resources,
Wuhan University of Science and Technology, Wuhan, Hubei, China

Received: 2 May 2019

Accepted: 21 August 2019

Abstract

Complex soil pollution is a major part of soil pollution, and heavy metals and organic pollutants coexist in the soil in most cases. With the common pollutants phenol and heavy metals (Cu and Cd) as the target pollutants, this study investigated the principles and mechanism of electro-kinetic (EK)-laccase combination technology for repairing contaminated soils by developing an EK repair device. Additionally, the migration of heavy metals and migration and the degradation of phenol in complex environments were analyzed. The results showed that the removal efficiency of phenol in soil with Cd²⁺ in the cathode region was 27.6% lower than that with Cu²⁺ in the cathode region, while the removal efficiency of phenol in the anode region exceeded 90% in both cases. The removal efficiency of Cd²⁺ and Cu²⁺ in the soil were 56.7% and 61.2% respectively. Contaminants such as succinic acid and p-benzoquinone and catechol in the repaired soil were detected by GC/MS. In this repairing system, the degradation of phenol could be either electrocatalytic oxidation or laccase catalytic oxidation.

Keywords: laccase, electro-kinetic, composite pollutant, migration, degradation

Introduction

As an important component of the ecological environment, soil is one of the main resources that human beings rely on to survive, and also the material repository of bio-geochemical cycles [1-3]. Modern soil pollution has become increasingly complicated and the coexistence of organic pollutants and heavy metals in the soil is a common phenomenon [4-6]. Phenol is an

important industrial raw material and also a common organic pollutant [7, 8]. This compound is hazardous to human kidney and liver [9]. On the other hand, heavy metals are also widely found in soil [5]. Heavy metals can threaten human health by accumulating in the human body via the food chain [10, 11]. Currently, the complex contaminated soil was repaired by chemical, physical and biological methods [12]. Bioremediation technologies exhibit advantages such as reduced secondary pollution, high recovery efficiency, and no interferences to the ecological environment in soil, compared to physical and chemical remediation technologies [13-15]. It is broadly meaningful to pollutants in the environment, and especially important

*e-mail: dj_ren@163.com

Table 1. Main properties of the soil.

pH	Organic matter / (g·kg ⁻¹)	N / (mg·kg ⁻¹)	P / (mg·kg ⁻¹)	K / (mg·kg ⁻¹)	CEC (cmol·kg ⁻¹)	Air drying moisture content / (%)
7.55	2.90	70.00	10.95	9.58	15.50	2.00

for *in situ* soil remediation, which seeks to reduce exposure risks at contaminated sites [16].

In this study, laccase was used for biodegradation, which is characterized by a wide range of reaction substrates, no secondary pollution, and high effectiveness in repairing low-concentration organic matter [17, 18]. However, the transfer efficiency of pollutants in the soil is low due to the complicated soil environment, thus limiting practical applications of laccase in repairing contaminated soil [19]. Therefore, the electro-kinetics (EK) technology has been involved to improve the contact efficiency of exogenous microbial enzymes and pollutants through electromigration and electroosmotic flow, thereby improving the removal rate of pollutants [20-22]. In recent years, EK and bioremediation technology have been rapidly developed and EK-enhanced bioremediation technology has been an effective way to solve the issue of low mass transfer efficiency. However, few reports on the EK-Laccase combination repairing technology for complex contaminated soil have been published [23-25]. Therefore, it is necessary to further explore the effectiveness and mechanism of EK-laccase combination repairing technology in order to provide theoretical and technical references for repairing complex pollution of soil.

In this study, the EK-laccase combination repairing technology was employed to repair the contaminated soil. The variations of physical and chemical properties of soil, migration of heavy metals, and the degradation of phenol in a complex environment were studied. The effects of heavy metals on repairing phenol-contaminated soil by laccase in an EK system were investigated and the migration and transformation mechanism of heavy metals and organic matters under EK conditions was revealed.

Material and Methods

Materials and Instruments

ABTS (guaranteed reagent) and laccase (*Trametes versicolor*) were obtained from Sigma Aldrich, methanol (guaranteed reagent) was obtained from CNW Co. Ltd., and other reagents involved were of analytical grade and obtained from Sinopharm Chemical Reagent Co., Ltd.

Preparation of Soil Sample

Soil samples were collected from a flowerbed, dried naturally, ground, sieved by a 26-mesh sieve and stored in a cool and dry place. The physical and chemical properties of the soil sample were checked and the results are listed in Table 1. The pH of the resulting soil sample was 7.55; the organic matter content was 2.90 g·kg⁻¹; the N, P, and K contents were 70.00, 10.95, 9.58 mg·kg⁻¹; the CEC was cmol·kg⁻¹; and the air-drying moisture content was 2.00%.

800 g soil was weighed for each sample and contaminated soil samples were prepared according to Table 2. Deionized water was added so that the water contents in contaminated soil samples were 40% (dry substrate). Eventually, the homogeneously mixed soil filled the container layer by layer. Five groups of samples were involved. T1 was the control group, T2 and T3 were samples without laccase, and T4 and T5 were samples with laccase. The purpose was to study the effect of heavy metals on the degradation of phenol by laccase in the presence of different heavy metals (Cd²⁺ may inhibit the laccase activity, while Cu²⁺ may promote the laccase activity[26]). The specific experimental design is shown in Table 2.

Table 2. Experimental design of electro-kinetic remediation.

Number	Voltage gradient	Laccase /ABTS	Phenol / (mg·kg ⁻¹)	Cu / (mg·kg ⁻¹)	Cd / (mg·kg ⁻¹)	Time
T1	0.5 V/cm ²	15 mL·4 U/mL	200			96 h
T2	0.5 V/cm ²		200	600	600	96 h
T3	0.5 V/cm ²		200		600(Cd)	96 h
T4	0.5 V/cm ²	15 mL·4 U/mL	200	600	600(Cu)	96 h
T5	0.5 V/cm ²	15 mL·4 U/mL	200		600(Cd)	96 h

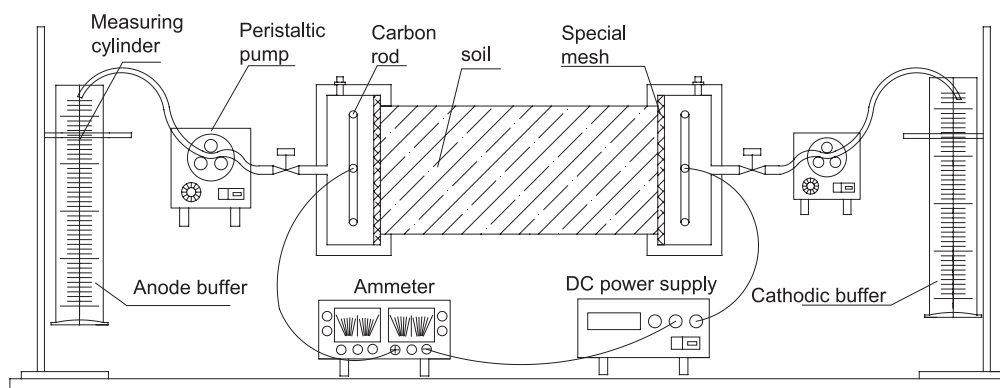


Fig. 1. Schematic diagram of electrodynamic device.

EK Repairing Device

Fig. 1 shows the experimental device. As observed, it consists of plexiglass ($12\text{ cm} \times 6\text{ cm} \times 6\text{ cm}$), a peristaltic pump, a measuring cylinder, a DC power supply, and an ammeter. Graphite vertical electrodes were used to generate a non-uniform horizontal electric field in which contaminant migration is more efficient. The voltage intensity was 1 V/cm^2 , the cathode buffer was 0.1 M acetic acid (HAc), and the anode buffer was 0.1 M sodium acetate (NaAc). The flow rates of anode and cathode buffer solutions were controlled by two constant current pumps and kept consistent. After 24 hours, 15 mL of 3 U/mL laccase solution was injected into the soil.

Sample Measurement

In order to reflect the removal rates of pollutants in different regions, six sampling areas were designed, as shown in Fig. 2. Points ① and ② are located in the cathode region, ③ and ④ are located in the transition region, and ⑤ and ⑥ are located in the anode region. At the end of each test, soil samples were collected from all areas and involved in consecutive measurements.

Measurement of Phenol

Phenol was extracted by ultrasonic extraction method. The soil in the area to be tested was stirred

evenly. 1 g of moist soil was added to 20 mL of methanol solution as an extract. After shaking for 2 h , the extract was transferred to a centrifuge tube and centrifuged for 10 min (rpm: 3000 r/min). After repeating the shaking and centrifugation operation twice, the supernatant was taken and the phenol concentration was measured by high performance liquid chromatography (HPLC, UltiMate 3000, DIONEX) under a Thermo SCIENTIFIC C18 reverse phase column ($250\text{ mm} \times 4.6\text{ mm I.D.}$, $5\text{ }\mu\text{m}$). The flow rate of 1.0 mL/min , the mobile phase was methanol and water (volume ratio = $3:1$), the detection wavelength was 275 nm , the column temperature was 35°C , and the sample volume was $20\text{ }\mu\text{L}$.

Measurement of Heavy Metals

The heavy metals in the soil to be tested were determined by atomic absorption spectrometry to determine the concentration of heavy metals after microwave digestion. Take 0.2 g of the experimental soil after drying, add 3 mL of concentrated hydrochloric acid, 1 mL of concentrated nitric acid, 0.5 mL of perchloric acid, and put it into the microwave digestion furnace (MDS-6G, Shanghai Xinyi Electronic Technology Co., Ltd.), and the contents of Cu or Cd in these samples were measured by atomic absorption spectrometry (NOVAA350, Analytik Jena AG).

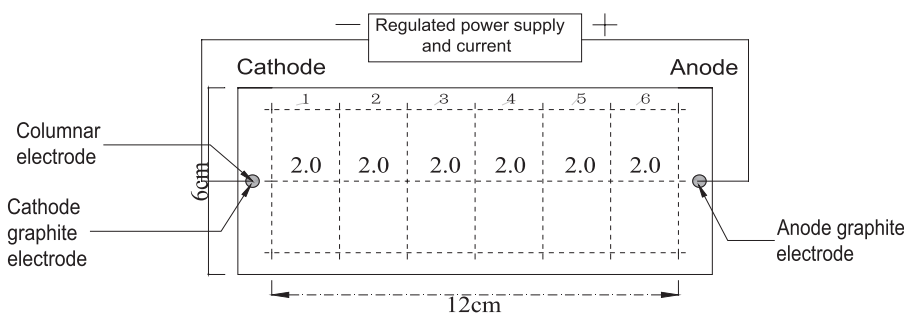


Fig. 2. Distribution map of sampling points.

Measurement of pH of Soil

2 g of mixed sample was added into 5 mL of 0.01 M CaCl_2 solution, vibrated for 3 h, and centrifuged. Then, the pH of the supernatant was measured.

Measurement of Conductivity

The conductivities of soil samples were measured using a portable conductivity meter. Finally, the intermediate products of phenol degradation were determined using GC/MS (5977B MSD, Agilent technologies).

Results and Discussion

EK Properties and Physical and Chemical Properties of Soil

Soil Current and Cumulative Electroosmotic Flow

As shown in Fig. 3a), the current intensity increased continuously at a decreasing rate. This can be attributed to increased ion concentration in the inter-spaces of soil caused by diffusion of Na^+ in the early anode and

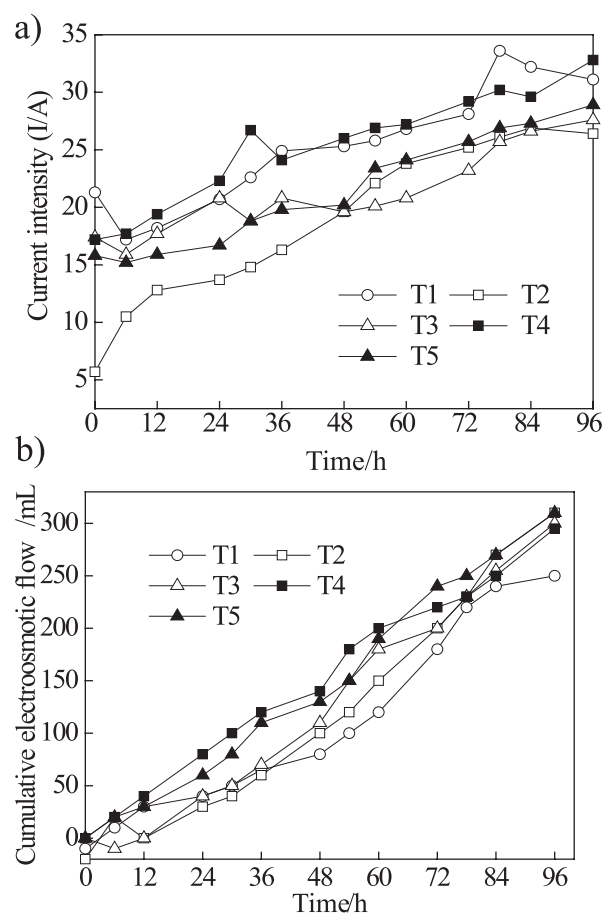


Fig. 3. Variation of soil electric current a) and electroosmotic b) flow with time.

cathode pools and OH^- and H^+ produced by electrolysis. At the late stage, resistance polarization and activation gasification were observed on graphite electrodes.

The electroosmotic flow was caused by a channel along the direction of the electric field, which can be attributed to a double electron layer generated by ions of pore water and negative charges on soil surface. Therefore, the magnitude of the cumulative electroosmotic flow and the current intensity play a key role in migration of contaminants. As shown in Fig. 3b), the cumulative electroosmotic flows increased in all cases. The cumulative electroosmotic flows and currents of T2 and T4 were significantly higher than those of other groups, while the cumulative electroosmotic flows of T3 and T5 were significantly higher than that of T1. This can be attributed to high molar concentration of Cu at a constant mass, indicating that the concentration of heavy metal ions in the soil is an important factor affecting the current intensity. Additionally, the cumulative electroosmotic flow was positively related to the current intensity.

Conductivity and pH of Soil

Fig. 4a) depicts the trend of soil conductivity as a function of distance from the anode. As observed, soil conductivity increased with distance. The conductivities

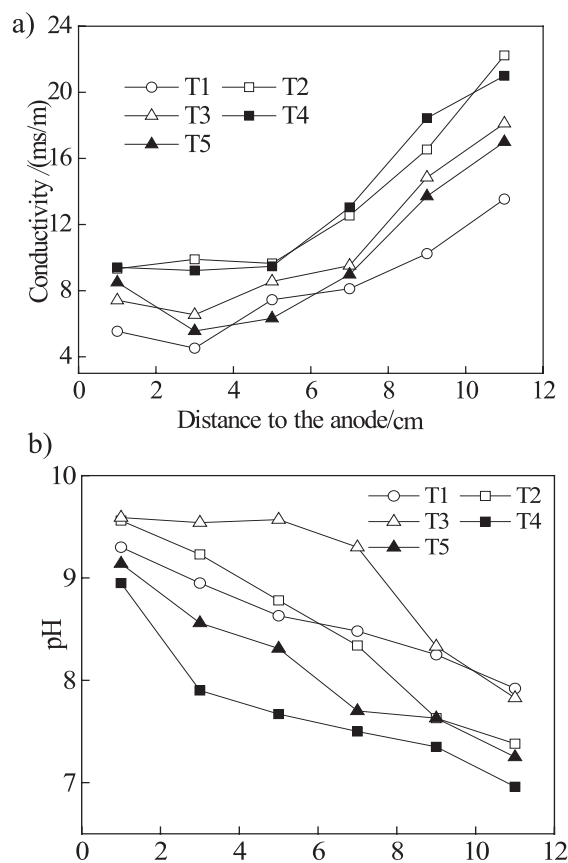


Fig. 4. Distribution of soil conductivity a) and pH b) after the tests.

of T2 and T4 were significantly higher than those of T3 and T5, especially in areas far from the anode. This can be attributed to the reactions of OH^- in the NaAc buffer in the anode cell with Cu^{2+} or Cd^{2+} in the soil, which led to reduced concentrations of heavy metal ions in the soil. Meanwhile, Cu^{2+} or Cd^{2+} in the anode region migrated toward the cathode due to electroosmotic flow and electromigration. In the cathode region, considerable H^+ ions were present in the buffer and $\text{Cu}^{2+}/\text{Cd}^{2+}$ migrated to this region, resulting in high conductivity.

In the process of soil repair by EK, anodic and cathodic hydrolysis continuously producing OH^- and H^+ has resulted in greatly varying acidity and alkalinity of the soil. Therefore, the pH of the soil is controlled by the HAc/NaAc buffer solution system. The pH at different locations of soil are summarized in Fig. 4b). As observed, the pH of soil was maintained at 7~10 to facilitate the migration of heavy metals.

Migration and Degradation of Pollutants

Fig. 5a) is a schematic diagram of migration and degradation of phenol-heavy metal contaminated soil by the EK-laccase system. Due to the action of the EK electric field, contaminants migrate to the anode under the action of electroosmotic flow. At the same time, laccase is uniformly added to the soil and participates in the catalytic degradation process of phenol. The laccase is a hydrophilic substance and migrates to the anode

under the action of the EK electric field. Therefore, the closer to the anode, the better the degradation of phenol. Heavy metal pollutants mainly participate in the process of migration to the anode, so the contents of Cu^{2+} and Cd^{2+} gradually increase as they approach the anode.

Migration and Degradation of Phenol in Soil

As shown in Fig. 5b), the removal rates of phenol in the anode regions of all samples exceeded 90%. This is mainly attributed to the migration of phenol to the cathode region under electromigration, as well as electro-catalysis of phenol by $\cdot\text{OH}$ and $\cdot\text{O}^{2-}$ in the anode region. In T2 and T4, the removal rates of phenol were relatively high (>90% in the anode region), indicating that the removal rate of phenol increased with the current intensity.

As shown in Fig. 5b), the residual concentrations of phenol in the cathode regions of T1, T4 and T5 were significantly lower than those of T2 and T3, indicating that laccase greatly contributes to the removal of phenol in the cathode region. It is worth noting that the removal rate of phenol in T5 was significantly lower than those of T1 and T4, which may be attributed to the inhibition of laccase by Cd^{2+} in soil. This is consistent with a previous study [27].

In the cathode region, the contents of phenol remained high. This can be attributed to the premature inactivation of laccase caused by the complex

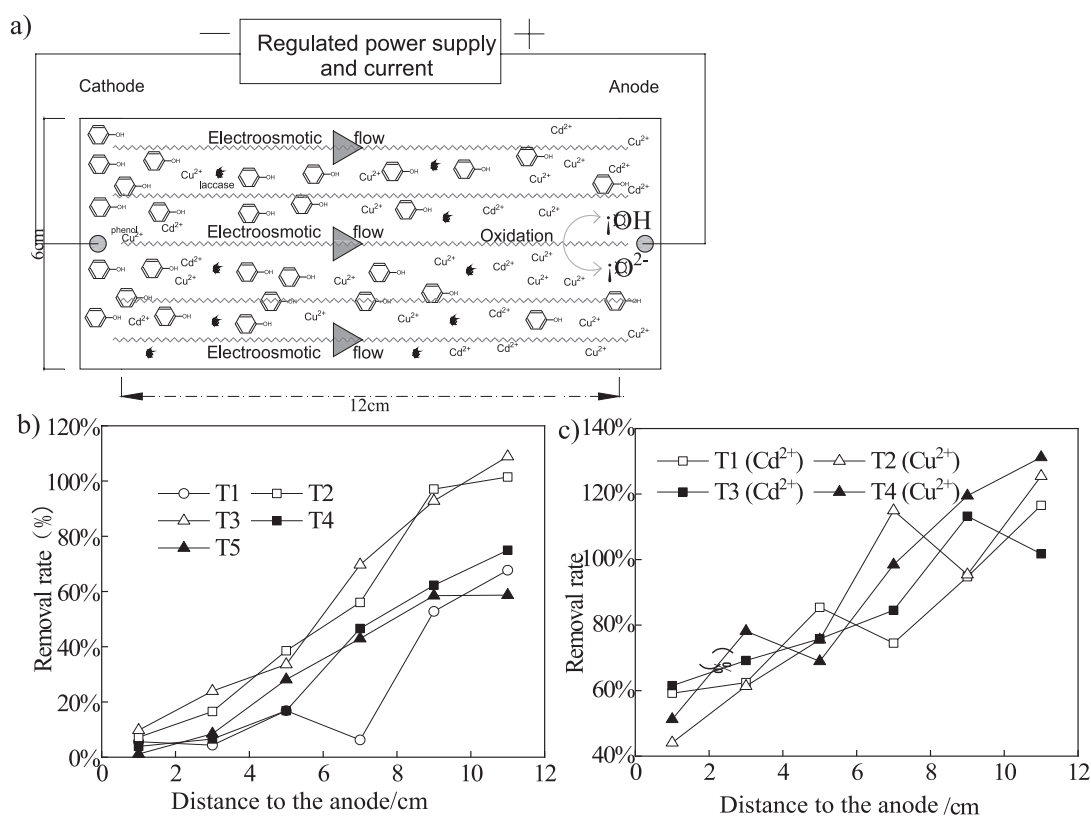


Fig. 5. Schematic diagram of pollutant migration and degradation a) and distribution of residual phenol b) and distribution of residual Cu^{2+} and Cd^{2+} c) in soil.

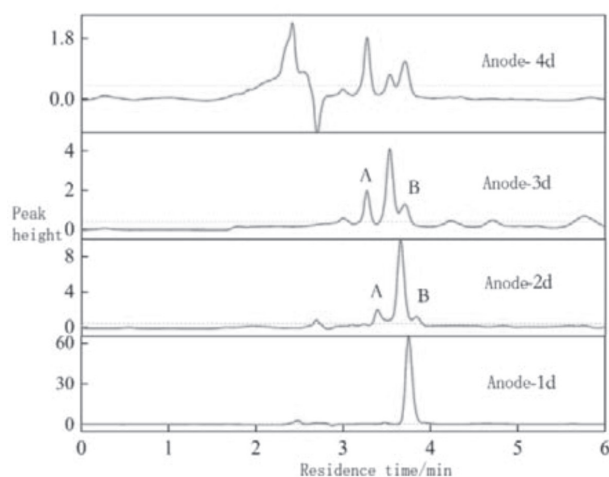


Fig. 6. HPLC chromatogram of soil residual phenol.

environment of soil, accompanied by the gradual migration of phenol from anode to cathode, resulting in a continuously increasing concentration of residual

phenol in the cathode region. In the anode region, however, the concentrations of residual phenol were below 10% and it is impossible to determine the effects of laccase on phenol degradation.

Migration of Heavy Metals in Soil

As shown in Fig. 5c), the concentrations of heavy metals in soil increased from anode to cathode. Based on concentrations of heavy metals in buffer, the removal rates of Cu^{2+} and Cd^{2+} in soil were calculated to be 56.7% and 61.2%, respectively. Meanwhile, the removal rate of Cu^{2+} in soil was significantly higher than that of Cd^{2+} , which is consistent with the trends of cumulative electroosmotic flow and current intensity. Additionally, the removal rates of heavy metals in groups without pH control were significantly lower than those of groups with pH control. This can be attributed to the migration of OH^- generated at the cathode toward the anode under the electric field. These ions would react with heavy metals migrating from the anode to produce precipitates, thus reducing the removal rates of heavy metals in soil.

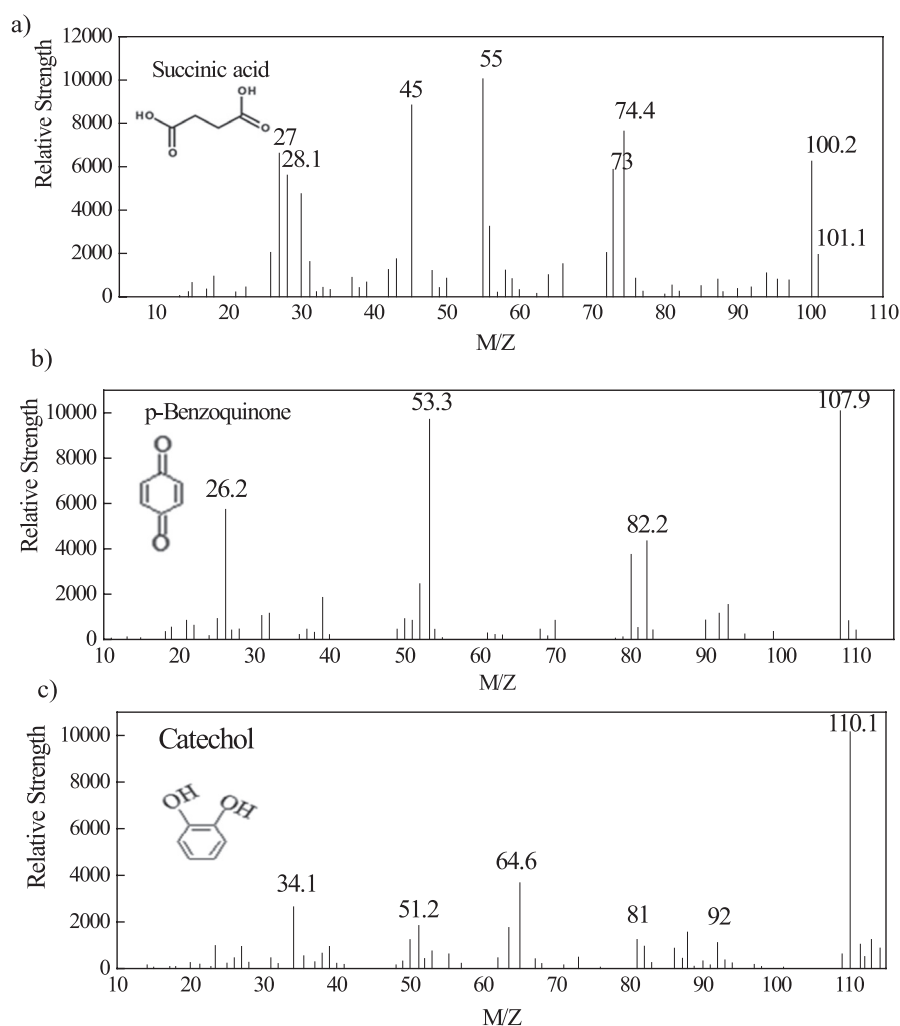


Fig. 7. Mass spectrum of the intermediate: Succinic acid a), p-Benzoquinone b) and Catechol c).

In this study, HAc and NaAc were used as a buffer to increase the solubility of metal ions, which is favorable for the removal of heavy metal ions in soil.

Analysis of Degradation Products of Phenol

Degradation Products of Phenol

Liquid chromatographic analysis Fig. 6 illustrates the liquid chromatogram of residual phenol in the anode region (Point ⑥) from Day 1 to Day 4. As observed, the peak area of phenol in the contaminated soil on the first day was 30.25. After the addition of laccase on the second day, the peak area of phenol rapidly decreased to 10.32, the content of phenol in the anode region (Point ⑥) decreased significantly, indicating that the EK-laccase combined repair system has higher efficiency for the degradation of phenol. Two minor peaks (denoted as A and B, respectively) were observed at different sides of the main peak corresponding to phenol. The area of these two minor peaks did not increase further, indicating that the substance corresponding to Peaks A and B may be an intermediate of phenol degradation. Hence, the degradation products of phenol were investigated by GC/MS.

GC-MS Analysis

The GC-MS results for the intermediates of phenol degradation are shown in Fig. 7. There are three

important intermediates in the degradation system: succinic acid, p-benzoquinone and catechol. There are two main degradation pathways for phenol in the EK-laccase system (as shown in Fig. 8). The first degradation pathway is direct catalytic oxidation by direct current, and the second is direct catalytic oxidation of phenol by laccase.

According to the product map of GC-MS, it is known that in the electrocatalytic oxidation pathway, electrons of phenolic hydroxyl groups in phenol start to transfer, and hydroxyl radical ($\cdot\text{OH}$) and superoxide radical ($\cdot\text{O}_2^-$) are generated. The free radical first attacks the para position of the phenolic hydroxyl group to form hydroquinone, and then further oxidizes hydroquinone to form p-benzoquinone – an important intermediate for the degradation of phenol [28]. In the subsequent degradation stage, the p-benzoquinone is deprived of an electron by the anode, and the carbon bonded to the oxygen double bond is attacked by the adjacent hydroxyl radical generated by the electrolysis of water to be opened. The open-ended p-benzoquinone is degraded into various carboxylic acids, possibly carboxylic acids such as fumaric acid and maleic acid, which are further completely oxidized to H_2O and CO_2 [28].

For another degradation pathway (laccase-catalyzed oxidation of phenol), one possible pathway is that the phenol is polymerized by the action of laccase to produce an oligomer of phenol, which is further oxidized to produce a dipolymer of phenol. The laccase then breaks the dipolymer in the presence of O_2 to produce

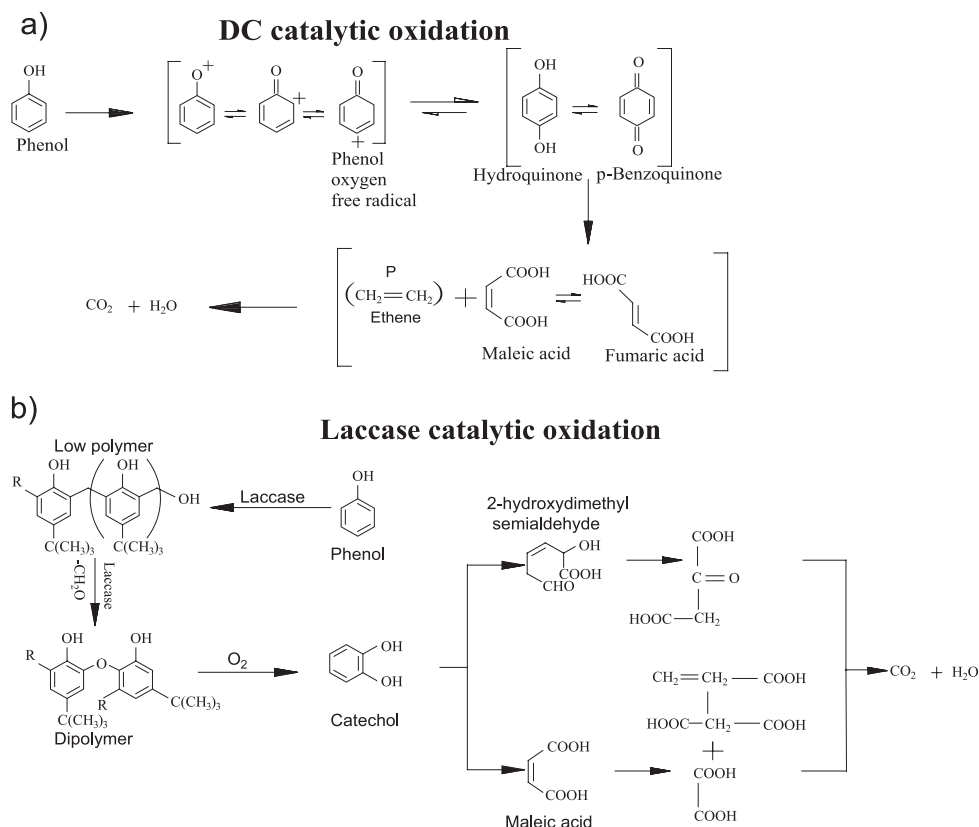


Fig. 8 Two degradation pathways of phenol: direct catalytic oxidation of phenol by direct current a) and laccase b).

the intermediate catechol [29]. Part of the catechol is further metabolized to maleic acid, and a part of the product of catechol cracking will be 2-hydroxymuconic acid semialdehyde, which will eventually degrade to H_2O and CO_2 [30].

Conclusions

Laccase can effectively enhance the removal rate of phenol in the cathode region, while the complex environments of soil tend to significantly affect the stability of laccase. In this study, Cd^{2+} in soil has a strong inhibitory effect on laccase activity, while Cu^{2+} has a positive effect on laccase activity. Therefore, the removal rate of phenol in the cathode region in the presence of Cd^{2+} was 27.6% lower than that in the presence of Cu^{2+} . Therefore, improvement of stability of laccase in complex environments is of great significance for the wide application of laccase.

The EK-laccase combination repairing technology can effectively repair complex soils contaminated by phenol and/or heavy metals. The removal of heavy metals was positively related to current intensity and cumulative electroosmotic flow, while the concentration of heavy metals in soil affects the EK properties and physical and chemical properties of soil. The extent of mutual influence is a key factor for the removal of heavy metals.

Acknowledgements

This work was supported by the Major Project of Science and Technology Research Program of the Hubei Provincial Department of Education (grant No. D20181101), the National Natural Science Foundation of China (41571306), the Project of Excellent Fund in Hubei (2018CFA067) and the Hubei Key Laboratory for Efficient Utilization and Agglomeration of Metallurgical Mineral Resources (2017zy003).

Conflict of Interest

The authors declare no conflict of interest.

References

- CHENG M., ZENG G.M., HUANG D.L., LAI C., XU P., ZHANG C., LIU Y. Hydroxyl radicals based advanced oxidation processes (AOPs) for remediation of soils contaminated with organic compounds: A review. *Chemical Engineering Journal*, **284**, 582, **2016**.
- BARABASZ W., ALBINSKA D., JASKOWSKA M., LIPIEC J. Biological effects of mineral nitrogen fertilization on soil microorganisms. *Polish Journal of Environmental Studies*, **11** (3), 193, **2002**.
- LU Y., SONG S., WANG R., LIU Z., MENG J., SWEETMAN A.J., JENKINS A., FERRIER R.C., LI H., LUO W. Impacts of soil and water pollution on food safety and health risks in China. *Environment international*, **77**, 5, **2015**.
- SONG B., ZENG G., GONG J., LIANG J., XU P., LIU Z., ZHANG Y., ZHANG C., CHENG M., LIU Y., YE S., YI H., REN X. Evaluation methods for assessing effectiveness of in situ remediation of soil and sediment contaminated with organic pollutants and heavy metals. *Environ Int*, **105**, 43, **2017**.
- VAN POUCKE R., AINSWORTH J., MAESELE M., OK Y., MEERS E., TACK F. Chemical stabilization of Cd-contaminated soil using biochar. *Applied Geochemistry*, **88**, 122, **2018**.
- ONDER S., DURSUN S., GEZGIN S., DEMIRBAS A. Determination of Heavy Metal Pollution in Grass and Soil of City Centre Green Areas (Konya, Turkey). *Polish Journal of Environmental Studies*, **16** (1), **2007**.
- LIU Y., LIU Z., ZENG G., CHEN M., JIANG Y., SHAO B., LI Z., LIU Y. Effect of surfactants on the interaction of phenol with laccase: Molecular docking and molecular dynamics simulation studies. *Hazard Mater*, **357** (5), 10, **2018**.
- OLESZCZUK P., BARAN S. Degradation of Individual Polycyclic Aromatic Hydrocarbons (PAHs) in Soil Polluted with Aircraft Fuel. *Polish Journal of Environmental Studies*, **12** (4), **2003**.
- WEI L., WANG S., ZUO Q., LIANG S., SHEN S., ZHAO C. Nano-hydroxyapatite alleviates the detrimental effects of heavy metals on plant growth and soil microbes in e-waste-contaminated soil. *Environ Sci Process Impacts*, **18** (6), 760, **2016**.
- LI Z., MA Z., VAN DER KUIJP T.J., YUAN Z., HUANG L. A review of soil heavy metal pollution from mines in China: pollution and health risk assessment. *Sci Total Environ*, **468-469**, 843, **2014**.
- PARK J.H., LEE S.J., LEE M.E., CHUNG J.W. Comparison of heavy metal immobilization in contaminated soils amended with peat moss and peat moss-derived biochar. *Environ Sci Process Impacts*, **18** (4), 514, **2016**.
- BOLAN N., KUNHIKRISHNAN A., THANGARAJAN R., KUMPIENE J., PARK J., MAKINO T., KIRKHAM M.B., SCHECKEL K. Remediation of heavy metal(loid)s contaminated soils – To mobilize or to immobilize? *Journal of Hazardous Materials*, **266** (4), 141, **2014**.
- MEGHARAJ M., NAIDU R. Soil and brownfield bioremediation. *Microb Biotechnol*, **10** (5), 1244, **2017**.
- SHARMA B., DANGI A.K., SHUKLA P. Contemporary enzyme based technologies for bioremediation: A review. *Environ Manage*, **210**, 10, **2018**.
- GHANEM K.M., AL-GARNI S.M., AL-ZAHRANI M.A. Bioremediation of Diesel Fuel by Fungal Consortium Using Statistical Experimental Designs. *Polish Journal of Environmental Studies*, **25** (1), **2016**.
- BUSHNAF K.M., MANGSE G., MEYNET P., DAVENPORT R.J., CIRPKA O.A., WERNER D. Mechanisms of distinct activated carbon and biochar amendment effects on petroleum vapour biofiltration in soil. *Environ Sci Process Impacts*, **19** (10), 1260, **2017**.
- VATS A., MISHRA S. Identification and evaluation of bioremediation potential of laccase isoforms produced by *Cyathus bulleri* on wheat bran. *Hazard Mater*, **344**, 466, **2018**.

18. KALKAN E., NADAROGLU H., CELEBI N., CELIK H., TASGIN E. Experimental Study to Remediate Acid Fuchsin Dye Using Laccase-Modified Zeolite from Aqueous Solutions. *Polish Journal of Environmental Studies*, **24** (1), **2015**.
19. WANG X., SUN S.Y., NI Z.J., LI Z.X., BAO J. Degradation of polycyclic aromatic hydrocarbons in contaminated soil by immobilized laccase. *Journal of the Serbian Chemical Society*, **83** (5), 549, **2018**.
20. GAO Y.-C., GUO S.-H., WANG J.-N., ZHANG W., CHEN G.-H., WANG H., DU J., LIU Y., NAIDU R. Novel *Bacillus cereus* strain from electrokinetically remediated saline soil towards the remediation of crude oil. *Environ Sci Process Impacts*, **25** (26), 26351, **2018**.
21. CHANG J.H., DONG C.D., SHEN S.Y. The lead contaminated land treated by the circulation-enhanced electrokinetics and phytoremediation in field scale. *Hazard Mater*, **368**, 894, **2019**.
22. KALKAN E., NADAROGLU H., DIKBAŞ N., TAŞGIN E., CELEBI N. Bacteria-Modified Red Mud for Adsorption of Cadmium Ions from Aqueous Solutions. *Polish Journal of Environmental Studies*, **22** (2), **2013**.
23. SONG Y., CANG L., FANG G., ATA-UL-KARIM S.T., XU H., ZHOU D. Electrokinetic delivery of anodic in situ generated active chlorine to remediate diesel-contaminated sand. *Chemical Engineering Journal*, **337**, 499, **2018**.
24. MA Q., LI J., LEE C., LONG X., LIU Y., WU Q.T. Combining potassium chloride leaching with vertical electrokinetics to remediate cadmium-contaminated soils. *Environ Geochem Health*, 1-11, **2019**.
25. MA Y., LI X., MAO H., WANG B., WANG P. Remediation of hydrocarbon-heavy metal co-contaminated soil by electrokinetics combined with biostimulation. *Chemical Engineering Journal*, **353**, 410, **2018**.
26. ZHOU C., DONG A., WANG Q., YU Y., FAN X., CAO Y., LI T. Effect of Common Metal Ions and Anions on Laccase Catalysis of Guaiacol and Lignocellulosic Fiber. *BioResources*, **12** (3), 5102, **2017**.
27. GARCÍA-DELGADO C., JIMÉNEZ-AYUSO N., FRUTOS I., GARRATE A., EYMAR E. Cadmium and lead bioavailability and their effects on polycyclic aromatic hydrocarbons biodegradation by spent mushroom substrate. *Environ Sci Pollut R*, **20** (12), 8690, **2013**.
28. AMMAR H.B., BRAHIM M.B., ABDELHÉDI R., SAMET Y. Green electrochemical process for metronidazole degradation at BDD anode in aqueous solutions via direct and indirect oxidation. *Separation Purification Technology*, **157**, 9, **2016**.
29. D'ACUNZO F., GALLI C., MASCI B. Oxidation of phenols by laccase and laccase-mediator systems: Solubility and steric issues. *European Journal of Biochemistry*, **269** (21), 5330, **2002**.
30. CATHERINE H., PENNINCKX M., FRÉDÉRIC D. Product formation from phenolic compounds removal by laccases: A review. *Environmental Technology Innovation*, **5**, 250, **2016**.

