Bioremediation of Cd contamination at smelting sites by sulphate-reducing bacteria (SRB)

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Abstract. The problem of cadmium pollution in the soil of non-ferrous smelting sites needs to be solved urgently. This study investigated the remediation of cadmium pollution in non-ferrous smelting sites using sulphate-reducing bacteria (SRB). The results showed that the leachate concentration of soil cadmium decreased significantly after remediation with SRB compared with the control group. The chemical form analysis of the soil in the control and remediation groups, respectively, after 36 days of reaction, revealed that the cadmium in the remediation group was transformed into a more stable organic matter bound fraction and residual fraction compared with that in the control group, which indicated that the application of SRB could effectively control the migratory transformation of cadmium. The addition of functional microorganisms in soil remediation promoted the increase of biodiversity. The composition of microbial communities in the control and remediation groups differed significantly, and the most abundant genera in the remediation system were *Pseudomonas*, *Macclibactoroides,* and *Trichococcus*. The continuous addition of sulphate-reducing bacteria enhanced the stable growth of indigenous sulphate-reducing bacteria.

Keywords: sulphate-reducing bacteria, cadmium, Non-ferrous smelting sites.

1 Introduction

1.1 A Subsection Sample

Cadmium (Cd) is abundant in the earth's crust, mainly in the divalent form in zinc ores and rocks. With industrial activities such as mining and smelting lead and zinc, manufacturing nickel-cadmium batteries, and producing semiconductor solar panels, Cd has migrated from the deeper layers of the soil to the surface layer. Due to the persistent

A. E. Abomohra et al. (eds.), *Proceedings of the 2023 9th International Conference on Advances in Energy Resources and Environment Engineering (ICAESEE 2023)*, Atlantis Highlights in Engineering 29,
https://doi.org/10.2991/978-94-6463-415-0_67

and non-biodegradable nature of heavy metals, Cd migrated to the surface layer of soil can be continuously enriched in the food chain, posing a health hazard. According to the US Agency for Toxic Substances and Disease Registry (ATSDR), cadmium (Cd) is ranked 7th[1, 2], which is an essential challenge for achieving the second Sustainable Development Goal (SDG) of the United Nations' 2030 Agenda for related to food safety [3, 4]. Meanwhile, the health threat of cadmium contamination is also an important issue in countries such as China [5], Bangladesh, India, and Pakistan [1, 4, 6].

The main factors affecting the transport and bioavailability of Cd in soil are pH, other cations, and soil organic matter [7]. Under acidic soil conditions at pH < 6.0, Cd and its compounds are relatively water-soluble, with the percentage of the Cd^{2+} ion-exchange state ranging from 40 to 60%. In weakly acidic soils, the rate of Cd^{2+} in the exchange state decreases to about 20%. Whereas under neutral or alkaline soil conditions, the formation of $CdCO_3$ leads to a decrease in the solubility of Cd. For the precipitation reaction of Cd^{2+}, competition from other cations in the response leads to the substitution of Cd^{2+} by other cations such as magnesium, zinc, and silica hydroxyl (Si-OH) [8, 9]. Soil organic matter, on the other hand, reduces the bioabsorption or bioavailability of Cd by converting soluble or exchangeable Cd into the soil organic bound fraction. Under the influence of acid rain precipitation in the south, cadmium in the soil around the non-ferrous smelting site can easily migrate to the surrounding soil, threatening the health and safety of residents.

Microbial remediation is an emerging development with eco-friendly and low-cost advantages. Microbial remediation uses microbial agents, mainly through fungi, archaea, or bacteria, to biosorb and enrich, oxidation-reduction, and biomineralization to solidify heavy metals in soil and water [10, 11]. The action of bacteria on heavy metals is divided into three aspects: external cell precipitation, cell surface adsorption, and internal cell detoxification.

The mechanism of heavy metal curing by sulphate-reducing bacteria (SRB) mainly includes precipitation and adsorption. Sulphate reduction products H_2S and S^{2-} form insoluble sulfide precipitates with extremely small Ksp with heavy metal ions. pH increases in SO_4^{2-} reduction by SRB, some heavy metal ions form hydroxide precipitates, and CO_2 decomposed from organic matter during metabolism reacts with some heavy metal ions to form insoluble carbonates [12]. Heavy metal ions are adsorbed and solidified on the extracellular polymers of SRB. The metabolic process of SRB can actively absorb, transform, and eventually accumulate heavy metal ions within the cell protoplasm to achieve removal.

2 Materials and Methods

2.1 Sampling collection

The soil used in this study was collected from the Zhuzhou Smelting Plant in Zhuzhou, Hunan Province, the south central of China. The soil samples were collected with a shovel from the contaminated soil approximately 0-1.5 m. Then, the pieces were placed into sterile bags and transported to the laboratory as soon as possible. XRF analysis of

the test sample soil, as shown in Tab. 1, revealed that the main metallic elements in the soil were Fe, Ca and Cd.

Table 1. Chemical properties of the sampled soils(ppm)

depth(m)	Fe	Ca	Cd
0-0.5	20.86%	5.39%	134
0.5-1	5.29%	3.22%	56
1-1.5	26.21%	7.25%	97

The remediation experiments of contaminated soil were carried out in a 250 mL Erlenmeyer flask, which contained 100 g soil and 250 mL medium. Samples (15 mL) from the remediation system were taken after 1, 7, 15, 21, and 36 days. Afterward, 5 mL of the leaching solution was tested for heavy metal concentration by a 0.22 μm filter, and the rest (10 mL) was used to monitor the microbial community's dynamic changes.

2.2 The Source of Bacteria and Medium

The SRB were isolated from activated sludge preserved in the National Engineering Laboratory of Biohydrometallurgy, China. The SRB was cultured in the Postage liquid medium with 1 g/L of Sodium Lactate, 0.5 g/L of Na_2SO_4, 2 g/L of $MgSO_4$, 0.5 g/L of K_2HPO_4, 0.1 g/L of $CaCl_2$ and 1.2 g/L of Yeast extract.

2.3 High-Throughput Sequencing

Amplification products complete high-throughput gene sequencing was performed in the Illumina Miseq platform (Majorbio BioPharm Technology Co., Ltd., Shanghai, China). The data were analyzed on the free online platform of Majorbio Cloud Platform (www. majorbio.com accessed on 7 July 2022).

2.4 Analytical Methods

Cell extracts were filtered using a 0.22 μm filter system before Cd concentration was measured; the total Cd concentration was determined by ICP-MS (Agilent Technologies 7700x, Palo Alto, CA, USA).

3 Results and Discussion

3.1 Removal Efficiency of Cadmium

To illustrate the effect of bioremediation, the Cadmium concentration was shown in Fig. 1 After 30 days of treatment, the average Cadmium concentration in soil leachate samples was 0.023 mg/L. By contrast, the cadmium concentration of samples without

SRB was about 0.623 mg/L, which exceeded the standard for groundwater category V [13] by 62.3 times. For the subsoil, the average concentration of cadmium in the leachate samples was 0.014 mg/L, and the average concentration of cadmium in the control leachate was 0.216 mg/L. SRB were highly efficient in reducing Cd concentration in leachate compared to the control group, mainly by creating an anaerobic environment and increasing the pH value, which can promote the conversion of Cd^{2+} to CdS [14].

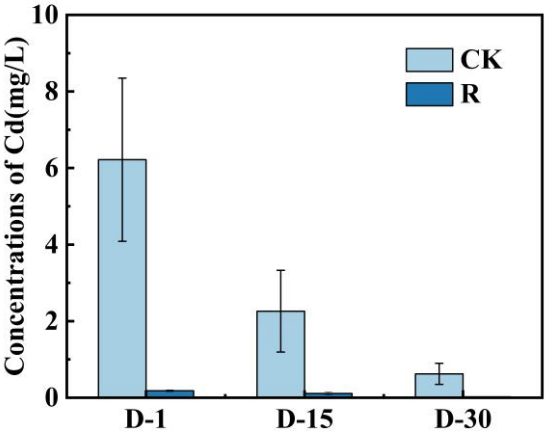

Fig. 1. The Cadmium concentration in contaminated soil remediation by SRB during different periods (1, 15, and 30 represent the experimental group at 1, 15, and 30 days, respectively).

3.2 Stabilization of Cd in soils by SRB

The percentage of different chemical forms of Cd in the control and remediation group were measured at reaction times of 36 days to investigate the transformation rule of Cd in the biological stabilization process. The Tessier morphology of Cd was analyzed in control (CK) and remediated (R) soils separately, and as shown in Fig. 2, the proportion of organically matter bound fraction and residual fraction increased significantly in the remediated group compared to the control group. Specifically, the balance of organically matter bound fraction in the remediation group increased by 1%, and the proportion of residual fraction increased by 5% compared with the control group at 0-0.5 m. The ratio of organically matter bound fraction increased by 9% at 0.5-1 m, and the proportion of residue state increased by 9% in the group of 1-1.5 m, which suggests that the presence of sulfate-reducing bacteria can effectively promote the transformation of cadmium to a stable state in the soil, as previously reported by Yang et al [15].

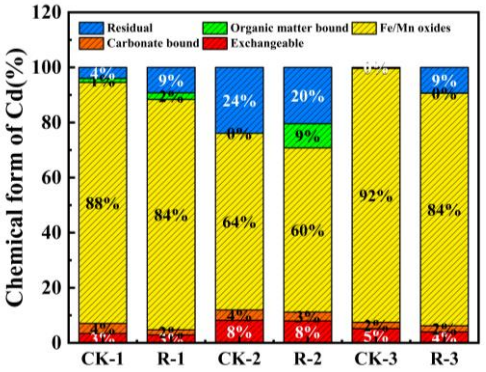

Fig. 2. Chemical form changes of Cd in soils during biological stabilization process (CK- control, R- biological stabilization, 1, 2, and 3 represent the experimental group at 0-0.5m, 0.5-1m, and 1-1.5m, respectively).

3.3 Microbial Diversity and Richness Assessment

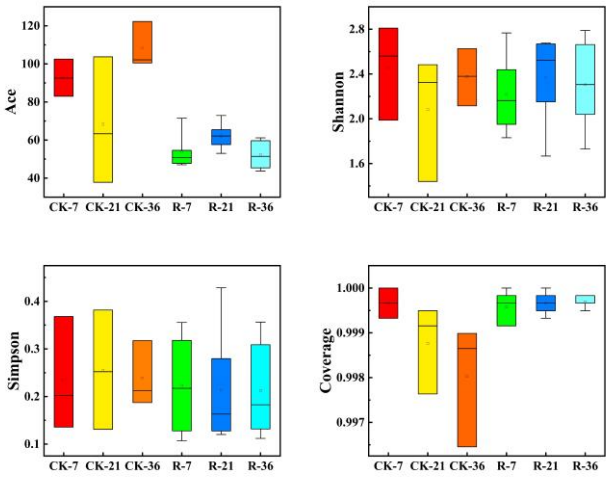

Fig. 3. Microbial succession in the remediation group at alpha index (CK-7, CK-21, and CK-36 represent the samples of soils after control for 7, 21, and 36 days, respectively; R-7, R-21, and R-36 represent the samples of soils after biological stabilization for 7, 21, and 36 days, respectively).

Alpha diversity was used to evaluate the richness and diversity of community diversity [16]. The Shannon and Simpson indices are a combination of richness and evenness, which are microbial diversity indices, with larger values of the former and smaller values of the latter indicating higher community diversity [17]. In contrast, ACE indices were used to evaluate richness. The coverage index responded to the community

coverage, and the coverage index of all samples was almost always above 0.999, proving that most of the sequences in the system were detected. The Ace, Shannon, Simpson, and Coverage diversity of different groups were shown in Fig.3.

The Ace indices of microorganisms were more in the control group than in the remediation group, which indicated that the abundance of anaerobic microorganisms was more in the control group than in the remediation group, probably due to the continuous addition of sulphate-reducing bacteria to the system during the reaction process. The sulphate-reducing bacteria adapted to the environment and colonized the soil environment as the dominant bacteria. The addition of exogenous microorganisms in the remediation group R increased the microbial abundance with a maximum abundance value of 64.17 at 21 days of reaction; as time increased, the competition of indigenous microorganisms was at a disadvantage, leading to a decrease in abundance at 36 days of reaction.

The Shannon index of the restored group R increased with the addition of SRB, while that of the CK group showed a decreasing and increasing trend. The Shannon index of soil samples without SRB decreased from 2.45 to 2.37 and increased from 2.45 to 2.72, respectively. The Shannon index of soil samples with SRB increased from 2.22 to 2.31 and from 2.18 to 2.35, respectively, indicating that adding group SRB increased the diversity of soil microorganisms.

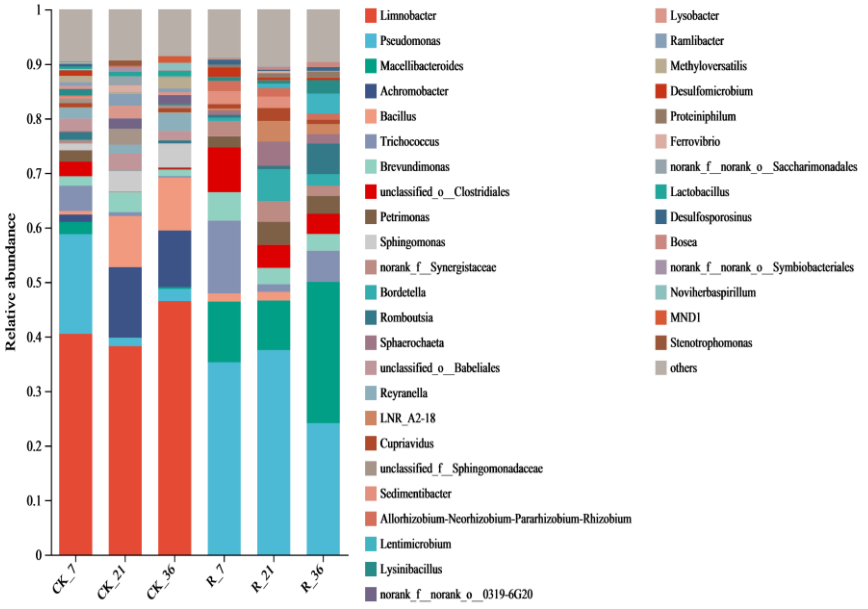

Fig. 4. Composition of bacterial communities at the phylum level (CK-7, CK-21 and CK-36 represent the samples of soils after control for 7, 21, and 36 days, respectively; R-7, R-21 and R-36 represent the samples of soils after biological stabilization for 7, 21, and 36 days, respectively).

Alterations in soil microbial communities were assessed at the genus level, as shown in Fig. 4. In the biological stabilization group (R), *Pseudomonas* was the most dominant genus with 35.3% at day 7 of restoration, and its proportion reached a maximum of 37.5% at day 21 of restoration, dropping to 24.1% later in the response, this indicates that Pseudomonas has high resistance to Cd-contaminated soil and is the most effective genus in Cd treatment of contaminated soil, and some studies have shown that Pseudomonas is an important genus in the remediation of heavy metal-contaminated soils [18, 19] and the pathway of remediation of Cd contamination is mainly through cellular adsorption [20]. In the control group (CK), *Limnobacter* had the highest relative abundance, which slowly increased over time from 40.5% to 46.5%, Pseudomonas showed a significant decrease from 18.3% to 2.3%, and *Macellibacteroides* showed an increase in proportion as the repair rection progressed, from 11.1% to 25.9%. *Trichococcus* decreased from 13.3% to 1.4% and then slowly increased to 5.7% as the reaction progressed, and *Bacillus* dropped below the detection line on day 36 of the reaction. The main genus sulfate-reduced bacteria of the biological stabilization group (R) were *Desulfomicrobium* and *Desulfosporosinus*, which increased and then decreased as the reaction progressed. *Desulfomicrobium* in the biological stabilization group (R) accounted for 1.67 % of the total at 7 days of reaction, decreasing and then stabilizing at about 0.4 % as the reaction progressed, while *Desulfosporosinus* decreased to 0.2 % as the reaction progressed and then increased to 0.6 % at 36 days of reaction. Xue et al [21] reported that *Desulfomicrobium* was found to be significantly associated with heavy metal content. Desulfosporosinus was used in techniques to assist in phytoremediation of heavy metal pollution [22]. The control group (CK) had 0.9% and 0.47% of *Desulfomicrobium* and *Desulfosporosinus* in the system at the beginning of the reaction, and the abundance gradually disappeared as the reaction progressed. This indicates that the indigenous sulphate-reducing bacteria of the original genus are weak in competition, and the continuous addition of sulphate-reducing bacteria enhances their stable colonization in the system for long-lasting and stable solidification of heavy metals.

4 Conclusion

Cd pollution from smelters causes serious harm to the surrounding soil, and in recent years, microbial remediation has attracted much attention in the removal of heavy metals. In this study, we applied sulphate-reducing bacteria to bioremediate Cd in non-ferrous smelters, and the Cd concentration in the bioremediation system was significantly reduced under the action of sulphate-reducing bacteria. Tessier results showed that Cd was transformed into a stable residue state by microorganisms, and by properly constructing the microbial community structure, functional bacteria became the dominant population in the remediation process. Based on the results, we propose that sulphate-reducing bacteria can be efficiently applied to the remediation of Cd pollution at non-ferrous smelting sites. In addition, microbial remediation technology matches the new concept of eco-friendly, low-cost, and stable pollution removal, which can be efficiently applied to treat industrial pollutants.

Funding

This research was funded by the National Natural Science Foundation of China [grant numbers 51974279], the National Key Research and Development Program of China [grant numbers 2018YFC18027].

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