論文の内容の要旨
Abstract of Dissertation

論文題目　Improved phytoextraction of cadmium from contaminated soil using microbial biopolymers

（微生物バイオポリマーを利用したファイトレメディエーションによる汚染土壌からのカドミウム除去方法の開発）

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（本文）(Abstract)

Due to the toxicity and non-degradable nature, heavy metals are of great environmental concern for both developing and developed countries. They tend to pose serious health hazard to human beings and animals while accumulating in soil by adsorption. Cadmium is one of the ubiquitous and highly toxic heavy metals. Once cadmium accumulates in body, it may cause renal dysfunction (“Itai-itai disease”). In Asian countries such as China, a large area of paddy soil needs cadmium remediation. It is really a challenging and essential task to clean up the contaminated sites with heavy metals. Conventional remediation techniques (e.g. soil flushing, solidification, excavation) seem expensive and destructive in nature, since they may destroy soil structure and decrease soil productivity.

Phytoremediation (plant-based remediation) is effective for the mitigation of large area surface soil contamination with regards to extraction efficiency, duration of cleanup and economy. Usually, phytoextraction needs long time and the efficiency is not high. An important reason is that a large proportion of metals in soil are unavailable for plant uptake. In order to enhance the metal availability and sustain adequate metals in soil solution for plant uptake,

various agents have been added to soil. Agents, such as EDTA, were reported to increase bioavailable lead in soil and bring greater accumulation in plants. However, the addition of chemical agents (e.g. EDTA, DTPA) provides limited benefit. Chemical agents inhibit plant uptake of essential elements, and are toxic to plant growth. Furthermore, they could pose a risk to soil and ground water environment. Thus, natural agents seem more promising than synthetic chemicals.

In this dissertation, the microbial biopolymers were extracted from activated sludge and utilized to cadmium contaminated soil. Microbial biopolymers produced from activated sludge process consist of polysaccharide, protein, RNA, and DNA. The proteinaceous biopolymers have been considered to be economical and were reported to play an important role in removing heavy metals from solution.

In the other hand, plants release root exudates (e.g. amino acid) containing biopolymers with the potential to enhance cadmium uptake, translocation and resistance. However, the concentration of the naturally excreted biopolymers is low. In this dissertation, high concentration of microbial biopolymers would be produced and used for cadmium removal by plant from contaminated soil.

There were four major objectives of this study: (1) to elucidate the heavy metal binding characteristics of microbial biopolymers obtained from activated sludge grown on different media; (2) to determine the cadmium extraction efficiency of microbial biopolymers from contaminated soil; (3) to investigate the influence of microbial biopolymers on phytoextraction of cadmium in hydroponic solutions; and (4) to evaluate the potential applications of microbial biopolymers in improving phytoextraction of cadmium as an effective method for soil remediation.

This dissertation consists of eight chapters. Background information and objectives of the present study as well as the structure of the dissertation are represented in Chapter 1. Basic information on heavy metal contamination, remediation of contaminated soils, microbial biopolymers and enhanced phytoextraction based on literatures are provided in Chapter 2. Materials and methods used in this research are described in Chapter 3. Chapter 4, 5, 6 and 7 are corresponding to the four objectives described above, respectively. Chapter 4 focuses on cadmium binding characteristics of microbial biopolymers in aqueous solution. Chapter 5 depicts the effectiveness of microbial biopolymers for cadmium extraction from soil. The influence of microbial biopolymers on phytoextraction of cadmium in hydroponic condition is documented in Chapter 6. The effectiveness of microbial biopolymers application in improving phytoextraction of cadmium from contaminated soil is shown in Chapter 7. Finally, the conclusions drawn from this study and further recommendations are outlined in Chapter 8.

Metal-binding biopolymers were reported to be induced with the existence of trace metals in growth media of activated sludge. In this research, microbial biopolymers were obtained from non-induced, copper-induced and cadmium-induced activated sludge culture, and named as ASBP, ASBPCu and ASBPCd, respectively. The adsorption of cadmium by microbial biopolymers was observed in solution. The adsorption of cadmium by microbial biopolymers was fast (10 min to reach equilibrium). The BET model fitted the best of isotherm adsorption data, with R^2 values of 0.991, 0.966 and 0.982 for ASBP, ASBPCu and ASBPCd, respectively.

Microbial biopolymers, as well as EDTA, citric acid and BSA, were tested for their efficiency to extract cadmium from contaminated soil. The results demonstrated that the inclusion of extracting agents increased cadmium extraction from the soil. Microbial biopolymers extracted more cadmium than 0.005 mM EDTA, however, less than 0.05 mM EDTA. Cadmium extraction with ASBPCd was slightly higher than ASBP and ASBPCu.

In the later step, the influence of microbial biopolymers on phytoextraction of cadmium in hydroponic solutions was examined in this study. Biopolymers provided higher cadmium uptake by plants (*Crassula lycopodioides v. pseudolycopodioides*), than EDTA. The cadmium uptake in the presence of biopolymers was 5.5~6.6, 24.5~31.9 and 141.7~190.1 μg/g, at the initial cadmium concentration of 0.5, 1.9 and 9.1 mg/l, respectively. The cadmium uptake in the presence of EDTA was 0.4~2.7, 8.9~26.4 and 34.9~152.8 μg/g, at the initial cadmium concentration of 0.5, 1.9 and 9.1 mg/l, respectively.

The potential application of biopolymers in improving phytoextraction of cadmium from contaminated soil was also investigated in this research work. Microbial biopolymers, compared to other agents, were found to be more effective in improving the phytoextraction of cadmium from soil. The existence of biopolymers allowed higher cadmium content accumulated in plant biomass, than other extracting agents. In ASBP, ASBPCu and ASBPCd, the cadmium content in plants was found to be 1.52, 1.63 and 1.33 μg (1.9, 2.0 and 1.6 times of the control), respectively. It was also found that in the presence of microbial biopolymers ASBP, ASBPCu and ASBPCd, 10.9%, 26.2% and 13.7% of exchangeable cadmium fraction was extracted from soil matrix to plant or liquid, higher than the control test (4.3%). Microbial biopolymers were effective in improving the available cadmium amount in the soil, thus providing a higher and subsequential potential of cadmium uptake by plants in long term phytoextraction.

The synergistic effects of the sorption of heavy metals onto plants as well as the dissolution of metals from solid phase would be the possible mechanism for the improved phytoextraction of cadmium from contaminated soil. Different mechanisms were observed between microbial biopolymers and EDTA, when they were applied to improve the phytoextraction of cadmium from contaminated soil. In this dissertation, it was found that in the existence of microbial biopolymers, the limiting step would be the extraction of cadmium from soil matrix to soil solutions. However, the transportation of EDTA-Cd complex from soil solution to plant root would be the limiting step while EDTA were used for improving the phytoextraction of cadmium from contaminated soil.

In this dissertation, microbial biopolymers, acting as a new environmentally safe extracting agent, were more effective in improving cadmium accumulation in plants than other chemical agents. Instead of current chemical agents, microbial biopolymers could significantly reduce the production costs. A further study on the characterization of heavy metal-binding biopolymers derived from protein cloning techniques would have higher potential for its application in phytoextraction of heavy metals in contaminated soils. Extensive field tests of hyperaccumulator plants with biopolymers deem vital before its large-scale implementation. Practical aspects of improved heavy metal phytoextraction by microbial biopolymers also need further research. This might include development of an economical method to prepare the microbial biopolymers in a large scale, and feasible techniques to treat plants accumulated with heavy metals after harvest, and so on.