学 位 論 文 内 容 の 要 旨/Dissertation Abstract

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Cyanoremediation of heavy metals from aqueous solution by live and dead biomass: An eco-sustainable technology
（生細胞と死細胞による水溶液からの重金属のシアノレメディエーション：環境に優しい持続可能な技術）

Water pollution by heavy metals (HMs) is a pivotal and environmental concern. Surface water pollution by toxic heavy metals is increasing in tandem with industrialization and development in the contemporary era. These HMs are widely recognized as one of the most preeminent environmental pollutants, owing to their intrinsic toxicity, persistence, and bio-accumulative proclivity within biota. Approximately 23 million people living on floodplains are impacted by toxic metal waste originating from historical and contemporary metal mining activities. Although numerous conventional physicochemical techniques exist for HMs removal from wastewater, these are expensive and sometimes produce secondary pollution. So, microbial-based bioremediation techniques are now in high demand due to the drawbacks associated with conventional treatment techniques and the global focus on achieving Sustainable Development Goals (SDGs).

Cyanoremediation technique for heavy metals (HMs) removal from wastewater using live cyanobacteria is promising to reduce pollution risk both for the environment and human health. Although it is a promising technology, the research with live cyanobacteria is limited and the initial working concentration of HMs is not well studied. To fill this gap this study was conducted with live and dead cyanobacteria. The cyanoremediation technique for toxic HM removal has been reviewed along with its working mechanism and efficiency in this thesis. Cyanobacteria produces extracellular polymeric substances (EPS) in harsh environments. This EPS increases the HM tolerance level of cyanobacteria. Live cyanobacteria can remove HMs through both biosorption and bioaccumulation processes, whereas dead biomass can only adsorb HM from the aqueous solution. To achieve sustainable and eco-friendly treatment, it is essential to reduce the usage of chemicals and adopt the biological treatment technique of cyanoremediation. Genetically modified HM-tolerant model cyanobacteria can enhance the efficiency of this cyanoremediation technique for HM removal.

Identifying appropriate live cyanobacterial strains for heavy metal removal is challenging. Not all strains can effectively tolerate and remove heavy metals from aqueous solutions. Some exhibit high tolerance to heavy metal concentrations, while others are more sensitive. Therefore, it is crucial to carefully select cyanobacterial candidates based on their tolerance levels and efficiency in metal removal. Eight cyanobacterial species were cultured and screened to get suitable candidates for HM removal at different initial concentrations and two efficient species *Anabaena variabilis* (NIES-2095), and *Synechocystis* sp. (NIES-3758) were found for cyanoremediation. *Scytonema* sp. was also a potential species. It removed approximately 76% of Cr (VI), 78% of Pb (II), 71% of As (V), and 26% of Cd (II) but due to its morphological characteristics, the effects of HMs on their growth were difficult to measure as they were not uniformly distributed in the aqueous solution.

In this study, two freshwater cyanobacteria, *Anabaena variabilis*, and *Synechocystis* sp. were used to explore their efficacy in HMs (As (V), Cd (II), Cr (VI), Pb (II)) removal. The different optimum adsorption conditions of pH were 8 and 7.5 for *A. variabilis, and Synechocystis* sp., respectively, but temperature (25 ^{0}C) and contact time (48 hours) were the same for both strains. Under these specified conditions, *A. variabilis* exhibited the capability to remove 25% of As (V), 78% of Cd (II), 54% of Cr (VI), and 17% of Pb (II), whereas *Synechocystis* sp. removed 77% of As (V), 57% of Cd (II), 91% of Cr (VI), and 77% of Pb (II) at different initial concentrations. Metal diversity interfered negatively with cyanobacterial growth, especially Cd (II) and As (V), regarding OD_{730}, dry biomass, chlorophyll a, and carotenoid production for both strains. Fourier transform infrared spectrum (FT-IR) analysis revealed the existence of diverse surface binding sites for HMs adsorption, stemming from proteins and polysaccharides. Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDS) confirmed the presence of HMs on the surface of cyanobacterial cells. The SEM image of *A. variabilis* and *Synechocystis* sp., after being exposed to four different heavy metals, played variations in morphology, as indicated by alterations in filament length, the dimension of individual vegetative cells, and the appearance of exopolysaccharidic precipitates. The cell size of *A. variabilis* exhibited more significant changes after Cd (II) adsorption and *Synechocystis* sp. exhibited a haphazard crystal-coated appearance after As (V) adsorption. This random crystal or EPS secretion played an important role in the HM removal from the solution. Morphological changes in the cyanobacterial cells after HM adsorption were confirmed by EDS analysis. The EDS spectrum showed the qualitative and quantitative existence of HMs on the cell surface.

Finally, the zeta potential results indicating alterations in surface negative charges elucidated the adsorption mechanisms involved in the HMs removal by both cyanobacteria. The zeta potential of *Synechocystis* sp. *and A. variabilis* was measured and a negative value was recorded. This negative charge on the surface of cyanobacterial cells is derived from the existence of acidic compounds, including amines, acidic polysaccharides, and carboxylic acids, which signifies the cyanobacterial cell's ability to absorb cationic metals into their surfaces. The zeta potential of the control (before HM contamination) has a higher negativity because of the presence of negative ions, and after HM adsorption, the total negativity decreases, which validates the mechanism of HM adsorption on the cyanobacterial cell surface. In both cyanobacterial strains, the zeta potential value or negativity decreases upon HM adsorption

A biosorbent derived from the dry biomass of *Synechocystis* sp. was prepared for the bioremoval of highly concentrated Cd (II) from aqueous solutions which showed around 79 % of Cd (II) removal efficiency at 10 mg L^{-1} initial concentration. Biosorbent mass was optimized from 50 mg to 500 mg range and 200 mg was found to be efficient. Solution pH influenced Cd (II) removal efficiency. Both lower and higher pH levels reduced the metal removal efficiency, with the highest Cd (II) removal efficiency observed at pH 7. A range of solution temperatures from 20^{0}C to 40^{0}C exhibited a similar effect on Cd (II) removal efficiency and most adsorption occurred within the first 30 minutes.

Cyanobacteria have the potential to tolerate HMs at a moderate level. However, this tolerance limit is species and dose-dependent which requires more advanced research using the cyanoremediation technique. To make this technique sustainable more research should be conducted using live cyanobacteria in parallel with its dead biomass. The findings of this study can provide insight into establishing a possible reference standard for the initial metal concentration in future research on cyanoremediation. These results also provided a comprehensive understanding of HMs adsorption mechanism by cyanobacteria, offering valuable theoretical insights that can be extrapolated to enhance our comprehension of cyanoremediation mechanisms by various other cyanobacterial strains.