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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)

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Introduction

Water pollution by heavy metals (HMs) is a pivotal and environmental concern. Surface water pollution by toxic HMs 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 HMs removal from wastewater using live cyanobacteria is promising to reduce pollution risk both for the environment and human health. 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. Although cyanoremediation 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.

Chapter 1: Cyanoremediation of heavy metals from aqueous solution and its mechanism

Water contamination caused by HMs stands as a critical environmental issue due to their inherent toxicity, and prolonged presence in the environment. The escalation of surface water contamination by hazardous HMs is concomitant with the ongoing processes of industrialization, urbanization, mining, and development activities in the modern era which has become a challenge for researchers and scientists. Within living organisms, certain HMs serve as essential micronutrients, contributing to vital biological processes while others exert negative effects and can be detrimental to health and the environment when present in excess concentrations. Conventional methods for HM removal from wastewater tend to be costly and occasionally result in secondary pollution so efficient, cost-effective nature-based systems (NBS) are being recognized as a promising technological option aligning with the objectives of the SDGs. Cyanoremediation is comparatively a new technique to remove HMs from an aqueous solution by using different cyanobacteria.

Cyanoremediation is a technique to remove toxic compounds of organic materials or HMs from a contaminated environment of aqueous solution by biological agents or microorganisms with biosorption, bioaccumulation, and biotransformation processes. By this biological process, contaminated media can be brought back to their original or nearly original state without affecting the surrounding environment. Biosorption is a mechanism to remove HMs or metalloid ions from aquatic solution or wastewater by the surface of the biological adsorbents. These biological adsorbents may be of natural biomass or cyanobacteria, fungi, algae, bacteria,

and plants. Although both dead and live biomass can be used to remove these HMs even at lower concentrations of parts per billion, dead biomass is frequently being used. The cell wall of cyanobacteria, fungi, and bacteria makes these microorganisms suitable for HM adsorption on their surface. The mechanism for biosorption is simply the interaction of HM ions to the surface binding sites of anionic functional groups present on the surface of cyanobacteria or dead biomass. Simple electrostatic interactions or Van der Waals interactions between the surface binding sites and HM ions make this biosorption possible. The other technique of microorganisms to remove HMs from the abiotic environment (soil, water, air) is to absorb these toxic metals through cellular uptake directly or indirectly with their trophic transfer or dietary source (food, and nutrients) and accumulate inside the cells called bioaccumulation. These toxic HMs are trapped inside the live cells by different metal-binding proteins in the cytoplasm. In this metal bioaccumulation process at first HMs need to come in contact with the cell surface to be attached and the second step is to enter the cells by active metabolism. HMs can be accumulated within intracellular space by lipid bilayer translocation route as well as proteins, and peptide ligands.

Biotransformation is a mechanism involved in the conversion of toxic HMs into their non/less toxic state to reduce the lethal effects of HMs on the cells for their survival. Algae, cyanobacteria, and fungi show higher efficiency for HM bioremediation. Living microorganisms often use HMs as their nutrient or energy source through the enzyme-catalyzed pathway and convert or mineralize these HMs into less/non-toxic metal ions state. This biotransformation is also one of the strategies of microorganisms to adapt to HM contaminated environment resulting in the detoxification of contaminants. Transformation of metal ions state occurs inside the cells. HMs enter the cyanobacterial cells through the uptake system (phosphate/sulfate transporter), then translocated by metal-binding proteins finally converting into a less toxic state.

Cyanobacteria have negative binding sites on their surface and demonstrate varying levels of tolerance limits to different HM. They also exhibit distinct binding ability to different HM ions and can alternatively form complexes with different metal ions. EPS plays a crucial role in HM removal from aqueous solution. EPS plays an essential role in protecting from harmful environmental factors the microbial community residing in biofilms.

In this chapter, the cyanoremediation technique for toxic HM removal has been reviewed along with its working mechanism and efficiency. Further, the role and synthesis mechanism of cyanobacterial EPS in HM removal has been discussed. Additionally, this review chapter attempts to address the question of whether live or dead cyanobacteria are more effective for HM removal from a sustainable perspective to provide a foundational understanding to beginners and a pathway to experts in the selection of better techniques for future research in this field.

Chapter 2: Screening of the potential cyanobacterial candidates for heavy metal removal from an aqueous solution

A diverse group of cyanobacteria have high photosynthetic efficiency, growth rate, and tolerance to wastewater toxicity which make them a suitable candidate for biomass production coupled with wastewater treatment. Cyanobacteria cultivation in a wide range of wastewater (such as municipal, agricultural, and industrial wastewater) can effectively remove N, P, and metal ions. The major removal mechanisms employed by the cyanobacteria are bioaccumulation and bioadsorption which are eco-friendly and economically feasible as compared to the conventional methods. Different operational strategies and multidiscipline integration have been incorporated to increase cyanobacteria cultivation and limit the growth-inhibiting parameters in wastewater. Indeed, identifying suitable cyanobacterial candidates especially live cyanobacteria for HM removal presents a significant challenge. Not all cyanobacterial strains possess the capability to effectively tolerate and remove HMs from

aqueous solutions. Some strains may exhibit higher tolerance to HM concentrations, while others may be more sensitive. Thus, careful selection of cyanobacterial candidates based on their tolerance levels and metal removal efficiency is essential. Additionally, optimizing culture conditions, such as temperature, light intensity, and nutrient availability, plays a crucial role in enhancing the HM removal capacity of cyanobacteria. By systematically evaluating different cyanobacterial strains and their culture conditions, researchers can identify optimal candidates for HM remediation applications.

The objective of this chapter is to screen and identify cyanobacterial strains that are suitable for the removal of HMs from aqueous solutions. Through systematic evaluation and screening processes, the goal is to identify strains with high tolerance to HMs and efficient removal capabilities. By selecting the most promising cyanobacterial candidates, this research aims to contribute to the development of effective cyanoremediation strategies for HM-contaminated water bodies.

This study has identified two cyanobacterial strains, *Anabaena variabilis* (NIES-2095), and *Synechocystis* sp. (NIES-3758), capable of effectively removing four HMs from aqueous solutions. Interestingly, *Scytonema* sp. ((NIES-4073) showed good performance in removing all four metals (As, Cd, Cr, and Pb). This versatility suggests that *Scytonema* sp. could be a valuable candidate for cyanoremediation efforts targeting multiple HMs in contaminated water sources. This finding suggests that these strains possess inherent characteristics that enable them to tolerate and efficiently sequester HMs, highlighting their potential for use in cyanoremediation applications aimed at mitigating HM pollution in the aquatic environment.

Chapter 3: Cyanoremediation of heavy metals by live cyanobacteria, *Synechocystis* sp., and *Anabaena variabilis*

Cyanoremediation technique for HMs removal from wastewater using live cyanobacteria is promising to reduce pollution risk both for the environment and human health. In this study,

after screening 8 species, two widely recognized 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 °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 OD₇₃₀, 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. Finally, the zeta potential results indicating alterations in surface negative charges elucidated the adsorption mechanisms involved in the HMs removal by both cyanobacteria. The empirically supported conclusions suggest that two scrutinized live cyanobacteria *Synechocystis* sp. and *A. variabilis* exhibit substantial potential, showcasing numerous prospective applications in the realm of cyanoremediation as they promote the reduction of toxic metals concentrations such as As (V), Cd (II), Cr (VI), and Pb (II) from aqueous solution with almost 50% of cell viability. This result demonstrates that cyanobacteria exhibit variable binding affinities toward distinct HM ions, allowing for their alternate association with these metal ions. This finding further elucidates that the main mechanism underlying the HM removal involves surface adsorption of cyanobacteria. These results 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.

Chapter 4: Biosorption of Cd (II) heavy metal by dry biomass of cyanobacteria *Synechocystis* sp. (NIES-3758)

The objective of this chapter was, to remove Cd (II) from an aqueous solution by dry biomass of *Synechocystis* sp. and to decipher the biosorption mechanism of Cd (II) removal from the aqueous medium.

Cd is one of the most toxic substances encountered in aquatic ecosystems. This metal has a propensity to accumulate in photosynthetic plants and fish, ultimately transferring to humans through the food chain, thereby causing various diseases. Dry biomass of *Synechocystis* sp. was utilized for Cd (II) removal in a batch experiment due to its high biomass production capability within a short time frame. The biosorbent was prepared after 60 days of culture on a large scale. Around 15.64 g lyophilized dry biosorbent was prepared from a total of 30 L culture. Surface characterization of the biosorbent was analyzed by determining the pH point of zero charge. Following a 30 min exposure to an initial concentration of 10 mg L⁻¹, approximately 79.08% of Cd (II) was adsorbed. This outcome suggests that *Synechocystis* sp. holds significant promise as a candidate for removing Cd (II) from aquatic environments. The process is not only feasible and reliable but also eco-friendly.

General discussion

Cyanoremediation is a comparatively new technique to remove HMs from an aqueous medium but could be a potential, sustainable, and green technique depending on the suitable live cyanobacterial candidate selection for specific HM. *Anabaena variabilis*, *Synechocystis* sp., and *Scytonema* sp. showed notable removal efficiency of As (V), Cd (II), Cr (VI), and Pb (II) by surface adsorption. To use live cells, it is important to maintain cell viability with metal

concentration. Living cells will be adapted to metal contamination and reproduce new cells to receive HM on their surface which will continue in a circular way reducing the operational cost for wastewater treatment. Despite the prevalence of dead cells in the wastewater culture, it is crucial to acknowledge that the biosorption process can take place on both the surfaces of dead and live cyanobacterial cells. This phenomenon contributes to the removal of HMs from the wastewater. Dry biosorbent of *Synechocystis* sp. can remove higher concentrations of Cd (II) compared to live cells. However, continuous addition of this dry biomass is necessary for the treatment process. Cyanobacteria were cultured at a fixed pH as both high and low pH can exert a substantial impact on HMs adsorption. The optimum pH of 7.5, and 8.0 were used for *Anabaena variabilis*, and *Synechocystis* sp., respectively, because these two strains were cultured in MDM and BG-11 medium. To ensure maximum growth of live cyanobacteria we used these pH values and it is also known that these cyanobacteria can't tolerate extreme pH. In low pH solutions (pH<2.5), an abundance of H⁺ ions compete with the HMs on the cell surface, generating a repulsive force between the HMs and the binding sites during the adsorption process. Functional groups bearing negative charges undergo deprotonation at high pH levels such as pH > 8.5. This deprotonation process amplifies the electrostatic attraction between these negatively charged functional groups and positively charged HM ions, thus augmenting the biosorption capacity.

HMs removal mode was adsorption as the intracellular removal mechanism requires long time cultivation in contaminated water. After 7 days of cultivation, only 7-15% of Cd accumulated intracellularly by *Anabaena doliolum*. Moreover, the biotransformation mechanism of HMs by enzymatic activity inside the cyanobacterial cells can contribute to HMs removal which requires a transition of metallic charges. Metal homeostasis is important but how metals transport in cyanobacteria is still not fully clear. Cyanobacterial cells can uptake a trace amount of intracellular HMs to maintain the concentration below the toxic level. In biotransformation,

chemical and structural differences of metals can create specific intracellular effects of protein denaturation and uncoupling of oxidative phosphorylation thus these oxidative state changes can limit the amount of HMs adsorption by cyanobacteria and finally intracellular concentration of HMs can be observed after a long period of cultivation. These all support the results of this study that the main mode of HMs removal was mainly surface adsorption by different binding sites on the cell surface and in future cyanobacteria specifically *A. variabilis*, *Synechocystis* sp., and *Scytonema* sp. can be used for HM removal from aqueous medium in a sustainable way.

The dry biomass of *Synechocystis* sp. removed Cd (II) efficiently at specific working conditions of pH, biosorbent mass, contact time, initial cadmium concentration, and temperature. pH is a crucial parameter, as lower pH levels lead to excess H⁺ ions competing with cadmium ions, reducing cadmium removal efficiency. Conversely, higher pH levels cause metal precipitation from the solution. It is also essential to optimize the initial metal concentration and biosorbent mass to determine the saturation level of the available biosorbent sites.