Bioremediation of Heavy Metals Using Extremophile *Galdieria sulphuraria* from Multi-Metal Systems Aqueous Solutions.

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Abstract: Discharging untreated wastewater containing toxic heavy metals into water and wastewater ecosystems is widespread. Beyond permissible limits, elevated HM concentrations threaten both living organisms and the environment, underscoring the critical need for their removal. This study explores the efficiency of removing HMs from a multi-metal system aqueous solution using the extremophilic red alga *Galdieria sulphuraria*. The experimental setup introduced a combination of seven HMs (Pb, Cd, Cu, Fe, Mn, Ni, and Zn), each at a concentration of 10 mgL^{-1}, into the algal growth media. Conducted in quintuples over nine days, the experiments revealed an initial decrease in growth on the first day, followed by continuous growth until the ninth day in the HM-containing media. Simultaneously, ammonia and phosphorus levels in the growth media decreased by the ninth day. ICP-AES analysis of the supernatant demonstrated G. sulphuraria's effective sorption of HMs, showcasing substantial removal values ($8\% - 40\%$) throughout the growth experiment. This research shows *G. sulphuraria* as a promising bioremediation pathway, demonstrating the efficient removal of HMs from a multi-metal system.

Keywords: bioremediation, microalgae, Galdieria sulphuraria, heavy metal removal.

Introduction

The release of heavy metals (HMs) into the environment has become a major environmental issue in recent years. HMs are discharged into the environment by numerous sectors, including but not limited to metal mining, the oil and textile industry, metallurgy, iron and steel, electroplating, galvanization, electric appliance manufacturing, and metal surface treatment [1, 2]. These HMs can enter the human body in a variety of different ways, and they can cause a wide range of adverse health impacts from moderate to severe, including but not limited to headaches, arthralgia, mental disorders, altered liver and kidney function, and even cancer [3]. The removal of HMs from contaminated water and wastewater sources has been approached using various techniques, such as electrochemical technology, reverse osmosis, nano/ultrafiltration, precipitation, and adsorption [4]. The application of traditional techniques has been restricted because of financial, technical, and sustainability issues [5]. Compared to conventional physiochemical techniques, phycoremediation has been investigated as a more sustainable, effective, environment-friendly, and cost-effective method over the past few decades. The phycoremediation process utilizes the ecological function of microalgae to eliminate impurities from water and wastewater [6].

Galdieria sulphuraria (G. sulphuraria) is

a unicellular red alga that can survive in extremely low pH (0.0-3.0) and high temperatures (37-55o C) [7, 8]. Additionally, *G. sulphuraria* demonstrates metabolic versatility by effectively surviving on over 50 distinct carbon sources [9]. These extreme conditions make *G. sulphuraria* a good candidate for HM tolerance studies and phycoremediation [10]. G. sulphuraria has been effectively used to treat produced water, landfill leachate, and municipal wastewater to remove carbon and nutrients ($NH_4 - N$and $PO_4 - P$) [11-13].

Municipal wastewater treatment systems are vulnerable to receiving nutrient-rich wastewater containing HMs in minute quantities, which are discharged by various industrial and mining activities [14]. Furthermore, as the treatment system usually gets a wide variety of metal ions, studies on the effects of various metal combinations are more predictive of practical treatments. This study aims to assess the effectiveness of using *G. sulphuraria* in a multi-metal system aqueous solution for bioremediation of seven HMs: lead (Pb), copper (Cu), cadmium (Cd), manganese (Mn), nickel (Ni), zinc (Zn), and iron (Fe) while simultaneously removing nutrients. In addition, this work seeks to quantify the inhibitory effect of various HMs on *G. sulphuraria'*s growth and biomass yield.

Materials and Methods

Culturing of algal strain and HM solution preparation

This study utilized *G. sulphuraria* CCMEE 5587.1, a red microalga obtained from the Culture Collection of Microorganisms from Extreme Environments at the University of Oregon. This microalgal strain was cultivated at a controlled temperature of $40°C$ under constant illumination of 4000 lumens in an incubator (Percival, IA, US) in the Cyanidium Medium (CM). The following media recipe was used to prepare CM: $(NH_4)_2SO_4$, 1.32 gL^{-1}; KH_2PO_4, 0.27 gL^{-1}; $NaCl$, 0.12 gL^{-1}; $MgSO_4$Âů7H_2O, 0.25 gL^{-1}; $CaCl_2$Âů2H_2O, 0.07 gL^{-1}; Nitch's trace element solution, 0.5 $mL\ L^{-1}$; $FeCl_3$ (solution = 0.29 gL^{-1}), 1.0 $mL\ L^{-1}$. The pH of the medium was maintained at 2.5 by adding 10 N H_2SO_4.

The corresponding salts of each tested metals were dissolved in distilled water to produce stock solutions of HMs. All the chemicals used were analytical-grade, and all the glassware were autoclaved before they were used. Synthetic wastewater solutions were prepared by adding stock solutions of HMs to CM to achieve $10 mgL^{-1}$ concentrations of each HMs.

B.Bioremediation experiment

G. sulphuraria was isolated during its exponential growth phase and centrifugated at $4°C$ for 10 minutes at 3000 rpm using a Centrifuge 5920R (Eppendorf AG 22331 Hamberg, Germany). Following centrifugation, the supernatant was discarded while the biomass was resuspended in the media that were exposed to HM.

The experiment was carried out using four cases with different media compositions, each with five replicates. A variety of sources can provide nutrients to *G. sulphuraria* because it is mixotrophic. Adding a carbon source other than CO_2 can accelerate the growth. Consequently, two media compositions were supplemented with 25 mM of glucose for an additional carbon source.

All the tests were carried out in Erlenmeyer flasks with a capacity of 125 ml, each containing 50 ml of the experimental sample. A New Brunswick Innova 2050 platform shaker (Eppendorf, Edison, NJ, USA)) was used to rotate all the flasks at a speed of 120 rpm for nine days.

Figure 1: Experimental setup used in the study.

C. Sample analysis

Algal biomass density was calculated by measuring the optical density at the 750 nm wavelength using a spectrophotometer (HACH, Colorado, USA). The biomass density was evaluated in terms of ash-free dry weight (AFDW) gL^{-1}, which was correlated to OD at 750 nm by using the following equation:

$$AFDW = 0.4775 * (OD@750nm) - 0.0163$$

$$n = 12, r^2 = 0.997$$

Ammoniacal nitrogen and phosphate were measured using a HACH DR 3900 (HACH, Loveland, CO, USA) spectrophotometer. The concentration of HM was measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES) (SHIMADZU ICPE 9820 simultaneous ICP atomic emission spectroscopy). The HM removal efficiency was calculated by using the following equation:

$$Removal(\%) = \frac{(c_I - c_f)}{c_i}$$

C_i and C_f represent the HM concentrations in the media on Day 0 and Day 9.

Results and Discussion

A. Growth of G. Sulphuraria

The growth profiles of *G. sulphuraria*, cultured in different media compositions for 9 days, are shown in Figure 2. Adding glucose to the media significantly increased the algal biomass density compared to adding no glucose. Adding HM to the media caused a growth inhibition on Day 1 before the algal biomass density began to increase. This implies that the presence of HMs could impede microalgae growth in the initial days. The reason for this could be that *G. sulphuraria* needs some time to adjust to environments that are contaminated with HMs.

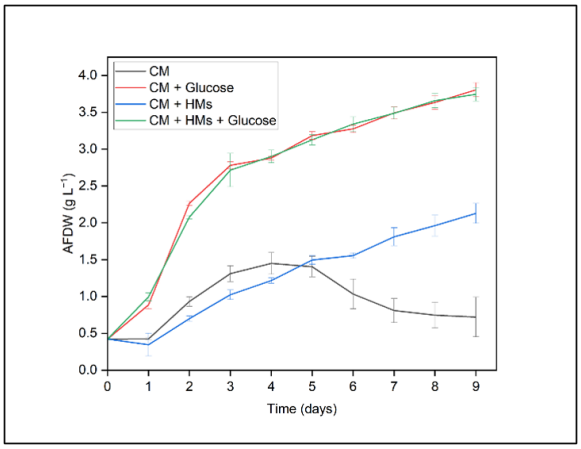
B. HM Bioremediation

After nine days of treatment with live cells, *G. sulphuraria* was able to remove HM ions from the media to some extent. Figure 3 shows the findings of an ICP-AES analysis that was carried out on both the initial and final days of the experiment. A slightly higher removal efficiency was obtained in the case of media without the addition of glucose. The removal efficiency is in the order of Pb (40.61%) > $Zn(33.38\%) > Cd(28.46\%) > Ni(27.19\%) > Cu(26.77\%) > Mn(26.31\%) > Fe(8.91\%)$.

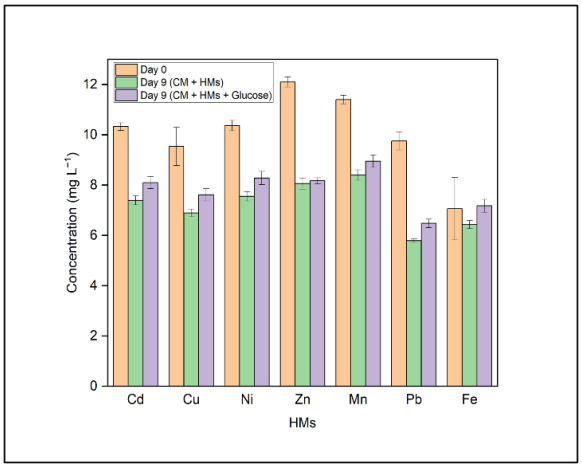
Nutrient removal

The removal of ammoniacal nitrogen (NH_4-N) and phosphate phosphorous ($PO_4 - P$) by *G. sulphuraria* in the different media compositions

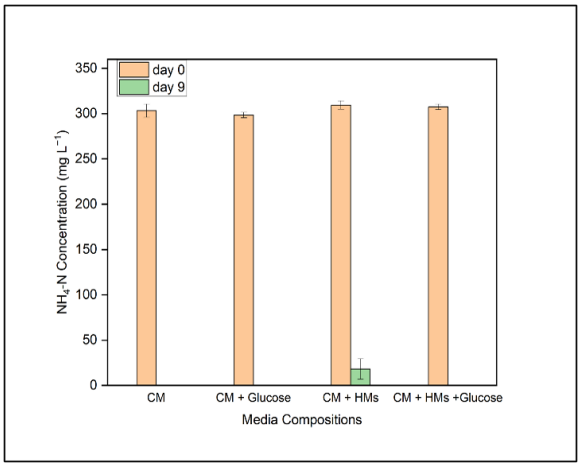
are shown in Figures 4 and 5. In all the media except the one with added HMs with no glucose, the ammoniacal nitrogen removal was around 100%. The addition of glucose to media compositions also resulted in a higher phosphorus removal (about 50%). The removal patterns observed for both nutrients followed a similar trend for growth profiles.



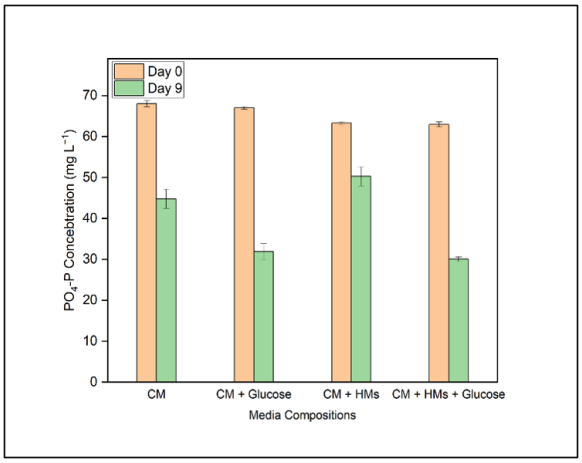
(a) Figure 2: Growth profiles of G. sulphuraria in different media compositions. Data points represent average SD of n=5 biological replicates.



(b) Figure 3: HM concentration before and after the treatment in different media compositions. Data points represent average SD of n=5 biological replicates.



(c) Figure 4: Ammoniacal nitrogen concentration before and after the treatment in different media composition. Data points represent average SD of n=5 biological replicates.



(d) Figure 5: Phosphorus concentration before and after the treatment in different media composition. Data points represent average SD of n=5 biological replicates.

Conclusions

Overall, this study evaluated the potential of using *G. sulphuraria* for the bioremediation of HMs from the multi-metal system aqueous solution with simultaneous removal of nutrients. Results obtained from the study showed that

G. sulphuraria can grow in the HM-containing media with initial growth inhibition. At the end of the cultural period, HM ions are effectively removed from the aqueous solution, along with a significant amount of nutrients. This finding presents an intriguing possibility for the application of *G. sulphuraria*in the field of bioremediation of HMs.

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