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REUSABILITY OF *NOSTOC MUSCORUM* Meg 1 BIOMASS FOR Cd^{2+} REMOVAL

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ABSTRACT: The present study examined sorption-desorption of Cd^{2+} by *Nostoc muscorum* Meg 1 biomass in order to establish the repeated usability of same microbial biomass for removal of Cd^{2+}. Cd^{2+} removal by the cyanobacterium in a trial run using a 500 mL conical flask was 94% at the end of 24 h when cells were exposed to 0.5 ppm Cd^{2+} supplemented medium. For medium scale removal of Cd^{2+}, photo-bioreactor and mini pond bioreactor were designed. In these two experiments, *Nostoc muscorum* Meg 1 was able to remove 90% of Cd^{2+} in the photo-bioreactor while the removal percentage was 86% in the mini pond bioreactor within 24 h. Desorption of Cd^{2+} from *Nostoc muscorum* Meg 1 cell surfaces was analysed under treatment with 0.1 M HNO_3/HCl/ H_2SO_4/ EDTA/NH_4Cl. Next, the reusability of the biomass in Cd^{2+} removal was checked by conducting a repeated sorption desorption experiment. Maximum Cd^{2+} desorption (89%, 86%, 82% and 77%) was possible in presence of HNO_3 after first, second, third, fourth cycles. Among the five desorption agents tested, least desorption (74% 68%, 65% and 61%) was achieved in the presence of NH_4Cl. This study thus indicated that the same *Nostoc muscorum* Meg 1 biomass can be effectively used at least four times for the removal of Cd^{2+} from Cd^{2+} contaminated wastewater.

Keywords: Cd^{2+}, Biomass reuse, desorbing agents, sorption-desorption, bioreactors.

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1.INTRODUCTION

The biosorption of Cd^{2+} by the cyanobacterium *Nostoc muscorum* Meg 1 has been studied in laboratory in detail [1]. Water samples were collected from the rice fields adjacent to coal mines in Sohra, Meghalaya. The Cd^{2+} concentration was found in the water sample was 0.22 ppm that was far higher than the permissible limits (i.e. 0.22 ppm). The concentration of Cd^{2+} was ~ 44 folds higher than the recommended limit of U.S. EPA (2000) [2]. However, Cd^{2+}content in contaminated fields may change from time to time depending on the amount of coal residues that enter these rice fields. As the region boast of heavy rains, Cd^{2+} contaminated water enters almost all crop fields especially during rainy season which enhances level of Cd^{2+} in these fields to toxic proportion. Result of such an event is increased accumulation of Cd^{2+} in the grains [3,4] and further up-scale movement of toxic Cd^{2+} in the food chains creating health risk in animals and human beings [5,6]. This calls for the employment of remediation measures for clean-up of such pollutants from the contaminated environment. Many scientist have tried methods such as chemical treatment, solvent extraction, evaporation upturn, application of ion-exchange and membrane technologies for removing heavy metal burden from polluted sites [7,8]. In recent times, metal removal by biological resources such as plants, algae, and bacteria is gaining attention due to issues related to cost and degradation efficiency. Microorganisms can lessen heavy metal load from the adjacent environment by mechanisms such as biosorption, bioaccumulation, biotransformation or bio-mineralization [9,10]. Among microbes, cyanobacteria offer attractive possibilities as they are both photosynthetic and nitrogen fixing requiring input of simple nutrients. This makes the process cost-efficient and eco-friendly. Selecting fast-growing cyanobacteria provides opportunity for higher biomass production that reflects availability of larger surface area for metal binding. Many such rapidly multiplying cyanobacterial strains also produce mucilage / exo-polysaccharides that additionally aid in metal removal [11–13]. *In-situ* removal of Cd^{2+} by cyanobacteria is desirable as it reduces the risk of spreading Cd^{2+} contamination. The reclamation of the used cyanobacterial biomass through desorption of the metal ion from the biosorbent after sorption process is saturated is an important attempt to keep costs low and develop the possibility to recover the extracted metals from the liquid phase [14]. Thus the aim of this study was to evaluate the possibility of using live *Nostoc muscorum* Meg 1 cells for removal of Cd^{2+}. Special emphasis was placed on evaluation of reusability of *Nostoc muscorum* Meg 1 biomass in removal of Cd^{2+} from contaminated water. Reusability of the cyanobacterial biomass for Cd^{2+} sorption was determined by the sorption-desorption study. Sorption of Cd^{2+} in medium scale level in batch photoreactor and pond reactor were also studied.

2. MATERIALS AND METHODS

2.1. Growth and maintenance of cyanobacterium *Nostoc muscorum* Meg 1

Ten days old (mid-log phase) cyanobacterial cultures were used for experiments and the culture concentrations was kept constant at 3 μg/mL chlorophyll *a* concentrations throughout the study. The pH of the BG-11_0 medium was maintained at 7.5. All the experimental set-ups were kept in a culture room under light (PAR) at a photon fluence rate of 50 $\mu mol/m^2/s^1$ with continuous illumination and the temperature of 25 ± 2 °C and a shaking rate of 50 rpm [15].

2.2. Cd^{2+} treatment

$3CdSO_4 \cdot 8H_2O$ was used as the source of Cd^{2+} and the concentration was calculated only for Cd^{2+}, not for the salt. Experimental Cd^{2+} solution was prepared by diluting the 100 ppm stock solution with BG-11_0 medium. The Cd^{2+} concentration was chosen for the study was 0.5 ppm Cd^{2+}.

2.3. Analysis of various desorbents for recovery of Cd^{2+}

HNO_3, H_2SO_4, HCl, EDTA, and NH_4Cl were assessed for desorption of Cd^{2+} ions from the cyanobacterial biomass. At the end of 24 h of Cd^{2+} treatment, the cyanobacterial biomass was collected by centrifugation and washed with milli-Q water to remove excess Cd^{2+} ions from the sample. Next, the biomass was kept in desorbing solutions for 10 min under shaking condition. The solution was centrifuged and the supernatant was used for the estimation of the amount of Cd^{2+} desorbed from the cells [16–18]. Percent desorption was calculated using the following formula:

$$\%\ \text{Desorption} = \frac{\text{amount of } Cd^{2+} \text{ desorbed}}{\text{amount of } Cd^{2+} \text{adsorbed}} \times 100 \qquad (1)$$

2.4. Cd^{2+} removal study using batch bioreactors

One photo-bioreactor of 3 L capacity and one pond bioreactor of 5 L capacity were designed to study Cd^{2+} removal potential of the cyanobacterium in bulk scale. In both bioreactors, 0.5 ppm of Cd^{2+} was added to the medium. At the end of the incubation period of 24 h, 20 mL of treated cultures were taken, centrifuged at 2500 rpm for 3 min and the supernatant was transferred into new vials for estimating Cd^{2+}. The % Cd^{2+} removal was calculated using formula (2).

$$\text{Percent } Cd^{2+} \text{ removal} = \frac{C_F - C_I}{C_F} \times 100 \qquad (2)$$

2.5. Statistical analysis

Experimental data were obtained in triplicates. All the data were expressed as mean ± SD. Data were analyzed by applying one-way analysis of variance (ANOVA) following the Dunnett's multiple comparison tests. Statistical significance levels were: * = $p < 0.05$; ** = $p < 0.01$ and *** = $p < 0.001$. All the statistical analyses were performed using IBM SPSS Statistics 24 program (IBM Corporation, Somers, NY, USA).

3. RESULTS AND DISCUSSION

3.1. Screening for the effective Cd^{2+} desorbing agent(s)

Ahad et al. in 2017 [1], reported that the cyanobacterium showed significant biosorption capacity, (Q_{max} = 71.4 mg/g) indicating its strong potential in industrial application for large scale bioremoval of Cd^{2+} from contaminated waters. Thus, it became enormously important to understand its metal sorption/desorption behavior. Suitable desorbing agents could remove sorbed metal ions from the biomass and thus open up the possibility of re-using the same biomass multiple times in sorption/desorption process. Alternately, once desorbed, the cyanobacterial biomass could be allowed to recover and grow and then be harvested for newer cycles of Cd^{2+} biosorption. Five different desorbing agents i.e. 0.1 M each of HCl, HNO_3, H_2SO_4, EDTA, and NH_4Cl were tested for desorption of Cd^{2+} from the *Nostoc muscorum* Meg 1 biomass. Four rounds of sorption and desorption were studied using the same biomass. Figure 1 presents the percentages of Cd^{2+} ions recovered from the cyanobacterial biomass under treatment with different desorbing agents. Maximum Cd^{2+} desorption was 89 %, 86 %, 82 % and 77 % in presence of HNO_3 after first, second, third, and fourth cycles of desorption. However, the least desorption of 74 %, 68 %, 65 % and 61 % after first, second, third, and fourth cycle was achieved in the presence of NH_4Cl. The Cd^{2+} desorbing capacity of the solutions was found in the following order for first, second, third, and fourth cycle: HNO_3 > H_2SO_4 > HCl > EDTA > NH_4Cl. Sorption in presence of HNO_3, H_2SO_4, HCl, EDTA, and NH_4Cl was reduced by 14 %, 16 %, 20 %, 22 % and 28 % in the fourth cycle compared to first sorption cycle. Like sorption, desorption was also declined by 12 %, 13 %, 15 %, 13 %, and 14 % in fourth cycle in comparison to the first desorption cycle. Dixit and Singh, (2013) [13] reported that in *Nostoc muscorum*, maximum Cd^{2+} desorption was achieved in presence of HNO_3 which agrees with our study. Similarly, Mishra (2014) [16] reported that the recovery of Cd^{2+} ions from brown alga *Ecklonia maxima* using HNO_3, H_2SO_4, HCl acids and NaCl salt that were more effective than chelator EDTA and carbonate salts. Zhou et al. (1998) [19] reported that use of HCl and EDTA solutions were very effective for desorbing biosorbed metal ions from macroalgae. In their study, the biomass after treatment with HCl and EDTA showed high desorption performance suggesting the possibility of using HCl and EDTA for desorption of metal ions from biomass. The above-mentioned studies indicated that different desorbing agents act differently on different organisms. Therefore, for a particular organism under study both sorption and desorption agents and parameters need to be optimized for best results.

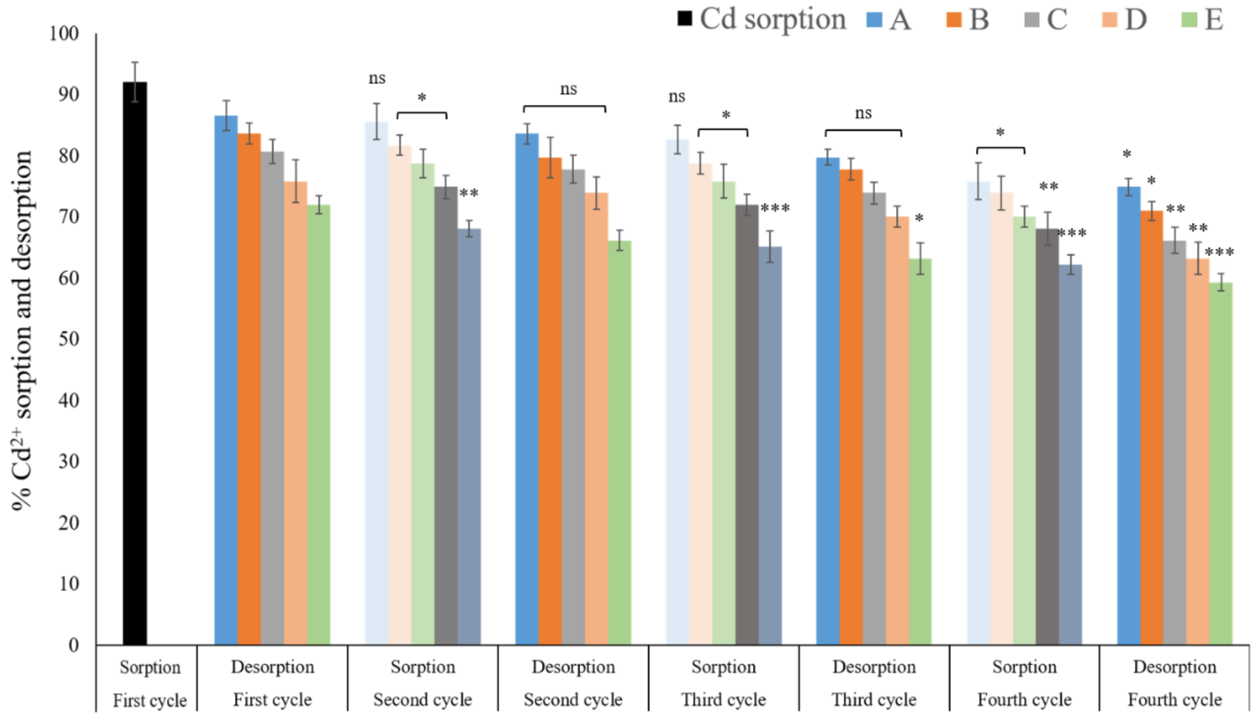

Fig. 1: Recovery of Cd^{2+} ions using different desorbing agents (HNO_3, H_2SO_4, HCl, EDTA, and NH_4Cl) from the *Nostoc muscorum* Meg 1 cells after four consecutive sorption and desorption cycles. Cd^{2+} concentration: 0.5 ppm. Duration of exposure of desorbing agents: 10 min. Cd = Cd^{2+}; A = HNO_3; B = H_2SO_4; C = HCl; D = EDTA, and E = NH_4Cl. All the values are expressed as mean ± SD, (N =3). Statistical analysis was performed using one way ANOVA (Dunnet's multiple comparison test) by comparing sorption cycle with second, third, and fourth sorption cycle as well as first desorption cycle with second, third, and fourth desorption cycle. Significance levels were expressed as ns = no significance, $*p < 0.05$, $*p < 0.01$ and $** p < 0.001$.

3.2. Batch bioreactor study

A simple photo-bioreactor (3 L capacity) and a mini pond bioreactor (5 L capacity) were designed and developed to study medium scale Cd^{2+} removal efficiency of the cyanobacterium *Nostoc muscorum* Meg 1 (Fig. 2a; b). The study was conducted to assess the efficiency of the organism in removing Cd^{2+} from larger volume of solution. Both bioreactors were kept in a culture room whose optimum temperature was maintained at 25 ± 2 °C. A final Cd^{2+} concentration of 0.5 ppm was added in the bioreactors containing the cyanobacterium with 3 µg/mL chlorophyll *a* concentration. The aeration facility in the photo-reactor was provided by allowing air to pass through various air openings made on the lid of the reactor. Aeration was ensured in the pond reactor by keeping gap in the lid of the set-up. Homogeneity of culture within the photo-reactor was maintained by stirring the culture using magnetic stirrer while the pond bioreactor was kept on a rocking shaker. The experimental time was 24 h. At the end of the experimental period, 100 mL cultures were removed from both the reactors and remaining Cd^{2+} ions were estimated in these solutions by GF-AAS in order to calculate the amount of Cd^{2+} ions removed by the organism [20]. The results obtained showed that the *Nostoc muscorum* Meg 1 was able to remove 90 % and 86 % Cd^{2+} within 24 h in the photo-bioreactor and the mini pond reactor, respectively (Fig. 2c; d). These removal percentages were very similar to that of the organism's biosorption capacity (90 %) when the experiment was conducted within the confinement of 50 mL conical flasks. The study, therefore, points out that medium scale Cd^{2+} removal practices are feasible for the removal of metal ions from the wastewater using photo-bioreactors.

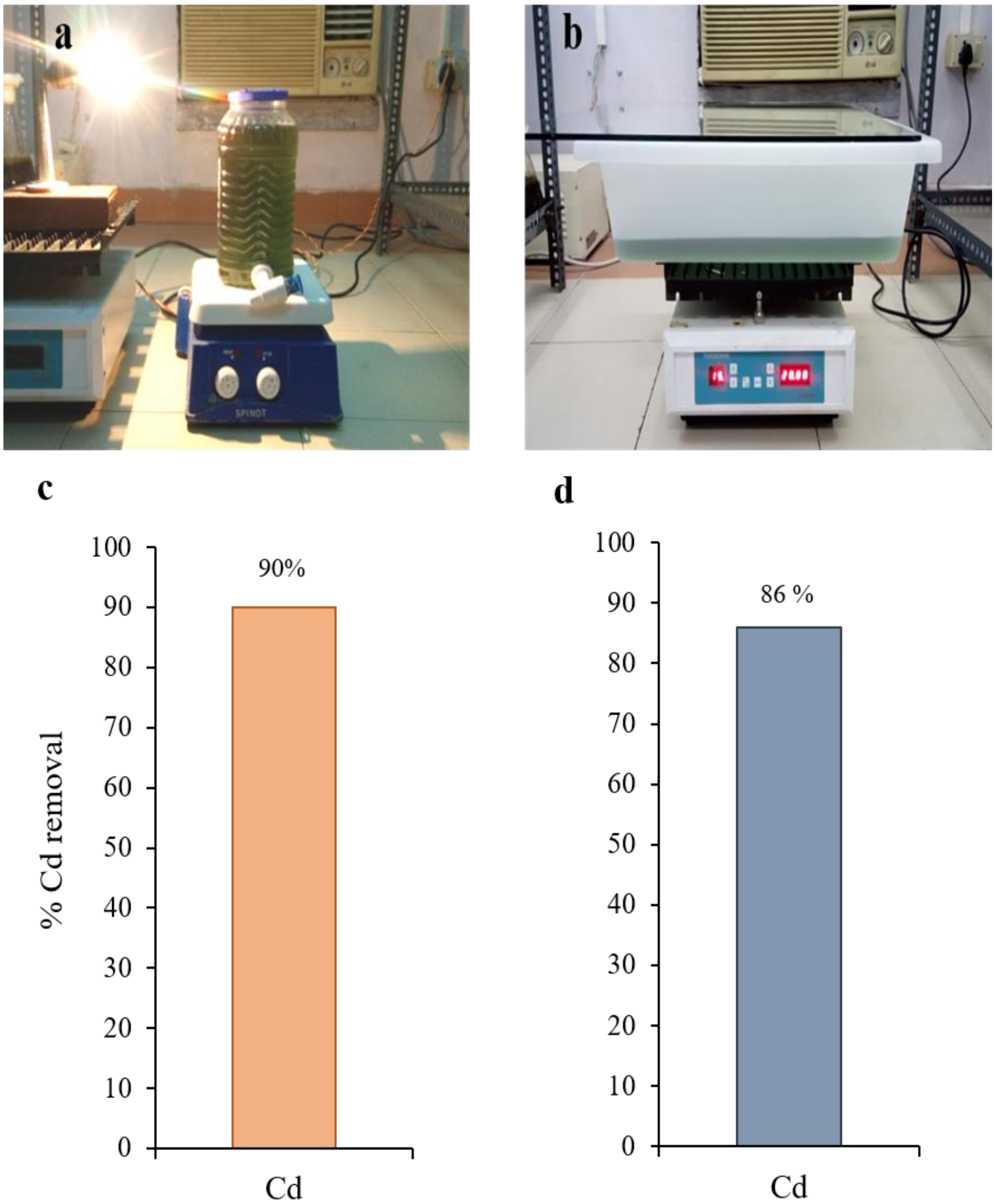

Fig. 2: Percent Cd^{2+} removal by the *Nostoc muscorum* Meg 1 after 24 h in batch photo-bioreactor and pond bioreactors under optimum conditions. **a:** image of the photo-bioreactor; **b:** image of the pond bioreactor; **c:** % Cd^{2+} removal in the photo-bioreactor, and **d:** % Cd^{2+} removal in the pond bioreactor. Cd = Cd^{2+} ions.

In recent times, due to urbanization and industrialization, use of heavy metals and their derivatives have polluted environments especially soil and water bodies [21]. This has prompted widespread research into finding different ways to remediate the contaminated surroundings. The use of various plants and microbes (fungi, algae, bacteria including cyanobacteria) has gained popularity in the context of bioremediation of degraded environment [22-25] . In this connection, microbes are preferable as they have a simple nutrient requirement and short generation time that produce higher cell surface for metal binding. Among microbes, cyanobacteria present exciting opportunities being both carbon and nitrogen-fixing in nature [26-27] that brings down their nutrient requirements and consequently the cost of their production as biosorbents. Cyanobacterial cell surfaces contain large number of negatively charged groups suitable for binding positively charged metal ions and thus higher biomass production ensure increased metal removal [28-31]. However, developing technology for metal removal using cyanobacteria requires in depth study in understanding metal-cyanobacteria interactions and metal tolerance of the organism(s). With the realization that indigenous strains of cyanobacteria native to metal-polluted sites would already be adapted to high metal pollution and therefore could be suitable for metal bioremediation. These various aspects of metal sorption using cyanobacterial biomass need thorough investigation. However, in recent times there are a number of bioremediation companies (Pluton Biosciences (USA), Australia's Commonwealth Scientific and Industrial Research Organization (Australia), Tatva group (India) etc.) that offer their services for remediation of contaminated waters using microbes such as cyanobacteria.

4. CONCLUSION

The present findings showed that the *Nostoc muscorum* Meg 1 biomass can be used for Cd^{2+} sorption. The same biomass can be reused for at least four cycles of Cd^{2+} sorption following desorption of the sorbed metal ions after every cycle. The best agent for Cd^{2+} desorption was found to be HNO_3. The study further pointed out that medium-scale Cd^{2+} removal practices are feasible from wastewater using photo and pond bioreactors.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The author confirms that the data supporting the findings of this research are available within the article.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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