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ORIGINAL ARTICLE

Biosorption and equilibrium isotherms study of cadmium removal by *Nostoc muscorum* Meg 1: morphological, physiological and biochemical alterations

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Abstract Rice fields of Meghalaya especially in the coal mining belt receive water contaminated by effluents from mines that are known to carry harmful heavy metal ions such as Cu, Fe, Zn, Ni, Cd, As, Pb, Cr, etc. Cd exposure was analyzed in the cyanobacterium *Nostoc muscorum* Meg 1 isolated from a contaminated rice field in Sohra, Meghalaya, India. Toxicity study established 0.5 ppm on day 3 to be the LD_{50}. At LD_{50} chlorophyll *a* and total protein concentration was reduced by 50.9 and 52.5%, while nitrogenase and glutamine synthetase activities were inhibited by 40.8 and 38.4%. EDX and FTIR analyses confirmed Cd binding and participation of hydroxyl, carbonyl, carboxyl and phosphate groups in biosorption of Cd onto the cell surfaces. SEM study established morphological changes. At pH 8.0 and temperature 25 ± 2 °C, the cyanobacterium removed 92% Cd within 24 h. Of this, 91% Cd was adsorbed on the cell surface while 4% was internally accumulated. The energy required for internal accumulation of Cd was partly provided in the form of ATP synthesized during active photosynthesis. The Langmuir isotherm was found best fitted with a R^2 value 0.98 when compared to Freundlich and Temkin adsorption isotherms. The maximum sorption capacity, Q_{max}, of the organism was 71.4 mg of Cd per g of biomass. R_L value of 0.29 indicated favorable interaction between cyanobacterial biomass and Cd. The adsorption intensity, *n* value 7.69 g/L obtained from Freundlich isotherm showed that the organism possessed high Cd sorption capacity.

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Introduction

In recent times, metal pollutants are increasing in the environment with increase in anthropogenic activities including mining and release of industrial and agricultural effluents (Gupta and Rastogi 2008). Metals are harmful and non-degradable with the possibility of accumulation and bio-magnification in organisms (Dietz et al. 2000; Gallego et al. 2012). Although metals such as Cu, Fe, Zn, Mg, Mn, Ni and Mo are essential for living organisms, they can be toxic at elevated levels (DRI reports 2001). Many others such as Cd, Hg, Cr, As and Pb have no known biological importance and are highly toxic to living organisms even at very low concentrations (Qaiser et al. 2007). Among these, Cd is a common pollutant that is a potent mutagenic and carcinogenic agent. Exposure to this metal can damage lungs, bones, liver, nervous tissue, gastrointestinal tract and kidney (Jin et al. 2003; Waalkanes 2003).

There are methods such as chemical treatment, solvent extraction, evaporation upturn, application of ion-exchange and membrane technologies that have been investigated for removing heavy metal burden from polluted sites (Babu and Gupta 2008; Haritash and Kaushik 2009). In recent times, metal removal by biological materials such as plants, algae and bacteria is gaining popularity due to issues related to cost and degradation efficiency. Microorganisms can reduce heavy metals load from the surrounding by mechanisms such as biosorption, bioaccumulation, bio-transformation or bio-mineralization (Umrania 2006; Xiao et al. 2010). Among microbes, cyanobacteria offer attractive possibilities as they are both photosynthetic and

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diazotrophic requiring a simple nutrient input with fast growth rate that provides large surface area for metal binding. Many produce mucilage that also aids in metal removal process (Gupta and Rastogi 2008; Latifi et al. 2009; Dixit and Singh 2013). Metal remediation by living organisms could be tricky as different organisms show differential tolerance towards same metal and different metals may not result in similar effects on the organisms (Deniz et al. 2011; Gaber et al. 2012; Dixit and Singh 2013; Goswami et al. 2015a, b). Thus, for metal remediation technology to be effective, individual organisms need to be assessed for their metal removal potential. Many microbes in the presence of toxic heavy metals develop different strategies such as metal exclusion, sequestration of metals in polyphosphate bodies, metal chelation by producing metallothioneins and phytochelatin to overcome their harmful effects (Keasling and Hupf 1996; Cobette 2000). However, Mallick and Rai (1994) had shown that high concentrations of Cd disrupt cyanobacterial metabolism either by inactivating the photosynthetic machinery or enzymatic pathways. Cd at high concentration causes substantial changes in physiological and biochemical parameters and leads to enhanced reactive oxygen species production (Atri and Rai 2003).

The cyanobacterium *Nostoc muscorum* Meg 1 taken for this study was isolated from a rice field in Sohra, Meghalaya, India that receives contaminated water from coal mine effluents. Its existence in contaminated fields established that the organism was tolerant towards various contaminants including heavy metals. An atomic absorption spectroscopic study of the soil sample from the site recorded the presence of various metal ions (0.32 ppm Fe; 0.22 ppm Cd; 0.16 ppm Cr; 0.14 ppm Zn; 0.06 ppm Pb and 0.05 ppm Cu). Of these, the Cd and Pb were two non-essential metal ions with Cd concentration being much higher than that of Pb. One must understand that the concentration of various contaminants may change from time to time depending on the amount of coal residues that enters the rice fields with water, especially during rainy season. Heavy metals including Cd gets accumulated in crops (Murakami et al. 2008; Zhang et al. 2014) and further gets concentrated along the food chain creating threat of chronic exposure in animals and humans producing adverse affects as mentioned above. Rice fields provide conducive atmosphere for cyanobacterial growth in terms of availability of light, water and nutrients whereas in turn, these organisms provide fixed nitrogen on their turn over to the rice crop (Prasanna et al. 2014). In addition to this biofertilizer potential, many researchers have also established cyanobacteria's metal binding abilities (Hazarika et al. 2015; Khan et al. 2016). Thus, when present in rice fields, in addition to providing fixed nitrogen, by binding metal ions from surrounding these organisms reduce the harmful metal load that the crop plants get exposed to.

In our laboratory, we are looking into the effects of various metal ions on different cyanobacteria as well as metal removal efficiency of this group of microbes. The high concentration of Cd in the rice field from where the organism was isolated presented an intriguing proposal to study Cd removal efficiency and Cd induced changes in the organism *Nostoc muscorum* Meg 1. In the article, a comprehensive study of Cd-induced morphological, physiological and biochemical alterations to the organism is presented. Also, kinetics of Cd biosorption and equilibrium isotherms were investigated in detail.

Materials and methods

Identification and maintenance of the organism

Soil sample was collected from a rice field of Sohra adjacent to a coal mining site, Meghalaya, India and was diluted in double-distilled water prior to inoculating in freshly prepared BG 11_0 medium (pH 8) to allow growth of cyanobacteria (Ripkka et al. 1979). After a period of 10 days, the growing cyanobacterial filaments were purified by repeated plating on 1.5% agar supplemented media (Rippka 1988). MiniPrep bacterial genomic DNA method (Ausubel et al. 1999) was used to extract genomic DNA from the cyanobacterium. 16S rRNA gene from the isolated DNA was amplified by PCR and sequenced. DNA sequencing reaction was carried out in Xceleris Laboratory, Ahmedabad, India with CY106F (5′-CGG ACG GGT GAG TAA CGC GTG A-3′) and CY781R {equimolar mixture of CY781R(A) 5′-GAC TAC TGG GGT ATC TAA TCC CAT T-3′ and CY781R(B) 5′-GAC TAC AGG GGT ATC TAA TCC CTT T-3′} primers using BDT v3.1 cycle sequencing kit on 3730 × l Genetic Analyzer (Applied Biosystems, USA) (Nubel et al. 1997). Related sequences were retrieved from NCBI Genbank database using BLAST and first 15 sequences were used based on maximum identity score and aligned using multiple alignment software program Clustal W. Distance matrix was generated using RDP database and the phylogenetic tree was constructed using MEGA 5 (Tamura et al. 2011). The isolate was maintained inside a culture room at a pH 8, temperature (25 ± 2 °C) and light at a photon fluence rate (50 μmol/m^2 s^1) (Hazarika et al. 2015). Ten-day-old exponential phase cyanobacterial cells at initial culture concentration of 3 μg/mL chlorophyll *a* were used for all experiments carried out.

Chemicals and reagents

All the chemicals procured in this study were of analytical grade supplied by Sigma-Aldrich, India, Sisco Research Laboratory and HiMedia Laboratories Pvt. Ltd.

Metal treatment

$3CdSO_4.8H_2O$ was used as the source of Cd for all the experiments. Experimental Cd solution was prepared by diluting a stock solution of 100 ppm with medium. To establish the upper limit of Cd concentration for the study, an initial Cd removal study was carried in cultures supplemented with 0.1, 0.2, 0.3, 0.5, 1.0, 1.5, 2, 2.5, 3, 3.5, 4, 5 and 10 ppm of Cd. The maximum removal found in 3 ppm Cd-supplemented medium and beyond which it remained almost constant. Hence, maximum Cd concentration up to 5 ppm is presented in this study.

FTIR spectroscopy analysis

Five mL of control and Cd-treated cultures was transferred after 24 h treatment in 0.5 ppm of Cd onto Petri-plates and dried in an oven at 40 °C. Dried samples were mixed with desiccated spectroscopic-grade potassium bromide, 1:10 (w/w) to make pellets. Fourier transform infrared spectroscopic analysis was performed using Perkin Elmer, 400 FTIR/FT-FIR Spectrometer; model: SP400 operating in the range of 4000–450 cm^{-1} to determine various cell surface functional groups in metal binding.

EDX analysis

EDX analysis was done using INCA Penta FETX3 in combination with SEM, JEOL-JSM-6360; JEOL, Tokyo, Japan. The raw samples were pre-treated with 4% glutaraldehyde for 24 h at 4 °C followed by washing in 0.1 M sodium cacodylate buffer thrice at an interval of 15 min. Dehydration was carried out in acetone (30, 50, 70, 80, 90, 95 and 100%). The dehydrated samples were mounted on brass stubs and gold coated before viewing.

Cadmium removal and its distribution in the cell

Metal removal was analyzed using GF-AAS (Analytik Jene AG Vario 6) (Nongrum and Syiem 2012). 20 mL cultures treated for 24 h with 0.5, 1.0, 1.5, 2.0, 2.5, 3, 3.5, 4.0 and 5 ppm Cd were centrifuged at 3000 rpm for 10 min and the amount of metal in the supernatant was analyzed. Percent cadmium removal was calculated using the Eq. 1.

$$\% \text{ Cd removal } = \frac{(C_I - C_F)}{C_I} \times 100 \qquad (1)$$

where C_I is the cadmium concentration initially present in the medium; C_F is the remaining Cd concentration in the supernatant.

To the pellet, 20 mL BG-11_0 medium was added, vortexed for 2 min and centrifuged at 3000 rpm for 5 min. The supernatant was analyzed for metal precipitated on the cell surface. Next, 20 mL of 0.1 M HCl solution was added to the pellet and left for 2 min before vortexing vigorously to desorb surface bound metal ions. Desorbed amount of Cd ions was determined in the supernatant collected after centrifugation. After this step, the residual pellet was resuspended in 20 mL medium, ruptured by ultrasonication (Sonics Vibra cell sonicator, VC-505, USA fitted with a microprobe) for 3 min and the resulting solution was used for determination of intracellularly accumulated Cd.

Provision of energy for cadmium accumulation

The energy requirement, if any, for the process of biosorption and intracellular accumulation was studied by setting up four experiments. Cyanobacterial cells (3 µg/mL) were added in 20 mL BG-11_0 medium containing 0.5 ppm Cd and incubated at 25 ± 2 °C for 24 h in the following conditions: (a) continuous light (b) dark (c) continuous light in presence of a photosynthetic inhibitor DCMU, (10 µM) and (d) in dark in the presence of ATP, (10 µM). After 24 h incubation, solutions were centrifuged at 3000 rpm for 3 min and supernatants were taken for Cd analysis by AAS.

Biosorption isotherms

Cd biosorption was analyzed using—Langmuir, Freundlich and Temkin isotherms (Aharoni and Ungarish 1977; Volesky and Holan 1995). Cells incubated in 0.5, 1.0, 1.5, 2.0, 2.5, 3, 3.5, 4 and 5 ppm Cd for 24 h were centrifuged and the supernatant was analyzed using AAS.

Langmuir isotherm

The amount of Cd taken up by the *Nostoc muscorum* Meg 1 cells was expressed as q (mg/g) and was calculated from C_I, C_F, v and w where C_I, C_F, are the initial and final metal concentrations, respectively, v is the volume of medium in mL and w is the biomass weight in g.

$$q = \frac{(C_I - C_F) \times v}{w} \qquad (2)$$

Langmuir equation is based on the following assumptions: (a) adsorbent has finite numbers of energetically uniform sites, (b) interaction among the adsorbed species are not found and (c) adsorbed species

always form a monolayer on the adsorbent surface beyond which no further adsorption takes place. The Langmuir's isotherm can be expressed as:

$$q_F = \frac{Q_{max} \times K_L \times C_F}{1 + K_L \times C_F} \tag{3}$$

where q_F is the amount of metal adsorbed in mg per g of biosorbent at equilibrium, Q_{max} is the maximum adsorption capacity per unit weight per g for the metal ions needed for complete formation of monolayer on the biosorbent surface and K_L refers the adsorption equilibrium constant related to the binding affinity.

Using the Langmuir equation, the separation factor (R_L) was calculated by the following Eq. 4.

$$R_L = \frac{1}{1 + K_L \times C_I} \tag{4}$$

R_L is a dimensionless equilibrium parameter which can determine the nature of interaction of the biomass with the metal ions. If R_L value is > 1, it indicates the unfavorable interaction whereas $R_L = 1$ indicates linear interaction between metal and the biosorbent and $R_L < 1$ shows favorable interaction. An $R_L = 0$ indicates irreversible interaction (Oguz 2005).

Freundlich isotherm

Freundlich adsorption isotherm that is used to describe adsorption on a heterogeneous surface assume that enthalpy of adsorption is independent of the metal adsorbed. Freundlich isotherm can be expressed as:

$$q_F = K_F \times C_F^{1/n} \tag{5}$$

where K_F and n are the Freundlich constant related to sorption capacity and intensity.

Temkin isotherm

Temkin isotherm model relates to interaction between adsorbent and adsorbate and takes into account that the free energy of adsorption is a function of surface coverage. It presumes that adsorption of all the molecules in the layer decreases linearly and not logarithmically.

Temkin isotherm can be expressed as:

$$q_F = \frac{RT}{b_T} \ln(A_T C_F) \tag{6}$$

$$B = \frac{RT}{b_T}$$

$$q_F = B \ln A_T + B \ln C_F \tag{7}$$

where R is the universal gas constant (J/mol/K), T is the temperature in K, b_T is the Temkin isotherm constant (J/mol), A_T is the Temkin isotherm equilibrium binding constant (L/mg) corresponding to the maximum binding energy and C_F is the equilibrium concentration (mol/L) of the adsorbate and B is the constant related to heat of sorption (J/mol).

Morphological, physiological and biochemical changes

Changes in all the morphological and biochemical parameters upon Cd treatment were studied in LD_{50} (0.5 ppm Cd).

Morphological studies

Morphological changes were observed under SEM when cells were exposed for 3 days. Samples for SEM were prepared following pre-fixation and dehydration procedure described under EDX analysis.

Estimation of chlorophyll a

Three mL of experimental culture was centrifuged and to the pellet 3 mL methanol was added. This was kept at 4 °C overnight for chlorophyll a extraction. At the end of the incubation, the solution was centrifuged and absorbance was read at 663 nm using UV–Vis spectrophotometer (Smart Spec Plus; Bio Rad, USA) and chlorophyll a concentration was calculated using the formula described by MacKinney 1941.

Protein estimation

Cyanobacterial culture (3 mL) was centrifuged at 2500 rpm for 3 min and the pellet resuspended in milliQ water (volume to 3 mL). The cells were disrupted by ultrasonication and 0.5 mL of the resulted solution was taken and the volume was made up to 1 mL with milliQ water. 5 mL of solution C which was made by mixing 98 mL Solution A (2% Na_2CO_3 in 0.1 N NaOH) and 2 mL solution B (0.5% $CuSO_4$ and 1% $C_4H_4KNaO_6 \cdot 4H_2O$ in the ratio of 0.5:5) was added to each test tube and incubated for 20 min in room temperature. This was followed by immediate addition of 0.5 mL 1 N Folin–Ciocalteu's phenol reagent to the test tubes and kept for 10 min. The developed color solution was read at 750 nm against reference blank and the concentration was calculated using a standard curve (Lowry et al. 1951).

Heterocyst frequency and nitrogenase activity

Heterocysts frequency of the cyanobacterial cells was calculated according to Wolk (1965). At least 500 cells

were counted for each calculation under Olympus light microscope BX-53.

Nitrogenase activity was measured by following the acetylene reduction assay method described by Stewart et al. (1967). In a 15 mL vial, 5 mL of cyanobacterial culture was added and air phase was replaced by 10% (v/v) acetylene. This was followed by incubation of all the vials at 25 ± 2 °C for 1 h in the presence of light with continuous shaking. Gas chromatography (Varian 3900, The Netherlands) fixed with a Porapak T column (stainless steel column 6′ × 1/8″, Porapak T of mesh size 80/100) and a flame ionization detector was used to estimate the amount of reduced acetylene. Activity of enzyme nitrogenase was expressed as nmol of C_2H_2 reduced/μg chl a/h.

Glutamine synthetase (transferase) activity

Cyanobacterial culture (3 mL) was centrifuged and the pellet was washed twice with 50 mM Tris–HCl buffer, pH 7.5 and resuspended in the same buffer. The cells were disrupted using ultra-sonicator to release the enzyme into the buffer. To 0.5 mL of the sonicated cultures, 0.5 mL of assay mixture that contained 40 mM Tris–HCl buffer (pH 7.5), 3 μM $MnCl_2$, 20 μM of potassium arsenate, 0.4 μmol of ADP (sodium salt), 60 μmol of hydroxylamine and 30 μmol of glutamine were added and incubated at 30 ± 2 °C for 10 min in dark. 2 mL of stop mixture (10% $FeCl_3$, 24% TCA, 6 M HCl in distilled water) was added to the above solution to stop the enzymatic reaction. This was followed by centrifugation at 2000 rpm for 5 min and the supernatant collected was read at 540 nm. The γ-glutamyl hydroxamate produced in the reaction was estimated by plotting the absorbance value in a standard curve described by Sampaio et al. (1979). Activity of GS was expressed as nmol of γ-glutamyl hydroxamate produced/μg of protein/min.

Results

Identification of the organism

Under light microscope (Olympus BX-53), the isolated organism was identified as Nostoc muscorum (Desikachary 1959). Its phylogenetic analyses carried out by comparing amplified partial sequences of 16S rRNA gene with similar sequences deposited in GenBank (NCBI) showed high although not 100% similarity to Nostoc muscorum 19 and was thus positioned near Nostoc muscorum 19 and named as Nostoc muscorum Meg 1 (GenBank Accession No. KM596855).

FTIR analysis

FTIR analysis showed significant spectral variations in the Cd-treated cells in comparison to control cultures (Fig. 1). A shift in the frequency of the FTIR spectra reflects change brought about by binding of the metal ion on the functional groups. Shift seen in the region $3830 \rightarrow 3849$ cm^{-1} established involvement of hydroxyl group. A shift towards the lower frequency from $3431 \rightarrow 3444$ cm^{-1} and from $2271 \rightarrow 2294$ cm^{-1} indicated the possible participation of O–H stretching and phosphate group in form of phosphine ligands for metal binding. Shift in frequency from $1456 \rightarrow 1468$ cm^{-1}, $1400 \rightarrow 1408$ cm^{-1}, $1191 \rightarrow 1238$ cm^{-1} and $1079 \rightarrow 1094$ cm^{-1} corresponded to involvement of O–H (phenol ring), carboxyl, acyl/phenyl C–O group and alcohol C–O stretch. Change in frequency from $501 \rightarrow 518$ cm^{-1} and $475 \rightarrow 482$ cm^{-1} reflected the participation of phosphate group in Cd binding.

EDX analysis

Energy-dispersion spectra of the characteristic EDX of control and 0.5 ppm Cd-treated cells for 24 h are presented in Fig. 2. The presence of a Cd peak in the Cd-treated sample (Fig. 2b) and its absence in the control culture provided evidence of Cd binding on the cell surfaces of the treated cells.

Metal removal and its cellular distribution

Nostoc muscorum Meg 1 cells at a concentration of 3 μg/mL chlorophyll a were treated with 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 and 5 ppm Cd for 24 h to record Cd removal by the cells (Fig. 3). Removal of Cd ions was found to be a concentration-dependent process; the amount removed was directly proportional to the initial Cd concentration provided in the medium. The amount of Cd removed within 24 h increased from 0.46 to 3.27 ppm when initial Cd concentration in the medium was raised from 0.5 to 5 ppm. However, increase in percent Cd removal was very nominal (92–96.3% in the medium supplemented with 0.5–3 ppm). Beyond 3 ppm, the percent Cd removal declined to 65.4% in 5 ppm Cd-supplemented medium. This result indicated that when Cd concentration beyond 3 ppm is raised in the medium, the Cd on to the cell surfaces of the organism reached a saturation limit and any increase in externally supplied concentration in the medium could not be taken up proportionally by the cells.

Figure 4 represents the distribution of Cd in the Nostoc muscorum Meg 1 cells treated with 0.5 ppm Cd supplemented medium for 24 h. 92% of the supplemented Cd amounting to 0.46 ppm Cd was removed by the Nostoc

Fig. 1 FTIR spectra of *Nostoc muscorum* Meg 1 cell surface of control and Cd-treated cells
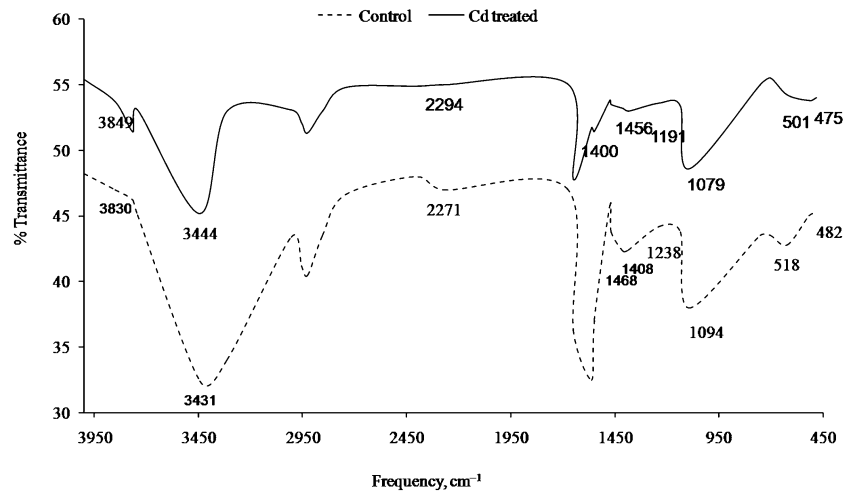

Fig. 2 Energy-dispersion spectra of the characteristic EDX in control and Cd-treated cells of *Nostoc muscorum* Meg 1. **a** Control, **b** cells treated with 0.5 ppm Cd. *Arrow* indicates Cd peak in the EDX spectra
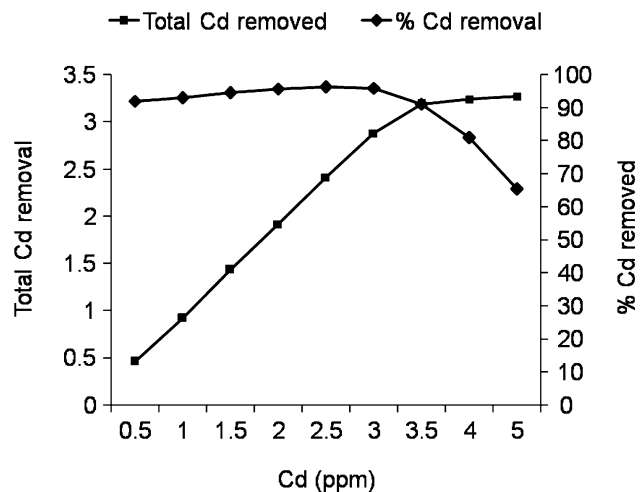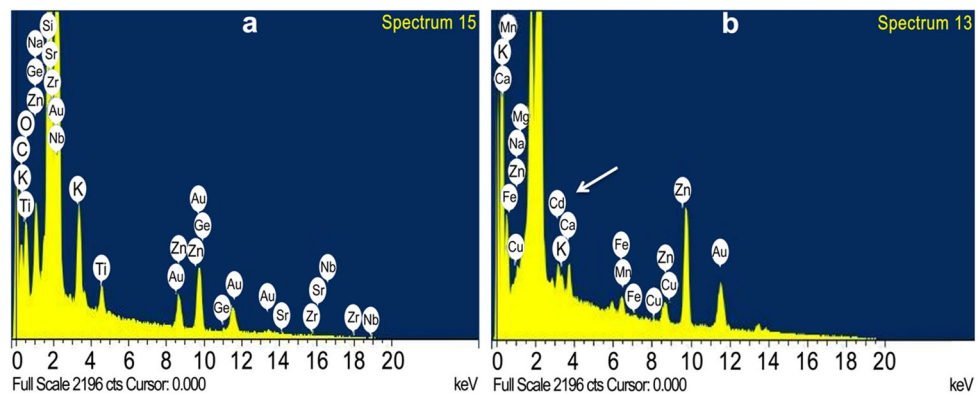

Fig. 3 Total and percent Cd removal by *Nostoc muscorum* Meg 1 cells within 24 h

muscorum Meg 1 cells during this period (Fig. 4a). Of this total Cd removed, 91% was found adsorbed on the cell surface indicating the Cd removal process to be primarily biosorption. Further analysis showed 4% of the removed Cd precipitated on the cell surface that could be easily removed by shaking and 4% was internally accumulated that could be accessed only upon disruption of cells (Fig. 4b).

Energy provision

As shown in Fig. 5, the cyanobacterium was able to remove 92% of Cd when incubated for 24 h in continuous light from the medium containing 0.5 ppm Cd. Same experiment conducted in dark resulted in 88.2% of Cd removal. When photosynthetic electron transport chain was blocked by DCMU in the experiment conducted in the presence of continuous light, *Nostoc muscorum* Meg 1 cells were able to remove 87.9% Cd. The almost similar amount of Cd removed (88.2% in dark and 87.9% in the presence of DCMU in light) indicated that Cd removal did not require energy that could have been provided by photosynthetic electron transport chain activity in the form of ATP. However, addition of ATP in the experiment carried out in dark resulted in 92.4% Cd removal—a value similar to that obtained in the experiment carried out in the presence of continuous light where photosynthesis was active in culture and ATP and reductants were produced in the light reaction of photosynthesis for carbon fixation. In both

Fig. 4 **a** Percent Cd removal; and **b** distribution of removed Cd in the *Nostoc muscorum* Meg 1 cells. Medium was supplemented with 0.5 ppm Cd and the duration of the treatment was 24 h
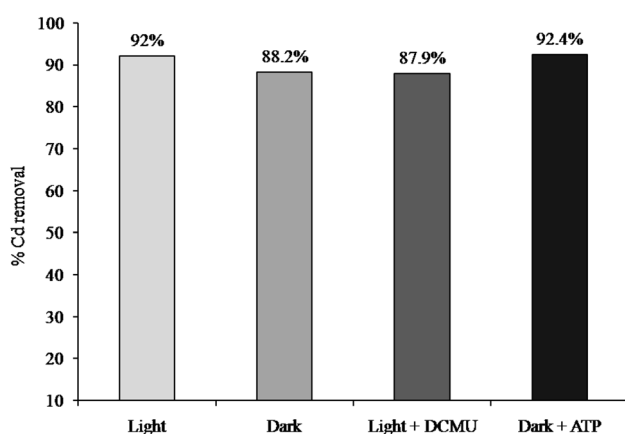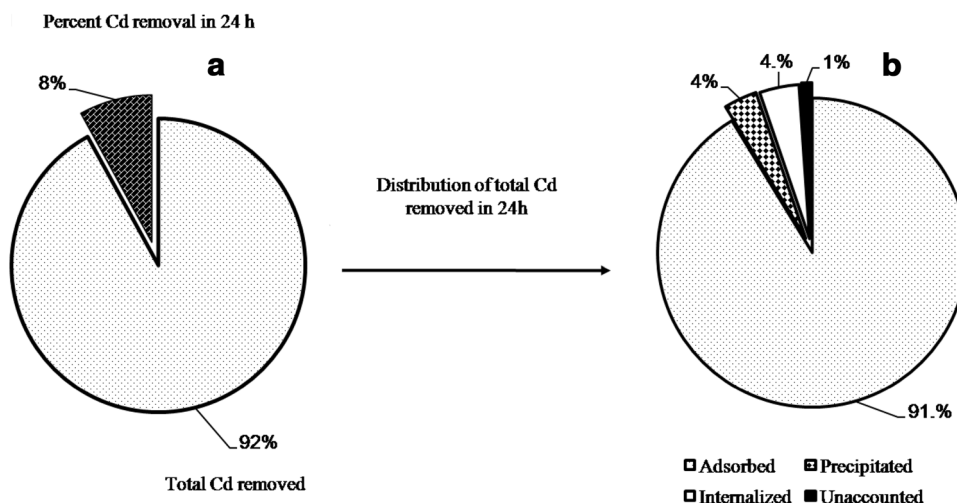

Fig. 5 Percent Cd removal under four different conditions: **a** light; **b** dark; **c** light + DCMU; and **d** dark + ATP. Cd concentration in the medium was 0.5 ppm and the duration of the treatment was 24 h

cases, availability of ATP during the experiment carried out in light or ATP externally supplied during the experiment carried out in dark resulted in an increase of ∼ 4% Cd removal from the Cd supplemented medium. This must be the 4% internally accumulated Cd that was recorded in cellular distribution study (Fig. 4b). This experiment provided evidence that although Cd removal was primarily sorption to the cell surfaces, the presence of high amount of Cd in the surrounding medium led to internal accumulation that required energy for transmembrane transport.

Biosorption isotherm studies

Biosorption of Cd was studied by fitting the data in the Langmuir, Freundlich and Temkin biosorption isotherm models (Fig. 6). The various isotherm parameters of Langmuir, Freundlich and Temkin are enlisted in Table 1. Among the isotherms, Langmuir was best fitted with an R^2 value of 0.98 as against that of Freundlich R^2 value of 0.94

and Temkin R^2 value of 0.86. The maximum adsorption capability Q_{max} of the cyanobacterium for Cd was found to be 71.4 mg of Cd taken up per g of biomass. The value of n (7.69 g/L) showed the favorable Cd sorption by the cyanobacterium. Temkin isotherm constant, B and A_T found to be 0.92 L/mg and 55.07, respectively, indicated the interaction between adsorbent and adsorbate was a favorable physical adsorption process. R_L value (separation factor) of 0.29 signified favorable interaction between the cyanobacterial cells and the Cd ions.

Morphological, physiological and biochemical analysis

Scanning electron microscopic studies

Control and Cd-treated *Nostoc muscorum* Meg 1 cells were viewed under SEM (Fig. 7). Extensive deformations with definite breaks in the filaments at several places were noticeable in the filaments. Cd at 0.5 ppm concentration caused cell enlargement, elongation and shriveling in the cells within 3 days of exposure (Fig. 7b–d).

Chlorophyll a: a measure of growth

Growth in terms of chlorophyll *a* showed a decreasing trend with increase in Cd concentration ranging from 0.1 to 1 ppm from day 1 to day 3 (Fig. 8). The growth of control cultures increased by 14.5% on first day and 44.1% on third day. However, there was noticeable decline in chlorophyll *a* concentration in all Cd-treated cultures. The percent reduction of chlorophyll *a* content on very first day upon 0.1, 0.2, 0.3, 0.5 and 1 ppm Cd exposure was found to be 1.2, 9.3, 13.1, 20.3 and 30.1%, respectively, in comparison to control cultures. Cd at 0.5 ppm concentration showed ∼51% reduction in chlorophyll *a* within 3 days of Cd exposure.
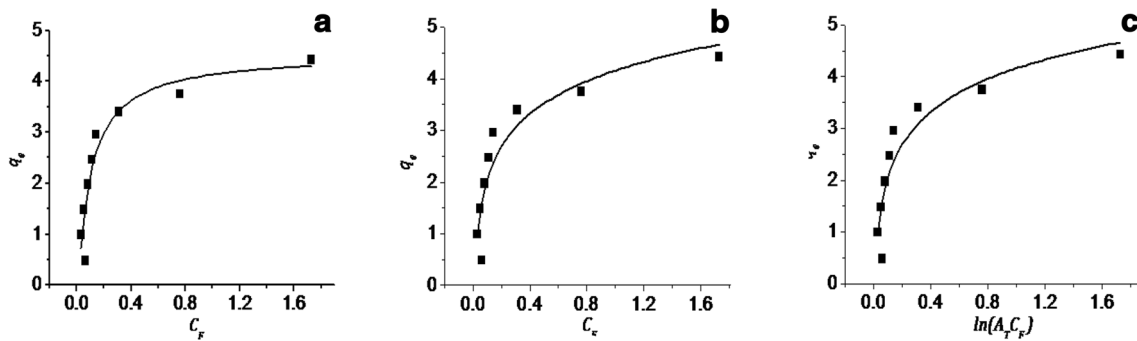
Fig. 6 Isotherm models; **a** Langmuir; **b** Freundlich; and **c** Temkin for Cd biosorption onto the cell surface of the cyanobacterium *Nostoc muscorum* Meg 1 at 25 ± 2 °C

Table 1 Langmuir, Freundlich and Temkin adsorption isotherm parameters of Cd-treated *Nostoc muscorum* Meg 1

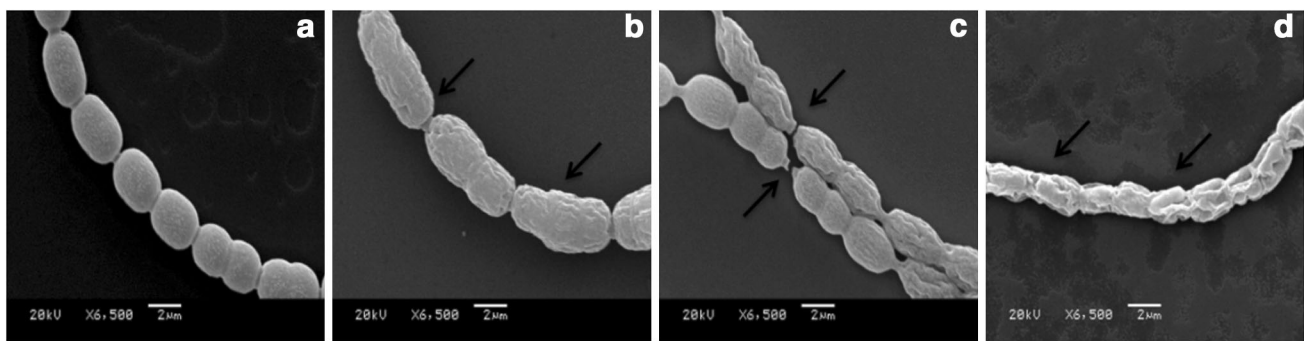| Metal	Langmuir				Freundlich			Temkin		
	Q_{max} (mg/g)	K_L	R^2	R_L	K_F	n	R^2	K_T	R_T/b_T	R^2
Cd-treated cultures	71.4	4.94	0.98	0.29	0.39	7.69	0.94	55.07	0.92	0.86

Fig. 7 Scanning electron micrographs of control and 0.5 ppm Cd-treated *Nostoc muscorum* Meg 1 cells. Duration of treatment: three days. **a** control; **b–d** Cd-treated cells (*arrows* in **b** indicate cell elongation and enlargement; *arrows* in **c** indicate broken filaments; *arrows* in **d** indicate cell deformation and shriveling)

Fig. 8 Chlorophyll *a* concentrations of control and Cd-treated *Nostoc muscorum* Meg 1 cells. Duration: 3 days. Cd concentration: (0.1–1 ppm). All the values are mean ± SD
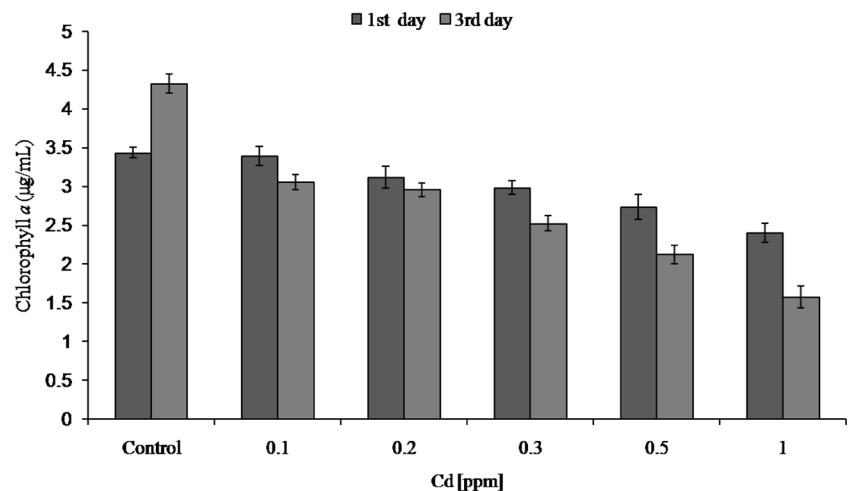
Protein content

Protein content of the cyanobacterium under Cd treatment displayed similar trend seen for chlorophyll *a* study (Fig. 9). Untreated cells showed an increase of ~ 16 and 49% in protein concentration on day 1 and day 3, respectively. However, adverse effect of Cd exposure was visible from day 1 itself on the organism. There was decrease in protein content by 9.7, 17, 30, 36 and 48.7% in the cultures treated with 0.1, 0.2, 0.3, 0.5 and 1.0 ppm Cd, respectively, when protein content was measured after completion of one day treatment. A reduction of $\sim 53\%$ in protein content was seen after day three on exposure to 0.5 ppm Cd emphasizing the toxic nature of Cd exposure.

Nitrogen metabolizing machinery

Heterocysts frequency, nitrogenase and glutamine synthetase activities

Exposure to 0.5 ppm Cd inhibited heterocyst frequency by 44% at the end of third day indicating that Cd could break down existing heterocysts in the filaments (Fig. 10a).

The activity of the N_2-fixing enzyme nitrogenase was reduced by $\sim 40\%$ and the glutamine synthetase (transferase) activity of the enzyme which assimilates fixed NH_4^+ into amino acids was reduced by 38%, within the same period of time (Fig. 10b).

Discussion

Many microbes including cyanobacteria have been shown to be present in polluted environments where there is predominance of various pollutants including heavy metals (Ozturk et al. 2014; Hazarika et al. 2015). Occurrence of
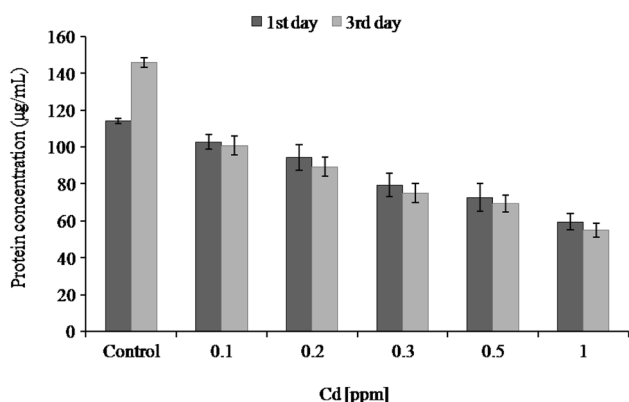
Fig. 9 Protein content of control and Cd-treated *Nostoc muscorum* Meg 1 cells. Duration: 3 days. Cd concentration: (0.1–1 ppm). All the values are mean $\pm$ SD

microbes in contaminated areas indicates their ability to acclimatize to such adverse conditions (Gadd 2010). One of the common heavy metal contaminants is Cd which is of growing concern due to its many known adverse effects on all living organisms. Jung (2001) and Goswami et al. (2015b) have reported the presence of Cd up to the level of ~ 0.3 ppm in coal mine-contaminated waters which is higher than the normal (0.1–0.2 ppm) occurrence of Cd in uncontaminated environment. A water analysis of the rice field from which the organism was purified recorded the presence of ~ 0.22 ppm Cd. The justification for exposing the organism to higher Cd concentration (0.5 ppm) is that we wanted to see its tolerance to Cd, its Cd removal ability and various morphological and physiological changes that have been brought about from its continuous exposure to high levels of Cd in its surrounding.

Our study during optimization of growth parameters established that best growth of *Nostoc muscorum* Meg 1 was at pH 8 and temperature 25 ± 2 °C. $3CdSO_4 \cdot 8H_2O$ supplemented at this pH did not result in any metal precipitation in the medium. Since biomass is an important factor for metal removal and there was no metal hydroxylation at this pH, pH 8 was chosen for the study. The state of Meghalaya is a hilly area and temperature generally remains below 25 °C throughout the year except during summer season where temperature sometimes goes up to 27–28 °C. Combined with the fact that this temperature $(25 \pm 2$ °C) was found to be ideal for maximum biomass production and the organism was isolated from an environment where daily temperature remains in the range of 20–28 °C during rice growing season, 25 ± 2 °C was chosen for the study. The initial metal removal study also provided evidence that at this temperature and pH, very high percentage (92%) of Cd was removed by the organism within 24 h of 0.5 ppm Cd exposure. FTIR and EDX studies confirmed Cd binding on to various cell surface groups. Carboxylic, carbonyl, hydroxyl, phenolic and phosphate moieties present in the cell surface of the cyanobacterium were found to participate in the Cd binding. In our study, we used three different isotherm models: Langmuir, Freundlich and Temkin to study biosorption of Cd on the cyanobacterium. Among these, Langmuir isotherm model is based on monolayer adsorption while Freundlich isotherm model is based on multi-layer adsorption. We also have included Temkin isotherm model which contains a factor that explicitly takes into account the adsorbent adsorbate interaction. These isotherms studies showed that the biosorption process fitted best in the Langmuir isotherm with R^2 value 0.98. The maximum Cd adsorption capacity, Q_{max}, of the organism was 71.4 mg/g. A comparative study showed that the *Nostoc muscorum* Meg 1 reported in this study had higher Q_{max} for Cd than *Pseudomonas veronii* 2E., *Spirogyra insignis*, *Oscillatoria*

Fig. 10 **a** Heterocysts frequency (%), **b** nitrogenase and glutamine synthetase activities in control and Cd-treated *Nostoc muscorum* Meg 1 cells. Duration: 3 days. All the values are mean ± SD. Nitrogenase activity (nmol of C_2H_2 reduced/µg of chl *a*/h), GS activity (nmol of γ-glutamyl hydroxamate produced/µg of protein/min)
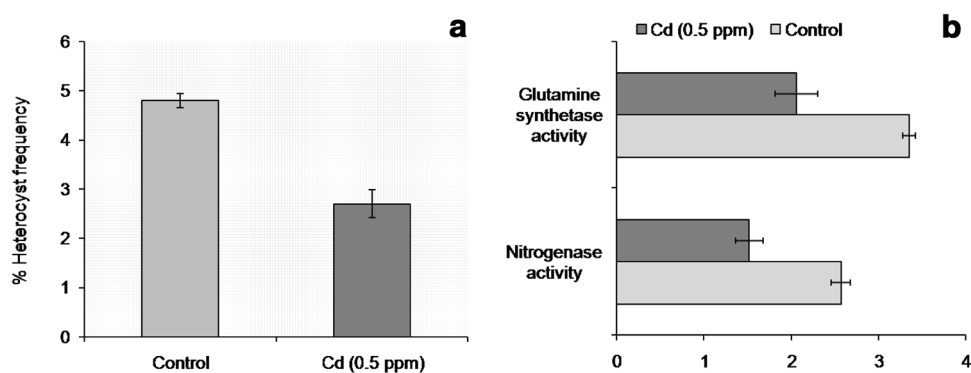
limosa and *Gloeothece magna* indicating its potential towards bioremediation of Cd from contaminated waters (Table 2). That this organism could play a significant role in Cd removal was also seen from the R_L value obtained from Langmuir isotherm discussed in the result. An R_L value between 0 and 1 (in this case 0.29) signified favorable interaction between the organism and Cd. The *n* value, 7.69 g/L obtained from Freundlich isotherm again pointed towards the favorability of Cd sorption by the organism. The high percentage of Cd removal (92% within 24 h) corroborated the finding of Langmuir and Freundlich isotherms, i.e., favorable binding leading to high Cd removal. Toxicity of Cd on the organism was evident as within 3 days of exposure; the adverse effects of Cd were visible on its morphology. SEM pictures established the fact that on continuous exposure, Cd could induce cell elongation and enlargement, filament breakage as well as cell deformation. Cell enlargement was also reported by Khan et al. (2016) where they have attributed this phenomenon to biosorption of Cd in the bacterium *Salmonella enteric* 43C.

That the organism was able to grow in the contaminated rice fields established its ability to tolerate presence of Cd in its vicinity. The high percent removal (92%) achieved by the organism from the medium supplied with 0.5 ppm Cd further established its high Cd sorption ability. Our study further established that the maximum amount of Cd removal was a surface phenomenon in this cyanobacterium and did not require involvement of energy. The 4% of total removed Cd that has internally accumulated using energy

may have compromised various enzymatic activities by virtue of being cationic in nature and producing oxidative stress. These adverse changes reflected in various physiological parameters such as growth measured by the content of chlorophyll *a* and proteins; heterocysts frequency, nitrogenase and glutamine synthetase activities that were partially inhibited between 40 and 50% when exposed to 0.5 ppm Cd, a value higher than that was found (0.22 ppm) in Cd-contaminated rice fields in coal mining areas. Cd initiated break down of existing heterocysts as well as production of new ones. This in turn led to decline in N_2-fixation and subsequent NH_4^+ assimilation by glutamine synthetase. Whether the inhibition of nitrogenase and glutamine synthetase activities was a more direct affect of Cd could only be assessed with antibodies and immuno-electron microscopic studies that we plan to do in near future.

All these above findings established the fact that the presence of this cyanobacterium in the contaminated rice fields where 0.22 ppm Cd concentration was recorded is beneficial to the rice crop as the organism could remove a large percentage of Cd, effectively reducing the toxic load of Cd in the water. All in all, although, Cd exposure adversely affected the well-being of the organism, 0.5 ppm Cd concentration was not lethal. Thus in future, *Nostoc muscorum* Meg 1 could prove to be an attractive organism for biotechnological application performing dual function of bioremediation and biofertilizer in contaminated crop fields aiding to green chemistry.

Table 2 Cd biosorption of *Nostoc muscorum* Meg 1 and various other microbes reported in the recent past: a comparative analysis

Name	Cd biosorption capacity (mg/g)	References
Nostoc commune	126.3	Morsy et al. (2011)
Anabaena sphaerica	111.1	Abdel-Aty et al. (2013)
Nostoc muscorum Meg 1	71.4	Present study
Pseudomonas veronii 2E	54	Vullo et al. (2008)
Spirogyra insignis	22.9	Romera et al. (2007)
Oscillatoria limosa	10.8	Mahalakshmi and Premkishore (2015)
Gloeothece magna	4	Mohamed (2001)

Conclusion

The Cd removal by *Nostoc muscorum* Meg 1 was a concentration-dependent process and was primarily by cell surface binding. The organism registered high Cd removal potential (92%) and the ability to tolerate Cd in its surrounding. The maximum Cd adsorption capacity, Q_{max} (71.4 mg/g) of the organism was higher than many other microbes including *Pseudomonas veronii* 2E., *Spirogyra insignis,* and *Oscillatoria limosa.* The ubiquitous presence of this cyanobacterium in the rice fields indicated that it plays a significant role not only as biofertilizer but also as a bioremediator of heavy metals particularly Cd, thereby reducing toxic Cd accumulation in rice plants and further in the food web. Overall, this study proved the tremendous potential of *Nostoc muscorum* Meg 1 in Cd removal from contaminated water.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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