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Copper uptake by free and immobilized cyanobacterium

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1. SUMMARY

Copper uptake in free and immobilized cells of the cyanobacterium *Nostoc calcicola* has been examined. The immobilized cells invariably maintained a higher profile of Cu intake rate (12.7 nmol mg^{-1} protein min^{-1}) over the free cells (6.0 nmol mg^{-1} protein min^{-1}). The total Cu uptake in immobilized cells was almost two and a half-times more than their free cell counterpart under identical experimental conditions. Also, the immobilized cells showed a stronger positive correlation between Cu adsorption and uptake. The results have been discussed in terms of improved metabolic efficiency of immobilized cells.

2. INTRODUCTION

While there are many demonstrations that free cyanobacterial cells take up heavy metals involving a rapid initial reaction (adsorption), followed by the slower metabolism-dependent metal intake [1–4], no direct comparisons of free-living and immobilized cells have been reported in this regard. Immobilization of cyanobacteria improved functional longevity and higher yields of nitrogen fixation, hydrogen evolution and ammonia production [5–7], and the observed efficiency has led to bioreactor concept for exploiting such photosynthetic prokaryotes for generation of NH_4^+ biofertilizers [8]. Recent advances in the area of immobilized photosynthetic microorganisms have been reviewed quite extensively [9].

The present study deals with a comparison of Cu-intake by the immobilized and free-cells of the diazotrophic cyanobacterium *Nostoc calcicola.*

3. MATERIALS AND METHODS

Nostoc calcicola, a local isolate was grown in Allen and Arnon's combined nitrogen-free medium [10] with A_6 trace elements (devoid of Cu). The cultures were maintained in culture room at $24 \pm 1°C$, illuminated with cool daylight fluorescent tubes (14.4 W m^{-2}) for 16 : 8 h as light-dark regime.

3.1. Cell immobilization

The exponential phase cyanobacterial cells (400 μg protein ml^{-1}) obtained by centrifugation and repeated washings, were suspended in 5% (w/v) solution of alginic acid (Sodium salt, Fluka) prepared in the growth medium as above. The mixture was pumped out dropwise into 0.2 M $CaCl_2$

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solution aseptically in a Laminar-flow cabinet. The beads thus formed, containing cyanobacterial cells to a tune of 55 μg protein bead^{-1}, were subsequently harvested, washed several times with sterile tripple glass distilled water and resuspended in a 20 ml volume of growth medium contained in a 250 ml cotton-stoppered culture flask. The monolayer of beads spread over the bottom of the flask was light-incubated for Cu-uptake measurements.

Likewise, a free cell suspension of the cyanobacterium (400 μg protein ml^{-1}) was also exposed to Cu. Both sets were subjected to constant, gentle shaking during Cu exposure (0–180 min).

3.2. *Cu-uptake*

The saturating Cu concentration (40 μM) as determined previously for the same organism [11] was also applied here to compare Cu uptake by immobilized and free cells.

The free cells (after centrifugation) as well as beads recovered simultaneously at different time intervals of Cu exposure (initial 5, and subsequent 10 min) were suspended in aqueous EDTA (10 μM; disodium salt, BDH, U.K.) to account for the EDTA-washable metal fraction (adsorption). Cu depleted from the medium, for both the sets, was estimated in a Perkin-Elmer AAS 2380 atomic absorption spectophotometer at 324.7 nm. Cellular Cu intake has been expressed as the difference of Cu adsorption and the amount of the metal depleted from the uptake medium in which the free cells or beads remained suspended during Cu exposure.

3.3. *Estimation of protein*

Protein was estimated by the method of Lowry et al. [12] modified by Herbert et al. [13] using lysozyme (Sigma, U.S.A.) as standard.

3.4. *Statistical analysis*

A correlation between Cu-intake and adsorption was calculated by using the equation

$$\gamma = \frac{\Sigma X \cdot \Sigma Y - \Sigma X \cdot Y/N}{\sqrt{\left[\Sigma X^2 - \frac{(\Sigma X)^2}{N}\right]\left[\Sigma Y^2 - \frac{(\Sigma Y)^2}{N}\right]}}$$

where X = Cu uptake, Y = Cu adsorption and N is number of samples.

4. RESULTS AND DISCUSSION

The initial rapid binding of cations to the negatively charged groups on the cell surface followed by their energy-dependent transport across the cell membrane has been reported in yeast and eukaryotic algae [14–16]. Similar observations also exist for cyanobacteria involving cations such as Zn [17], Cu and Pb [2], Cd, Cu and Zn [1], Cd [3], Cu [18], Al [19] and Ni [4]. These investigations have revealed the crucial role of light in regulating metal intake in such photosynthetic prokaryotes as the dark incubated cells did not show metal bioaccumulation with a few exceptions [18–19]. A comparison of Cu adsorption and uptake by the free- and immobilized cyanobacterium at similar light irradiance indicates the greater efficiency of immobilized cells (Fig. 1a, b). Cu-uptake kinetics in free *N. calcicola* cells exposed to saturating Cu concentration (40 μM) showed a curvilinear increase in metal uptake attaining a maximum of 96.69 nmol Cu mg^{-1} protein within 60 min (Fig. 1a). The faster metal uptake rate for the first 5 min, however, was not maintained, declining almost 3.7-fold (1.61 nmol Cu mg^{-1} protein min^{-1}) at 60 min of metal exposure. A negligible difference in Cu-intake values at 50 or 60 min of metal exposure suggests that the saturation occurred before 1 h.

Similar saturating Cu concentration (40 μM), when adopted to assess metal uptake by immobilized *N calcicola* cells, reflected an initial increase in the rate to 12.7 nmol Cu mg^{-1} protein min^{-1}, thus corresponding to a 2-fold increase over the free cells. Also, the immobilized cells accumulated 2 and a half-times more Cu (242.15 nmol mg^{-1} protein) over that by free cells during 1 h. A comparison of Cu uptake and adsorption pattern in the immobilized and free cells (Fig. 2a, b) shows that these events had a positive correlation with each other for both the treatment conditions. A stronger correlation as observed for immobilized cells ($r = 0.99$, *df* 5, $p < 1\%$) compared to suspension cultures ($r = 0.98$, *df* 5, $p < 1\%$) indi-

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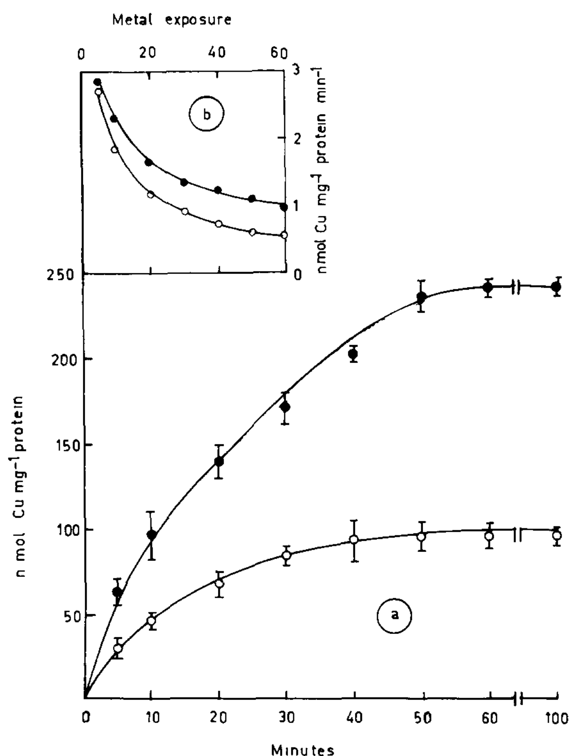

Fig. 1. (a) Time-course pattern of Cu uptake in free (○——○) and immobilized (●——●) cells of *N calcicola* exposed to 40 μM Cu (saturating concentration). (b) Rate of Cu adsorption in free (○——○) and immobilized (●——●) cyanobacterial cells at different intervals of metal-exposure

cates the overall efficiency of the former over the latter. Since the energy-dependent metal intake in cyanobacteria, to a larger extent, depends on photosynthetically generated energy [3,20], the relatively higher Cu uptake in immobilized cells could be attributed to their enhanced photosynthetic activity as reported earlier [8,21]. Analogous reports are also available on *Botryococcus braunii* as the immobilized cells showed higher net O_2-evolution compared to free-living cells [22]. Immobilized technique has been successfully applied to achieve sustained hydrogen production in marine photosynthetic bacteria [23] and cyanobacteria [24]. Similarly, high rates of hydrogen production by immobilized *Chromatium* cells led to the suggestion that energy production and removal of sulphide pollution could be simultaneously achieved through immobilization [25].

The apparent superiority of immobilized cells of *N. calcicola* in Cu intake over the free cells suggests that such a system could be successfully applied to scavenge heavy metals in repeated cycles without loss of cells.

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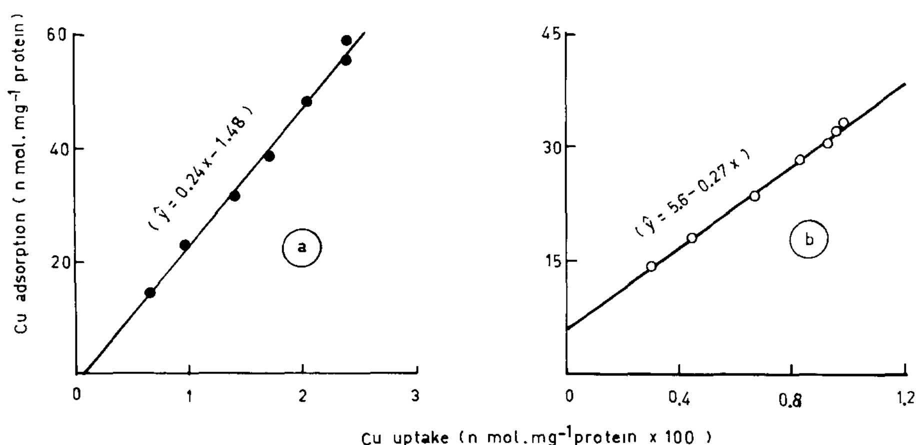

Fig. 2. (a) Regression between Cu intake and adsorption for immobilized *N calcicola* cells exposed to Cu (40 μM) for different times; X = Cu uptake and $\hat{Y}$ = Cu adsorbed. (b) Regression between cellular Cu intake and adsorption for free cyanobacterial cells under conditions as in 2a, X = Cu uptake and $\hat{Y}$ = Cu adsorbed.

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