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Toxicity of nickel, zinc, and cadmium to nitrate uptake in free and immobilized cells of *Scenedesmus quadricauda*

Mamta Awasthi[a,*], Lal Chand Rai[b]

[a]*Department of Zoology, Arunachal University, Itanagar 791 112, India*
[b]*Department of Botany, Banaras Hindu University, Varanasi 221005, India*

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Abstract

We examined the influences of three trace metals on the accumulation of a major nutrient (NO_3^-) in S*cenedesmus quadricauda*. A comparative study on metal–nutrient interaction in free and immobilized states of algal cells was conducted. The effect due to interaction between different variables (cell state type, metal type, and metal dose) was studied to assess the variation in the nitrate uptake by free and immobilized cells. The results analyzed by ANOVA (three-way) (components: cell state type, metal type, and metal dose) confirmed that the inhibition of nitrate uptake by test metals was highly significant ($P<0.001$). Free and immobilized states of S. *quadricauda* responded differently ($P<0.05$, ANOVA) to the types of metal added. Uptake kinetics was studied by monitoring short-term uptake rates at different nutrient levels. Free and immobilized cells of the organism displayed noncompetitive modes of inhibition for Ni and Zn while a competitive mode of inhibition by Cd was observed in both free and immobilized states of the organism.

Keywords: Heavy metal; Immobilized; *Scenedesmus quadricauda*; Nitrate uptake

1. Introduction

Freshwater ecosystems are influenced by heavy metal pollution (Baun et al., 1998). The chemical forms of these metals in water are accessible to the biota through significant accumulation in the food chain. Toxicity of heavy metals to algae has been reviewed earlier (Gaur and Rai, 1994). In addition to heavy metal pollution, excess nitrate discharge is also attracting attention. The nitrate threat to ground water comes from various sources including nitrogen-based fertilizers, waste from dairy and other livestock operations, and septic tank systems, both residential and industrial (Bier, 2002).

Many organisms including algae possess the ability to incorporate nutrients very rapidly from the external medium (Forni et al., 2001). For total removal of nitrogen, a biological process is more suitable than any conventional technique. However, the use of algae as a biological material is restricted since it is difficult to harvest algae for its use in sewage treatment. Recently, this problem was overcome by the use of immobilized algae (Lau et al., 1998). Immobilized biomass offers many advantages including better reusability, high biomass loading, and minimal clogging in continuous flow systems (Zhang et al., 1998; Tam et al., 1998).

Nitrate uptake is an interaction between cells and substrate similar to the interaction between enzymes and substrate in any biochemical process. In the present study, nitrate serves as a substrate and is converted into amine form ($-NH_2$). During this process, many substances (including heavy metals) may alter the uptake of nutrients by combining with them in a way that influences the binding of substrate by algal cells thus they act as inhibitors. An inhibitor can compete directly with the substrate for an enzymatic-binding site

*Corresponding author.
E-mail address: awasthi6@rediffmail.com (M. Awasthi).

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(competitive inhibition) or can bind to either the free enzyme or the enzyme–substrate complex (noncompetitive inhibition).

The study of nitrate uptake by algae becomes important since algae can be used as an excellent nutrient stripper, if properly managed. The present analysis will enable assessment of the extent and mode of inhibition of nitrate uptake in the immobilized state of algal cells by heavy metals. A comparative study of nutrient uptake kinetics in free and immobilized cells is also significant for assessing the superiority of immobilized cells, if any, over free cells. The presence of heavy metals together with excess nutrients can cause interference in the nutrient removal process by algae. The study was designed to evaluate whether metals present in effluents inhibit NO_3^- uptake by imbedded algae more so than that by free cells, thereby creating problems with effluent treatment using imbedded cells.

2. Material and methods

2.1. Isolation, purification, and culture

Eukaryotic green algae *Scenedesmus quadricauda* (local isolate, Banaras Hindu University) was grown in modified CHU-10 medium (Gerloff et al., 1950). The pH was maintained at 7.0 by using 2.0 mM Tris (hydroxymethyl) methylene/HCl. Cultures from the logarithmic phase were used for toxicity tests. Stock solutions of $NiCl_2 \cdot 6H_2O$, $ZnSO_4 \cdot H_2O$ and $CdSO_4 \cdot H_2O$ were filter-sterilized by passing through Millipore membrane filters (0.45 μm) before addition to the culture medium. Test metals were freshly prepared and the actual metal concentrations used were the LC_{50} values determined previously by the plate/colony count method based on the survival of the test alga to 50%. The concentrations were as follows:

Ni, 1.10, 2.10 (LC_{50}), and 3.10 μmol L^{-1}; Zn, 1.40, 2.40 (LC_{50}), and 3.40 μmol L^{-1}; Cd, 1.30, 2.30 (LC_{50}), and 3.30 μmol L^{-1}.

The culture received 72 mmol photons m^{-2} s^{-1} PAR light intensity at $24 \pm 2\,^{\circ}C$. Standard microbial techniques were employed for selection, isolation, and cloning of algae in pure culture.

Protein value was estimated following the method of Herbert et al. (1971).

2.2. Bubble column reactor

A glass tube containing a ground-glass filter at the bottom and sealed with a rubber stopper at the top was used. A hollow rod was inserted through the stopper to reach the bottom of the column. The tube was used to pump air in the reactor. The column was aerated at 250–300 cc/min with an air bubbler. A hollow tube was also inserted sideways at the bottom to remove the solution from the reactor at different time intervals.

2.3. Cell immobilization

Most methods for immobilization of biomaterials in alginate beads basically involve two main steps. First, there is the internal phase where the alginate solution containing biological materials is dispersed into small droplets. Secondly, the droplets are solidified by gelling or membrane formation at the droplet surface. This is termed the dialysis/diffusion method (diffusion setting) as the alginate solution is gelled by diffusion of gelling ions from an outer reservoir. The procedure is as follows: Dissolve 30 g of sodium alginate in 1 L to make a 3% solution. Mix approximately 10 mL of desired protein value algal culture with 10 mL of 3% (wt.) sodium alginate solution. The concentration of sodium alginate can be varied between 6% and 12% depending on the desired hardness. The beads are formed by dripping the polymer solution from a height of approximately 20 cm into an excess (100 mL) of stirred 0.2 M $CaCl_2$ solutions with a syringe and a needle at room temperature. Pump pressure and the needle gauge can control the bead size. A typical hypodermic needle produces beads of 0.5–2 mm in diameter. Other shapes can be obtained by using a mold whose wall is permeable to calcium ions. Leave the beads in the calcium solution to cure for 0.5–3 h.

In the present study, exponentially grown algal cells (500 μg protein/mL) obtained by centrifugation and repeated washings were suspended in 3% (w/v) solution of sodium alginate (Sigma). The mixture was pumped dropwise into $CaCl_2$ (0.2 M) solution. The beads thus formed contained algal cells in an amount of 60 μg protein per bead. The beads were washed several times with sterile deionized double-distilled water and resuspended in a 200-mL growth medium for autotrophic growth under culture room conditions. Free cells were also cultured under similar conditions at the same time.

2.4. Estimation of NO_3

For studying the effect of metals on NO_3^- uptake, cultures were incubated in KNO_3 (1.5–20.0 μM). A bubble column reactor was used for NO_3^- uptake. The column was aerated at 250–300 cc/min with an air bubbler. Beads were placed into a bubble column reactor in 500 mL growth medium for 24 h. At that time, the medium was pumped out and the beads were washed with 500 mL of double-distilled water. The column reactor was exposed to a NO_3^- uptake medium for 30 min; 3-mL samples were taken every 10 min and tested for NO_3^- depletion. NO_3^- in the medium was estimated spectrophotometrically by