Short Communication

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Nitrate and Phosphate uptake by immobilized cells of *Gloeocapsa gelatinosa*

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Original Article

Abstract

The use of algae to treat wastewater has been under investigation for decades. Laboratory experiments were performed to study the growth rate and nitrate and phosphate uptake of free and immobilized cells of saline tolerant, nitrogen fixing cyanobacterium *Gloeocapsa gelatinosa*. The immobilization was carried out in calcium alginate matrix. The immobilized cells were found to be highly effective in the removal of nutrients. 93% of nitrate was absorbed by immobilized cells within 24 hours whereas algae- free beads and free algal cells absorbed only 46% and 70% respectively. 80% of phosphate was also absorbed by immobilized cells within 24 hours. The results suggest that *G. gelatinosa* is a promising biological agent for nutrient removal from wastewater. Bioreactors with immobilized cells of this alga could be developed for the removal of nutrients from aquatic system.

Keywords: *Gloeocapsa gelatinosa, calcium alginate, immobilization, micro algae, nutrient removal.*

Introduction

The use of algae to treat wastewater has been under investigation for decades. A high nitrate and phosphate content and an alkaline pH (7.5-8.5) of sewage enhance the growth of algae. The cultivation of algae in wastewater offers the combined advantages of treating wastewater and simultaneously producing algal biomass, which can further be exploited for protein complements and food additives (aquaculture and animal feed), energy such as biogas and fuels and in agriculture (fertilizers, soil conditioners). In comparison to the entirely heterotrophic systems like activated sludge plants, the primary attraction of algal ponds lie in the low grade technology and in saving of energy, since photosynthetic oxygen production can replace mechanical aeration (Mallick, 2002).

A major drawback of algal ponds is the difficulty and associated cost of harvesting micro algae (Huntley *et al*., 1989). Recently research efforts have increasingly focused on the use of non suspended algae, either attached or immobilized as a valid method that avoid harvesting problem (Gonzalez *et al*., 2000), in this context immobilization of algal cells for wastewater treatment has been proposed for circumventing the harvest problem as well as for retaining the high value algal biomass for further processing (de la Noue and de Pauw, 1998). Application of immobilization technology to algal wastewater treatment provides more flexibility in the reactor design, when compared to conventional suspension systems. Moreover, accelerated reaction rates due to increased cell density, increased cell wall permeability, no washout of cells and better operational stability are the additional advantages

of immobilized cells over their free living counterparts (Brouers *et al*., 1989). For immobilization of algae, alginate is the most frequent polymer used. Studies have adequately verified cell viability in alginate matrix (Hertzberg and Jensen, 1989; Vilchez *et al*., 2001).

The present study presents the results of experiments carried out to investigate nitrate and phosphate uptake by saline tolerant nitrogen fixing cyanobacterium *Gloeocapsa gelatinosa* immobilized in calcium alginate beads.

Material and methods

Organism and culture conditions: *Gloeocapsa gelatinosa* culture maintained in the Department of Marine Biology, Microbiology and Biochemistry, was used for the study. Stock cultures of *G. gelatinosa* were raised in 1000 ml Erlenmeyer flasks, containing 500 ml Allen and Nelson Medium (Allen and Nelson, 1910). Illumination was provided by cold white fluorescent light of 2000 lux for a light/ dark period 12:12 hours. The room temperature was maintained at 25± 1^{0}C.

Immobilization: Algal culture in logarithmic phase of growth was used for immobilization. 100 ml of the culture was centrifuged at 3000 rpm for 10 min, the supernatant was discarded and the cells were resuspended in 100 ml autoclaved distilled water. Into that, algal suspension 4% (W/V) sodium alginate was added with continuous manual shaking for 20 min and sieved with two layered cheese cloth. Beads of 4 mm dia were obtained by dropping the alginate algal mixture into 100 ml, 2.5% $CaCl_2$ solution in distilled water, at room temperature in sterile condition. The beads were washed several times in autoclaved distilled water to remove any remains of calcium chloride and later stored at 4^{0}C in autoclaved distilled water. Calcium alginate beads without *G. gelatinosa* are also produced using the same procedure, but without incorporating the algae.

Growth analysis: Growth was measured by estimating the concentration of chlorophyll a in the culture. One ml of algal culture was taken at regular intervals from free cell cultures and fourteen (approximately equal to 1 ml of free cell culture) beads were taken regularly from immobilized cell cultures. Both the samples were suspended in 10 ml of 0.1 M trisodium citrate separately. The cells were released from the beads after 15 min and then the samples were filtered through GF/C filter. The filter was soaked in 10 ml of 90% acetone and incubated for 24 hours in dark. After incubation, the samples were centrifuged at 5000 rpm for 10 min, absorbance of the supernatant was noted using 90% acetone as blank at 750, 665, 645 and 630 nm in a Hitachi U 2001 spectrophotometer and Chl a concentration was calculated (Strickland and Parsons, 1972).

Nutrient analysis: 200 ml of *G. gelatinosa* culture was taken, out of that 100 ml of the culture was immobilized as stated previously and the rest 100 ml was centrifuged at 3000 rpm for 10 min and the cells were isolated. The immobilized algae, algae- free calcium alginate beads and free cells were introduced separately into 100 ml modified Allen and Nelson medium containing 100 mg/L nitrate and 100 mg/L phosphate, in 250 ml conical flasks in triplicate. The cultures were incubated at 25±1^{0}C. Illumination was provided continuously by cold white fluorescent light of 2000 lux. Samples were collected from the cultures at 6 hours interval from each flask and filtered using GF/C filter. The filtrate was collected and used for the estimation of nitrate and phosphate. Nitrate was estimated photometrically by Brucine sulphuric acid method (Nicholas and Nason, 1959) and Phosphate using Stannous chloride method (APHA, 1985).

Statistical analysis: Statistical analysis was done with SPSS Version 11. One way analysis of variance (ANOVA) was done for mean values; Chl a at 15th day and for other parameters, values at 24th hours were considered. Mean values were compared using Tukeys test ($p<0.05$) for nutrient parameters

Results and discussion

Growth was measured in terms of Chl a concentration. There was slight variation in concentration of Chl a in free and immobilized cells of *G. gelatonisa* (Fig.1). Not much difference in Chl a values of free and immobilized cells were noted up to 9th day. But from 9th day onwards the immobilized cells produced more Chl a than its free living counterpart. On 15th day the Chl a value reached up to 4.4 μg/ml in immobilized cells and in free cells the value was 4 μg/ml ($r = 0.041$, $P> 0.05$), similar results were reported by many workers. (Mitsui *et al*., 1985; Rai & Mallick, 1992; Thakur and Kumar, 1999; Jimenez *et al*., 2004). However, a decrease in growth rate in immobilized cells than in free cell culture were observed by Bailliez *et al.* (1985) and Robinson *et al.* (1985).

Microscopic studies of sliced gel beads revealed that cells were randomly distributed throughout the gel bead

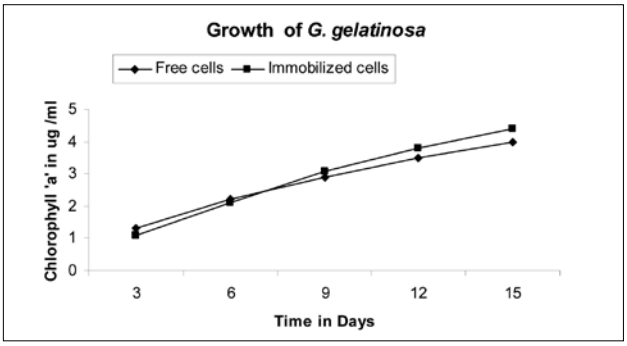

Fig.1. Growth of *G. gelatinosa* in free and immobilized condition

immediately after immobilization. After an incubation period of 9-12 days in growth medium, beads showed dense green colour and thick cell layer near their surface. The size of the cells was comparatively smaller in immobilized beads than that of free living cells. But the number of cells were high, producing small colonies in the matrix, similar observations were made by other workers also. (Kuhn *et al.*, 1991; Jimenez *et al.*, 2004). Calcium alginate entrapped *Chlorella,* usually a unicellular organism tend to form small colonies (8-30 cells) when released from immobilized gel (Trevan and Mak, 1998).

Immobilized cells of *G. gelatinosa* were more efficient in nitrate removal. It absorbed 93% of nitrate from the medium within 24 hours, whereas algae-free beads and *G. gelatinosa* cells absorbed 46% and 70% respectively ($r = 0.000$, $P < 0.05$) (Fig. 2). There was a gradual release of absorbed nitrate into the medium by all the three groups after 24 hours. Phosphate was also effectively absorbed by the immobilized cells of *G. gelatinosa* (Fig. 3). Immobilized cells absorbed 80%

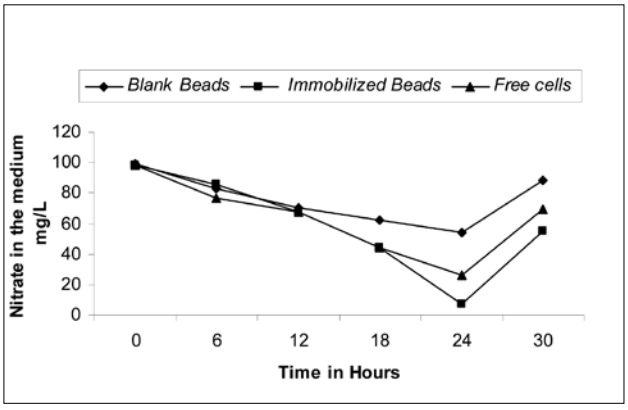

Fig.2. Nitrate uptake by free and immobilized cells of *G. gelatinosa*

of the phosphate from the medium within 24 hours but a gradual release of phosphate into the medium was observed afterwards. Algae-free beads were also effective in phosphate adsorption; it adsorbed 78% of phosphate from the medium within 24 hours, and found no statistical variation with immobilized cells ($r = 0.499$, $P < 0.05$) but *G. gelatinosa* free cells absorbed only 58% of phosphate even after 30 hours ($r = 0.000$, $P < 0.05$).

In the present study immobilized cells showed better ability for the uptake of nutrients than that of free cells; this may be due to the mass aggregation of cells inside the bead matrix. Similar results were shown by alginate immobilized *Chlorella vulgaris* and *Scenedesmus bijugatus* (Megharaj *et al.*, 1992), *Chlorella* and *Anabaena* (Rai and Mallick, 1992), *Chlamydomonas reinhardtii* (Vilchez and Vega, 1994), *D. salina* (Thakur and Kumar, 1999) and Ca-alginate immobilized

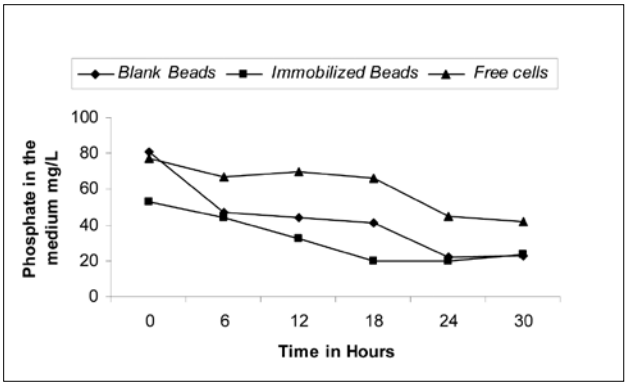

Fig.3.Phosphate uptake by free and immobilized cells of *G. gelatinosa*

Chlorella cells packed in transparent PVC (Tam and Wong, 2000). However, Faafeng *et al.* (1994) and Lau *et al.* (1997) reported that immobilization carries a disadvantage of restricted nutrient diffusion. Reduction in the phosphate level in the medium containing beads without algae may be due to the precipitation of phosphate by Ca-alginate matrix (Jimenez *et al.*, 2004). The present study shows that immobilized cells could be employed for nutrient removal from closed systems.

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