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Utilization of Cyanobacteria in Photobioreactors for Orthophosphate Removal from Water

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Abstract

The effectiveness of photosynthetic free-living and polyurethane foam (PU) immobilized *Anabaena variabilis* cells for removal of orthophosphate (P) from water in batch cultures and in a photobioreactor was studied. Immobilization in PU foams was found to have a positive effect on P uptake by cyanobacteria in batch cultures. The efficiency of P uptake by immobilized cells was higher than by free-living cells. A laboratory scale photobioreactor was constructed for removal of P from water by the immobilized cyanobacteria. The photobioreactor was designed so that the growth medium (water) from a reservoir was pumped through a photobioreactor column with immobilized cyanobacteria and back to the reservoir. This created a closed system in which it was possible to measure P uptake. No leakage of cells into the photobioreactor medium reservoir was observed during the operation. The immobilized cells incorporated into a photobioreactor column removed P continuously for about 15 d. No measurable uptake was demonstrated after this period. Orthophosphate uptake efficiency of 88–92% was achieved by the photobioreactor.

Index Entries: Orthophosphate; water clean-up; immobilization; cyanobacteria; photobioreactor.

Introduction

Inorganic phosphorus (orthophosphate and phosphate) is an essential element for the growth of plants and animals, but it could be harmful for the environment. High levels of phosphorus in rivers and lakes owing to pollution can cause a negative environmental impact through the process

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of eutrophication *(1)*. Eutrophication results when an excess of inorganic phosphorus enters a waterway. Algae and aquatic plants grow fast, choking waterways and consuming large amounts of dissolved oxygen *(2)*. The rapid and uncontrollable growth of aquatic organisms will cause decay and, eventually, destruction of the aquatic ecosystem. Removal of phosphorus from municipal and industrial wastewater is required to protect water quality. According to federal government standards, phosphate levels in water should not exceed 0.01–0.1 mg/L *(3)*. There are several chemical and physical methods for removing phosphorus from water, such as distillation *(4)*. Treatment plants have been designed to remove phosphorus, often by using chemicals *(4)*. Chemical and physical phosphorus-removing methods require a great deal of energy to operate efficiently and are high-maintenance systems. Studies have shown that cyanobacteria are good candidates for use in removal of phosphorus from water *(5,6)*. They can use orthophosphate for their growth (formation of photosynthetic adenosine triphosphate) with solar light as their energy source.

The goal of the present study was to compare the effectiveness of photosynthetic free-living and polyurethane (PU) foam–immobilized *Anabaena variabilis* cells for the removal of orthophosphate from various sources of water in batch cultures and in a photobioreactor. Sources of water included a cyanobacterial standard medium, municipal tap water, and water from a local lake. Capabilities of orthophosphate uptake by ammonia excreting a mutant and a wild-type cyanobacterium were compared as well. The ultimate goal of this study was to develop and operate a photobioreactor with PU foam–immobilized cyanobacterial cells for the removal of orthophosphate from polluted water. One of the important advantages of the immobilized cells is the very large surface-to-volume ratio, which enhances mass removal of orthophosphate by the cells. In addition, cyanobacteria, when immobilized in matrices such as agar, cotton, polyurethane, or polyvinyl foams, stabilize and increase their physiologic functions *(7)*.

A. variabilis was chosen for the current study of orthophosphate removal from water because higher efficiencies of inorganic nitrogen and phosphate removal by this cyanobacterium in a hollow-fiber photobioreactor were observed in preliminary studies *(8)*. Because hollow-fiber photobioreactors might currently be too expensive for water treatment, other options for a photobioreactor design (PU foam immobilization) were considered. Immobilization of *A. variabilis* on different substrates has been studied thoroughly *(9)*. The immobilization of *A. variabilis* on hollow fibers led to stabilization of H_2 photoproduction for several months *(9)*.

Materials and Methods

Chemicals

Chemicals were purchased from Fisher (Atlanta, GA) and Sigma (St. Louis, MO).

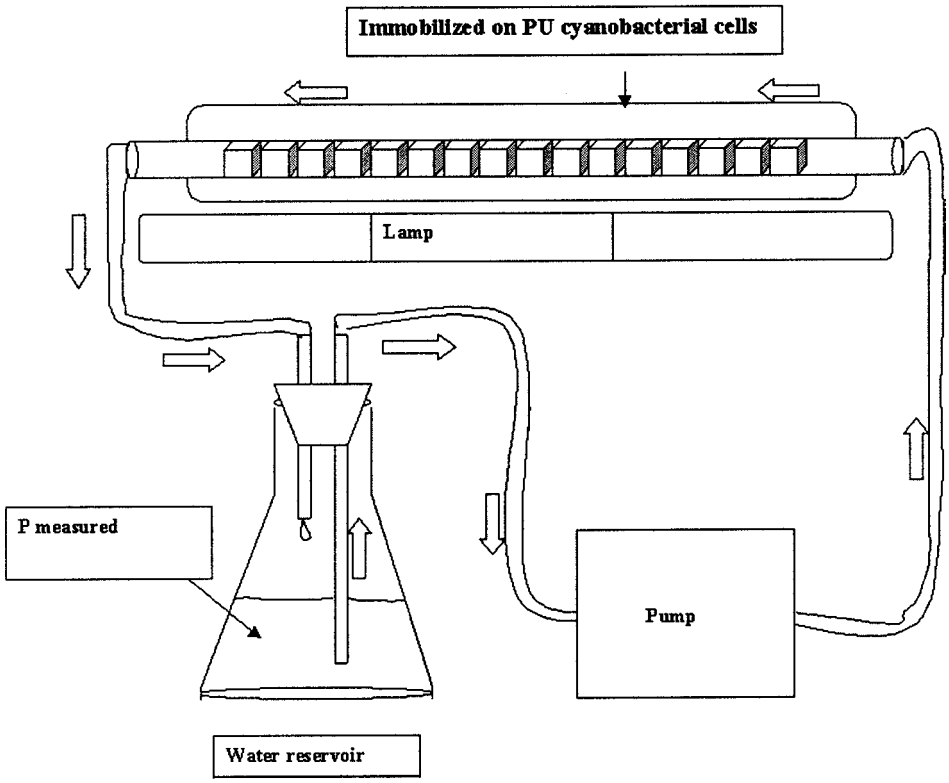

Fig. 1. Schematic diagram of the photobioreactor with PU foam–immobilized cyanobacteria for removal of orthophosphate from water.

Culture Growth

Cyanobacterium *A. variabilis* (wild-type SA-0 and ammonia-excreting mutant SA-1) was obtained from Dr. K. T. Shanmugam (University of Florida). Batch cultures of the cells were grown in the medium of Allen and Arnon *(10)*, municipal tap water, or water from J. Percy Priest Lake, Nashville, TN, at 26–28°C. Continuous light was provided by cool white fluorescent lamps (30–50 ft candles [15–25 μmol/m^2s] on the surface of the culture) in an incubator as described previously *(11)*. Cell concentration for batch cultures during inoculation was 0.65 mg of cell dry wt/mL and increased because of cyanobacterial growth.

Photobioreactor Design

A laboratory-scale photobioreactor was constructed for the removal of orthophosphate from water (Allen-Arnon medium) by *A. variabilis* SA-0 cells (Fig. 1). The photobioreactor glass column (19 cm long × 7 cm diameter) was filled with PU foams. Cyanobacterial cells (0.65 mg of cell dry wt/mL) were added to the photobioreactor column under sterile conditions. Cyanobacterial growth medium was continuously returned to

a water reservoir that created a closed system in which it was possible to measure the uptake of orthophosphate. The column was maintained at room temperature and illuminated continuously with a cool white fluorescent lamp at the bottom. The irradiance above the column was measured at approx 25 ft candles and below the column at approx 30 ft candles (15 $\mu mol/m^2s$).

Cell Immobilization

Cyanobacterial cells were immobilized by adsorption on PU foam (1-cm cubes). Before inoculation with cells, foams were washed with distilled water. Seven pieces of PU foam were added to each flask. The flasks were autoclaved (121°C for 15 min) and cooled to room temperature. Then cyanobacterial cells were added.

Orthophosphate Assay

Orthophosphate content was measured regularly using a modified ascorbic method from *Standard Methods for Examination of Water and Wastewater (12)*. Samples (0.5 mL) were obtained from each flask and the reservoir of the photobioreactor. Samples were then diluted in 100 mL of distilled water. Eight milliliters of the combined reagent was added to half of each sample. The combined reagent was mixed as follows: 50 mL of H_2SO_4, 5 mL of potassium antimony tartrate solution, 15 mL of ammonium molybdate solution, and 30 mL of ascorbic acid solution. The mixture was allowed to stand for at least 10 min, but no more than 30 min. The samples were read on a Hitachi U-2000 Spectrophotometer at 880 nm, providing a 1-cm light path.

Orthophosphate Uptake in Batch Cultures

For each experiment, water samples were taken from 20 flasks (10 flasks for each cyanobacterial strain and 5 flasks each for free-living or immobilized cells) and analyzed for orthophosphate content by the ascorbic acid method just described.

Orthophosphate Uptake in Photobioreactor

Water samples were collected from the water reservoir of the photobioreactor and analyzed for orthophosphate content by the ascorbic acid method just described.

Calculation of Orthophosphate Uptake Efficiency

Orthophosphate uptake efficiency (E) was defined as: $E = [(I - F)/I] \times 100\%$, in which I and F are the initial and final concentrations of orthophosphate, respectively *(5)*. An efficiency value of 100% was obtained when no orthophosphate appeared in the water (i.e., $F = 0$).

Biomass

Biomass was determined after filtering and drying the cell suspension at 90°C to a constant weight.

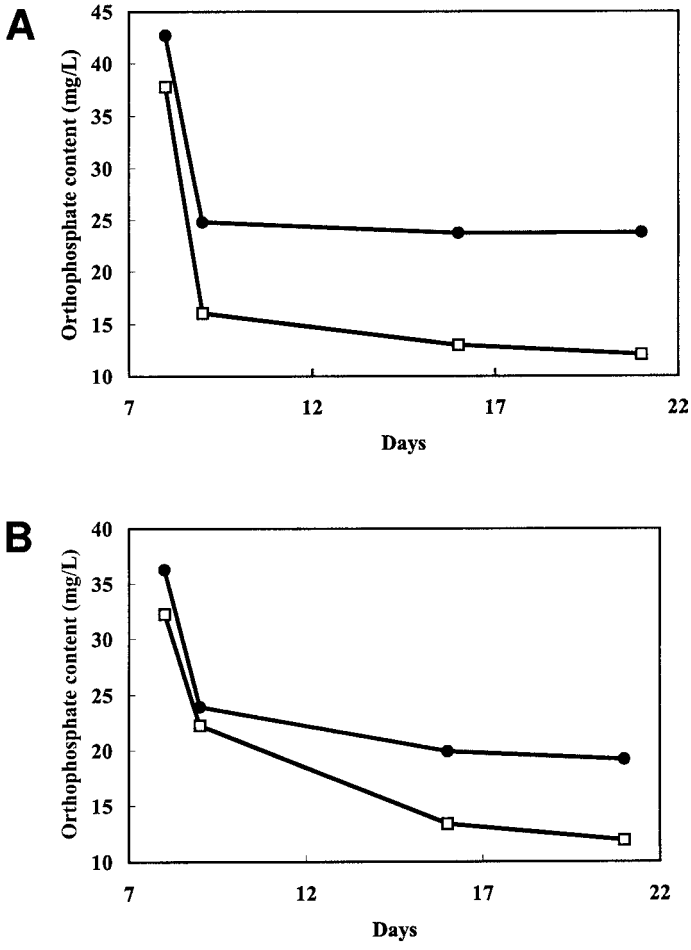

Fig. 2. Orthophosphate uptake from standard cyanobacterium medium by free-living (●) or PU foam–immobilized (□) cyanobacterium *A. variabilis* wild type SA-0 **(A)** or *A. variabilis* mutant SA-1 **(B)** in batch cultures.

Results

Orthophosphate Uptake in Batch Cultures

Both immobilized and free-living cells absorbed orthophosphate from a variety of water sources used in experiments (Figs. 2–5). In general, PU foam–immobilized cells absorbed orthophosphate faster than free-living cells during the experiment, regardless of the water source. There was practically no difference in orthophosphate uptake from water by cells of *A. variabilis* wild strain (SA-0) or ammonia-excreting mutant *A. variabilis* SA-1.

Orthophosphate Uptake from Standard Cyanobacterium Medium

Orthophosphate uptake by free-living and immobilized cells of *A. variabilis* SA-0 and SA-1 is presented in Fig. 2. Initially, orthophosphate

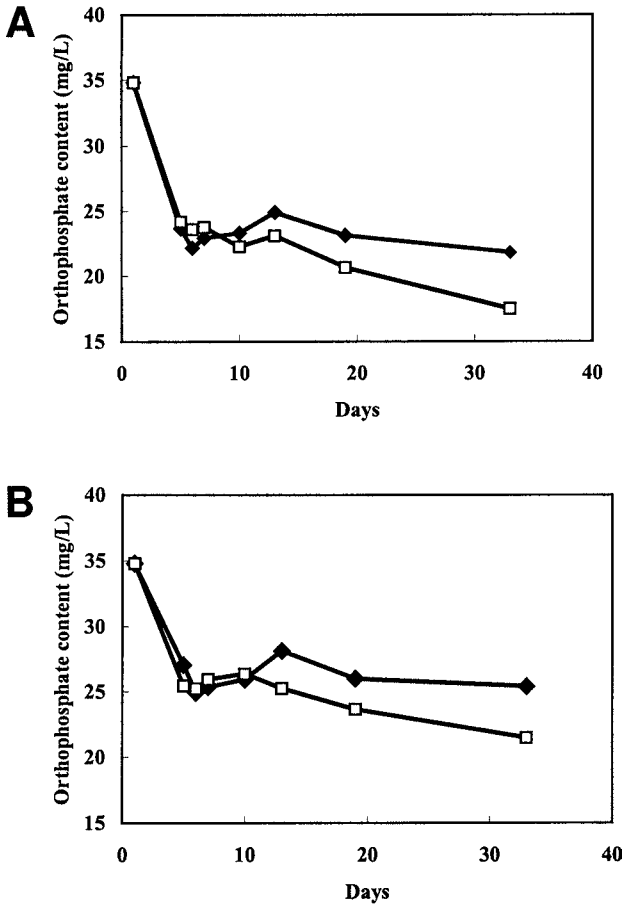

Fig. 3. Orthophosphate uptake from lake water by free-living (◆) or PU foam–immobilized (□) cyanobacterium *A. variabilis* SA-0 **(A)** and SA-1 **(B)** in batch cultures.

uptake by cyanobacterial cells was faster, but it subsequently decreased to a lower steady uptake. The decrease in the orthophosphate uptake down to the steady uptake in batch cultures was apparently owing to the age of the cyanobacterial culture and lack of nutrients other than orthophosphate. The efficiency of orthophosphate uptake by immobilized cells was higher than by free-living cells (up to 30% higher).

Orthophosphate Uptake from Lake Water

Inorganic phosphorus was not found in water from J. Percy Priest Lake at the time of sample collection (October 1999). For experiments, orthophosphate was added at concentrations similar to those for a standard cyanobacterial medium. Cyanobacterial cells, both free living and immobilized, were found to remove orthophosphate better when they were added to the standard cyanobacterial medium rather than to lake water, probably owing to the difference in ion compositions (Fig. 3). The efficiency

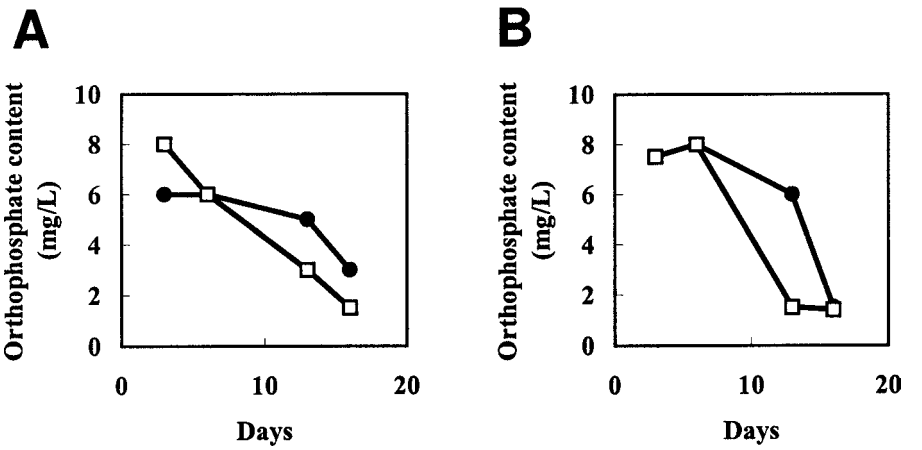

Fig. 4. Orthophosphate uptake from municipal tap water by free-living (●) or PU foam–immobilized (□) cyanobacterium *A. variabilis* SA-0 **(A)** and SA-1 **(B)** in batch cultures.

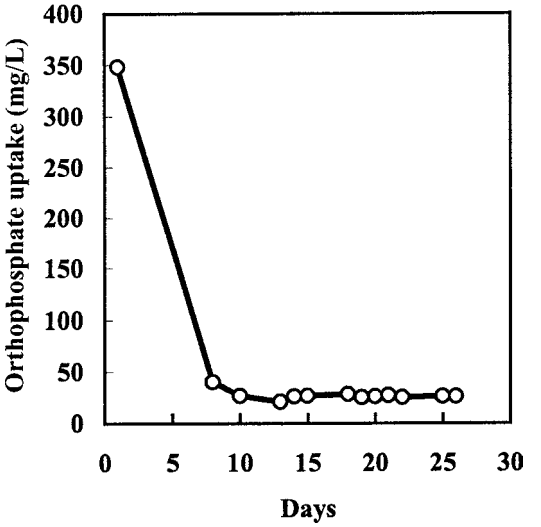

Fig. 5. Orthophosphate uptake by *A. variabilis* SA-0 immobilized on PU foam in a photobioreactor.

of orthophosphate uptake from lake water by immobilized cells was higher than by free-living cells (up to 12% higher).

Orthophosphate Uptake from Municipal Tap Water

Orthophosphate was found in municipal tap water (up to 8 mg/L). The concentration of orthophosphate in tap water was not stable and varied from day to day. However, this orthophosphate content was lower as compared to standard cyanobacterial medium. At low initial orthophos-

phate concentrations, cyanobacterial cells were able to almost completely utilize orthophosphate from tap water (Fig. 4). At these concentrations, there was no big difference in orthophosphate uptake by free-living or PU foam–immobilized cyanobacteria.

Orthophosphate Uptake in Photobioreactor

The batch experimental results, which showed higher orthophosphate uptake by PU foam–immobilized cells compared to free-living cells, allowed us to select PU foam–immobilized *A. variabilis* for photobioreactor studies. The photobioreactor was run continuously for 26 d after inoculation with a cyanobacterial suspension. Orthophosphate uptake was measured, as shown in Fig. 5. Orthophosphate uptake in a photobioreactor was very similar, with batch cultures showing a higher initial orthophosphate uptake rate and a slower steady uptake days later. The efficiency of orthophosphate uptake was calculated. After 8 d of photobioreactor operation, the efficiency of orthophosphate uptake was 88%. After 26 d of orthophosphate uptake in the photobioreactor, the efficiency had reached 92%. After 27 d, no significant improvement in the efficiency of orthophosphate uptake was demonstrated.

Discussion

The results presented herein demonstrate that there is potential for the use of PU foam–immobilized cyanobacteria in photobioreactors for removing excess inorganic phosphorus from water to prevent eutrophication. It was found that immobilized cyanobacterial cells take up orthophosphate faster than free-living cells in batch culture during the experimental period, regardless of the water source. Each batch culture was inoculated with the same amount of cyanobacterial cells, so any increases in biomass are owing to cyanobacterial growth. There was no statistical difference in biomass amount (biocatalyst amount) during cyanobacterial growth between free-living and immobilized cells. Thus, it is cell immobilization, not biocatalyst amount, that increases the orthophosphate uptake. Similar results were found by Garbisu et al. *(4)*, in which polyvinyl foam–immobilized cyanobacteria took up phosphate from cyanobacterial medium faster than free-living cells during the incubation period. At low orthophosphate concentrations (which were found in tap water), cyanobacterial cells were able to completely consume orthophosphate from water in a batch culture (100% efficiency). A photobioreactor with a continuous flow of water, described herein, was also operated with higher efficiencies of orthophosphate uptake by PU foam–immobilized cyanobacteria (88–92%), despite a higher concentration of orthophosphate. These efficiencies were similar to previously reported efficiencies for phosphate uptake by chitosan-immobilized *Phormidium* in continuous cultures *(13)*. Efficiencies of orthophosphate uptake in a photobioreactor with PU foam–immobilized cyanobacteria and the low cost of PU foam make this photobioreactor suitable for future prac-

tical applications. In addition, we observed other advantages of immobilized cells compared to free-living cells during the operation of the photobioreactor. When the immobilized cyanobacterial cells were used in the photobioreactor to remove orthophosphate, no significant leakage of cells into the photobioreactor effluent was seen. This eliminates any problem of recovering the microorganisms from the treated effluent during the operation with PU foam–immobilized photobioreactors.

Acknowledgments

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