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SHORT COMMUNICATIONS

Intracellular Phosphorus Pool of the Cyanobacterium *Spirulina platensis*

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Abstract—An intracellular phosphorus pool in a monoculture of the cyanobacterium *Spirulina platensis* was assessed using radioactive and nonradioactive phosphorus. The derived dependence of specific growth rate on the intracellular content of mineral phosphorus can be presented in the form of the Droop equation. It was found that the stage of replenishment of the intracellular phosphorus pool may affect the phosphorus turnover estimation in aquatic environments from the results of short-term measurements of phosphorus uptake.

Phosphorus turnover in aquatic environments is extensively studied, since mineral phosphorus is the main factor limiting the development of phytoplankton [1–3].

Natural microbial communities consist of species whose phosphorus requirements are different. Moreover, phosphorus requirements of particular species depend on their functional activity. These and some other factors undoubtedly affect the intensity of phosphorus turnover in the "phytoplankton–aquatic environment" system and complicate its investigation. To gain better insight into this problem, we performed a long-term laboratory experiment in which phosphorus turnover was studied using a monoculture of the cyanobacterium *Spirulina platensis* and radioactive phosphorus. This approach allowed the processes of phosphorus uptake and release, as well as of its incorporation into cellular structures, to be studied with confidence at different growth stages. Using preliminarily limited (phosphorus-starved) cyanobacterial cultures enabled us to estimate the influence of the stage of phosphorus accumulation on the dynamics of its uptake.

Our earlier long-term laboratory experiments with a monoculture of *Spirulina platensis* have shown that the moments of the maximum accumulation of radioactive phosphorus and biomass do not coincide [3]. Thus, microbial cells took up almost all radioactive phosphorus from the medium within two days, whereas biomass was maximum only on the fourth day of the experiment. These findings pointed to the existence of a stage of intense phosphorus accumulation by phosphorus-starved *Spirulina* cells and to the formation of an intracellular phosphorus pool. The growth of the microscopic alga after the depletion of phosphorus from the medium occurred at the expense of this pool.

The aim of this work was to study the dynamics of the intracellular phosphorus pool of the cyanobacterium *Spirulina platensis*.

MATERIALS AND METHODS

The cyanobacterium *Spirulina platensis* used in this study was obtained from the Institute of Medical and Biological Problems, Moscow. The cyanobacterium was grown in a modified Zarruk medium [4] without phosphorus. The culture thus obtained was diluted with phosphate-free Zarruk medium to reduce the phosphorus content of the culture filtrate to almost zero. The diluted culture was dispensed into conical flasks in 0.4-l amounts and supplemented with radioactive phosphorus in the form of $KH_2{}^{32}PO_4$ at concentrations varying from 5 to 50 μg ^{32}P/l (control flasks contained equivalent amounts of the stable phosphorus isotope). The flasks were placed in a luminostat with an illuminance intensity of 20 W/m^2 and cultivated for up to 1032 h at a temperature of 31 ± 1°C. Samples in amounts of 5 ml were taken from the flasks immediately after the addition of phosphorus (zero time of the experiment), 1, 2, 9, and 24 h afterwards, and then at 24-h intervals. The samples were filtered through membranes with a pore size of 0.15–0.25 μm, and the radioactivity of the filters and filtrates was determined using an MST-17 end-window counter. At some moments, *Sp. platensis* cultures in the control and experimental flasks were additionally supplemented with nonradioactive phosphorus.

Cyanobacterial biomass was assessed by determining the dry weight of the biomass on filters. The concentration of soluble reactive phosphates (SRP) in culture filtrates was determined with ammonium molybdate.

RESULTS AND DISCUSSION

The intracellular phosphorus pool was assessed using the cyanobacterial culture grown in phosphorus-free medium. It was found that the radioactive phosphorus added to the phosphorus-starved culture was consumed within about 9 h of cultivation. The increase in biomass was insignificant and independent of the

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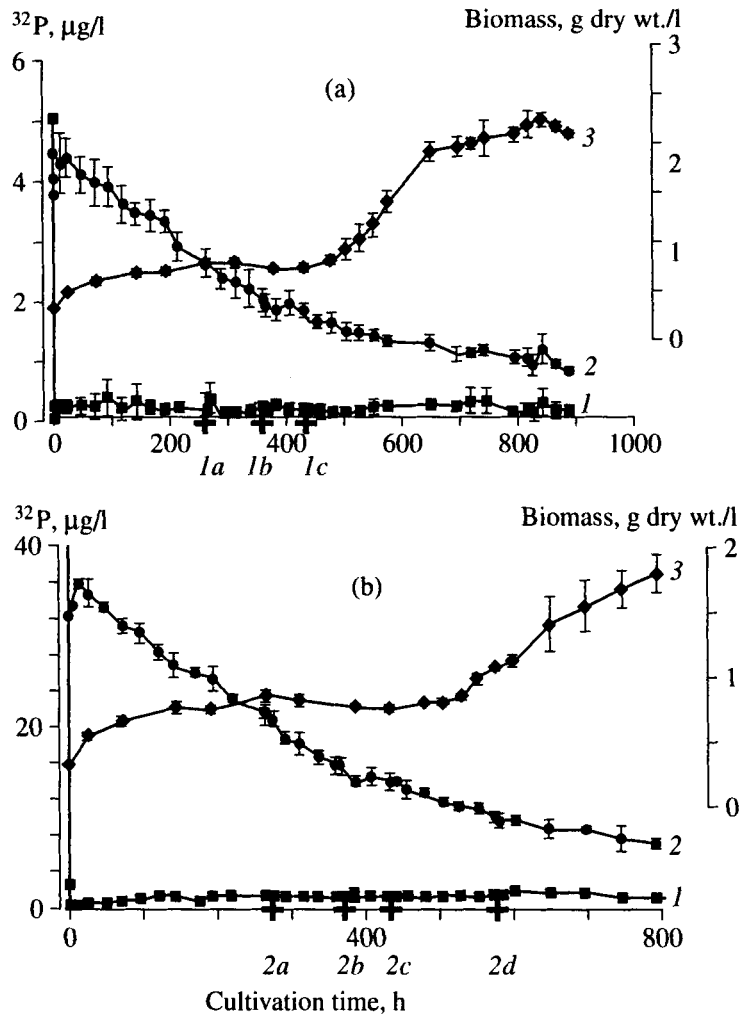

Fig. 1. (*3*) Growth of *Sp. platensis* and the dynamics of radioactive phosphorus content in (*1*) medium and (2) cells after the addition of (a) 5 and (b) 50 μg ^{32}P/l at zero time. Symbols "+" on the abscissa axis indicate the moments of addition of nonradioactive phosphorus in amounts of (*1a*) 150, (*1b*) 300, (*1c*) 1500, (*2a*) 300, (*2b*) 600, (*2c*) 750, and (*2d*) 1000 μg P/l.

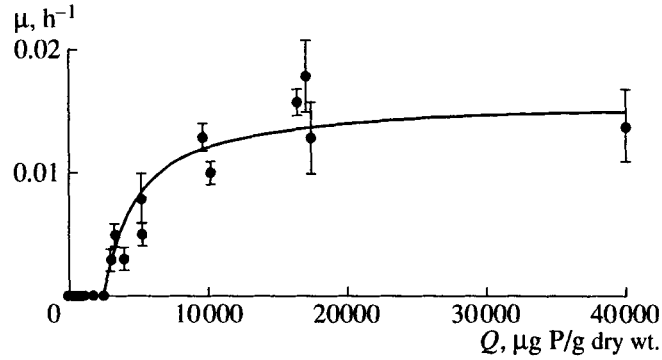

Fig. 2. Dependence of the specific growth rate of *Sp. platensis* on the intracellular phosphorus content.

amount of the radioactive phosphorus added to the medium, which was indicative of the complete exhaustion of this bioelement. When biomass reached a stationary level, the *Spirulina* culture was supplemented with nonradioactive phosphorus to resume its growth. As can be seen from Figs. 1a and 1b, the sequential addition of phosphorus in 150 and 300 μg P/l amounts or in 300 and 600 μg P/l amounts failed to induce culture growth, indicating that the replenishment of the intracellular phosphorus pool was incomplete. Only after the single addition of stable phosphorus in an amount of 1500 μg P/l (Fig. 1a) or the total addition of

about 2000 μg P/l (Fig. 1b), when the intracellular phosphorus pool was evidently replenished, was the growth of *Spirulina* resumed. These experiments confirmed the existence of the intracellular phosphorus pool in *Sp. platensis* and showed that its growth becomes possible only after the pool is completely replenished. In other words, the intracellular content of phosphorus is a limiting factor for the growth of *Spirulina* cultures.

We also derived the dependence of the specific growth rate of *Sp. platensis* on the intracellular phosphorus content, which can be presented in the form of the Droop equation:

$$\mu = \mu_{max}(1 - q/Q),$$

where μ_{max} is the maximum specific growth rate, Q is the intracellular phosphorus content, and q is the existence constant, which is equal to the intracellular phosphorus concentration at $\mu = 0$. The parameters of the Droop equation—$\mu_{max} = 0.016\ h^{-1}$ and $q = 2500$ μg P/g dry biomass—most adequately fit the experimental data (Fig. 2).

The occurrence of the intracellular phosphorus pool in some species of alga [5] and cyanobacteria offers them some advantages in competition for limited amounts of this bioelement in aquatic habitats. The amount of the intracellular phosphorus pool and the degree of its completeness may influence the rate of phosphorus uptake by aquatic microorganisms and the rate of phosphorus turnover in aquatic environments. The rapid uptake of phosphorus observed by many investigators in early terms after its addition may result from the active replenishment of the intracellular phosphorus pool; therefore, the calculation of the phosphorus turnover rate based on only the rate of its uptake will be incorrect.

The use of the cyanobacterial monoculture for the assessment of phosphorus turnover showed that this process is complex. In particular, studies of phosphorus turnover in aquatic environments should take into account the existence of the intracellular phosphorus pool in *Sp. platensis* cells and its dependence on the growth phase [3].

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