Russian Journal of Plant Physiology, Vol. 43, No. 1, 1996, pp. 131–136. Translated from Fiziologiya Rastenii, Vol. 43, No. 1, 1996, pp. 149–155.


APPLIED RESEARCH

# Closed Photobioreactors for Microalgal Cultivation

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Received June 9, 1995

**Abstract**—The novel design of two closed photobioreactors, LUXFORS (8–12 l in volume) and Priboi (6–14 l), including their biological and technical characteristics, and the dynamics of the growth and productivity of *Chlorella*, *Spirulina*, *Porphyridium*, and *Dunaliella* cultures grown in these reactors are described. The productivity of these photobioreactors under conditions of 24-h illumination (sodium-discharge lamp DNaT-400) was 4.5 g/(l day) for LUXFORS and 4.2 g/(l day) for Priboi, and the efficiency of light use was 11.8 and 12.5%, respectively. *Chlorella* grown under natural sunlight in Moscow on clear days from June 1 to 5 exhibited a productivity of 64 g/m$^2$, which significantly exceeds that attained with other reactors under similar conditions. The LUXFORS photobioreactor, which is equipped with electronics from the firm INFORS HT, is more suitable for laboratory research, while Priboi is easy to scale up and can be used as a model for developing industrial-scale closed photobioreactors.

*Key words: microalgae - cyanobacteria - photobioreactor - productivity - efficiency of light use*

## INTRODUCTION

Unicellular green algae and cyanobacteria attract considerable interest as producers of diverse physiologically active substances (proteins, vitamins, valuable metabolites, and medicinal and veterinary preparations); they can also be used for the production of additives to food and fodder. Existing industrial and laboratory reactors for the cultivation of microalgae do not comply with the requirements for the mass production of biomass and cannot realize a high productivity of microalgae; this restrains the development of biotechnological methods for algal biomass production on an industrial scale. Thus, there is considerable interest in the development of new types of photobioreactors for the cultivation of unicellular photosynthesizing microorganisms; tubular [1, 2], planar [3, 4], high-biomass-density [5] reactors, and types in which light is directed into and distributed within a reactor through light guides [6] have been designed. To develop high-performance photobioreactors for algal cultivation, the following specific problems must be resolved:

(1) The reactor design should be universal and permit the cultivation of various unicellular photosynthesizing organisms.

(2) In order to ensure a high efficiency of light use by the culture, the cultivator design must provide for the uniform illumination of the culture surface and the fast mass transfer of $CO_2$ and $O_2$.

(3) Cells of microalgae are highly adhesive, which results in the rapid fouling of the light-transmitting surfaces of reactors. This necessitates photobioreactors to be frequently shut down for their mechanical cleaning and sterilization. The reactor design must prevent or minimize this fouling of the reactor, particularly of its light-transmitting surfaces.

(4) High rates of mass transfer must be attained by means that neither damage cultured cells nor suppress their growth.

(5) The photobioreactor must function normally under conditions of intense foaming, as often occurs in reactors with high rates of mass transfer.

(6) In order to attain high productivity, the volume of the nonilluminated parts of the reactor should be minimized.

(7) For the industrial-scale production of biomass, the energy consumption required for mass transfer and the arrangement of the light-receiving surface of the algal suspension must be reduced to its minimum possible level.

This work describes two new closed photobioreactors for the cultivation of unicellular photosynthesizing microorganisms and presents the results of growing microalgae and the cyanobacterium *Spirulina* in these reactors. We found solutions to overcome the difficulties listed above and used them in designing these photobioreactors.

## LUXFORS LABORATORY PHOTOBIOREACTOR

This photobioreactor has been developed by the firms DOKA (Zelenograd, Russia) and INFORS HT (Bottmingen, Switzerland) and the Institute of Plant Physiology of the Russian Academy of Sciences (Mos-

Abbreviation: PCV—packed cell volume

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cow). The photobioreactor uses centrifugal force to form the light-receiving surface of the algal suspension, a principle first suggested by V.A. Zhavoronkov (Institute of Chemical Engineering, Moscow).

The photobioreactor (Fig. 1) consists of a hermetizable vessel (1) made of stainless steel, which houses a double-walled water-cooled quartz flask (2) containing the light source, and a rotor (3), whose rotation leads to the formation of the light-receiving surface and enhances mass transfer in the culture. The reactor's housing has several openings for inserting sensors (4) and windows for the visual monitoring of the cultivation process (5). The algal suspension is thermostated by water circulating between the water jacket (6) and a thermostat.

A separate rack (7) contains the thermostat, the rotor drive, and the units that control and measure the rotation speed, temperature of the algal suspension, rates of gas flows, $pO_2$, and pH. This rack also contains the emergency and warning systems.

The volume of algal suspension can be varied from 8 to 12 l.

Intensive mass transfer is provided by the rotor, which is designed to avoid suspension foaming and prevent damage even to microalgal cells lacking cell walls. Centrifugal force spread the algal suspension (8) over the inner surface of the photobioreactor, thus preventing contact of the suspension with the light-transmitting surface of the quartz flask and providing for the uniform irradiation of the suspension and the efficient use of light energy. Any lamp of not more than 65 mm in diameter and 300 mm in length can be used as the light source (9). In the experiments reported below, we used a 400-W sodium-discharge lamp (DNaT-400, Russia). The reactor design and the materials used allow chemical or ultraviolet (ozone) sterilization of the reactor. Prior to sterilization, the light source is replaced by an UV lamp; this is why the flask that houses the light source is made of quartz. The water jacket (10) used for cooling the lamp must be emptied before the sterilization procedure.

Diverse photosynthesizing microorganisms were grown under optimum conditions determined in preliminary experiments. The organisms were cultured under 24-h/day illumination; air containing 2% $CO_2$ was supplied to reactor at a rate of 2 l/min. *Chlorella* was also grown under 100% $CO_2$ (at a flow rate of 0.01–0.04 l/min). $CO_2$ was supplied to reactor at a flow rate calculated from the expected growth rate of the culture. $CO_2$ flow was adjusted depending on the pH of the culture medium. The flow rate was lowered when the pH of the algal suspension decreased below 7.5. In all experiments, the volume of the algal suspension was 10 l.

Figure 2 shows the growth curves of several algal cultures in the LUXFORS photobioreactor; the curves show no signs of growth suppression. No negative effects on the physiological state of algal cells were observed during cultivation.

Judging by the packed cell volume (PCV) (Fig. 2), the maximum density of the *Chlorella* culture (strain IPPAS C-1) reached 20–22 ml/l within 60–70 h of culturing, which is equivalent to about 8–10 g biomass per liter. The maximum growth rate corresponding to the linear portion of the growth curve was 4.5 g dry wt/(l day) or 45 g per photobioreactor. The growth rate averaged over the growth cycle was 3.1 g/(l day), provided that the inoculum was 0.01 g/l.

The maximum biomass densities (g/l) were 9.6 for *Spirulina*, 6.2 for *Porphyridium*, and 5.5 for *Dunaliella*; the maximum growth rates were 4.2 for *Spirulina*, 1.7 for *Porphyridium*, and 1.5 g/(l day) for *Dunaliella*.

Figure 3 shows changes in pH and $pO_2$ in a *Chlorella* culture grown in $CO_2$-enriched air. The pH of the culture medium increased from 6.6 at the onset of culturing to 8.0 at the end of the cultivation period, remaining within the physiological limits. The oxygen partial pressure increased from 21% to 28% at the beginning of cultivation, when the highest growth rates were observed; then, the oxygen partial pressure gradually decreased to 23%.

The cell density of the *Chlorella* culture grown for 74 h in 100% $CO_2$ (Fig. 4) was as high as 10.6 ml/l PCV (4.2 g biomass per liter). The maximum rate of biomass increase was 2.7 g/(l day) or 27 g/day per photobioreactor, which is 1.7 times lower than in culture grown in $CO_2$-enriched air.

When cells were grown in 100% $CO_2$, the concentration of dissolved oxygen in the algal suspension attained 80% or more at the initial stages of culturing and then decreased to 40% because of the retarded growth rate of the culture. When high $pO_2$ values were attained and the growth rate was slowed down, the algal suspension was bubbled for 30 min with ambient air at a rate of 2 l/min. As a result, the concentration of dissolved oxygen declined to 24–30%, and the culture resumed growth. The eventual cell concentration was equivalent to 18 ml/l PCV, which is close to that of cultures grown in $CO_2$-enriched air. It should be noted that, even in the early period of growth, when the concentration of dissolved oxygen was relatively low, the growth rate of the algae was lower at 100% $CO_2$ than at 2% $CO_2$. It is likely that high $CO_2$ partial pressure inhibits culture growth in the absence of an inert carrier gas (nitrogen) [7, 8].

These experiments allowed us to determine the principal biological and technical characteristics of the photobioreactor; our calculations refer to the most productive culture of *Chlorella*:

(1) Algal suspension volume: 8–12 l.

(2) Maximum biomass production by a liter of algal suspension per day: 4.5 g (dry wt).

(3) Total biomass production by the photobioreactor: 45 g/day.

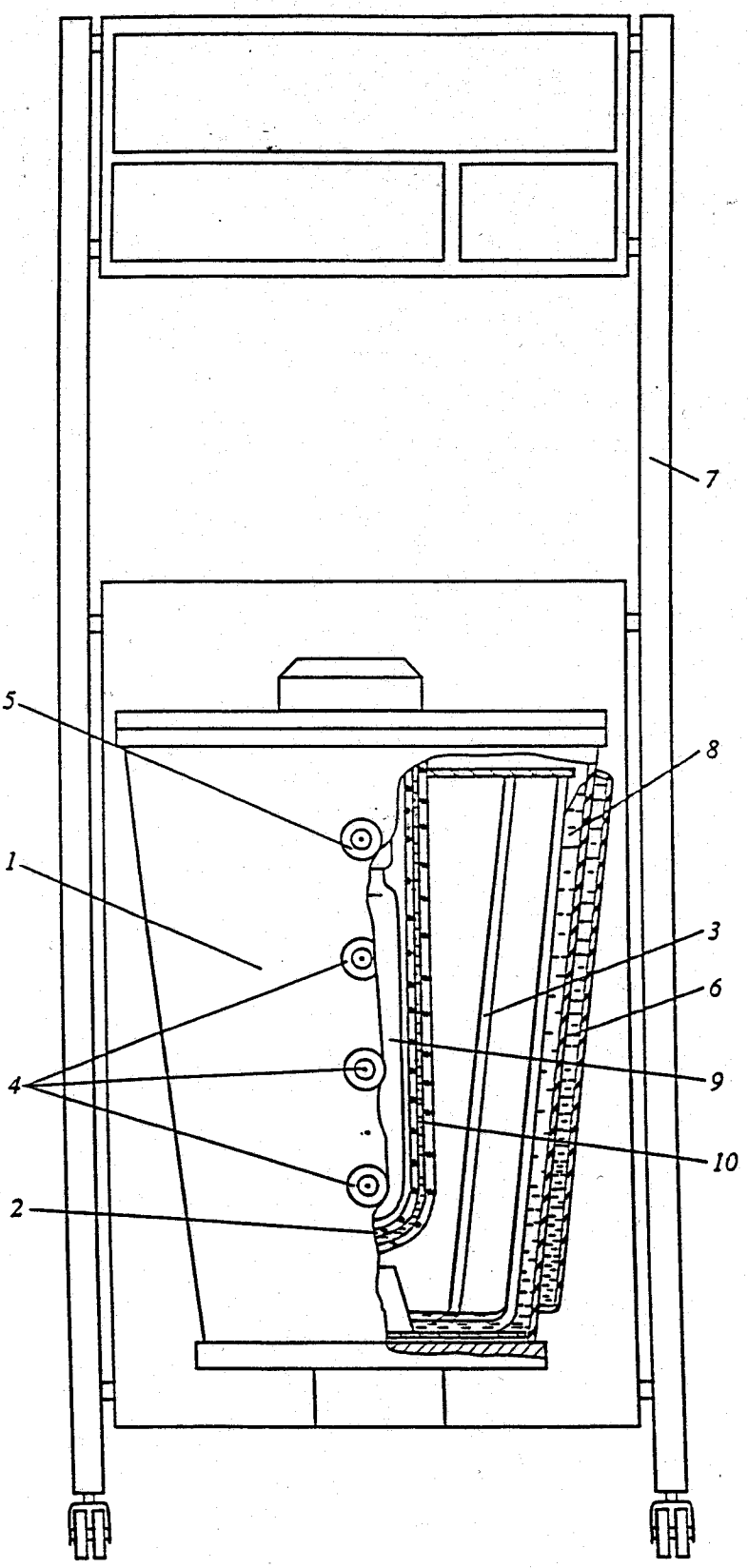


**Fig. 1.** Schematic diagram of the LUXFORS photobioreactor:
(*1*) hertmetizable housing of the photobioreactor; (2) water-cooled quartz flask; (*3*) rotor that enables the formation of the light-receiving surface and enhances mass transfer in the culture; (*4* and *5*) openings for inserting sensors and monitoring the cultivation process; (*6*) thermostating jacket of the photobioreactor; (*7*) rack housing the rotor drive and control and recording systems; (*8*) position of the algal suspension in an operating photobioreactor; (*9*) light source; (*10*) cooling water for the light source.

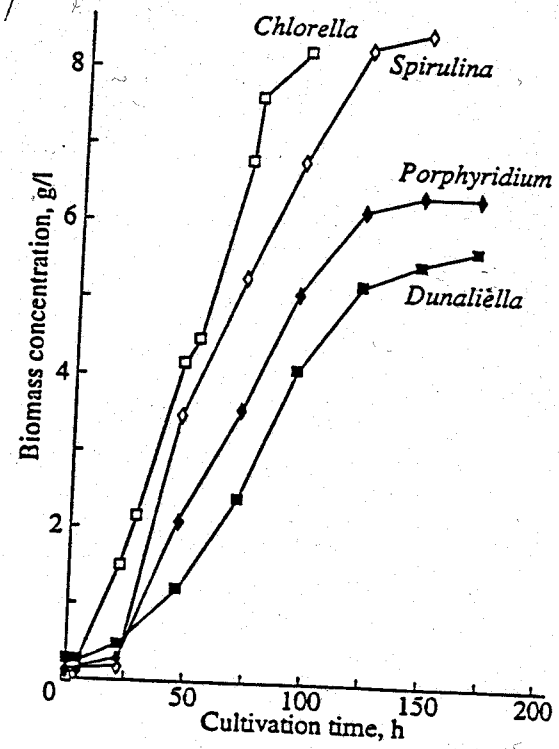


Fig. 2. Curves of algal culture growth in the LUXFORS photobioreactor.

(4) Biomass production per 1 $m^2$ of illuminated surface: 136 g/day.

(5) Maximum density of algal cells in the photobioreactor: 22 ml/l (PCV) or 8.8 g/l (dry wt).

(6) Average thickness of the suspension layer: 27 mm at a rotor speed of 200 rpm.

(7) Area of the illuminated surface: 0.33 $m^2$.

(8) Average photon flux density at the level of suspension surface: 1750 μmol/($m^2$ s).

(9) Efficiency of light energy use: 11.8%.

To date, the LUXFORS photobioreactor has run more than 3000 h without any loss in performance.

## PHOTOBIOREACTOR PRIBOI

The photobioreactor Priboi was designed for the laboratory cultivation of a broad range of unicellular photosynthesizing organisms. However, its design allows the apparatus to be scaled up for industrial biomass production.

This photobioreactor (Fig. 5) has a flat horizontal light-receiving surface (1) that is convenient for solar and artificial illumination. The algal suspension (2) is poured into a stainless steel cuvette (3). The thickness of the suspension layer may vary from 3 to 7 cm, which

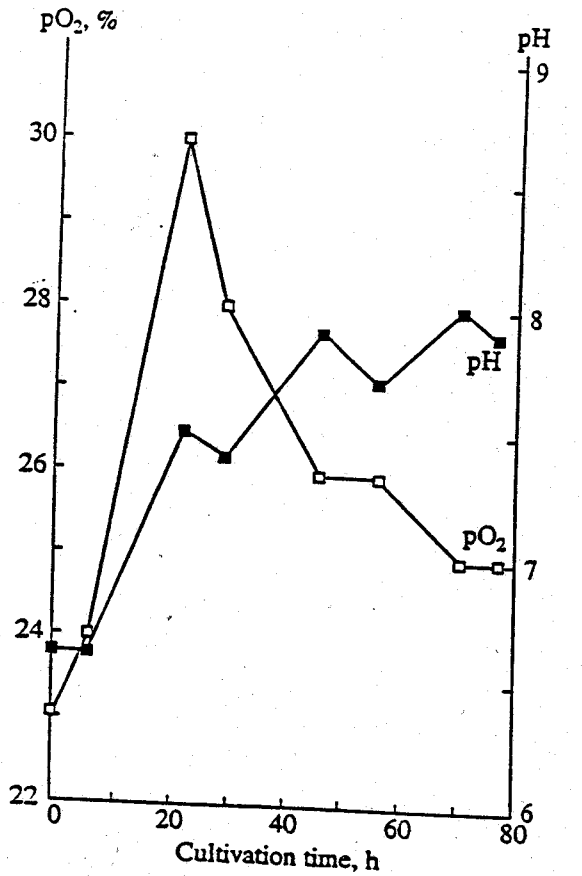


Fig. 3. Changes in $pO_2$ and pH in *Chlorella* culture during its growth in the LUXFORS photobioreactor.

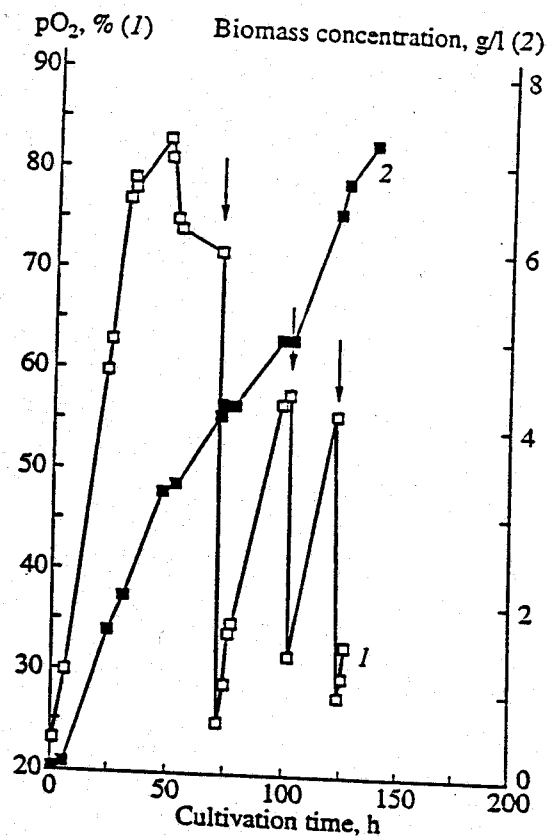


Fig. 4. Dynamics of biomass production and $pO_2$ changes in *Chlorella* culture grown in the LUXFORS photobioreactor in 100% $CO_2$. Arrows indicate the 30-min periods of bubbling the suspension with air flow.

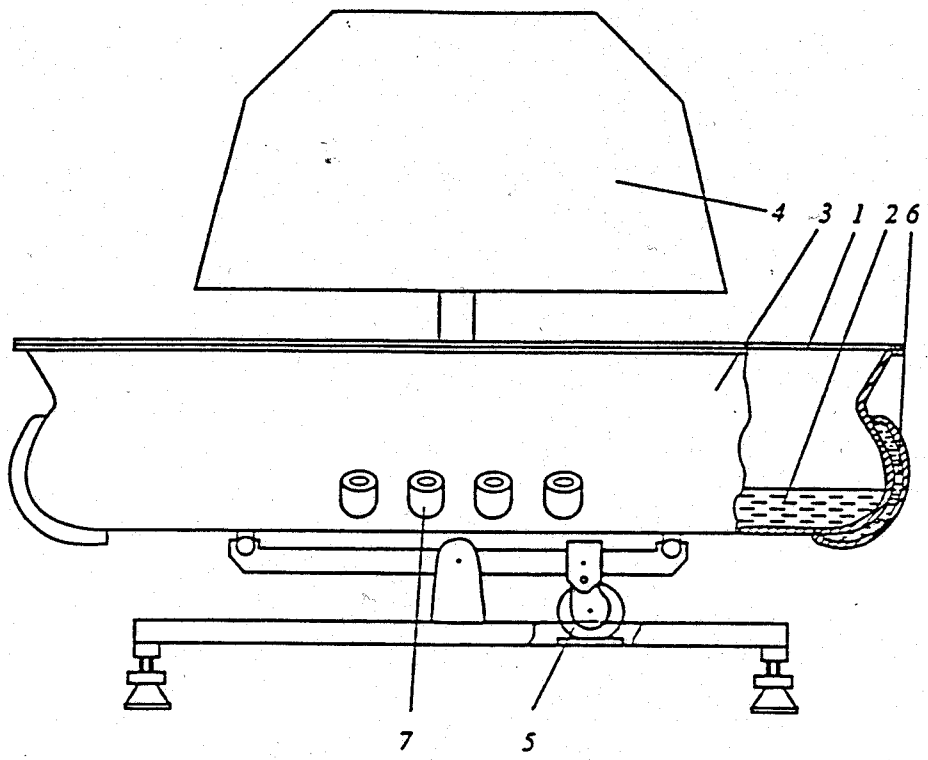


Fig. 5. Schematic diagram of the Priboi photobioreactor:
(*1*) light-receiving surface of the photobioreactor; (*2*) suspension of microalgae; (*3*) cuvette; (*4*) light source with a DNaT-400 lamp; (*5*) drive for the oscillatory motion of the cuvette; (*6*) thermostating water jackets; (*7*) openings for sensors monitoring characteristics of the cultural medium.

corresponds to a suspension volume from 6 to 14 l. In the laboratory, algal suspensions were illuminated by a detachable light source (4) containing a DNaT-400 lamp. The height of the lamp above the light-receiving surface can be adjusted, allowing variable light fluence rates at the suspension level. The cuvette is mounted on a drive (5), which moves the cuvette in an oscillatory motion along the cuvette's longitudinal axis. The oscillation frequency is adjusted to generate a swash effect in the cuvette, which provides an intense mixing and mass transfer of $CO_2$ and $O_2$. The suspension is thermostated with water running through the water jackets (6) connected to a separate thermostat. A stream of $CO_2$-enriched air or 100% $CO_2$ was directed into the space between the suspension and the light-receiving surface; the air easily penetrated the suspension because of the intense mass transfer in the cuvette. The photobioreactor has openings (7) for sensors to monitor characteristics of the culture medium.

Figure 6 shows the growth curves of diverse photosynthesizing microorganisms grown in this photobioreactor under artificial light. The volume of culture suspension made up 6 l. The photon flux density at the level of the light-receiving surface was 1511 μmol/(m² s) for *Chlorella* and *Spirulina* and 695 μmol/(m² s) for *Porphyridium* and *Dunaliella*.

*Chlorella* was also grown under natural sunlight in Moscow (June). The culture volume was 10 l. The photobioreactor was exposed to direct sunlight for 11 h per day. During the rest of daylight, the reactor was shaded, and the light intensity was not more than 220 μmol/(m² s). During the experiment, the midday air temperature changed between 29 and 32°C, and the highest light intensity varied from day to day within the range of 1250–1720 μmol/(m² s). The corresponding growth curve is shown in Fig. 7.

The rate of biomass production under these conditions was 1.3 g/(l day) or 13 g/day per photobioreactor. Biomass production, calculated per 1 m² of illuminated surface, was 64 g/day.

Biological and technical characteristics of the Priboi photobioreactor for *Chlorella* culturing were as follows.

(1) Algal suspension volume: 6–14 l.

(2) Maximum biomass production under artificial light by one liter of algal suspension per day: 4.2 g (dry wt).

(3) Total biomass production by the photobioreactor: 25 g/day.

(4) Biomass production under artificial light per 1 m² of illuminated surface: 125 g/day.

(5) Biomass production under sunlight: 64 g/day.

(6) Maximum density of algal cells in the photobioreactor: 24 ml/l (PCV) or 9.2 g/l (dry wt).

(7) Area of the illuminated surface: 0.21 m².

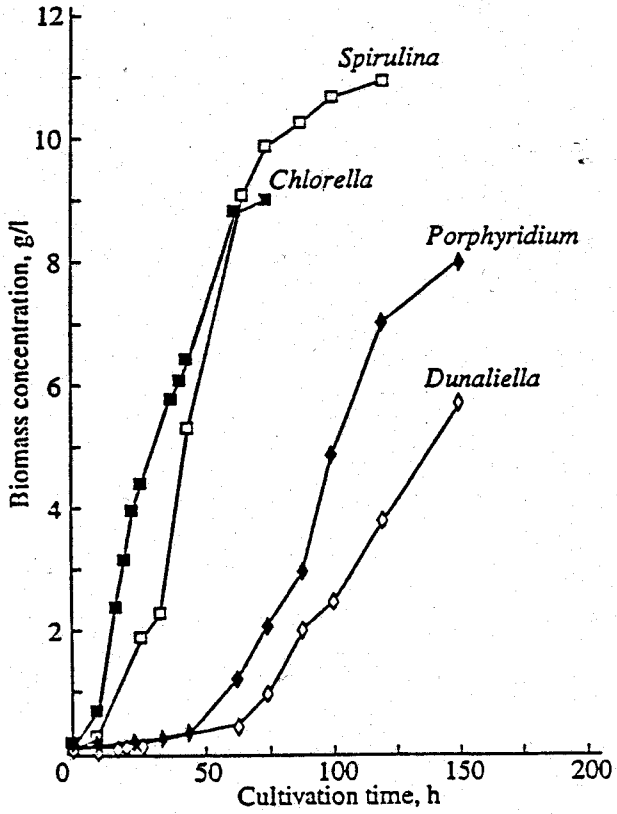


Fig. 6. Growth of microalgal cultures under artificial illumination in the Priboi photobioreactor.

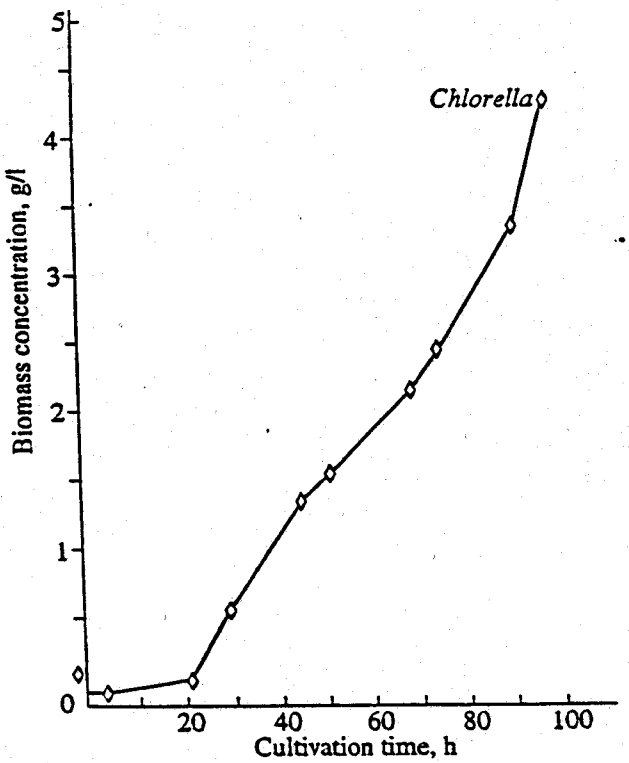


Fig. 7. Growth of *Chlorella* culture under sunlight in the Priboi photobioreactor.

(8) Maximum photon flux density at the suspension surface under illumination with a DNaT-400 lamp: 1511 μmol/($m^2$ s).

(9) Efficiency of light energy use: 12.5%.

(10) Power consumption to provide the swash effect: 1.2 W.

Our data show that these two photobioreactors have similar biological and technical characteristics. The LUXFORS photobioreactor, equipped with electronics from the firm INFORS HT, is more convenient for laboratory investigations and preparing inoculum destined for the industrial cultivation of microalgae. The Priboi reactor is easy to scale up; therefore, it can be used as a model for developing a closed photobioreactor designed for the industrial production of microalgal biomass.

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