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OBTAINING AN INOCULUM OF THE MICROALGA DUNALIELLA SALINA

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Abstract. The cultivation of microalgae is of increasing interest due to the growing demand for algal biomass and biologically active compounds. The productivity of Dunaliella salina cultures depends on a combination of cultivation parameters, including the composition of the nutrient medium, illumination, temperature, pH, carbon dioxide supply, and photobioreactor design. The aim of this study was to improve the cultivation technology of Dunaliella salina in order to increase cell concentration and biomass productivity through the use of a modified film-type photobioreactor. The object of the study was Dunaliella salina isolated from the Aral Sea. Cultivation was carried out in a 600 mL glass vessel containing 500 mL of nutrient medium under periodic illumination and continuous air bubbling. Cell growth was preliminarily assessed by direct counting using a Goryaev counting chamber. The stationary growth phase was reached on the fifth day, when the cell concentration reached 1.00 million cells/mL. Microscopic analysis of the sediment demonstrated the presence of reproducing microalgal cells. After one week of moderate illumination, new microalgal cells appeared from the sediment, and the resulting material was subsequently used as inoculum. A film photobioreactor equipped with ten transparent polyurethane plates was developed to increase the surface area available for microalgal growth and improve exposure of the culture film to light and carbon dioxide. Cultivation in the proposed photobioreactor increased the cell titer from 400 thousand cells/mL to 1.6 million cells/mL within 7 days, whereas the control reached 1.2 million cells/mL within 8 days. Thus, the proposed photobioreactor design demonstrated the potential to intensify Dunaliella salina cultivation and increase the final culture density.

Keywords: Dunaliella salina, microalgae, inoculum, microalgal biomass, photobioreactor, film cultivation, cell titer, cultivation technology, Aral Sea.

Introduction. The cultivation of microalgae is attracting increasing attention worldwide because of the wide range of applications of algal biomass and biologically active compounds. Green microalgae of the genus Dunaliella are widely used as objects of large-scale cultivation and applied research. Their biomass is of interest for the production of biologically active substances and other valuable products. Therefore, increasing the productivity and efficiency of microalgal cultivation remains an important scientific and technological task.

The productivity of microalgae depends on a combination of technological and environmental factors, including photobioreactor design, nutrient medium composition, carbon dioxide concentration, pH, temperature, and illumination intensity [1]. An appropriate combination of these parameters can provide favorable conditions for cell growth and maximum biomass accumulation. A considerable body of research has focused on the cultivation of Dunaliella, the production of biologically active substances, and the investigation of their properties [2].

For intensive accumulation of microalgal biomass, film-type bioreactors are widely considered as promising cultivation systems [3–4]. However, existing film-type bioreactor designs do not always provide sufficient surface area for the development and reproduction of microalgal cells. A

limited cultivation surface can reduce the efficiency of biomass accumulation. In addition, an inefficient distribution of the gas-air mixture during operation may result in increased specific energy consumption and reduced cultivation efficiency.The development of an effective inoculum is also an important stage in the cultivation process.

The physiological condition and concentration of the inoculum directly affect the subsequent development of the culture. In the present study, particular attention was therefore paid to the growth dynamics of Dunaliella salina, including the formation of sediment and the possibility of using microalgal cells from the sediment as inoculum.

The aim of this study was to improve the technology of Dunaliella salina cultivation in order to increase cell titer and biomass yield by obtaining an effective inoculum and developing a modified film-type photobioreactor.

Materials and Methods. 2.1. Biological material. The object of the study was the microalga Dunaliella salina isolated from the Aral Sea.

2.2. Nutrient medium. The following nutrient medium was used for the cultivation of Dunaliella salina:

- NaCl — 116 g/L;
- $MgSO_4 \cdot 7H_2O$ — 50 g/L;
- KNO_3 — 2.5 g/L;
- K_2HPO_4 — 0.2 g/L;
- trace-element solution — 1 ml.

The composition of the cultivation medium was selected according to the previously described approach [5].

2.3. Cultivation conditions. Initial cultivation was performed in a glass vessel with a total capacity of 600 mL, a height of 16.5 cm, and a diameter of 7.0 cm. The volume of nutrient medium was 500 mL, corresponding to a liquid height of approximately 9 cm.

The culture was cultivated under an illumination intensity of 1000 lx with air bubbling through the nutrient medium. The light/dark cycle consisted of 16 h of illumination followed by 8 h of darkness. The temperature of the culture medium was maintained within the range of 22–27°C.

When the inoculum reached the stationary growth level and the cell concentration reached 1.00 million cells/mL on the fifth day, the inoculum-to-nutrient-medium ratio was 1:5.

2.4. Determination of cell concentration. A preliminary assessment of microalgal growth was performed by direct counting of cells using a Goryaev counting chamber. The dynamics of cell concentration were used to determine the stage of culture development and the appropriate time for obtaining the inoculum.

2.5. Obtaining the inoculum from the sediment. Under periodic illumination and continuous air bubbling, the culture reached the stationary growth phase with maximum culture density on the fifth day. On the sixth day, a decrease in culture density was observed, which was associated with cell aggregation and sedimentation.

The sediment was examined microscopically. The analysis demonstrated that microalgal reproduction continued in the sediment. The sediment containing microalgal cells was collected and maintained under moderate illumination of 1000 lx. After one week, small green microalgal cells began to appear from the sediment. The resulting material was subsequently introduced into the bioreactor and used as an inoculum.

2.6. Design of the film photobioreactor. A modified film-type photobioreactor was developed for intensive cultivation of Dunaliella salina (Fig. 1).

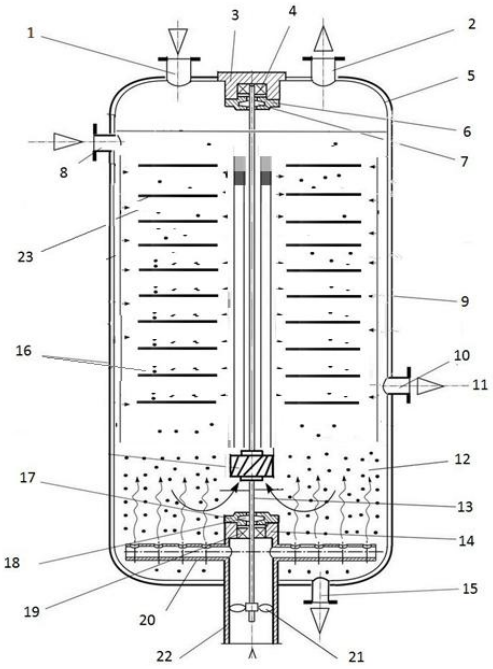


Fig.1. Developed photobioreactor design

1 – photobioreactor housing; 2 – mirror surfaces; 3 – incandescent lamp; 4 – bubbling device; 5 – nozzle; 6 – shaft;7–8 – bearings; 9 – bearing housing; 10–12 – bearing cover; 12–13 – seals; 14 – impeller; 15 – inner surface (culture liquid); 16 – plates; 17 – fitting for introducing the culture liquid (microalgal suspension); 18 – fitting for withdrawing the culture liquid (finished biomass); 19 – fitting for introducing cooling air; 20 – fitting for withdrawing cooling air; 21 – fitting for withdrawing the spent carbon dioxide-air mixture; 22 – rotary blower.

The main structural feature of the developed bioreactor is the presence of transparent horizontal plates. The use of these plates increases the available surface area for microalgal development while simultaneously providing exposure of the culture liquid film to light energy and carbon dioxide.

The presence of a gas-air mixture pressure regulator in the suspension inlet section is intended to contribute to more rational consumption of CO_2 within the apparatus.

To increase the surface area available for cells settling into the sediment, ten transparent polyurethane plates with a radius of 7 cm were placed inside the bioreactor at a spacing of 1 cm.

Results. Growth dynamics and inoculum formation. During cultivation under periodic illumination and air bubbling, the maximum culture density was observed on the fifth day. At this stage, the cell concentration reached 1.00 million cells/mL, corresponding to the stationary phase of growth.

On the sixth day, a decrease in culture density was observed. This decrease was associated with cell aggregation and subsequent precipitation. However, microscopic examination of the sediment demonstrated that the sediment was not biologically inactive: microalgal reproduction continued within the sediment.

The collected sediment was maintained under moderate illumination of 1000 lx. After one week, small green microalgal cells began to emerge from the sediment. When this material was introduced into the bioreactor as an inoculum, an increase in culture titer was observed.

These observations indicate that the sediment formed during cultivation may contain viable Dunaliella salina cells capable of further development and may therefore be considered a potential source of inoculum.

Cultivation in the developed photobioreactor. The developed film-type photobioreactor was designed to provide a larger surface area for the development and reproduction of microalgal cells. Ten transparent polyurethane plates were incorporated into the apparatus.The cultivation results demonstrated an increase in microalgal cell concentration from 400 thousand cells/mL to 1.6 million cells/mL within 7 days when using the proposed photobioreactor. Under the control conditions, the cell concentration reached 1.2 million cells/mL within 8 days.

Thus, the proposed cultivation system resulted in a higher cell titer and a shorter cultivation period compared with the control conditions.

As a result of cultivating microalgae in the proposed photobioreactor, it was possible to increase the microalgal cell titer from 400 thousand cells/mL to 1.6 million cells/mL within 7 days compared with the control (1.2 million cells/mL within 8 days), which consequently made it possible to increase the microalgal biomass yield. The obtained results showed that when microalgae were cultivated using 10 plates, the cell titer in the culture medium volume (500 mL) increased twofold.

When ten transparent plates were used, the cell titer in the 500 mL culture volume increased substantially. Compared with the control, the proposed photobioreactor provided a higher final culture density and enabled the accumulation of a greater amount of microalgal biomass.

Discussion. The obtained results demonstrate the importance of both inoculum preparation and photobioreactor configuration for the cultivation of Dunaliella salina. The observation that viable microalgal cells remained in the sediment after the culture density decreased suggests that sedimentation does not necessarily indicate the complete termination of culture development. Under appropriate illumination conditions, the sediment can retain viable cells capable of subsequent reproduction.

The use of the sediment-derived material as inoculum was accompanied by an increase in culture titer after its introduction into the bioreactor. This observation is relevant for the practical organization of microalgal cultivation because the preservation and subsequent use of viable cells may contribute to maintaining the culture and obtaining a sufficiently concentrated inoculum.

The design of the proposed film photobioreactor provides additional surfaces for the development of microalgal cells. The ten transparent polyurethane plates increase the surface area available for the culture and facilitate simultaneous exposure of the culture film to illumination and the supplied gas-air mixture. The use of a coaxial lamp arrangement and mirror surfaces also creates conditions for more uniform illumination during culture recirculation.

The experimental results showed that the developed photobioreactor allowed the cell titer to reach 1.6 million cells/mL within 7 days, whereas the control culture reached 1.2 million cells/mL within 8 days. This difference indicates that the proposed design can intensify the cultivation process under the studied conditions.

The results are consistent with the general principle that microalgal productivity depends on the combined effects of illumination, nutrient availability, gas exchange, temperature, and cultivation-system configuration [1–4]. At the same time, the results obtained in this study are specific to the investigated Dunaliella salina culture and the cultivation conditions described above.

An additional advantage of the proposed design is the possibility of reducing the overall dimensions of the cultivation apparatus by replacing a multistage system with a single-stage configuration. The increased surface area created by the transparent plates can potentially improve

the efficiency of biomass accumulation without requiring a proportional increase in the total volume of the apparatus.

Conclusion. The study demonstrated the possibility of obtaining an inoculum of Dunaliella salina from sediment formed during cultivation. Microscopic examination showed that viable microalgal cells continued to reproduce in the sediment. After one week of moderate illumination at 1000 lx, small green microalgal cells appeared and were subsequently used as inoculum.

A modified film-type photobioreactor containing ten transparent polyurethane plates was developed to increase the surface area available for microalgal development and to improve the exposure of the culture film to light and carbon dioxide.

Under the investigated cultivation conditions, the use of the proposed photobioreactor increased the cell titer from 400 thousand cells/mL to 1.6 million cells/mL within 7 days, compared with 1.2 million cells/mL within 8 days under control conditions.

The proposed photobioreactor provides several technological advantages:

- increased final biomass yield due to the additional surface area provided by the transparent plates;
- improved conditions for microalgal development and reproduction;
- more uniform illumination of the culture liquid during recirculation;
- more efficient use of the available cultivation volume;
- the possibility of reducing the overall dimensions of the apparatus through a single-stage design.

Therefore, the developed approach can be considered a promising method for intensifying the cultivation of Dunaliella salina and increasing the concentration of microalgal biomass.

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