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A New Approach to Spirulina Cultivation

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ABSTRACT

This paper presents an overview of a novel approach to spirulina microalgae cultivation. The most optimal design of a photobioreactor with compact dimensions and, at the same time, a large production area is proposed. The research areas that can significantly impact increasing spirulina cultivation productivity are outlined. Results of studies confirm the relatively high productivity of spirulina cultivation, which is more than 30 g/l of dry biomass per 1 sq. m per a day.

Introduction

Nowadays, microalgae are gaining popularity in worldwide market, including spirulina and Arthrospira genera. The relevance of research related to the cultivation and use of spirulina and its processed products is due not only to the content of many valuable substances such as proteins, vitamins, and some essential minerals (www.fdc.nal.usda.gov) but also to the possibility of their use in many areas of human activity such as agriculture, medicine (Nawal, et al., 2022), food industry (www.fao.org), crop production, environmental protection, etc. (Muzafarov and Taubaev, 1984).

Spirulina is a blue-green algae belonging to the oscillatoraceae-class of cyanobacteria. It is about 500 million years old and is one of Earth`s first photosynthetic life forms. A detailed mention of spirulina dates back to 1964 when J. Leonard discovered an aboriginal tribe in the African forests near Lake Chad that ate spirulina cakes. Unlike other African inhabitants, they had a long life expectancy, well-preserved teeth, and thick hair, and they rarely got sick. In 1974 spirulina was recognized by the World Food Conference of the UN as possibly the most healthy food for the future. Spirulina production for food purposes is gaining momentum in overpopulated countries such as China and India. In these countries, spirulina is officially recognized as the "national food" (www.un.org).

Taking into account the drawbacks of cultivation of spirulina in different types of photobioreactors, in particular equipment complexity and reliability and relatively low efficiency and productivity, we conducted R&D to determine the optimal structure of photobioreactors and designed and constructed the first modular prototype model, which was successfully tested and showed promising results. The core objective is to have developed technology for the production of spirulina with a productivity of more than 20 g/l of dry biomass per 1 sq. m a day.

Materials and methods

The current research and experimental works were carried out in the R&D department of Smart Technology Systems, JC Armenian Branch. This department is equipped with laboratory and production facilities such as press filters, centrifuges, and water treatment units based on reverse osmosis and deionization sections. The R&D object was the design and creation of an industrial-scale modular prototype photobioreactor for spirulina cultivation based on affordable and technically feasible materials with a productivity of more than 20 g/l of dry biomass per 1 sq. m a day (Meshcheryakova et al., 2015), which is higher than in conventional cases.

It was considered that the existing traditional methods for cultivating spirulina biomass have several drawbacks; in particular, they are not environmentally sound or cost-effective. Also, it's worth noting that the industrial large-scale production of phototrophic microorganisms, in general, faces problems related to the control, monitoring, and aseptic cultivation of technological processes at all stages, which complicates the production of the desired product with a predetermined biochemical composition as it also depends largely on lighting conditions (Bondarev and Muzafarov, 2016). The design of the proposed photobioreactor system is carried out to ensure the optimal light intensity for the microalgae cultivation, as it is one of the key factors in photobioreactor design together with nutrients and pH (Rajendran and Anderson, 2014).

From the beginning, there were several geometrical types of photobioreactor cells, such as spheric, cuboid, cylindrical, rhomboid, and other forms. Based on that, the existing operating photobioreactors are diverse, including: 1. Tubular photobioreactor; 2. Flat-panel photobioreactor; 3. Bubble columnar (bubbling) photobioreactor, and 4. Film photobioreactor.

The most important aspect of any photobioreactor is to provide efficient light intensity. In particular, the photobioreactor design should provide intense and uniform lighting for the culture. It is very important to use a combination of artificial and natural light sources here; otherwise, the cost of the resulting product will be uncompetitive. To uniformly supply light to each cell in the required amount, it is convenient to use sources evenly distributed in the culture liquid. Microalgae use light as an energy source for the synthesis of protoplasm. The microorganism growth rate is maximum when intensive light saturation is reached and decreases if it is less. As mentioned, light can be of different natures (natural or artificial), intensities, and wavelengths, and these parameters can improve or worsen growth conditions.

The listed photobioreactors, despite their design, do not always meet the desired requirements since they do not have a compact size or a sufficiently extended layer of two-sided lighting for a liquid mass of up to 10 cm. Large dimensions worsen not only the light intensity but also heating, cleaning, sterilization, harvesting, occupying a large area, etc.

The experience showed that the cuboid type of photobioreactor cell is preferable as it has several advantages: it is technically easy to carry out, there is sufficient light penetration or intensity across the photobioreactor cell, efficient distribution of carbon dioxide and salts in the whole volume, which are key factors for microorganism growth (Zarrouck, 1966) together with temperature control of the photobioreactor cell's liquid (Badger and Price, 2003), easy maintenance, etc. The growth process in the proposed photobioreactor system was carried out in 2021 inside the industrial building of the company in Yerevan, in multiple rounds to get accurate results and also to handle any problems that occurred during technological processes. For high spirulina productivity in a photobioreactor system, the following conditions are required: carbon dioxide supply, water supply, optimal temperature, nutrients supply, efficient light intensity, culture density, pH levels, gas exchange, and aeration. Fully grown, concentrated spirulina cultures are required for inoculum preparation and culture maintenance. The chosen spirulina mother culture must have a high proportion of coiled filaments and clear green color. In the technological line, mixing, aeration, centrifugation, and filtration are applied (Rajendran and Anderson, 2014).

Results and discussions

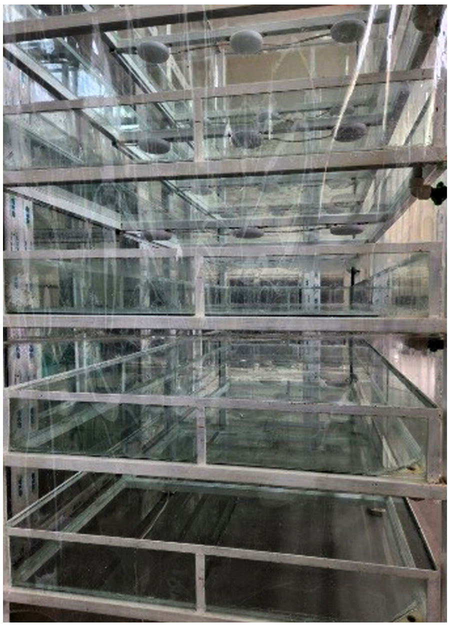
As a result of several years of studies of spirulina behavior under laboratory cultivation conditions, we have determined optimal growth conditions. Numerous experiments conducted in our modular prototype photobioreactor showed that spirulina can be cultivated efficiently indoors throughout the calendar year and cost-effectively. The designed and constructed modular photobioreactor is compact but, large.

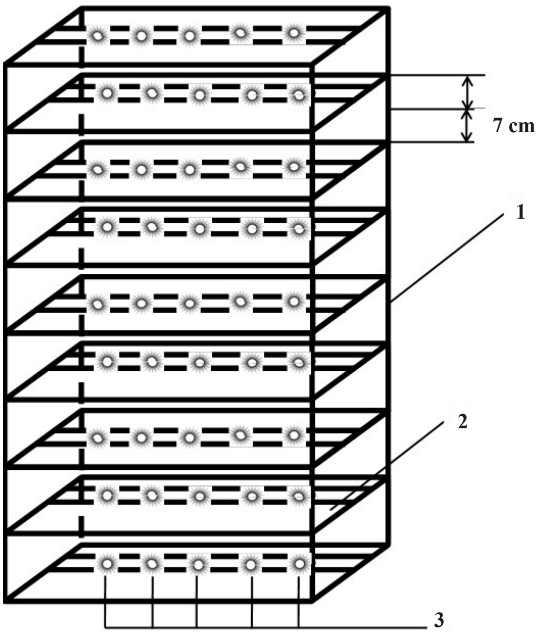
To create a photobioreactor with compact dimensions and a large production surface, we were guided by the simple idea that the existing design should include a 10- 40 cm depth of a cell, which will be assembled as a 2 - N number of cells in a vertical arrangement one above the other, thus increasing productivity by the same number of times in the area where the cell was located previously. The next step was to use plain glass in the photobioreactor design. As a result, we got cuboid-shape cells located on top of each other, in which spirulina is cultivated. All this made it possible to radically improve the light intensity throughout the cells. This is very important for intensive spirulina cultivation.

Requirements for the photobioreactors design used for the spirulina cultivation are more stringent than for chlorella or other similar microalgae. The fact is that spirulina consists of thin spiral threads, trichomes, and contains damage that leads to microorganism death. Therefore, the next important requirement for a photobioreactor is to mix the liquid mass in such a way so as to avoid damage to the microorganism trichomes. To solve the problem, technology for microbubbles was tested and implemented in a photobioreactor. In particular, a hydraulic pump takes liquid from the photobioreactor after a 1-μm-pore filter, where a certain amount of air is simultaneously supplied. At the outlet of the pump, a soft flow of liquid rich in air microparticles is obtained. This makes it possible to periodically mix the culture liquid without damaging microorganisms.

The modular prototype of the photobioreactor (Picture) is a structure with glass shelves arranged vertically, one above the other. The height of each shelf is calculated so that the depth of the loaded suspension does not exceed 14 cm. Depending on the building height, the number of photobioreactor shelves located vertically, one above the other, can be at least 5 and not more than 30. The optimal shelf width is 1 meter.

The length is based on the building parameters and can be from 1 meter to 100 meters or more. Glass shelves are attached in a vertical position using a metal frame. The strength of the metal structure depends on the number of shelves and their dimensions. LED lamps are installed between the shelves (LEDs are a real alternative to traditional sources, including for transferring light energy to the cells of microorganisms during cultivation) to provide the necessary amount of light at night and in cloudy conditions while providing the heat necessary for optimal growth of the culture (Fontoura, et al., 2018). The glass structure of the shelves allows the lighting of the 14-cm-deep pendant layer from above and below and the lighting of the 7-cm-deep suspension layer necessary for the intensive growth of spirulina. The best range of growth for spirulina is 1500 lux to 4500 lux (Sukenik, et al., 1991). In our system, we achieve light intensity up to 4200 lux, which ensures favorable conditions for the microorganisms' intensive growth.





Picture. Cuboid cell photobioreactor (photo and general layout of structured designed and constructed in the company). *1- The metallic frame of a photobioreactor, 2- plain glass, 3- LED lighting system (composed by the authors).*

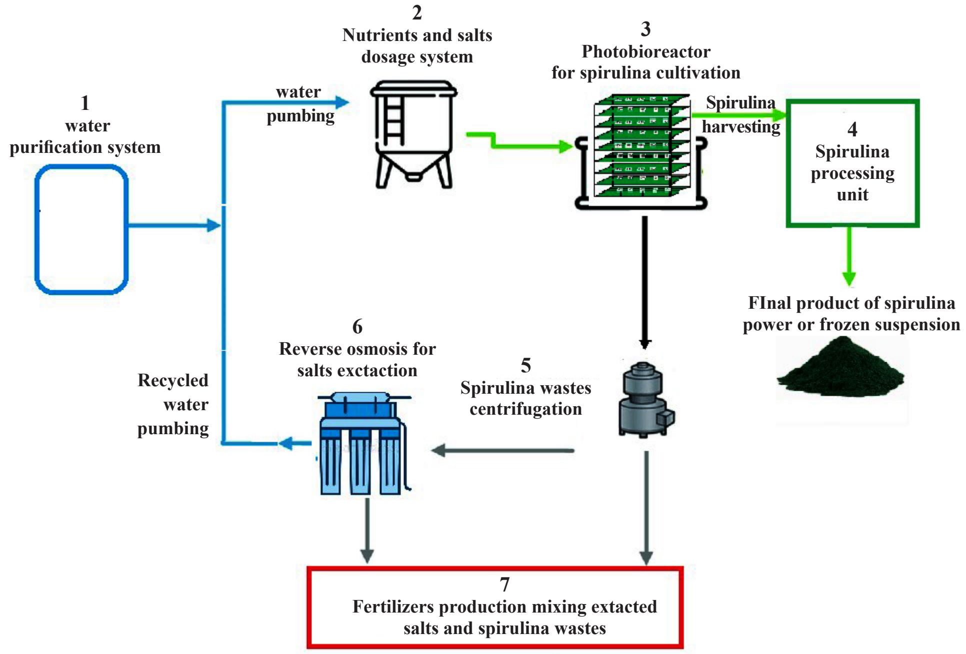


Diagram. The general layout of the full technological line for production of spirulina *(composed by the authors).*

The photobioreactor design provides easy sterilization to guarantee the high quality of the products. It is also important to ensure nutrient salt supply, periodic renewal of working suspension, periodic mixing of suspension, and the collection of ripened spirulina. All this is directly related to the photobioreactor and, in particular, to its design.

Throughout the entire period of research, the design of the photobioreactor has been constantly improved and adapted to the requirements of automatic control and adjustment of the pH of the suspension poured into the photobioreactor shelves, and optimal salt content (Belousov and Gurevich, 2005), frequency of salt mixing and working a suspension renewal, temperature and lighting. Special attention is paid to the complete automation of sterilization and harvesting ripened spirulina. The only human factor in the maintenance of the photobioreactor is that the operator monitors the technological processes of cultivating spirulina. This is done by applying special software.

Using the proposed approach, we have achieved the productivity of spirulina cultivation of 30–100 g/l of dry biomass per 1 sq. m a day. Ensuring optimal conditions such as pH >9, T-30 ^{0}C, for a 24-hour spirulina cultivation cycle, we got a minimum of 5 and a maximum of 30 g/l dried product.

Conclusion

R&D in spirulina commercial cultivation is of great practical importance for the development of the microbiological industry. The proposed technological line (Diagram) and model of the photobioreactors (representing a structure with glass shelves located vertically on top of each other), with possible further improvements, being modular can be scaled to an important industrial level. The availability of efficient equipment and analytical data based on our experience allow the development of microalgae production in a profitable and environmentally friendly way.

To sum up, the following can be stated with confidence.

- Based on modern scientific and technical achievements and more than 70 years of experience in microalgae cultivation, we have designed and constructed a photobioreactor necessary for the intensive cultivation of the spirulina microorganism with the best properties.
- Common and inexpensive materials – iron and glass – were used to manufacture the photobioreactor.
- Photobioreactors can be placed indoors with as much natural light as possible.
- Photobioreactors can be arranged from 1 to N number of parallel lines at a distance of 70 cm from each other.

- The length and number of photobioreactors depend on the length and width of the room, and the number of shelves depends on the room height.
- Photobioreactors are isolated from each other, sealed, and provide a high degree of sterilization.
- The design of photobioreactors allows the maximum use of natural light.
- The design allows automation of the entire process (from sterilization to harvesting) of spirulina cultivation.
- The design guarantees an unprecedented output of up to 100 g/l of dry biomass a day per 1 sq. m of production area in the form of the highest-quality finished product with competitive characteristics.

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