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BIOTECHNOLOGICAL PROSPECTS OF MICROALGAE

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The current state and perspectives of biotechnological use of microscopic algae were analyzed. The main directions of algobiotechnology, due to the physiological and biochemical features of these organisms, the volume of algae production in the world, the types of microalgae that had already been used or had practical prospects, ways of biomass obtaining and productivity increasing of industrial algae cultivation were given. The state of this problem, expediency of algobiotechnology development and prospects of microalgae cultivation in Ukraine were discussed.

Key words: algobiotechnology, microalgae, industrial cultivation, aquaculture.

Microscopic algae are considered as a new agricultural crop due to their valuable biochemical composition, high rate of reproduction and lability of metabolism. The industrial production of microalgae biomass — algobiotechnology — is a prospect for obtaining the renewable raw materials of various purposes, including replenishment of food and feed resources [1–7]. A significant advantage of this technology is that it does not increase the load on natural ecosystems, most of which are currently significantly depleted. The United Nations Organization [8] emphasizes the importance of enhancing the food security for the introduction of new technologies without sacrificing the environment, including the adjustment of production and use of food microalgae. At the same time, these organisms can find application not only as a food resource but also in many other areas.

Areas of use and perspective species of microalgae

In world practice, algae are used quite widely due to their physiological and biochemical characteristics. The most famous and investigated are *Chlorella vulgaris* Beijer., *Spirulina platensis* (Gomont) Geitler (*Arthrospira platensis* Gomont), *Dunaliella salina* Teod. Meanwhile, many other algae species are also suitable for practical application.

High content of proteins, carbohydrates, lipids, pigments, vitamins, polyunsaturated fatty acids, including essential, provides nutritional value of these organisms. Proteins content in the cells of algae is up to 45–65%, however they are well balanced by the content of essential amino acids and can be used for the enrichment of the amino acid composition of food.

Some of the microalgae have therapeutic and preventative effect in violation of the activity of the immune, endocrine, digestive, cardiovascular and nervous systems of animals and humans, having antitumor, antidiabetic, radioprotective and immunomodulatory activity. They are used in the medical and pharmacological fields, in diet nutrition, therapeutic cosmetology, in the production of biologically active supplements.

In a number of microalgae, regenerative properties were identified, so they are used to treat wounds and burns. In particular, in the Institute of Hydrobiology of the National Academy of Sciences of Ukraine, a method of chlorophyll-carotene paste obtaining from the algae as the basics of the medicinal preparation “Algofin”, an ointment with regenerative and anti-inflammatory properties, was developed [9].

In general, microalgae are a promising raw material for the production of antioxidants, vitamins, biomarkers, β-carotene, phycocyanin and others. These organisms are a rich source of natural food dyes that are used, in particular, in baby foods. Algae ability to direct biosynthesis of certain biologically active substances such as iodine-containing compounds of hormonal nature, alkaloids, steroids, etc. contributes to increasing an interest of their use.

Microscopic algae have prospects in the field of creating natural balanced feed for livestock, poultry and fish farming, including the cultivation of fish and invertebrates in aquaculture. Inland aquatic ecosystems undergo often significant anthropogenic changes. Loss and degradation of hydrobionts habitat, water pollution, overexploitation and intake, the introduction of alien species endanger the sustainability, biodiversity of hydro-ecosystems and formation of biological resources, which necessitates their artificial reproduction [10]. The use of green algae in fish farming increases the productivity of fishponds and the forage base for other aquatic organisms, as well as prevents their "flowering" and improves the hydrochemical status [11].

The advantages of microalgae use in livestock are animals productivity increase by improving immunity and cost reduction of veterinary products, feed consumption increased efficiency, the possibility of year-round feed enrichment with vitamins and natural biologically active compounds. The latter is of particular importance, as a large number of products are currently manufactured using food substitutes and synthetic preparations, which is a significant danger to living organisms consuming them.

Chlorella vulgaris and *Arthrospira platensis* are the most widely used for the needs of livestock [2, 3, 6, 7, 12–15]. More effective is introduction into the feed of a native suspension of algae, which contains a significant amount of valuable extracellular substances — the antibiotic chlorelin, arachidonic acid, amino acids, vitamins, enzymes, especially in the initial stages of culture growth [16].

In livestock and poultry farming, positive results were obtained when using other algae — *Chlorococcum, Spirogyra, Scenedesmus, Navicula, Nitzschia* and others [17]. It should be added that the biomass of the algae can be enriched with iodine, selenium or other essential elements [18, 19].

Microalgae in the form of dry powder, paste or suspension can be used in crop production to increase soil fertility and microbial activity, increase crop yields and accelerate their vegetation, reduce application standards of fertilizers, pesticides and growth regulators [20]. Algae have the most positive effect on crop yield in temperate zones and non-irrigated agriculture.

For soils recultivation, especially irrigated soils, it is advisable to use a suspension of cells of green algae (*Chlorella vulgaris*, representatives of the genus *Scenedesmus*) or nitrogen-fixing cyanobacteria (*Tolypothrix tenuis, Nostoc punctiforme, Anabaena cylindrica*). At one time, the effectiveness of seston using as a valuable organic fertilizer during "flowering" of the Dnieper reservoirs was proven [21]. Seston can also become a basis for the production of eco-friendly pure glue "Fitton", developed with the participation of specialists from the Institute of Hydrobiology of the National Academy of Sciences of Ukraine [22, 23], which is promising for agricultural plant seed pelleting. It should be added that some cyanobacteria (*Lyngbya majuscula* Harvey ex Gomont.) produce toxins that are active against phytopathogenic fungi [24], which may also find a use in plant production.

A considerable amount of research is related to the possibility of algae biomass usage to create alternative fuel types — biodiesel, bioethanol, hydrogen, methane [25–27]. It is commonly known that microalgae contain neutral and polar lipids. Polar lipids are mainly synthesized under favorable conditions, are characterized by high biological activity and are commonly used as food and dietary supplements. Neutral lipids are accumulated more in unfavorable conditions or under stress, are the main reserve substances of cells and are promising for the production of biofuels, biopolymers, etc. [28, 29]. Such biofuel is CO_2-neutral and its use will reduce the amount of gaseous emissions contributing to global climate change.

In order to improve biofuel production technologies, the search for promising species and strains of microalgae, ways of optimizing their cultivation and increasing the amount of lipid fraction, the methods of algae mass processing, in particular, methods for destruction of a cell membrane and extraction of lipid substances, development of photobioreactors structure, etc. are still under way [30–34]. Improving the mode of thermal treatment of algae biomass allows to reduce its duration and to convert from 50 to 65% of the raw material to so-called Biocrude, "artificial oil". This technology does not require predehydration of biomass, whereas usually high moisture content impedes complete phase separation and reduces the efficiency of lipid extraction. Additional catalytic treatment of microalgae biomass allows including proteins and carbohydrates in the biofuel production process, the destruction of which increases the yield of the product [35].

A number of microalgae species characterized by high lipid content were proposed as feedstock for alternative bioenergy: *Chlorella* sp., *Neochloris oleoabundans*, *Nannochloropsis* sp., *Botryococcus braunii*, *Dunaliella tertiolecta*, *Scenedesmus* TR -84 [35, 36]. In addition, autotrophic cyanobacteria and green algae are considered as promising objects capable of producing hydrogen for hydrogen energy [37], and many of the carbohydrate compounds can be used as a substrate for bioethanol production [38].

Work in the field of "green energy" is carried out in Ukraine. At the National Technical University of Ukraine "Igor Sikorsky Kyiv Polytechnic Institute", the scientific and technological bases for the conversion of algae biomass into biofuel are developed [39]. At the Kremenchuk Mykhailo Ostrohradskyi National University, it is offered to receive biogas from seston during "flowering" of Dnieper reservoirs. The developers are convinced that the invention can help to clean up the Dnieper and solve energy problems, in particular for the heat supply of small settlements [40].

Microalgae are one of the most important components in the system of biological treatment of domestic and industrial wastewater. As it is known, in Ukraine, most wastewater treatment plants use traditional biotechnologies that do not provide effective removal of phosphates and nitrates. At the same time, microalgae are able to use for growth only biogenic compounds, in addition, they saturate water with oxygen, which accelerates the oxidative processes and mineralization of organic impurities in wastewater [41, 42]. The possibility of using green algae for bioremediation of the aquatic environment contaminated with petroleum products and waste from pulp and paper enterprises was demonstrated [43, 44].

The cultivation of green algae on the runoff of livestock complexes enables to remove the excess of organic matter, to normalize odor and color, with a considerable part of Nitrogen returned to algae biomass and again to animals feed [45].

Algae cultivation allows the use such by-products of technological processes as heat and carbon dioxide excess, reducing their flow into the atmosphere. In intensive conditions of cultivation, algae are capable of 70% of CO_2 removing within eight hours [46]. In particular, the possibility of cultivation of some green chlorococcal algae using CO_2 concentrations in gas-air mixture from 0.2 to 16% was shown [47]. Our own research shown that green microalgae, in particular, representatives of the genus *Chlorella* and genus *Desmodesmus* had significant carbon dioxide assimilation potential, significantly increasing the growth rate [48–49].

It is known that in the formation of 1 kg of phytomass, microalgae absorb more than 1.8 kg of CO_2 from the surrounding air space, in addition, they are able to assimilate nitrogen oxides with partial conversion into gaseous nitrogen, as well as other mineral compounds, which include biogenic elements Sulfur, Potassium, Magnesium, Calcium, etc. [50]. In this regard, it is important to look for algae strains with increased ability to assimilate CO_2 and resistant to Sulfur and Nitrogen oxides [36].

Detailed studies of the tolerance limits and adaptive capacity of algae have considerable practical promise. For example, it is known that for many species the influence of temperatures above 35–40 °C is critical and is usually accompanied by loss of cell physiological activity. At the same time, at the incubation of the strain *Acutodesmus obliquus* (Turpin) Hegewald & Hanagata Syko-A Ch-055-12 IPPAS isolated from the activated sludge of the pulp and paper enterprise aerotanks, at 40–45 °C, some cells survived and continued vegetation, which made it possible to recommend a strain for sewage treatment in the temperature range from +15 to + 41 °C [51].

Nostoc muscorum Elenkin, *Scenedesmus acutus* Meyer, *Chlorella vulgaris* Beijer. species of *Neospongiococcum* genus can be used to treat sewage of the forestry complex, *Acutodesmus obliquus* — for urban wastewater treatment. These algae reduce the biochemical consumption of Oxygen in the effluents; accumulate ferrum, capable of decomposing phenolic compounds, etc. [52, 53].

Diatomic algae capable of synthesizing fats and fat-like substances as a basis for biodiesel production, mucopolysaccharides, and some unusual pigments (e.g. marennin) have considerable biotechnological potential [54]. Thus, *Cylindrotheca closterium* (Ehremberg) Lewin & Reimann is characterized by high content of polyunsaturated fatty acids and carotenoids, in particular fucoxanthin, which has antioxidant, antimutagenic and anticancerogenic properties and is used as a feed supplement for bivalve molluscs [55].

It was shown that representatives of genus *Euglena* produce protein, *Chlamidomonas* genus — carbohydrates, *Ankistrodesmus* genus — lipids, *Dunaliella* genus — carotene and tocopherol [56]. To obtain the ketocarotinoid astaxanthin, an extremely valuable preparation for aquaculture, the content of which reaches 4% in the dry matter of cells, in Japan (Fuji Chemical Industry) and the USA (Cyanotech), cultivation of *Haematococcus pluvialis* Flotow., thoroughly researched at one time by the specialists of the lapsed Institute of South Sea Biology of the National Academy of Sciences of Ukraine, has been mastered [57, 58].

The filamentous green algae *Cladophora* and *Rhizoclonium* are noteworthy, which develop abundantly in low-flowing water reservoirs. The cell envelope of these algae contains a significant amount of fiber that can be used in the production of various grades of paper and building materials.

Thus, microalgae have considerable potential for practical use. In this regard, algobiotechnology requires increased attention and expansion of biotechnological work directions, which involves the search for new strains and a detailed study of their biochemical characteristics and physiological properties.

Depending on the ultimate goal of algobiotechnologies, algae must first of all, grow and produce significant biomass in well-defined conditions — at the required temperature, light, pH value, medium composition, etc. Secondly, they must differ in some matters of metabolic features. Thus, to obtain feed materials, species with the optimal ratio of proteins, carbohydrates, lipids, biologically active substances (vitamins, carotenoids, coenzymes, etc.), with high nutritional quality and digestibility are required. For energy raw materials, it is necessary to select species with a high content of energy components, first of all, lipids. If it is necessary to treat sewage, algae should be tolerant of high concentrations of organic or mineral biogenic substances or contaminants (phenols, metal oxides, carbon dioxide, etc.). The selected species should also be characterized by stability of the main used characteristics, while having a labile metabolism and the ability to programmatically respond to external influences.

Ways to obtain biomass and increase the efficiency of industrial microalgae culture

In the southern latitudes, the cultivation systems for microalgae cropping can be placed in open areas, and in more moderate conditions — indoors. For the needs of animal husbandry, crop production, wastewater treatment, energy production, open systems (ponds, trays, and pools) can be used. For food or medical and pharmaceutical needs, where there are high requirements for algological and microbiological purity and product composition, closed photobioreactors can be used. In the first case, the process of algae growing is relatively uncomplicated and the cost of the biomass produced is low. Yet there is greater need for areas and high quality of production is not guaranteed. In the second case, product value increases significantly due to using the special equipment and more sophisticated technology. In such case the size of the occupied space decreases, and the complete control of the cultivation conditions ensures stable predicted quality of the product.

Despite considerable advances in the field of optimization and intensification of algae cultivation [59–64], the work is ongoing on improving the structures of closed photobioreactors and finding new materials [49, 65]. Thus, the use of polyethylene film has become widespread, it has been proposed to grow algae in tris-acetate-phosphate-pluronic, which is capable of being transformed from liquid into gel and vice versa when the temperature changes [16]. This improves lighting conditions, facilitates harvesting, and reduces energy consumption and duration of the cultivation process.

The use of genetic engineering methods to create highly productive algae strains capable of actively synthesizing certain compounds is becoming widespread. Thus, with the help of point genetic engineering, the new strains of microalgae were obtained on the basis of the genome of the freshwater chlorococcal *Acutodesmus dimorphus*, which should combine the best features of several planktonic species [66].

Despite the long history of biotechnology research, the potential of this field is not yet fully exploited. This applies both to the range of “new crops” and to the ways in which they are used and how to increase the content of valuable components. For example, according to the known patterns, the amount of lipids and carbohydrates increases at the stationary stage of algae growth or under stressful conditions. In this regard, in order to enhance the yield of these compounds, it is recommended to use two-stage technology: first to create optimal conditions for high crop yields, in the second stage algae should be stressed [29]. It was observed that lipid accumulation was facilitated by a decrease in the concentration of available nitrogen compounds, enhanced carbohydrate synthesis — by phosphate deficiency. However, such techniques significantly complicate the technology. At the same time, our studies shown that some algae had not only a high content of lipids, but also maintained it throughout the life cycle under normal cultivation conditions [67, 68]. Thus, active algobiotechnological studies would help to expand the range of promising algae species, optimize and reduce the cost of algae technologies.

Volumes of microalgae biomass production in the world

Analysis of scientific literature, press and internet publications shows that the production of microalgae biomass is gradually becoming traditional in many countries of the world. Symposia of the European Society of Microalgae Biotechnologies regularly take place in Hungary, analyzing new developments and current industry challenges [69]. In the United States, the first microalgae growing plants in artificial ponds were established in 1977, and industrial production of microalgae is gradually increasing [8, 26]. The largest capacities are concentrated in the USA, China, India, Japan, Thailand, Germany, Australia, and Israel. Well-known microalgae biomass producers are Royal, DutchShell (Hawaii), AlgaeBioFuels and Solazyme (USA), Aquaflow Bionomic Corporation (New Zealand), Mitsubishi (Japan) companies. In Europe Ingrepro B.V. (Netherlands) company offers the technological schemes for lipid-enriched biomass obtaining of microalgae.

In Europe and America, a variety of chlorella products are known, “Japan Chlorella” company produces its biomass for food purposes; about 1.5 thousand tonnes of dry biomass are produced annually in Taiwan; Malaysia and Philippines consume for food needs over 500 tonnes of algae. In Africa and Mexico, a significant amount of protein concentrates are produced from spirulina, using alkaline lakes to grow them. Italy develops spirulina cultivation technology in seawater and in closed-type cultivators.

There are some small enterprises in Russia (OOO, limited liability companies under the laws of Russian Federation, such as “Ecofactor”, “Legion Center”, “Solixant”) that produce chlorella as a pure suspension or with lactic and bifidobacteria for livestock, and spirulina, as well as Omega-3 polyunsaturated fatty acids and carotenoids from freshwater and marine algae. “Energotehnoprom” company (Kazan) produces bioreactors of different capacity for growing chlorella [70]. It is traditional to grow microalgae for agriculture in Central Asia — for animal husbandry, crop production, fur farming and silk production [56, 64]. There is growing interest in this problem in Belarus, where a large complex of biotechnological works and patenting of development is being performed [63].

Prospects for microalgae cultivation in Ukraine

In Ukraine, active cultivation of microalgae and their use in animal husbandry began in the 1970s [71]. Significant achievements in this area were obtained by scientists of the Institute of Hydrobiology of the National Academy of Sciences of Ukraine, headed by prof. L.Ya. Sirenko. At the Institute, up to now there is a collection of living microalgae cultures created by Lydia Yakimivna (Fig. 1). The staff of the Institute performed a large complex of biotechnological works to find new directions for algae biomass using, ways of seston utilization of Dnieper reservoirs, development of microalgae cultivation technology in tubular photobioreactors (Fig. 2) and introduction of it into the department of microalgae industrial cultivation on the basis of Ladyzhinskaya Thermal Power Plant for the needs of livestock and fish farming, etc. [21, 61, 62, 72–76].

At present, unfortunately, in Ukraine the market for this product is almost not filled, the needs are met mainly due to foreign supplies (partly from Europe and mostly from China) and only a few companies supply biomass of domestic algae. In particular, the limited liability company under the laws of Ukraine, “Mercury-II”, in the framework of a joint scientific project with of the lapsed Institute of South Sea Biology of the National Academy of Sciences of Ukraine, started growing *Spirulina platensis* (the trademarks “Living Spirulina AlgaeLife” and “SpirulinaLive”) since 2007 in the Kharkiv region to implement it in Ukraine and abroad, continuing to further develop and improve the technology. Within this company, the scientific-production firm “Prombiotechnika” (Odesa) offers bioreactors for the cultivation of microalgae. In Vinnitsa region Bar branch of the company “Tsukorpromvodonaladka” grows *Chlorella* and *Scenedesmus* for the treatment of sewage of food industry enterprises. “Chlorella Ukraine” (Bila Tserkva) private enterprise offers chlorella

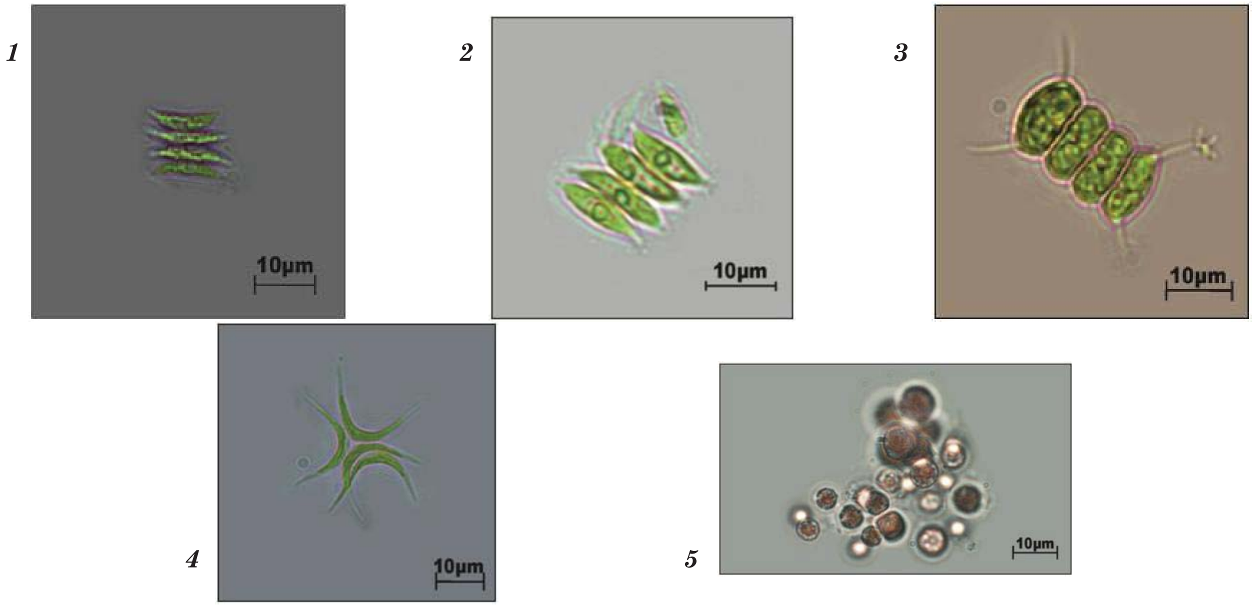

Fig. 1. **Microscopic algae from the collection of the Institute of Hydrobiology of the National Academy of Sciences of Ukraine HPDP**: *1 — Tetradesmus dimorphus* (Turpin) M.J. Wynne HPDP–108 (=*Acutodesmus dimorphus* (Turpin) P. Tsarenko); *2 — Tetradesmus obliquus* (Turpin) M.J. Wynne HPDP–104 (=*Acutodesmus obliquus* (Turpin) E. Hegew. et Hanagata); *3 — Desmodesmus communis* (E. Hegew.) E. Hegew. HPDP–109; *4 — Messastrum gracile* (Reinsch) T.S. Garcia HPDP–115 (=*Selenastrum gracile* Reinsch); *5 — Porphyridium purpureum* (Bory) K.M. Drew et R. Ross HPDP–141 (=*Porphyridium cruentum* (Gray) Nägeli)

for various needs in the form of suspension, concentrate, paste or dry powder [78].

Recently, Ukrainian entrepreneurs are focusing on the development of aqua farming. For this purpose, they are given the opportunity to rent parts of reservoirs for fish breeding in seas, reservoirs, garden farms, create favorable conditions for investment and credit. The state is trying to promote aquaculture and mariculture development in accordance with world standards, with the aim of producing organic aquaculture products, in particular, the cultivation of a number of freshwater and marine fish and shellfish species. In this regard, it is also promising to grow microalgae as a component of fish and invertebrates feed.

A significant impediment to the implementation of algobiotechnology, especially in Ukraine, is the unwillingness of domestic entrepreneurs to make long-term investments and the high cost of algae production. Intensification of research activities aimed at increasing the productivity of algae and increasing the yield of target products, improving the methods of cultivation and integrated processing of biomass, as well as the use of local resources — waste heat from Thermal Power Plants, food production waste, CO_2 from flue gases of industrial enterprises, etc. can contribute to its reduction [29, 79].

The cultivation of microalgae is promising for the renewable raw materials obtaining of various purposes. The development of algobiotechnology research will promote the replenishment and improvement of the food base, the creation of medical products and new technical resources, and safety issue resolution of the environmental as well.

Algae production volumes are subject to UN Organization structures accounting and expansion, in particular through aquaculture. Meanwhile, the amount of biomass of freshwater microalgae produced is incomparably lower than that of marine algae. In particular, world production of *Spirulina* spp., concentrated in Australia, Israel, India, Malaysia, Myanmar, Japan according to available statistics from the Food and Agriculture Organization of United Nations (FAO), does not exceed 100 thousand tones, compared to hundreds and million thousands of tons for marine species [80].

Analysis of the achievements of algobiotechnology indicates the feasibility of creating a new and complete agriculture in Ukraine to solve various technological problems related to wastewater treatment, utilization of excess potential biogenic resources (biogenic compounds, organic substances, carbon dioxide), obtaining natural balanced feeds for animals, fish and poultry, as well as raw materials for further processing (production of biologically valuable compounds, biogas, biodiesel). For this purpose, given the considerable scientific and practical achievements of specialists of many organizations (academic institutes, universities, state and private enterprises), it is advisable to create a register (in the future — a bank) of algae cultures with different ranges of ecological valence and useful physiological properties, to study adaptive potential of different types of algae with respect to fluctuations of abiotic and biotic factors, to determine ways of purposeful regulation of productivity, biochemical composition and accumulation of certain valuable compounds in biomass.

Fig. 2. **Experimental tubular photobioreactor of biotechnological complex of the Institute of Hydrobiology of the National Academy of Sciences of Ukraine**

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БІОТЕХНОЛОГІЧНІ ПЕРСПЕКТИВИ МІКРОВОДОРОСТЕЙ

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Проаналізовано сучасний стан та перспективи біотехнологічного використання мікроскопічних водоростей. Наведено основні напрями альгобіотехнології, зумовлені фізіолого-біохімічними особливостями цих організмів, обсяги одержуваної в світі водоростевої продукції, види мікроводоростей, що вже набули застосування чи мають практичні можливості, шляхи одержання біомаси та підвищення продуктивності промислового культивування водоростей. Розглянуто стан цієї проблеми, доцільність розвитку альгобіотехнології та умови вирощування мікроводоростей в Україні.

Ключові слова: альгобіотехнологія, мікроводорості, промислове культивування, аквакультура.

БИОТЕХНОЛОГИЧЕСКИЕ ПЕРСПЕКТИВЫ МИКРОВОДОРОСЛЕЙ

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Проанализировано современное состояние и перспективы биотехнологического использования микроскопических водорослей. Приведены основные направления альгобиотехнологии, обусловленные физиолого-биохимическими особенностями этих организмов, объемы производимой в мире водорослевой продукции, виды микроводорослей, уже нашедшие применение или имеющие практические возможности, пути получения биомассы и повышения продуктивности промышленного культивирования водорослей. Рассмотрены состояние этой проблемы, целесообразность развития альгобиотехнологии и условия выращивания микроводорослей в Украине.

Ключевые слова: альгобиотехнология, микроводоросли, промышленное культивирование, аквакультура.