https://doi.org/10.15407/cryo35.02.057
UDC 57.086.13:602:582.263

N.A. Chernobai [1, 2, *], A.P. Gerilovych [1, 3], N.O. Shevchenko [1]
[1] Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, Kharkiv, Ukraine
[2] National Scientific Center "Hon. Prof. M.S. Bokarius Forensic Science Institute", Kharkiv, Ukraine
[3] Private Scientific Institution "One Health Scientific and Research Institute", Kharkiv, Ukraine
* nadiiachernobai@gmail.com

BIOTECHNOLOGICAL POTENTIAL OF MICROALGAE: STORAGE METHODS AND NON-CRYOGENIC FACTORS OF CRYOPRESERVATION EFFICIENCY

The review considers the prospects for the use and preservation techniques for microalgal collection samples. Microalgae have significant biotechnological potential in the food, pharmaceutical, environmental and energy sectors. The paper analyzes and summarizes current methods of maintaining microalgae cultures with a focus on their stability, preservation of biological properties, and long-term storage capabilities. The work includes a comparison of different approaches to microalgae preservation, including batch subcultivation, lyophilization, and cryopreservation. Particular attention is paid to cryopreservation as an effective method of long-term storage of genetically stable cultures, which minimizes the risk of losing valuable biotechnological characteristics. The influence of the stage of culture development, cell concentration in the suspension, cold adaptation, and centrifugation on the survival of microalgae after cryopreservation was determined. The results obtained are important for the development of biotechnological and environmental programs related to the use of microalgae.

***Key words*:** *microalgae, biotechnology, cryopreservation, lyophilization, cultivation, adaptation.*

Microalgae have a wide geographical and ecoclimatic distribution in the world, characterized by high levels of divergence of biological and genetic properties, different potential for endurance in aggressive environments (more than 70 thousand species with different resistance to pH, temperature and salinity have been described). Their considerable species diversity and ability to synthesize biologically active substances make them a promising research object in the fields of biotechnology, food and processing industry, medicine, agriculture, cosmetology, production of energy substrates, development of "green technologies", and organic production. They can also be a valuable model organism for toxicity studies.

Microalgae present a group of diverse photosynthetic microorganisms (cyanobacteria, diatoms, unicellular green algae, and some other types of algae) that inhabit soils, fresh and salt water bodies, and are an integral part of both moderately traditional and extreme biocenoses. These microorganisms can survive in difficult agroclimatic conditions and produce a number of compounds useful for biotechnology: lipids, proteins, carbohydrates, hor-

Reference: Chernobai NA, Gerilovych AP, Shevchenko NO. Biotechnological potential of microalgae: storage methods and non-cryogenic factors of cryopreservation efficiency. *Probl Cryobiol Cryomed*. 2025; 35(2): 57–67. https://doi.org/10.15407/cryo35.02.057

mones, vitamins, flavonoids, and others. They are also a source of indicator and bioactive pigments [5, 7, 9, 26, 28, 36, 45, 52, 53, 61, 62, 64, 72, 73]. The particular interest is presented by application of microalgae as organisms capable of accumulating solar energy during the process of photosynthesis, since their energy conversion efficiency is much higher than that of higher plants [61, 71, 73]. Also, their ability to absorb carbon dioxide from the atmosphere and release hydrogen has the potential to reduce greenhouse gases and generate alternative energy [31]. The study of microalgae, including extreme tolerant and extremophilic species, opens up new horizons for developing biotechnological approaches and new areas of their application. Current research focuses on ecological adaptation and studying the potential of using microalgae in biotechnology, emphasizing their ability to produce biologically active compounds under stress [11]. As reported by P. Leão *et al.* [40], the extremophilic microalgae can significantly increase biomass production and reduce the risks of environmental pollution in large-scale systems by biodegrading contaminants of organic origin. This suggests that extremophilic microalgae have great prospects for use in the food, pharmaceutical, cosmetic, and biofuel industries, offering sustainable and effective alternatives to traditional resources [60].

Currently, more than 72,500 potential microalgae species have been morphologically characterized, of which approximately 30,000 have been identified by molecular genetic profiles [24]. Their application for industrial, medical and environmental purposes is possible only due to the unique, genetically programmed characteristics of these biological objects [25, 31, 52]. Achievements in the industrial use of microalgae emphasize the importance of stable long-term preservation of their cultures in their original state (without phenotypic and genotypic modifications). This is due to the fact that genetic changes can lead to a distortion of the productivity and biological activity of these microorganisms, which, in turn, will cause a loss of their biotechnological value [39]. Throughout the history of biotechnological research, various methods of preserving microalgae have been developed, including batch subcultivation, lyophilization, and cryopreservation [24].

The aim of the study was to analyze current approaches to the biotechnological use of microalgae, to consider the methods of storing collection samples, and to assess the impact of non-cryogenic factors on cryopreservation efficiency. Particular attention was paid to the role of physiological state of cells and preparatory procedures to improve the post-thaw viability of microalgae.

The use of microalgae in biotechnology. The best-studied genera of microalgae include *Arthrospira, Chlorella, Chlamydomonas, Coccomyxa, Dunaliella*, and *Galdiera*. The biochemical composition of microalgae varies depending on the genus and species. A particularly important aspect of biotechnology is the production of β-carotene, a yellow terpenoid pigment, the demand for which is growing due to its wide range of applications on the market [27, 55, 60]. Among the known producers of β-carotene, the green algae *Dunaliella salina* should be noted. This species is extremely halotolerant, growing in the marine environment and inland salt lakes, making it the most halotolerant eukaryote discovered to date [33]. The production of carotenoids can reach a concentration of up to 14% of dry weight, in addition, microalgae of the genus *Dunaliella* contain astaxanthin and canthaxanthin) [7, 11, 28, 35]. Carotene-synthesizing microalgae include the freshwater psychrophilic species *Mesataenium berggrenii,* whose cells synthesize β-carotene, neoxanthin, violaxanthin, lutein, zeaxanthin, but not α-carotene. The use of microalgal carotenoids as textile dyes has expanded the biotechnological potential of both species beyond the food industry. This underscores the relevance of research on extremophilic microorganisms and illustrates their promising application in various industries.

The application of microalgal biomass in the food industry faces several challenges, including the need to comply with strict governmental food safety regulations, high production costs, scalability of processes, and consumer acceptance of [30]. Overcoming these obstacles requires significant efforts in biotechnology research.

An important aspect of the microalgae application is their antagonistic, antimicrobial, and pesticidal properties. This determines the prospects for their use in the field of pest control in compliance with organic production standards in the food and processing industry, agriculture, and health care [5, 64].

A promising area for the use of microalgae is the development of environmental safety assurance systems. Field trials have shown that microalgae in

industrial pools and meanders can remove up to 99% of dissolved and particulate metals [21]. It should be noted that wastewater treatment plants account for approximately 15% of the total 6.4 tons of cadmium that are released into the aquatic environment annually. Cadmium is a heavy metal described as an important environmental contaminant known for its high toxicity even at low concentrations. However, there are studies describing the extremophilic green microalgae *Chlamydomonas acidophila* to be tolerant and capable of sequestering cadmium in its vacuoles, which emphasizes its importance in phytoremediation of water resources contaminated with heavy metals [2, 3, 57].

Researchers also consider microalgae to be a source of biogas and other types of biofuels, which also determines their biotechnological prospects in the green technologies segment [2, 3, 9]. One of the biggest challenges is the development of efficient and cost-effective systems for the production and supply of energy from microalgae, which requires increasing the productivity and efficiency of biomass utilization [10].

The wide variety of applications of extremophilic and extreme-tolerant microalgae can lead to the creation of environmentally friendly products: biofuels, bioplastics, cosmetics, biofertilizers. Investing in research and biotechnological development based on microalgae and their products is promising for the development of many industries and the improvement of environmental safety standards [61].

In order to ensure the biotechnological value of microalgae strains and cultures, a deep and comprehensive study of their biological properties is required. In addition to the selection of producers of useful substances, it is important to preserve and maintain the stability of their productivity characteristics during the constant cultivation and storage of microorganisms. It is important to identify and certify the biological characteristics of industrial strains and clones, their biotechnologically promising traits, and to collect evidence of the stability of these characteristics in passages during cultivation [56].

METHODS OF STORAGE OF MICROALGAE COLLECTION SAMPLES

The most famous collections containing microalgal strains are: The Culture Collection of Algae and Protozoa (UK), founded in 1947; the Culture Collection of Algae at the University of Texas (USA), founded in 1976; the Culture Collection of Algae at the University of Göttingen (Germany), founded in 1953, and the Culture Collection of Algae at the Laboratory of Algology (Czech Republic), founded in 1979 [4].

The most common way to preserve microalgal cultures is to keep samples under controlled environmental conditions. Conventional batch seeding is performed using aseptic microbiological techniques and involves transferring the inoculum from the late stationary phase of the culture to fresh culture medium [45]. This ensures the maintenance of the culture in a metabolically active state. The purpose of storing collection cultures is to maintain them in healthy physiologically, morphologically, genetically, and biochemically homogeneous population. It should be borne in mind that different ages of subcultures can be characterized by different stages of the life cycle of a species (for example, in addition to green motile cells that divide at the early stationary phase, cultures of *Haematococcus pluvialis* Flowtow contain orange/red aplanospores) [53].

The main limitations of the constant reseeding of microalgal cultures are the selective and artificial origin of the nutrient medium and incubation regimes, aligned with their ecological characteristics. Cultivation under laboratory conditions can lead to the loss of important biological and biotechnological characteristics. Examples of instability include a decrease in the size of diatoms branches, changes in morphology in *Micractinium pusillum* Fresenius, and loss of normal pigment composition in many algae [1]. Additional limitations include the possibility of contamination and infection of the primary (axenic) culture, mislabeling, or other errors that can occur with constant manipulation of sterile samples [23]. Studies have shown that the continuous subcultivation of algae from different taxonomic groups led to a number of serious phenotypic changes and loss of the ability to produce commercially important metabolites [19], which is likely due to changes in microenvironmental factors and possible genetic rearrangements.

In continuous cultivation, conditions can change dramatically over time, even if the external environment remains unchanged and the algae culture has not exhausted any of the required nutrients. For example, the pH often changes if

there is no suitable buffer, or some nutrients are oxidized or otherwise gradually altered, especially during excessive light exposure [72]. In addition, conventional batch subculturing is a laborious and costly process that limits the ability to maintain a large number of collection samples and to maintain multiple microbial lines simultaneously. There are also risks of interlineage contamination and loss of pure cultures. To minimize those risks of conventional subcultivation of vegetative forms, alternative approaches to the *ex situ* preservation strategy for algal and cyanobacterial cultures have been developed, including freeze-drying and cryopreservation.

Lyophilization of microalgae includes 3 phases: freezing, primary drying, and secondary drying [25]. The cell culture is first exposed to temperatures of about –40 °C in the freezing phase, then subjected to vacuum drying at this temperature for 2 hours to induce freeze-drying to remove moisture, and finally, additional dehydration is performed in the secondary drying phase by increasing the temperature to 0 °C. The whole process is completed by replacing the atmosphere with inert gases such as nitrogen [34]. Cultures preserved in this way are stable at room temperature for a shelf life of up to 5 years and are highly resistant to bacterial and fungal contamination due to their low moisture content [14]. In addition, the samples have a small volume factor required for lyophilized cultures, and resources are used efficiently by reducing storage space, energy consumption, and transportation costs.

W. Daily and J. McGuire [17] laid the foundation for the preservation of microalgae by lyophilizing 32 species of microalgae, of which viability was observed for 24 species. While studying the preservation of microalgae after lyophilization, O. Holm-Hansen in 1963 conducted parallel work with cryopreservation of samples. He determined the effect of cooling rate and storage temperature on the viability of post thaw cultures. It was shown that for most microalgal cultures, slow cooling rates and storage temperatures not exceeding –100 °C were effective [32]. This was the first ever study related to the cryopreservation of microalgae, which allowed to obtain a viable culture of blue-green *Nostoc muscorum* after 5 years of storage. The importance of this discovery is that it was the longest storage period ever achieved at that time for *Cyanobacteria*. However, for green eukaryotic microalgae, the viability at the first stages of cryobiological research did not exceed 1–5% [48]. Therefore, since the 70s of the last century, research on long-term storage has been aimed at developing technologies for cryopreservation of microalgae.

CRYOPRESERVATION AS AN EFFECTIVE WAY OF LONG-TERM PRESERVATION OF GENETICALLY STABLE SAMPLES OF CULTURES AND PRODUCTION STRAINS OF MICROALGAE

Cryopreservation is a technology that is currently used to preserve biological objects in a viable state for a long time at cryogenic temperatures, usually from –80 to –196 °C. At these temperatures, a state is maintained in which any biological activity, including biochemical reactions that could lead to cell death, is effectively stopped [36, 37, 65]. The main objective of any preservation strategy is to guarantee that the organism is representative of its original forms, as far as possible, without changes in biological characteristics [16, 19, 20].

Over the past 40 years, centers for biological resources and biotechnologists have been developing the methods for cryopreservation of microalgae. This mostly concerns cyanobacteria, and for many eukaryotic algal taxa, cryopreservation has either not been applied at all or has not allowed for a high level of viability of cryopreserved samples [18, 66].

Some microalgae, especially *Cyanobacteria*, can be preserved frozen under uncontrolled cooling by directly placing samples in freezers at temperatures from –20 to –70 °C [27]. However, according to some researchers, the shelf life of all studied microalgae under such conditions does not exceed one month [24, 38]. The maximum shelf life of flagellated algae, such as *Chlorella minutissima, Chlorella stigmatophora, Isochrysis galbana, Dunaliella tertiolecta*, with the addition of 5—10% DMSO or methyl alcohol, does not exceed 5 days, and without the addition of cryoprotectants, they lose their viability within a few hours [67]. The work of K. Vozovik *et al.* [70] showed that storage of *Chlorococcum dissectum* was impossible in a household freezer (at –18 °C); after 24 hours, viable cells were completely absent. When a cell suspension of this microalgae was placed in freezers at –40 and –70 °C, a significant decrease in the ability of its cells to form colonies was observed; medium-term

storage of collection samples is possible for no longer than 20 days. It is possible that ice recrystallization and/or intracellular chemical activity cause a gradual decrease in viability, so that many strains lose viability within a few days of storage at low negative temperatures.

Chemical activity and crystal growth during the cooling phase virtually cease at temperatures below –130 °C [24]. It is generally accepted that a population of living organisms is able to survive cooling-heating processes and can be stored indefinitely at temperatures below the glass transition temperature without significant loss of viability [43]. There is little empirical data on the long-term low-temperature storage of microorganisms. It has been shown that bacteria and fungi can retain high viability after 30 years of continuous cryostorage [17, 20]. Most microalgae species, even with a high level of preservation immediately after the cooling-warming stage, are not viable after a year of liquid nitrogen storage [24].

There are a number of critical factors (those that ensure viability during storage and restoration of full functionality afterwards) that can affect the outcome of cryopreservation, and they differ from organism to organism [17, 19, 66]. Non-cryogenic parameters before and after low-temperature storage (culture age, cell concentration, culture temperature, osmotic potential, and composition of the growth/recovery medium) can affect viability in the same way as cryogenic parameters (cryoprotective mixture composition, cooling and thawing modes) [17, 19, 29]. Therefore, the development of effective cryopreservation protocols should include a detailed assessment of such factors.

INFLUENCE OF THE GROWTH STAGE OF MICROALGAE CULTURES AND CELL CONCENTRATION ON VIABILITY AFTER COOLING AND WARMING

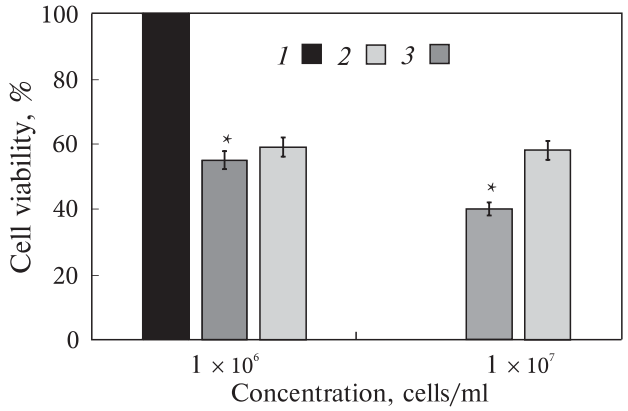
To obtain high viability rates of microalgal cells, a number of specific parameters must be taken into account, such as the age of the culture.

Intact cultures in the active phase of growth (early stationary phase) tend to show higher viability rates than those in the late stationary or decaying phase. At the same time, it was found that the age of microalgal cell culture is an important factor affecting the effective cryopreservation of many species. Out of 17 strains of the genus *Chlorococcales*, 11 showed high viability after thawing when cells at the stationary growth phase were used [46]. Similar results were obtained for 5 species of marine microalgae and for the freshwater *Chlorella vulgaris*. A higher level of viability was observed in post thaw cultures that were in the stationary phase of growth before cooling [50]. This fact may be due to the fact that older cultures often lack vacuoles, while the content of lipids, carbohydrates and other high molecular weight substances in the cells increases, which probably contributes to better results of sample cryopreservation [46].

Cryopreservation procedures for algae usually do not provide for a certain cell density in the culture during freezing. An analysis of scientific literature published before the 2000s revealed the absence of such information. However, recent articles have hightighted that the efficiency of cryopreservation of most species depends on cell concentration in the culture. No significant difference in the viability of cryopreserved samples of green microalgae at a concentration of 1 to 10×10^6 cells/ml was shown, but an increase in their number up 20 to 50×10^6 cells/ml led to a significant decrease in the studied indices. Currently, this is explained by the fact that during freezing and/or heating into the medium, one or more specific substances are released from the cells that reduce the viability of post thaw culture [8, 54]. Toxic substances are likely released from cell walls as a result of enzymatic processes. This assumption is supported by the fact that when cryopreserving strains with no cell wall, no correlation was observed between the number of cells in the sample and viability. Secondly, if an extract obtained from cultures with a cell wall damaged by repeated immersion in liquid nitrogen was added to the cell suspension before the cooling stage, then after heating, they showed a very low number of viable microalgae [8].

Preliminary data obtained by gel chromatography indicate that the harmful substances released by cells into the culture medium during cryopreservation are small (molecular weight <1.5 kDa), water-soluble, thermostable, organic compounds [57].

Our studies have demonstrated that the effectiveness of cryopreservation of cells of the halotolerant microalgae *D. salina*, which does not have a rigid cell wall, depends on both the cultivation conditions and their concentration in the suspension (Figure). At a concentration of 1×10^6 cells/ml, the survival rate was higher in the culture grown



The viability of *D. salina* microalgae cells after cooling to liquid nitrogen temperature depending on the initial concentration of cells in suspension on Ramaraj and 4M Ramaraj media (with an increased concentration of sodium chloride): *1* — control, *2* — Ramaraj media, *3* — 4 M Ramaraj media

in Ramaraj's medium [58] compared to the variant with a concentration of 1×10^7 cells/ml, respectively. When cells were cultivated in this medium with an increased sodium chloride content (up to 4 M), the cell concentration did not significantly affect the preservation level of the cryopreserved samples. The deterioration of the preservation rate may be due to an increase in cell density, which leads to uneven freezing, uneven ice formation, as cell clusters can cause the formation of large ice crystals that damage cell walls, changes in osmotic conditions during cooling.

It can be concluded that the optimal cell concentration of some species before cooling is an important factor for increasing their survival after heating. This should be taken into account when preserving microalgae collection samples for further use in biotechnology, the food industry, *etc*.

THE ROLE OF COLD ADAPTATION OF MICROALGAL CELLS IN THE DEVELOPMENT OF EFFECTIVE CRYOPRESERVATION METHODS

The ability of algae to adapt for extreme conditions has evolved over time. Recent metabolic studies indicate that plants have developed various strategies to restructure their metabolism under stressful conditions, but the full range of their adaptation mechanisms remains poorly understood [15, 41].

The cellular reactions that chlorophytes and other microorganisms use to survive at low temperatures have been actively studied only in recent years, mainly on psychrophilic species [13, 42]. It is obvious that different groups of algae share common genetically determined defense mechanisms that are activated in response to stress factors. Thanks to these mechanisms, they are able to withstand short-term exposure to unfavorable chemical and physical conditions, including low temperatures [44, 62]. Our results have shown that under the influence of cold stress on *Dunaliella salina* cell culture, carotenoids and lipid globules accumulate, which increases the efficiency of cryopreservation of this microalgae [11].

The main processes of adaptation include the synthesis of carotenoids, the production of cold shock proteins, and the activation of the pentose phosphate pathway [22, 63]. At the same time, even a short-term temperature drop of up to 3 °C can be lethal for the vegetative cells of the blue-green algae *Anacystis nidulans* and *Chlamydomonas reinhardtii* [59, 68]. It has been reported that the filamentous green alga *Cladophora sauteri* is not able to adapt to cold, as its ecological niche is water bodies where the temperature does not fall below 10 °C and does not rise above 25 °C [19]. Other algae (e.g., *Haematococcus pluvialis*) demonstrated the ability to tolerate desiccation and low temperatures (4—8 °C) during their normal life cycle. The freezing resistance of the freshwater alga *Klebsormidium flaccidum* was significantly increased by cold adaptation at 2 °C for 2—7 days. The cell viability after exposure to low temperatures (from –10 °C) was 55 and 85%, respectively, depending on the number of days of acclimatization, which significantly exceeded the viability rate (10%) obtained from the culture grown at 18 °C [51]. Biochemical changes accompanying freezing tolerance include the accumulation of soluble carbohydrates, amino acids, glycosides, as well as changes in the number and size of starch grains in chloroplasts, a decrease in vacuole size, and an increase in the size and number of chloroplasts and cytoplasmic space [19, 69]. On the contrary, cultivation in non-acclimatizing conditions or in conditions that promote deacclimatization can lead to a decrease in cryotolerance.

Thus, the ability of microalgae to tolerate low temperatures is determined by their taxonomic features and morphofunctional characteristics. The study of the impact of cold and other stressors on certain types of algae is extremely relevant, as, on the one hand, it can help increase the production of beneficial metabolites, and on the other

hand, it can help avoid cell death in culture due to excessively harsh conditions. According to the literature, researchers have shown the importance of cultivating algae at suboptimal temperatures (cold acclimatization), which led to better cell viability after cooling-warming of both freshwater [46] and marine microalgae [6]. In particular, G. Morris [47] suggested that the high level of viability of hardened samples after thawing might be due to an increase in the percentage of unsaturated fatty acids in the membrane phospholipids.

Thus, the cold adaptation of most algae affects their resistance to low temperatures, which can be used to improve the efficiency and effectiveness of cryopreservation.

Centrifugation of microalgae cells as one of the stages of preparation for cryopreservation. As one of the stages of sample preparation for cryopreservation, we should consider the method of sedimentation to concentrate cell biomass. In laboratory conditions, this cell sedimentation is performed by centrifugation. Samples of many microalgae can be centrifuged or, as in the case of filamentous taxa, thalli can be separated before treatment with a cryoprotectant [19]. The contribution of these preparatory steps is often overlooked by researchers. However, the common practice of concentrating cultures by centrifugation before freezing and/or removing cryoprotectants by pelleting cells before replacing the supernatant with fresh medium can cause significant stress to microalgal cells. Although the preparatory steps have not been shown to cause lethal stresses, it is believed that they can make the organism more susceptible to cryopreservation [18], as centrifugation creates certain shear forces that can damage algal cells after warming, when they are still very vulnerable [69]. In the paper of G. Morris [46], the elimination of the centrifugation step of tested samples increased cell viability by ~22% when the culture contained 1×10^6 cells/ml and no effect on the studied index at 50×10^6 cells/ml was described. This may be partly due to the treatment processes, in particular, to cell adhesion to the pipette tip and microcentrifuge tubes, which is more noticeable at lower concentrations. Therefore, when developing a cryopreservation protocol that involves the use of highly concentrated cryoprotectant solutions that require a washing step, the results described in this paper should be taken into account.

CONCLUSION

The analysis of reported data on biotechnological application of microalgae indicates their value for the food, pharmaceutical, cosmetic, and biofuel industries, offering sustainable and effective alternatives to traditional resources.

The preservation of biological characteristics, physiological and genetic stability and long-term storage of useful and predictable biotechnological properties of microalgae can be ensured by cryopreservation.

Studies demonstrate that the effectiveness of low-temperature storage depends on the age of the cultures, cell concentration in the samples, cold adaptation of the cultures, and the presence or absence of centrifugation step in preparation for cryopreservation.

Thus, microalgae are important for biotechnology, the food industry, and the preservation of biodiversity and ecosystem stability, and knowledge of their physiological characteristics and approaches to effective cryopreservation enbles developing effective methods of long-term storage and increasing the viability of rewarmed cultures.

REFERENCES

1. Abate R, Oon YL, Oon YS, et al. Diverse interactions between bacteria and microalgae: A review for enhancing harmful algal bloom mitigation and biomass processing efficiency. Heliyon [Internet]. 2024 Sep 15 [cited 17.04.2025]; 10 (17): e36503. Available from: https://www.cell.com/heliyon/fulltext/S2405-8440(24)12534-0
2. Abinandan S, Subashchandrabose SR, Pannerselvan L, et al. Potential of acid-tolerant microalgae, *Desmodesmus sp.* MAS1 and *Heterochlorella sp*. MAS3, in heavy metal removal and biodiesel production at acidic pH. Bioresour Technol. 2019; 278: 9—16.
3. Abinandan S, Subashchandrabose SR, Venkateswarlu K, et al. Acid-tolerant microalgae can withstand higher concentrations of invasive cadmium and produce sustainable biomass and biodiesel at pH 3.5. Bioresour Technol. 2019; 281: 469—73.
4. Arguelles EDLR, Martinez-Goss MR. Diversity of Philippine photosynthetic euglenophytes and their potential biotechnological uses: a review. Int J Emerging Technol. 2019; 10(4): 24—31.
5. Balasubramaniam V, Gunasegavan RD, Mustar S, et al. Isolation of industrial important bioactive compounds from

microalgae. Molecules [Internet]. 2021 Feb 10 [cited 18.04.2025]; 26(4): 943. Available from: https://www.mdpi.com/1420-3049/26/4/943
6. Bedard S, Roxborough E, O'Neill E, Mangal V. The biomolecules of *Euglena gracilis*: harnessing biology for natural solutions to future problems. Protist [Internet]. 2024 May 31 [cited 17.04.2025]; 175(4): 126044. Available from: https://www.sciencedirect.com/science/article/pii/S1434461024000361
7. Bortolini DG, Maciel GM, Fernandes IDAA, et al. Functional properties of bioactive compounds from *Spirulina spp*.: Current status and future trends. Food Chem Mol Sci [Internet]. 2022 Dec 30 [cited 13.03.2025]; 5: 100134. Available from: https://www.sciencedirect.com/science/article/pii/S2666566222000624
8. Bui TV, Ross IL, Jakob G, Hankamer B. Impact of procedural steps and cryopreservation agents in the cryopreservation of chlorophyte microalgae. PLoS One [Internet]. 2013 Nov 11 [cited 12.03.2025]; 8(11): e78668. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0078668
9. Carvalho RH, Callegari MA, Dias CP, et al. *Euglena gracilis* β-glucans (1,3): enriching colostrum of sow for enhanced piglet immunity. Animals [Internet]. 2023 Nov 12 [cited 14.04.2025]; 13(22): 3490. Available from: https://www.mdpi.com/2076-2615/13/22/3490
10. Chen Z, Dong Y, Duan S, et al. A chromosome-level genome assembly for the paramylon-producing microalga *Euglena gracilis*. Sci Data [Internet]. 2024 July 16 [cited 15.04.2025]; 11(1): 780. Available from: https://www.nature.com/articles/s41597-024-03404-y
11. Chernobai NA, Kadnikova NG, Vozovyk KD, et al. Temperature-salt stress increases yield of valuable metabolites and shelf life of microalgae. Biophys Bull. 2022; 48: 7—17.
12. Čížková M, Mezricky P, Mezricky D, et al. Bioaccumulation of rare earth elements from waste luminophores in the red algae, *Galdieria phlegrea*. Waste Biomass Valor. 2021; 12: 3137—46.
13. Collins T, Margesin R. Psychrophilic lifestyles: mechanisms of adaptation and biotechnological tools. Appl Microbiol Biotechnol. 2019; 103(7): 2857—71.
14. Corbett LL, Parker DL. Viability of lyophilized cyanobacteria (blue-green algae). Appl Environ Microbiol. 1976; 32(6): 777—80.
15. Cvetkovska M. Algae use the underwater light spectrum to sense depth. Nature. 2025; 637(8046): 553–4.
16. Dai JL, He YJ, Chen HH, Jiang JG. Dual roles of two malic enzymes in lipid biosynthesis and salt stress response in *Dunaliella salina*. JAFC. 2023; 71(45): 17067—79.
17. Daily WA, McGuire JM. Preservation of some algal cultures by lyophilization. Butl Univ Bot Stud. 1954; 11(8/17): 139—43.
18. Day JG, Brand JJ. Cryopreservation methods for maintaining microalgal cultures. In: Andersen RA, editor. Algal culturing techniques. London: Elsevier Academic Press; 2005. p. 165—87.
19. Day JG, Fleck RA. Cryo-injury in algae and the implications this has to the conservation of micro-algae. Microalgae Biotechnol. 2015;1(1):1—11.
20. Day JG, Watanabe MM, Turner MF. *Ex situ* conservation of protistan and cyanobacterial biodiversity: CCAP-NIES collaboration 1991–1997. Phycol Res. 1998; 46(s2): 77—83.
21. Dubey S, Chen CW, Haldar D, Tambat VS, Kumar P, Tiwari A, Singhania RR, Dong CD, Patel AK. Advancement in algal bioremediation for organic, inorganic, and emerging pollutants. Environ Pollut [Internet]. 2023 Jan 15 [cited 08.03.2025]; 317: 120840. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0269749122020553
22. Ermilova E. Cold stress response: an overview in *Chlamydomonas*. Front Plant Sci [Internet] Sep 03 [cited 28.02.2025]; 2020; 11: 569437. Available from: https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2020.569437/full
23. Fernandez-Valenzuela S, Chávez-Ruvalcaba F, Beltran-Rocha JC, et al. Isolation and culturing axenic microalgae: mini-review. Open Microbiol J. 2021; 15(1): 111—9.
24. Foo SC, Mok CY, Ho SY, Khong NM. Microalgal culture preservation: Progress, trends and future developments. Algal Res [Internet]. 2023 Feb 23 [cited 25.02.2025]; 71(1): 103007. Available from: https://www.sciencedirect.com/science/article/pii/S2211926423000401
25. Foo SC, Lee ZS, Yap MKK, Tan JW. The antioxidant, wound healing properties and proteomic analysis of water extracts from the tropical cyanobacteria, *Nostoc* NIES-2111_MUM004. 3Biotech [Internet]. 2023 Feb 02 [cited 17.04.2025]; 13(2): 71. Available from: https://link.springer.com/article/10.1007/s13205-022-03448-0
26. Gan S, Liang S, Zou Q, Shang C. Optimization of carotenoid extraction of a halophilic microalgae. PloS One [Internet]. 2022 Aug 2 [cited 15.04.2024]; 17(8): e0270650. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0270650
27. Garrido-Cardenas JA, Han X, Alonso DL, García-Maroto F. Evaluation and optimization of a methodology for the long-term cryogenic storage of *Tetradesmus obliquus* at –80 °C. Appl Microbiol Biotechnol. 2019; 103(5): 2381—90.
28. Gharajeh HN, Valizadeh M, Dorani E, Hejazi MA. Biochemical profiling of three indigenous *Dunaliella* isolates

with main focus on fatty acid composition towards potential biotechnological application. Biotechnol Rep [Internet]. 2020 June 2 [cited 11.03.2025]; 26: e00479. Available from: https://www.sciencedirect.com/science/article/pii/S2215017X20301235
29. Hamlaoui S, Yéprémian C, Duval C, et al. The culture collection of cyanobacteria and microalgae at the French National Museum of Natural History: A century old but still alive and kicking! Including in memoriam: Professor Alain Couté. Cryptogamie, Algologie. 2022; 43: 41—83.
30. Henchion M, Hayes M, Mullen AM, et al. Future protein supply and demand: Strategies and factors influencing a sustainable equilibrium. Foods [Internet]. 2017 July 20 [cited 13.03.2025]; 6: 53. Available from: https://www.mdpi.com/2304-8158/6/7/53
31. Hepburn C, Adlen E, Beddington J, et al. The technological and economic prospects for CO_2 utilization and removal. Nature. 2019; 575(7781): 87—97.
32. Holm-Hansen O. Viability of blue-green and green algae after freezing. Physiol Plant. 1963; 16(3): 530—40.
33. Hosseini A, Shariati M. Dunaliella biotechnology: Methods and applications. J Appl Microbiol. 2009; 107: 14—35.
34. Hosseinizand H, Sokhansanj S, Lim CJ. Studying the drying mechanism of microalgae *Chlorella vulgaris* and the optimum drying temperature to preserve quality characteristics. Dry Technol. 2017; 36(9): 1049—60.
35. Hyrslova I, Krausova G, Mrvikova I, et al. Functional properties of *Dunaliella salina* and its positive effect on probiotics. Mar Drugs [Internet]. 2022 Dec 15 [cited 09.03.2025]; 20: 781. Available from: https://www.mdpi.com/1660-3397/20/12/781
36. Jang HK, Kang JJ, Lee JH, et al. Comparison of annual biosynthetic calorie productions by phytoplankton in different southern Korean bays. Frontiers in Marine Science [Internet]. 2024 July 29 [cited 13.03.2025]; 11: 1367137. Available from: https://www.frontiersin.org/journals/marine-science/articles/10.3389/fmars.2024.1367137/full
37. Kihika JK, Wood SA, Rhodes L, et al. Cryoprotectant treatment tests on three morphologically diverse marine dinoflagellates and the cryopreservation of *Breviolum sp.* (Symbiodiniaceae). Sci Rep [Internet]. 2022 Jan 13 [cited 03.03.2025]; 12: 646. Available from: https://www.nature.com/articles/s41598-021-04227-2
38. Kugler A, Kumari P, Kokabi K, et al. Resilience to freezing in the vegetative cells of the microalga *Lobosphaera incisa* (Trebouxiophyceae, Chlorophyta). J Phycol. 2020; 56(2): 334—45.
39. Lam W, Lee TC, Tam NF, et al. Bacteria associated with *Karenia mikimotoi* in modulating its ichthyotoxicity. Mar Pollut Bull [Internet]. 2025 July 1 [cited 16.04.2025]; 216: 117939. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0025326X2500414X
40. Leão PN, Martins TP, Abt K, et al. Incorporation and modification of fatty acids in cyanobacterial natural products biosynthesis. Chem Comm. 2023; 59(30): 4436—46.
41. Margesin R, Miteva V. Diversity and ecology of psychrophilic microorganisms. Res Microbiol. 2011; 162(3): 346—61.
42. Marx JG, Carpenter SD, Deming JW. Production of cryoprotectant extracellular polysaccharide substances (EPS) by the marine psychrophilic bacterium *Colwellia psychrerythraea* strain 34H under extreme conditions. Can J Microbiol. 2009; 55(1): 63—72.
43. Mazur P. Freezing of living cells: mechanisms and implications. Am J Physiol. 1984; 247(3): 125—42.
44. Mock T. Algal model species for advancing biological sciences. J Phycol. 2023; 59(1): 1—3.
45. Mokrosnop VM, Zolotareva EK. [Accumulation of α-tocopherol in microalgae cells]. Microbiol Biotechnol. 2021; (2): 6—26. Ukrainian.
46. Morris GJ, Clarke A. The cryopreservation of *Chlorella*. 4. Accumulation of lipid as a protective factor. Arch Microbiol. 1978; 119(2): 153—6.
47. Morris GJ, McGrath JJ. Intracellular ice nucleation and gas bubble formation in *Spirogyra*. Cryo Letters. 1981; 2: 341—52.
48. Morris GJ. Cryopreservation of 250 strains of *Chlorococcales* by the method of two-step cooling. Br Phycol J. 1978; 13(1): 15—24.
49. Morris GJ. The cryopreservation of *Chlorella*. 2. Effect of growth temperature on freezing tolerance. Arch Microbiol. 1976; 107(3): 309—12.
50. Morschett H, Tenhaef N, Hemmerich J, et al. Robotic integration enables autonomous operation of laboratory scale stirred tank bioreactors with model-driven process analysis. Biotechnol Bioeng. [Internet]. 2021 Apr 19 [cited 16.04.2025]; 118(7): 275969. Available from: https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/10.1002/bit.27795
51. Nagao M, Ozaki T, Fukuda H, et al. Alkane biosynthesis gene expression and its increased production in recombinant cyanobacteria. FEBS Open Bio. 2025; 15(6): 949—62.
52. Ortiz-Martínez VM, Andreo-Martínez P, García-Martínez N, et al. Approach to biodiesel production from microalgae under supercritical conditions by the PRISMA method. Fuel Process Technol. 2019; 191: 211—22.
53. Oslan SNH, Shoparwe NF, Yusoff AH, et al. A review on *Haematococcus pluvialis* bioprocess optimization of green

and red stage culture conditions for the production of natural astaxanthin. Biomolecules [Internet]. 2021 Feb 10 [cited 13.03.2025]; 11(2): 256. Available from: https://www.mdpi.com/2218-273X/11/2/256
54. Piasecki BP, Diller KR, Brand JJ. Cryopreservation of *Chlamydomonas reinhardtii*: a cause of low viability at high cell density. Cryobiology. 2009; 58(1): 103—9.
55. Prieto A, Pedro Cañavate J, García-González M. Assessment of carotenoid production by *Dunaliella salina* in different culture systems and operation regimes. J Biotechnol. 2011; 151: 180—5.
56. Prieto-Guevara M, Alarcón-Furnieles J, Jiménez-Velásquez C, et al. Cryopreservation of the microalgae *Scenedesmus sp*. Cells [Internet]. 2023 Feb 09 [cited 11.03.2025]; 12: 562. Available from: https://www.mdpi.com/2073-4409/12/4/562
57. Puente-Sánchez F, Macías-Pérez LA, Campbell KL, et al. Bacterioplankton taxa compete for iron along the early spring-summer transition in the Arctic Ocean. Ecol Evol [Internet]. 2024 June 18 [cited 16.04.2025]; 14(6): e11546. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/ece3.11546
58. Ramaraj S, Niran J. Modified medium for enhanced growth of *Dunaliella* strains. Int J Curr Sci. 2013; 5: 67—73.
59. Raymond JA, Morgan-Kiss R, Stahl-Rommel S. Glycerol is an osmoprotectant in two Antarctic *Chlamydomonas* species from an ice-covered saline lake and is synthesized by an unusual bidomain enzyme. Front Plant Sci [Internet]. 2020 Aug 20 [cited 13.02.2025]; 11: 1259. Available from: https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2020.01259/full
60. Remias D, Procházková L, Nedbalová L, et al. Novel insights in cryptic diversity of snow and glacier ice algae communities combining 18S rRNA gene and ITS2 amplicon sequencing. FEMS Microbiol Ecol [Internet]. 2023 Dec 12 [cited 17.04.2025]; 99(12): 134. Available from: https://academic.oup.com/femsec/article/99/12/fiad134/7330195
61. Rojas-Villalta D, Rojas-Rodríguez D, Villanueva-Ilama M, et al. Exploring extremotolerant and extremophilic microalgae: new frontiers in sustainable biotechnological applications. Biology [Internet]. 2024 Sep 11 [cited 04.03.2025]; 13(9): 712. Available from: https://www.mdpi.com/2079-7737/13/9/712
62. Schulze PSC, Hulatt ChJ, Morales-Sánchez D, et al. Fatty acids and proteins from marine cold adapted microalgae for biotechnology. Algal Res. [Internet]. 2019 Sep 6 [cited 13.03.2025]; 42: 101604. Available from: https://www.sciencedirect.com/science/article/abs/pii/S22119264193012862
63. Song H, He M, Wu C, et al. Global transcriptomic analysis of an Arctic Chlorella-Arc reveals its eurythermal adaptivity mechanisms. Algal Res [Internet]. 2020 Jan 21 [cited 13.03.2025]; 46: 101792. Available from: https://www.sciencedirect.com/science/article/abs/pii/S2211926419309427
64. Soru S, Malavasi V, Concas A, et al. A novel investigation of the growth and lipid production of the extremophile microalga *Coccomyxa melkonianii* SCCA 048 under the effect of different cultivation conditions: Experiments and modeling. Chem Eng J [Internet]. 2019 Dec 1 [cited 13.03.2025]; 377: 120589. Available from: https://www.sciencedirect.com/science/article/abs/pii/S138589471832521X
65. Stock W, Willems A, Mangelinckx S, et al. Selection constrains lottery assembly in the microbiomes of closely related diatom species. ISME Commun [Internet]. 2022 Feb 02 [cited 16.04.2025]; 2(1): 11. Available from: https://academic.oup.com/ismecommun/article/2/1/11/7461074
66. Taylor R, Fletcher RL. Cryopreservation of eukaryotic algae – a review of methodologies. J Appl Phycol. 1998; (10): 481—501.
67. Tzovenis I, Triantaphyllidis G, Naihong X, et al. Cryopreservation of marine microalgae and potential toxicity of cryoprotectants to the primary steps of the aquacultural food chain. Aquaculture. 2004; 230(1—4): 457—73.
68. Valledor L, Furuhashi T, Hanak AM, Weckwerth W. Systemic cold stress adaptation of *Chlamydomonas reinhardtii*. Mol Cell Proteomics. 2013; 12(8): 2032—47.
69. Verspreet J, Schoeters F, Bastiaens L. The impact of non-concentrated storage on the centrifugation yield of *Microchloropsis gaditana*: a pilot-scale study. Life [Internet]. 2024 Jan 17 [cited 07.05.2025]; 14(1): 131. Available from: https://www.mdpi.com/2075-1729/14/1/131
70. Vozovyk K, Shevchenko N. [Effect of low temperature storage conditions on the viability of microalgae *Chlorococcum dissectum*]. The Journal of V.N. Karazin Kharkiv National University. Series «Biology». 2022; 39: 12—9. Ukrainian.
71. Yaremich AV, Karamushka Vl, Kramarenko AO. Remote control of micro algae cultivation under conditions of growth medium regulation. Hydrobiological Journal. 2021; 57(1): 48—54.
72. Zhang H, Yan Q, An Z, Wen Z. A revolving algae biofilm based photosynthetic microbial fuel cell for simultaneous energy recovery, pollutants removal, and algae production. Front Microbiol [Internet]. 2022 Oct 12 [cited 17.04.2025]; 13: 990807. Available from: https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2022.990807/full
73. Zolotarova OK, Mykhailenko NF, Syvash OO, Shniukova YeI. [Prospects for the use of microalgae in biotechnology.] Kyiv: Alterpres; 2008. 234 p. Ukrainian.

Received 17.03.2025
Accepted for publication 19.06.2025

*Н.А. Чернобай [1, 2 *], А.П. Герілович [1, 3], Н.О. Шевченко [1]*
[1] Інститут проблем кріобіології і кріомедицини НАН України
м. Харків, Україна
[2] Національний науковий центр «Інститут судових експертиз ім. Засл. проф. М.С. Бокаріуса»
м. Харків, Україна
[3] Приватна наукова установа «Науково-дослідний інститут єдиного здоров'я»
м. Харків, Україна
* nadiiachernobai@gmail.com

БІОТЕХНОЛОГІЧНИЙ ПОТЕНЦІАЛ МІКРОВОДОРОСТЕЙ: МЕТОДИ ЗБЕРІГАННЯ ТА НЕКРІОГЕННІ ЧИННИКИ ЕФЕКТИВНОСТІ КРІОКОНСЕРВУВАННЯ

В огляді розглянуто перспективи використання та методи збереження колекційних зразків мікроводоростей, які мають значний біотехнологічний потенціал у харчовій, фармацевтичній, екологічній та енергетичній галузях. У роботі проведено аналіз та узагальнено сучасні методи підтримання культур мікроводоростей з фокусом на їхню стабільність, збереження біологічних властивостей та можливості довгострокового зберігання. Дослідження охоплює порівняння різних підходів до збереження мікроводоростей, зокрема серійне субкультивування, ліофілізацію та кріоконсервування. Особливу увагу приділено кріоконсервуванню як ефективному методу довготривалого зберігання генетично стабільних культур, що дозволяє мінімізувати втрати цінних біотехнологічних характеристик. Визначено вплив стадії розвитку культури, концентрації клітин у суспензії, холодової адаптації, центрифугування на виживаність мікроводоростей після кріоконсервування. Отримані результати мають важливе значення для розвитку біотехнологічних та екологічних програм, пов'язаних з використанням мікроводоростей.

***Ключові слова*:** мікроводорості, біотехнологія, кріоконсервування, ліофілізація, культивування, адаптація.