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Microalgae cryopreservation techniques: choosing cryoprotectants and cooling regimens

This review was aimed to summarize and array current methods to cryopreserve microalgae, particularly, the approaches to selecting cryoprotectants and optimal cooling parameters, aimed at preserving their viability and functional activity after thawing. The paper analyses the key aspects of cryopreservation of microalgae, including the efficiency comparison of different classes of cryoprotectants as well as their impact on cells; mechanisms of cryodamage and strategies for minimizing it; optimal cooling and warming regimens for various types of microalgae. Special attention has been paid to the dependency of cryopreservation efficiency on the concentration and composition of cryoprotective solutions, which vary depending on the specific species and cultures. Some microalgae has been noted to maintain high viability using elevated concentrations of non-penetrating cryoprotectants. The necessity for further research using new and combined cryoprotective solutions has been emphasized to expand the possibilities of long-term storage of microalgae.

Key words: *microalgae, cryoprotectants, cryopreservation regimens, plant vitrification solutions, low-temperature storage.*

Cryopreservation of plant genetic resources at various levels of organization aims to ensure long-term storage of viable and genetically stable material at ultra-low temperatures using liquid nitrogen (–196 °C) or its vapor (from –150 °C). Under these conditions, cell division and metabolic activity in plant tissues are reduced to a minimum [6, 13, 20, 27, 56, 58, 59, 64, 77], that ensures the genetic integrity of biological material over long periods. Furthermore, kinetic energy is reduced, and molecular movement in living systems ceases, leading to the weakening of transport and enzymatic reactions and the slowing of the aging process [54, 64]. Consequently, the viability of plant samples is maintained for a theoretically unlimited period [20, 27].

It is important to note that the use of liquid nitrogen for the cryopreservation of biological objects offers many advantages, the most significant of which are chemical inertness, accessibility, and the independence of the temperature regimen from power outages (blackout periods) [20].

Nevertheless, cryopreservation protocols must be optimized for each specific species and type of object. This is due to the necessity of determining an effective method to prevent ice crystal formation and recrystallization within cells, particularly in the cytoplasm, which can lead to cell membrane rup-

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ture, loss of functionality, and, ultimately, cell death [1, 64, 69].

Ice formation is more critical in the intracellular space than in the extracellular one, as intracellular crystals can cause mechanical damage and the destruction of cellular structures. The formation of lethal intracellular crystals can be prevented by applying ultra-rapid cooling, which minimizes the time required for crystal formation, or by adding cryoprotectants for lowering the freezing point and limiting the availability of water for crystallization [54, 69]. The use of cryoprotective agents essentially initiated modern cryopreservation technologies [3].

Some cryoprotectants permeate into the cell and stabilize the intracellular environment, while others function primarily in the external environment, reducing the risk of extracellular crystallization. Due to these properties, cryoprotectants are widely used for the long-term storage of cells, tissues, and organisms involved in biotechnological processes, medicine, and agricultural science [8, 27, 32, 34, 59, 62, 71, 80].

For the cryopreservation of microalgae, permeating cryoprotectants (dimethyl sulfoxide (DMSO), ethylene glycol (EG), methanol, and glycerol) are the most ones which are commonly used to provide intracellular protection. Additionally, high-molecular-weight substances (sucrose, trehalose, polyethylene glycols of various molecular weights) are provided extracellular protection, as they cannot permeate the transmembrane [3, 8, 32, 34, 44]. Furthermore, the addition of compositions containing both non-permeating (trehalose) and permeating (glycerol) cryoprotectants leads to effective cell recovery after cryopreservation [32]. Non-permeating cryoprotectants are typically characterized by lower toxicity than their permeating analogues in identical concentrations [43—45].

Formamide (rarely used as a cryoprotectant for algae), EG, and DMSO can change the permeability of a cell membrane and its potential [72], as well as act as free radical scavengers [80].

Protective agents in high concentrations more effectively prevent ice formation in the cell environment during cryopreservation; however, they concurrently become more toxic and reduce the viability of samples during the storage and thawing of biological objects [43].

Thus, effective cryopreservation protocols must simultaneously minimize the harmful consequences of ice crystal formation and the risk of cryoprotectant toxicity [43—45]. Given the above, it is important to consider the primary biotechnological object of scientific interest for our experimental research — microalgae.

Many eukaryotic microalgae and cyanobacteria have been isolated from various natural environments (temperate, aggressive, extreme) [4, 24]. In our previous studies, we highlighted the broad biotechnological prospects for using these organisms. This is due to their metabolic characteristics, resilience, and ability to survive and regenerate under the influence of various unfavourable climatic and environmental factors [10, 11, 76]. Microalgae are characterized by many specific properties of research and commercial value. Their cultivation is carried out by various laboratories worldwide. Many collections of algal cultures have been established and maintained in research institutions and living biological repositories. In order to effectively maintain biodiversity and to involve the microalgae as test objects in scientific research and biotechnological developments, there is a need for their long-term storage while ensuring viability, purity, and genetic stability [7, 14, 17, 23, 46].

Low-temperature storage at –70...–80 °C is successfully used for depositing bacteria of various taxonomic groups as well as cyanobacteria [2, 17, 18, 24]; however, it is almost never applied to preserve complex eukaryotic microalgae [36]. The results of the study on the possibility of the unicellular green alga *Chlorococcum dissectum* storing showed that cooling the cells to –40 and –70 °C under uncontrolled temperature decrease conditions led to a threefold loss in viability. During the freezing of samples in a "Mr. Frosty" container (Freezing Container, Germany), which provides an average cooling rate of 1 degree per minute to both specified temperatures, the cells do not lose their ability to form colonies [76]. Cooling the algae *Desmodesmus spinosus*, *Chlorella sorokiniana*, and *Chlamydomonas biconvexa* to –70 °C led to a loss of cell viability [21]. At the same time, freezing the unicellular green alga *Lobosphaera incisa*, isolated from the snow slopes of Mount Tateyama in Japan, to –70 °C by direct placement in a freezer did not affect the culture reproductive capacity [41].

Some cultures of photoautotrophic organisms are maintained in laboratory conditions through serial subculturing [10, 17, 40]. However, this method has several disadvantages: increased risks of bacterial or cross-contamination of microalgae cultures

with other strains, as well as being labor-intensive and resource-demanding when working with large collections of unicellular algae.

Different species of algae react differently to low temperatures. This is likely related to their metabolic characteristics, specifically the composition of membrane lipids, the natural ability to accumulate cryoprotective compounds (sugars, glycerol, polyols), the level of antioxidant defense, and the activity of enzymes that maintain cell homeostasis during cooling. Additionally, differences in cellular water balance and the ability to synthesize cold-shock proteins may play a role.

In the context of selecting optimal cryopreservation conditions, it is important to choose effective cryoprotectants or their compositions, as well as cooling and warming regimens that would ensure maximal preservation of the algae's morphological uniformity and viability, allowing for rapid and efficient culture recovery and biomass accumulation for research and biotechnological purposes.

The aim of this review was to summarize the modern techniques of microalgae cryopreservation, specifically approaches to the selection of cryoprotectants and optimal cooling parameters to maintain the viability and functional activity of cultures after thawing.

SELECTION OF EFFECTIVE CRYOPROTECTANTS FOR MICROALGAE CELL CRYOPRESERVATION

The successful cryopreservation of biological materials depends on the type and concentration of the chosen cryoprotective compounds. Although cells of some *Chlorella* species maintain high viability (around 70 %) [30, 51, 52] after direct immersion in liquid nitrogen without cryoprotectants, most algae do not survive freezing without the addition of cryoprotective solutions. As reported by O. Holm-Hansen, 12 cyanobacterial and 17 green algal strains showed relatively high survival immediately after direct plunging into liquid nitrogen followed by warming; however, they were non-viable after one year of storage under liquid nitrogen conditions [30]. This is likely due to minor temperature fluctuations that may induce changes in the size and morphology of ice crystals formed during cooling. The addition of cryoprotective agents lowers the crystallization temperature, limits ice crystal growth under fluctuating temperatures, and provides additional protection for cell membranes against degradation and structural changes during the cooling–warming process [15, 23].

The effect of freezing *Chlorococcum dissectum*, which has a rigid cell wall, to liquid nitrogen temperatures without cryoprotectants demonstrated a significant reduction in colony-forming ability, almost tenfold [76]. For *Dunaliella salina*, which lacks additional cell coverings, no viable cells were observed. The use of different cryoprotectants helps to preserve viability and proliferative capacity of microalgae cells after low-temperature storage. However, different algal species require individual optimization of the type and concentration of cryoprotective mixtures.

DMSO as a cryoprotective agent provided the highest recovery of viability in *Tetradesmus obliquus* cells after freezing [25]. Cryopreservation of green algae *D. spinosus*, *C. sorokiniana*, and *C. biconvexa* under protection of various agents showed that for colonial coenobia, 5% DMSO was effective, whereas for unicellular coccoid and colonial palmelloid species, a combination of 5% glycerol and 5% polyethylene glycol 400 was most suitable [21]. Using protective solutions such as 10% DMSO, 10% EG, or 10% glycerol was ineffective for cryopreservation of 2 freshwater *Chlorella vulgaris* strains and two marine species, *Nannochloropsis oculata* and *Tetraselmis tetrathele*. Application of a mixture of 5% DMSO, 5% EG, and 5% proline achieved approximately 50% viability in these samples [55, 79].

Cryopreservation of *Scenedesmus* sp. was effective using 5% DMSO and 5% methanol [61]. Although no significant differences were observed during equilibration with 10—15% DMSO, D.J. Kihika *et al.* [37] recommended using the lowest effective concentration for cryopreservation. P. Ali *et al.* [1] reported that *Synechococcus* sp., *Microcystis aeruginosa*, *Scenedesmus obliquus*, and *C. vulgaris* cells cryopreserved with low concentrations of cryoprotectants exhibited higher viability than the cells equilibrated under high concentrations.

In a study by A. Shin *et al.* [71], *Stichococcus* microalgae were cryopreserved using a combination of linear polyglycerol and DMSO. The method was tested on 3 *Stichococcus* species (*S. bacillaris*, *S. deasonii*, and *S. minor*), demonstrating that this combination maintained viability and recovery rates under low temperatures for extended periods, compared to DMSO alone. While DMSO alone provided a high recovery after one week of low-

temperature storage, viability after 26 weeks dropped below 50%. Treatment with the combined solution increased recovery rates to 92% for all 3 *Stichococcus* species.

Our results also demonstrate that the viability rate of *C. dissectum* microalgae cells significantly decreased over a year of storage at liquid nitrogen temperature compared to data obtained immediately after the cooling-warming stage [76]. This fact can be attributed to the occurrence of slow processes even in liquid nitrogen that led to cell damage, such as gradual protein denaturation, oxidative reactions of residual oxygen, changes in the state of membranes and water within microdomains, and the accumulation of structural defects during storage.

Studies on halophilic microalgae, including *Dunaliella tertiolecta*, showed low effectiveness of DMSO, with cell survival not exceeding 2% [74]. Numerous observations have demonstrated the potential of 10% glycerol (often in combination with sucrose or raffinose) to achieve high viability levels in marine algae after warming [35, 60, 79]. For multicellular green algae *Gonium* sp., 6% dimethylformamide and 5—10 % methanol proved to be effective cryoprotectants for maintaining cell viability at all developmental stages [57].

Since microalgae differ in their halotolerance, the presence and structure of the cell wall, and their capacity for dehydration, it should be noted that these differences can influence the effectiveness of a particular cryoprotectant. According to literature data, most freshwater and soil algae possess a rigid cell wall that allows cells to withstand short-term swelling. Based on this evidence, they are better cryopreserved under the protection of monohydric alcohols, whereas most marine species often lack a strong wall, making the use of DMSO more effective for them [7, 38].

Permeating cryoprotectants are toxic at high concentrations. The prolonged exposure to methanol at concentrations of 5—10% is toxic for *Euglena gracilis*, and even short-term (20-minute) exposure at concentration above 15% can be harmful [22]. Monohydric alcohols, DMSO, and EG denature enzymes at room temperature, and DMSO additionally destabilizes the proteins of cells undergoing cryopreservation. However, C.J. Malajczuk *et al.* [47] showed that DMSO can protect isolated enzymes during freezing. This seeming contradiction is explained by temperature-dependent hydrophobic interactions between DMSO and non-polar protein fragments. At temperatures below –22 °C, low concentrations of rapidly permeating cryoprotectants can act as cryosensitizing agents, thereby accelerating damage to cell membranes [67]. Permeating cryoprotectants should be added to the culture immediately before freezing and promptly removed after warming. Occasionally, cryoprotective mixtures are added after cooling the culture to 0 °C or lower to minimize intracellular toxicity [39]. For algal strains that have not yet demonstrated high viability levels after cryopreservation, it is necessary to determine not only the type but also the concentration of protective agents.

It is advisable to establish their tolerance to a specific cryoprotective medium before investigating the influence of cooling regimens. As a rule, concentrations of low-molecular weight cryoprotectants below 2% are ineffective for cell preservation at low temperatures, whereas concentrations exceeding 12% are often toxic and vary significantly across different microalgae species, sometimes even among closely related strains. Despite the fact that many microalgae strains in collections have been successfully cryopreserved using 5% methanol or 5—8% DMSO, the most effective concentrations for individual strains must be determined empirically [78].

Non-permeating cryoprotectants (polyvinylpyrrolidone, hydroxyethyl starch, and polyethylene glycol) are rarely used for microalgae cryopreservation. G. Morris conducted cooling of *Chlorella* sp. under the protection of 10% polyvinyl alcohol; however, a higher viability rate was observed after using the penetrating cryoprotectant DMSO [50]. The cryoprotective properties of non-penetrating agents have been tested on a wide range of microalgae. It should be noted that a sufficient viability percentage has not yet been achieved for these cultures, as none of the investigated compounds provided the necessary level of cryoprotection [74].

P. Ali *et al.* [1] also indicate that non-penetrating cryoprotectants provide good protection for such strains of cyanobacteria and unicellular algae as *Scenedesmus* sp. HTB1, *Synechococcus* sp. CBW1003, *Synechococcus* sp. CB0101, and *Microcystis* sp. 7806. For all these strains, growth recovery was better when using non-penetrating cryoprotectants than

after adding 5% DMSO. Permeating cryoprotectants (EG, propylene glycol, DMSO, glycerol, and methanol) exhibit cytotoxic activity toward the studied cultures.

Therefore, before cryopreserving biological material for long-term storage, it is crucial to determine the type and concentration of the cryoprotectant for each individual strain and species of microalgae, as the viability of biological objects during their use and subsequent biomass recovery vary significantly across different strains and cultures [65].

The Alamar Blue test is one of the rapid methods for determining the damaging effects of cryoprotectants on unicellular algae cells; it allows for the assessment of cellular metabolic activity based on the fluorescence intensity of reduced resazurin [40, 75]. As reported by K. Vozovyk *et al.* [75], the effects of cryoprotective solutions of EG, DMSO, ethyl alcohol, glycerol, and the vitrification solutions PVS1 (22% glycerol + 13% 1,2-propylene glycol + 13% EG + 6% DMSO + 0.4 M sucrose) and PVS2 (30% glycerol + 15% EG + 15% DMSO + 0.4 M sucrose) at concen-trations of 50 and 75% were investigated on cells of the freshwater alga *C. dissectum* and the halophilic *D. salina*. Based on the obtained data, it was established that *D. salina* cells were more resistant to all studied cryoprotective solutions compared to *C. dissectum* cells. The cryoprotectant EG, even at a concentration of 5%, reduced the metabolic activity of *C. dissectum* cells by almost half. Exposure of cells to a 10% glycerol solution showed the least pronounced effect on the cells of this alga among all investigated cryoprotectants.

Treatment of samples with 5—15% solutions of ethanol and DMSO reduced the metabolic activity of cells by 31—33% compared to the control. Incubation of *C. dissectum* cells with vitrification solutions showed that the modified PVS1 solution at a 50% concentration had the lowest damaging effect, with a 33% reduction in metabolic activity compared to the control. Ethyl alcohol proved to be the most toxic cryoprotectant for the *D. salina* culture. Exposure of cells to 5—15% solutions of DMSO, EG, and glycerol reduced metabolic activity by less than 25%, while in PVS1 and PVS2 solutions, the reduction was 5%.

Thus, for the cryopreservation of various microalgae species, the use of glycerol, DMSO, monohydric alcohols, and EG has been proven to be effective [7, 12, 25, 26, 53, 57, 63, 78].

SELECTION OF OPTIMAL COOLING AND WARMING RATE PARAMETERS FOR MICROALGAE SAMPLES DURING CRYOPRESERVATION

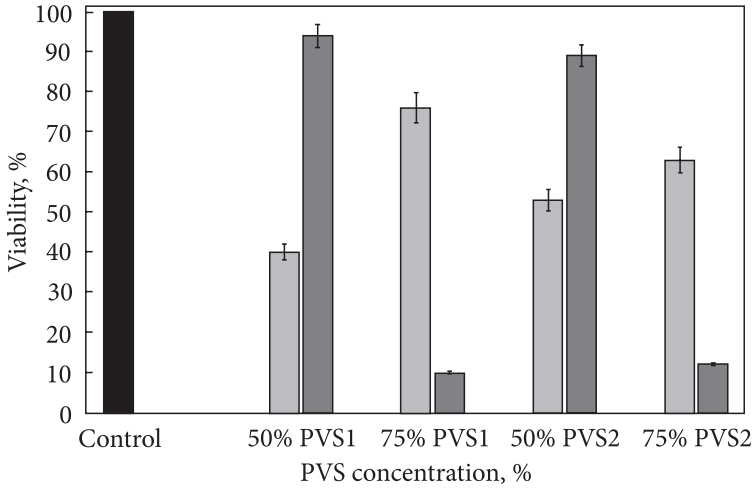
The development of effective and biotechnologically acceptable cryopreservation protocols aims to minimize intracellular ice formation and the impact of excessive osmotic-induced changes in cell volume, as either of these factors during cryopreservation can lead to irreversible physical and/or chemical damage to microalgae [15, 22, 23, 48].

In a study conducted by G.I. Morris, the cryovials containing microalgae were held at –30 °C for 15 minutes before being immersed in liquid nitrogen. This simulation of a two-step cryopreservation regimen was successful for the storage of 252 out of 284 tested strains, with a viability level of > 50% 24 hours after cooling; however, only 8 strains remained viable after one year of low-temperature storage [50].

To achieve high microalgae viability rates, it is necessary to select the correct cooling-warming regimen. According to the data presented by A. Ben-Amotz [5], a relatively high preservation level (around 30 %) can be obtained for microalgae of the genus *Chlorella* sp. through cryopreservation by direct immersion in liquid nitrogen under the protection of DMSO. A study on the viability of 8 eukaryotic algae species from the class *Chlorohyceae* after 15 years of storage at –196 °C, which were cooled following exposure to 5—10% DMSO, glycerol, and methanol, showed between 34 and 72 % viable cells, depending on the species [14, 19, 33, 78].

At the same time, most researchers believe that two-step regimens are more effective for microalgae cryopreservation. These protocols involve adding a permeating cryoprotectant to the algal culture, followed by cooling at a controlled rate to a specific sub-zero temperature, after which the sample is immersed directly into liquid nitrogen. This approach to cryopreservation allows the cells time for dehydration and the uniform distribution of ice in the extracellular environment (the first step), while also preventing the formation of large ice crystals inside the cells (the second step), thereby reducing mechanical and osmotic damage.

When using programmable freezers, cooling rates of 0.5—10 °C per minute are most commonly applied during the first temperature reduction stage down to –40...–70 °C [16, 19, 31, 53, 58, 60, 66, 78].



Viability of microalgae cells after cryopreservation by vitrification with the addition of 50 and 75% PVS1 or PVS2: ▒ — *Dunaliella salina*, ▒ — *Chlorococcum dissectum*

The disadvantages of this approach include reliance on expensive equipment and high liquid nitrogen consumption during programmable cooling. Since the introduction of convenient "Mr. Frosty" freezing containers, they have become the most frequently used tool for cooling cell suspensions during the first stage before immersing the samples in liquid nitrogen [49, 68, 76].

The effectiveness of microalgae cryopreservation depends on the warming conditions of the frozen culture. It is known that during the recrystallization at the temperature increase stage, ice crystals can grow, which typically damages cells. In a study by J.P. Cañavate *et al.* [9], the effects of slow and rapid warming on the preservation of five marine microalgae samples under the protection of 15% DMSO were investigated at rates of 0.5, 6, and 16 degrees per minute. It was shown that under the slowest warming rate, all studied cultures exhibited viability levels no higher than 17%. Therefore, in cryopreservation practice, high warming rates are most commonly used for cooled suspensions to minimize the negative impact of recrystallization on the cells.

It should be noted that for many microalgae, especially flagellated species such as *D. salina*, effective cryopreservation protocols could not be developed using programmed two-step cooling or direct immersion in liquid nitrogen after incubation with 10% penetrating cryoprotectants. Therefore, it was necessary to develop methods based on the vitrification of both the intra- and extracellular environments. Vitrification is a process that alters the aqueous state of cells so that ice formation is inhibited under cryogenic temperatures; due to high viscosity, the liquid forms an amorphous, non-crystalline glassy state [28, 29, 42]. The figure shows that vitrification with the addition of 50 and 75% PVS1 or PVS2 allows for the preservation of up to 75% of *D. salina* cells and 90% of *C. dissectum* cells relative to the control after warming.

Vitrification is the "solidification" of a liquid in the absence of crystallization, where, in this state, it possesses physical and mechanical properties similar to those of a solid. From a physicochemical standpoint, this state is considered metastable, as devitrification may occur, leading to the formation of a solid crystalline structure. In the case of vitrifying aqueous solutions at low temperatures, relaxation and devitrification can result in ice formation during warming [29, 70]. Achieving a stable glassy state during cryopreservation is possible when the increase in viscosity corresponds to a decrease in the movement of water molecules, restricting their ability to rearrange into a crystalline form and making ice nucleation nearly impossible as the temperature drops. During cooling, the viscosity of highly concentrated solutes increases, and water molecule mobility practically ceases [28]. At this point, the liquid becomes a glass with an amorphous, non-crystalline state defined by the glass transition temperature. This temperature is used to characterize the point at which the physical properties (*e.g.*, heat capacity and optical properties) of the system change. Vitrification offers critical advantages for the cryogenic storage of biological samples because glass, unlike ice crystals, does not significantly alter the structure or composition of solutions [23, 28].

In the report of E. Zhang et al. [81], it was shown that using a vitrifiable solution (PVS2) in encapsulation-vitrification technology allows for a high viability level of the marine diatoms *Nitzschia clos-*

terium (73.8%) and *Chaetoceros muelleri* (48.2%). In studies by W. Stock *et al.* [73], cryopreservation of freshwater and marine diatoms was conducted under the protection of 5—20% PVS2. The authors demonstrated that at these concentrations, nearly all algae resumed growth after warming, and cell viability indices significantly increased with higher cryoprotectant concentrations in the cryoprotective medium.

Thus, the following conclusion can be drawn. Further research into the low-temperature storage of microalgae should aim to improve cryopreservation methods and standard operating protocols by replacing toxic cryoprotectants with more effective, non-toxic substances or their compositions. These measures will facilitate the development and industrial implementation of organic biotechnologies, as well as enhance their quality and safety standards.

CONCLUSIONS

Based on the analysis of current literature data regarding microalgae cryopreservation, it has been established that the effectiveness of long-term low-temperature storage is determined by a combination of physical freezing parameters and the biochemical properties of the cryoprotective medium, which must be selected individually for each species or strain.

It has been shown that the use of cryoprotectants is critically necessary to prevent lethal cryoinjury for the most of microalgae cells. Penetrating cryoprotectants (DMSO, glycerol, EG, monohydric alcohols) remain the most common and effective; however, their action is accompanied by dose-dependent cytotoxicity, which limits the possibility of using high concentrations. Non-penetrating cryoprotectants are characterized by lower toxicity but, in most cases, do not provide an adequate level of protection when used alone.

Published data analysis indicates that combined cryoprotective compositions, which integrate both penetrating and non-penetrating components, allow for increased cell preservation. The effectiveness of such compositions significantly depends on the morphophysiological characteristics of the microalgae, including the presence of a cell wall, halotolerance, water balance, and membrane composition. It has been established that for most microalgae, two-step cooling regimens — featuring a controlled cooling rate during the first stage followed by immersion in liquid nitrogen — are more effective than direct immersion. Rapid warming of samples after storage also plays a vital role by minimizing ice recrystallization and associated cell damage.

Vitrification-based methods represent a distinct and promising prospect in microalgae cryopreservation. These methods ensure cell preservation by transitioning the aqueous medium into an amorphous glassy state without ice crystal formation. The use of vitrification solutions, particularly modified plant vitrification media, demonstrates high viability rates for both freshwater and halophilic microalgae species.

To conclude, the further development of microalgae cryopreservation technologies should aim to optimize the composition of cryoprotective mixtures, reduce their toxicity, standardize cooling and warming regimens, and implement vitrification methods more broadly. Implementing these approaches will enhance the efficiency of long-term microalgae storage, ensure their genetic stability, and expand the possibilities for their use in scientific and biotechnological research.

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МЕТОДИ КРІОКОНСЕРВУВАННЯ МІКРОВОДОРОСТЕЙ:
ВИБІР КРІОПРОТЕКТОРІВ ТА ПАРАМЕТРІВ ОХОЛОДЖЕННЯ

У поданому огляді узагальнено та проаналізовано сучасні методи кріоконсервування мікроводоростей, зокрема підходи до вибору кріопротекторів та оптимальних параметрів охолодження, з метою збереження життєздатності та функціональної активності культури після розморожування. Крім того, розглянуто ключові аспекти кріозбереження мікроводоростей, включаючи порівняння ефективності різних класів кріопротекторів та їхній вплив на клітини; описано механізми та стратегії мінімізації кріопошкоджень; визначено оптимальні режими охолодження та нагрівання для різних видів мікроводоростей. Окрему увагу приділено встановленню залежності ефективності результатів кріоконсервування від складу та концентрації кріопротекторів у кріозахисних композиціях, які підбираються окремо для конкретних видів культур. Відзначено, що деякі мікроводорості зберігають високу життєздатність за використання підвищених концентрацій непроникних кріопротекторів. Підкреслюється необхідність проведення подальших досліджень із застосуванням комбінованих розчинів кріопротекторів для розширення можливостей довготривалого зберігання мікроводоростей.

Ключові слова: мікроводорості, кріопротектори, режими кріоконсервування, здатні до склування розчини, низькотемпературне зберігання.