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Article

Changes in Energy status of *Saccharomyces cerevisiae* Cells during Dehydration and Rehydration

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Anhydrobiosis is the state of life when cells get into waterless conditions and gradually cease their metabolism. In this study, we determined the sequence of events in *Saccharomyces cerevisiae* energy metabolism during processes of dehydration and rehydration. The intensities of respiration and acidification of the medium, the amounts of Phenyldicarbaundecaborane (PCB^-) bound to yeast membranes, and the capabilities of cells to accumulate K^+ were assayed using electrochemical monitoring system, and intracellular content of ATP was measured using bioluminescence assay. Mesophilic, semi-resistant to desiccation *S. cerevisiae* strain 14 and thermotolerant, very resistant to desiccation *S. cerevisiae* strain 77 cells were compared. After 22 h of drying it was possible to restore the respiration activity of very resistant to desiccation strain 77 cells, especially when glucose was available. PCB^- binding also indicated considerably higher metabolic activity of dehydrated *S. cerevisiae* strain 77 cells. Electrochemical K^+ content and medium acidification assays indicated that permeabilization of the plasma membrane in cells of both strains started almost simultaneously, after 8-10 h of desiccation, but semi-resistant strain 14 cells were longer keeping K^+ gradient and stronger acidifying the medium. For both cells, the fast rehydration in water was less efficient compared to reactivation in the growth medium, indicating the need for nutrients for the recovery. Higher viability of strain 77 cells after rehydration could be due to the higher stability of their mitochondria.

Keywords: yeast; anhydrobiosis; dehydration-rehydration; metabolism; mitochondria

1. Introduction

Anhydrobiosis is one of the special forms of cryptobiosis, in which the yeasts survive through adverse environmental conditions, in this case – the absence of water (desiccation). To survive, cells adapt to the changed environment temporarily suspending their metabolism [1–3]. Understanding of the mechanism of anhydrobiosis and the main determinants, important for cell viability after the transfer to the state of anhydrobiosis, knowledge of events during the subsequent processes of rehydration and reactivation are important for the fundamental science and could be applied in biomedicine and biotechnology. In biomedicine, understanding of anhydrobiosis is important for the research in translational medicine, the production of new vaccines. Further improvement of the quality of dry biopreparations is needed in biotechnology, including active dry yeasts for wine, beer, and ethanol production, as well as for production of various biologically active compounds [4,5]. Big companies provide high-quality preparations of dry bakers' yeast with very high level of cell viability. At the same time, such a result still cannot be achieved for other yeast species, as well as for other *S. cerevisiae* strains necessary for the practical goals. One of the approaches to understand

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reasons for these differences is the comparison of yeasts demonstrating various resistance patterns during dehydration-rehydration treatment. Previous studies showed [6], that osmotolerant halophilic yeasts *Debaryomyces hansenii* are more resistant to dehydration compared to *S. cerevisiae.* Being in the exponential phase of growth these cells can effectively transit into anhydrobiosis, when other actively growing yeast species are very sensitive to such treatment. Further research revealed essential differences in the chemical composition of their plasma membranes, which led to the lower phase transition temperature of the membrane lipids [7,8]. In another study, it was revealed that thermotolerant strains of *S. cerevisiae* are more resistant to dehydration compared to mesophilic strains [8]. Recently it was shown [9], that, also, psychrophilic yeast species are more resistant to dehydration than mesophilic ones. These results mean that yeast strains and species, able to survive in more extreme environmental conditions, possess also higher resistance to dehydration-rehydration treatment. Therefore, we expected that continuation of the studies directed to the comparison of strains with different resistance to dehydration-rehydration treatment may lead to the information, important for general biology, as well as might facilitate the development of technologies for improvement of the resistance of sensitive strains, important for current biotechnology.

The purpose of this study was to compare mesophilic and thermotolerant strains of *S. cerevisiae,* to determine the sequence of events in the energy metabolism of these cells during dehydration, and regeneration of these processes during the rehydration. The intensities of respiration and acidification of the medium, the amount of Phenyldicarbaundecaborane (PCB^-) bound to cellular membranes, and the capability of cells to accumulate K^+ were assayed using an electrochemical monitoring system. Results of our experiments indicated, that very resistant to desiccation strain 77 cells show the respiration activity after 22 h of drying, demonstrate stronger plasma membrane barrier to lipophilic anions and keep the higher content of intracellular ATP, although mesophilic strain 14 cells were longer keeping K^+ gradient during desiccation and stronger acidifying the incubation medium. It was concluded that the higher viability of rehydrated 77 strain cells could be due to the higher stability of their mitochondria. These results advance our understanding of the impact of dehydration on the energy metabolism in yeasts.

2. Materials and Methods

2.1. Strains and the cultivation conditions

The diploid isogenic yeast strains used in this study *S. cerevisiae* 14 and *S. cerevisiae* 77 were obtained from the Microbial Strain Collection of Latvia (http://mikro.daba.lv). The cells were grown in conical flasks, 1/5 volume filled with YPD medium (1% yeast extract (Acros Organics), 2% peptone (Oxoid), and 2% glucose (Chempur), in a thermostated shaker at 30 °C for 44 h shaking at 220 rpm (to the mid of stationary growth phase).

2.2. Preparation of cells for experiments and desiccation

For dehydration experiments, the cells were grown as described above, harvested by centrifugation (1000xg, 10 min), washed twice with 0.1 M sodium phosphate buffer, pH 7, then resuspended in 5 ml of the same buffer. After measuring OD_{600} of the diluted suspension, the exact volumes of the suspensions, containing $1.7x10^8$ of cells (at conditions used, OD_{600} 1 corresponded to $3x10^7$ cells/ml), were aliquoted into 1.5 ml Eppendorf type tubes. The cells were pelleted by centrifugation (1000xg, 3 min) and supernatants were carefully removed. Open tubes with the cell pellets were placed into an oven and desiccated at 30 °C for 22 h. After drying, open tubes with the dry yeast pellets 1-3 days were kept in an exicator, in the presence of silica gel. For the control of 0% humidity, the cells where incubated at 105 °C for 24h.

2.3. Cell rehydration and reactivation

50 µl of deionized water (for rehydration) or the same volume of YPD growth medium (for reactivation) was poured to the tubes with the dry yeast pellet, the suspension was vortexed and incubated at room temperature for defined periods of time. The content of two tubes was transferred

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into one vessel for electrochemical measurements. For K^+ measurement, to determine the amount of this ion inside the cells, after incubation in YPD, the cells were twice washed with 0.1 M sodium phosphate buffer, pH 7.

2.4. Electrochemical measurements

Several parameters of the metabolic activity of yeast cells were investigated: changes in the medium concentration of dissolved oxygen (respiration rate), ability of the cells to acidify the incubation medium (intensity of glycolysis), K^+ and Phenyldicarbaundecaborane (PCB^-) concentrations in the incubation medium (cells' capability to accumulate K^+ and to keep metabolism-dependent plasma membrane barrier to lipophilic anions). The intensities of respiration and acidification of the medium, the level of PCB^- binding and the amount of cell accumulated K^+ were assayed using an electrochemical monitoring system [10]. K^+ (Thermo Orion, model 9300BN), H^+ (Hanna Instruments, model HI1131B), and PCB^- (prepared as described in [10]) - selective electrodes and the dissolved oxygen probe (Thermo Orion, model 9708) were connected to electrode potential amplifying system with an ultralow-input bias current operational amplifier AD549JH (Analog Devices, Norwood, MA, USA). The data acquisition system PowerLab 8/35 (ADInstruments, Oxford, UK) was used to connect the amplifying system to a computer. Agar salt bridges were used for indirect connection of the Ag/AgCl reference electrodes (Orion model 9001; Thermo Fisher Scientific) with cell suspensions in the vessels. Content of two Ependorff tubes was transferred into thermostated and magnetically stirred vessels and filled with 0.1 M sodium phosphate, pH 7, up to 5 ml. For acidification studies the dry cells were resuspended and vessels were filled with the medium, containing 95 mM NaCl and 5 mM sodium phosphate, pH 7. Calibrations of K^+ and PCB^- selective electrodes were performed before the additions of cells. During measurements of dissolved oxygen and H^+ concentration in the cell suspensions, calibrations were performed at the end of experiment, adding solid $Na_2S_2O_4$ up to ~20 mM (0% of dissolved oxygen, when 100% is the concentration in the medium before addition of cells), or 10 µL of 0.1 M HCl, correspondingly. Glucose up to 0.8% was added to energize the cells and Nystatin up to 10 µg/ml - to achieve complete permeabilization of the cell plasma membrane. Measurements were performed at 30 °C.

2.5. ATP measurements

The intracellular amounts of ATP were determined using Modulus luminometer (Turner BioSystems, model 9200-003) and luciferin-luciferase method, taking samples for analysis directly from the vessels for electrochemical measurements [10] after 10 min of incubation in 0.1 M sodium phosphate buffer, pH 7. ATP Biomass Kit (BioThema) was used in these experiments. 5 µl of the cell suspensions from the vessels was mixed with 5 µl of the lysing reagent, and after 10 min 90 µl of luciferin-luciferase mixture was added. The recorded intensity of luminescence was evaluated using ATP calibration curve.

3. Results

3.1. Studies of the dehydration

Intensities of the respiration and acidification of the medium, the binding of PCB^- to cellular membranes, and the amount of cell-accumulated K^+ were determined using an electrochemical monitoring system. Cells of two *S. cerevisiae* strains were compared: mesophilic, semi-resistant to desiccation strain 14 and thermotolerant, very resistant to desiccation strain 77. Before the dehydration, strain 14 cells demonstrated higher energy status than strain 77 ones: the intensity of respiration of strain 14 cells was higher (Figure 1, A and B) and they accumulated more K^+ (Figure 1, C and D). Addition of glucose to the incubation medium induced a very strong decrease of the dissolved oxygen concentration in suspensions of both cells, but this effect gradually decreased with the increase of the duration of cell desiccation. Additions of glucose revealed differences between the studied strains. Glucose stimulated the respiration of *S. cerevisiae* 77 cells even after 22 hours of

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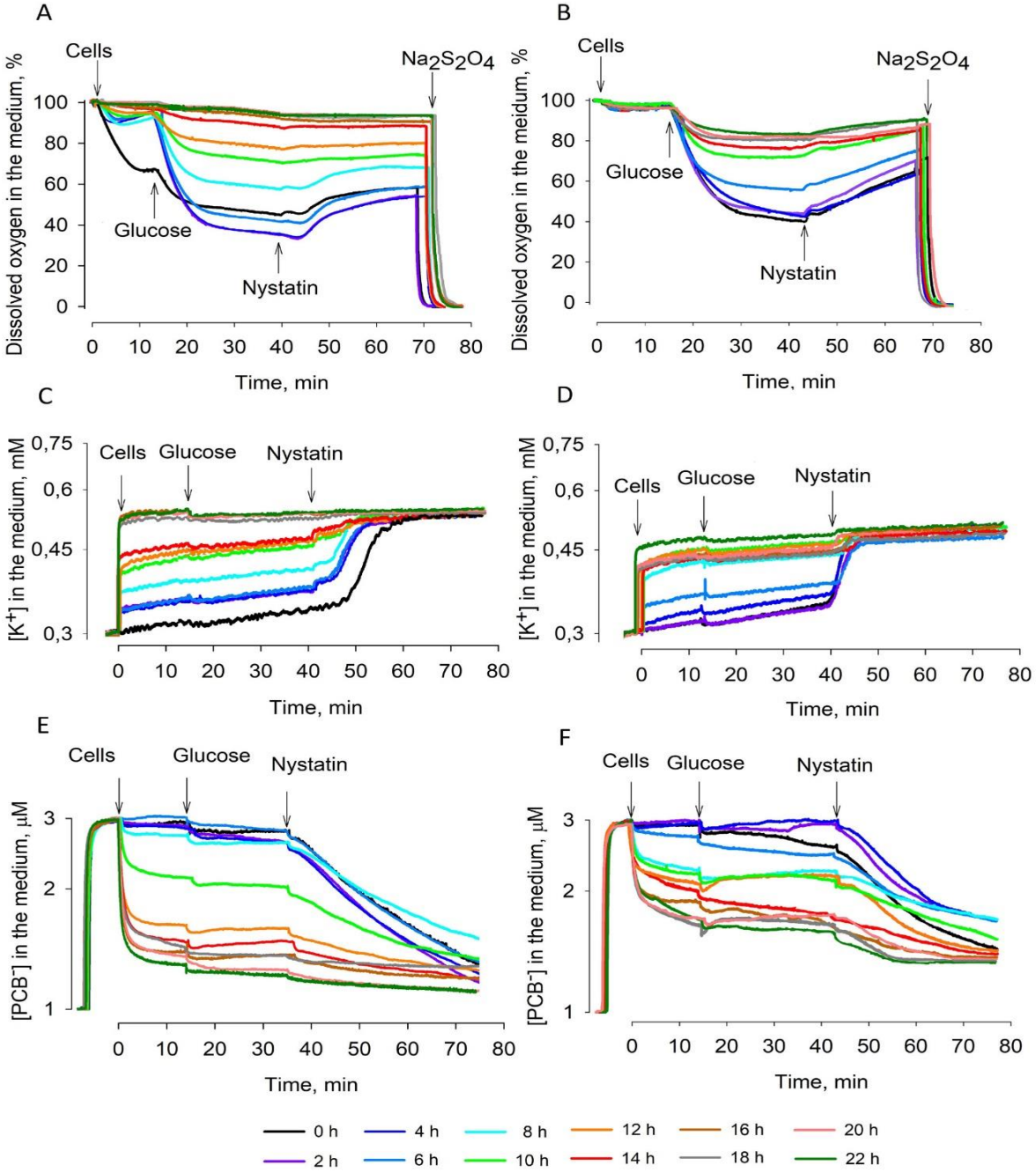
desiccation. Strain 14 cells earlier than strain 77 ones suspended the respiration during dehydration and after 14 h of drying stopped reacting to the additions of glucose.

Figure 1. Changes in the energy status of *S. cerevisiae* 14 (**A, C, E**) and *S. cerevisiae* 77 (**B, D, F**) cells during dehydration. A and B – activity of respiration, C and D – capability to accumulate K^+, E and F – binding of



PCB^- to the cells. Measurements were performed using magnetic stirring in thermostated glass vessels. The cells after certain periods of desiccation were resuspended in 50 µl of 0.1 M sodium phosphate, pH 7.0, and added to 5 ml of the same buffer in the vessels. Glucose was added to the final concentration of 0.8% and Nystatin - to 10 µg/ml, correspondingly. Monitoring dissolved oxygen, dry $Na_2S_2O_4$ was added to the final concentration of ~20 mM.

The studied cells differed not only by the accumulated amount of K^+, but also by the capability to keep these ions inside the cells during dehydration. Intensive K^+ leakage from strain 77 cells was observed till 6th h of desiccation and later only very slight K^+ flow was observed. Strain 14 cells were gradually releasing intracellular K^+ during the first 14 h of desiccation. After the addition of antifungal polyene macrolide Nystatin to the medium, the maximal amount of K^+ leaked from the

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cells. The studied strains differed in their sensitivity to this, pores in the yeast membranes forming compound. In suspensions of strain 77 cells, inhibition of the respiration and nystatin-induced release of K^+ started almost immediately after addition of this antifungal. In the case of strain 14 cells, 3-4 min lag-period was observed after the addition of this compound and the effect of nystatin on the respiration activity of these cells was considerably weaker compared to strain 77. It is worth to mention, that in the case of strain 77 cells, nystatin was releasing some amount of accumulated K^+ even after 20 hours of desiccation, although all accumulated K^+ leaked out of strain 14 cells in 16 h.

Lipid bilayers bind rather high amounts of lipophilic anions [11]. PCB^- accumulates in yeast membranes after metabolic inactivation of the cells [12,13]. During dehydration, the binding of PCB^- indicated considerably higher metabolic activity of *S. cerevisiae* 77 cells compared to strain 14. The maximal amount of PCB^- was bound to strain 14 cells after 12 h of dehydration. Accumulation of this lipophilic anion in strain 77 cells gradually increased during 22 hours of desiccation and nystatin induced an additional binding of this indicator to the cellular membranes (Figure 1, E and F).

The different rates of changes in energetical characteristics of the studied strains could be a result of different rates of desiccation. Control experiments showed that before desiccation strain 77 cells contained ~4% less of water than strain 14 ones, but were losing it at a similar rate. Cells of both strains 70-80 % of water lost during the first 11 hours of desiccation (Figure S1).

3.2. Effects of dehydration on acidification of the medium

The rate of acidification of the extracellular medium is frequently used to assay the rate of glycolysis, although there is another potential source of the extracellular protons – respiration [14]. Monitoring of the dissolved oxygen concentration in the medium demonstrated different effects of glucose on the respiration of studied cells. Before the drying, strain 77 cells were actively reacting to the addition of glucose. Strain 14 ones were actively consuming the dissolved oxygen before the addition of glucose, but their reaction to this substrate was less pronounced (Figure 1, A and B).

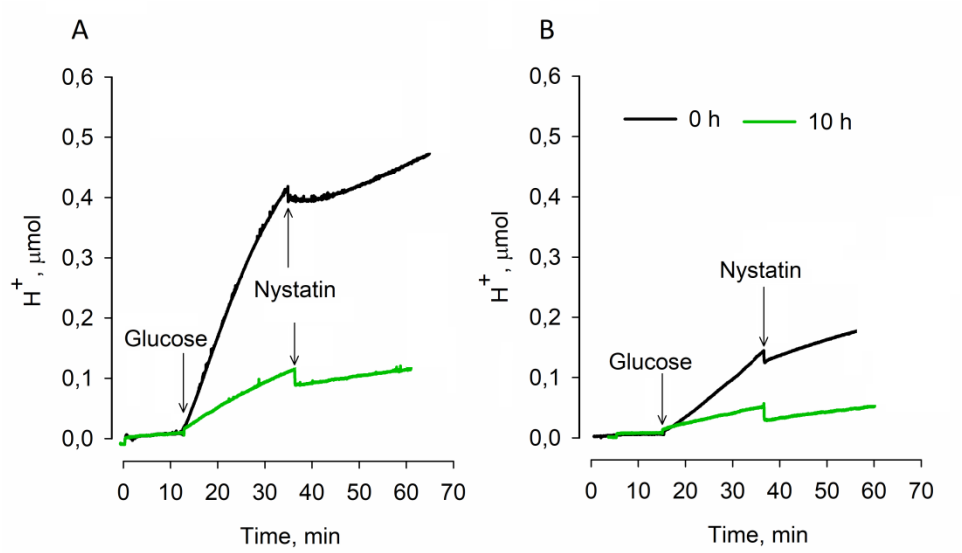

Figure 2. Effect of desiccation on the acidification of *S. cerevisiae* 14 (**A**) and 77 (**B**) cell suspensions. Measurements were performed at 30 °C in thermostated and magnetically stirred glass vessels, containing 5 ml of 95 mM NaCl solution, buffered with 5 mM sodium phosphate, pH 7. Cells were analyzed before the dehydration and after 10 h of drying. Glucose and nystatin were added to the final concentrations of 0.8% and 10 µg/ml, correspondingly.

Results of the medium acidification measurements showed (Figure 2) that responding to the addition of glucose before desiccation *S. cerevisiae* 14 cells were significantly stronger acidifying the medium compared to strain 77 ones. After 10 h of desiccation the intensity of acidification in the case of *S. cerevisiae* 77 cells decreased almost 4 times and of strain 14 cells - ~5 times compared to the initial

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ones before the drying. It should be mentioned that strain 14 cells twice more efficiently acidified the medium. These results indicate that at experimental conditions glycolysis is the main cause of medium acidification by the yeast cells.

3.3. Efficiency of rehydration

In the following experiments effects of rehydration (preincubation in deionized water) or reactivation (preincubation in YPD growth medium) on the energetics of the desiccated cells were analyzed. The rehydration slightly stimulated respiration of the dry *S. cerevisiae* cells and the effect on strain 77 cells was stronger compared to strain 14 ones (Figure 3, A and B). The respiration activities of both strains were stimulated by the addition of glucose: at the equilibrium conditions, the dissolved oxygen concentration in *S. cerevisiae* 14 cell suspension was 83-84%, and ~78% in the case of strain 77 cells. The addition of nystatin induced a weak inhibition of respiration in the case of 77 cells only. Strain 14 cells preincubated in the deionized water for 30-180 min did not consume any dissolved oxygen. In the case of strain 77, preincubation of dried cells in the deionized water also gradually inhibited consumption of the dissolved oxygen, and after 180 min of preincubation, the oxygen consumption was close to 0%.

Accumulation of PCB^- ions indicates the ability of cells to repair their plasma membrane: only the damaged cells bind high amounts of this indicator [10,13]. The desiccated cells directly added to the medium accumulated the highest amount of PCB^-. The amount of the indicator bound to cells slightly decreased after preincubation of the desiccated cells in the deionized water (Figure 3, E and F). Cells of both strains accumulated rather similar amounts of PCB^-. Dependence of the amount of PCB^- bound on the duration of preincubation was a bit stronger expressed in strain 14 cells, but strain 77 ones accumulated the higher amount of PCB^- after the addition of nystatin.

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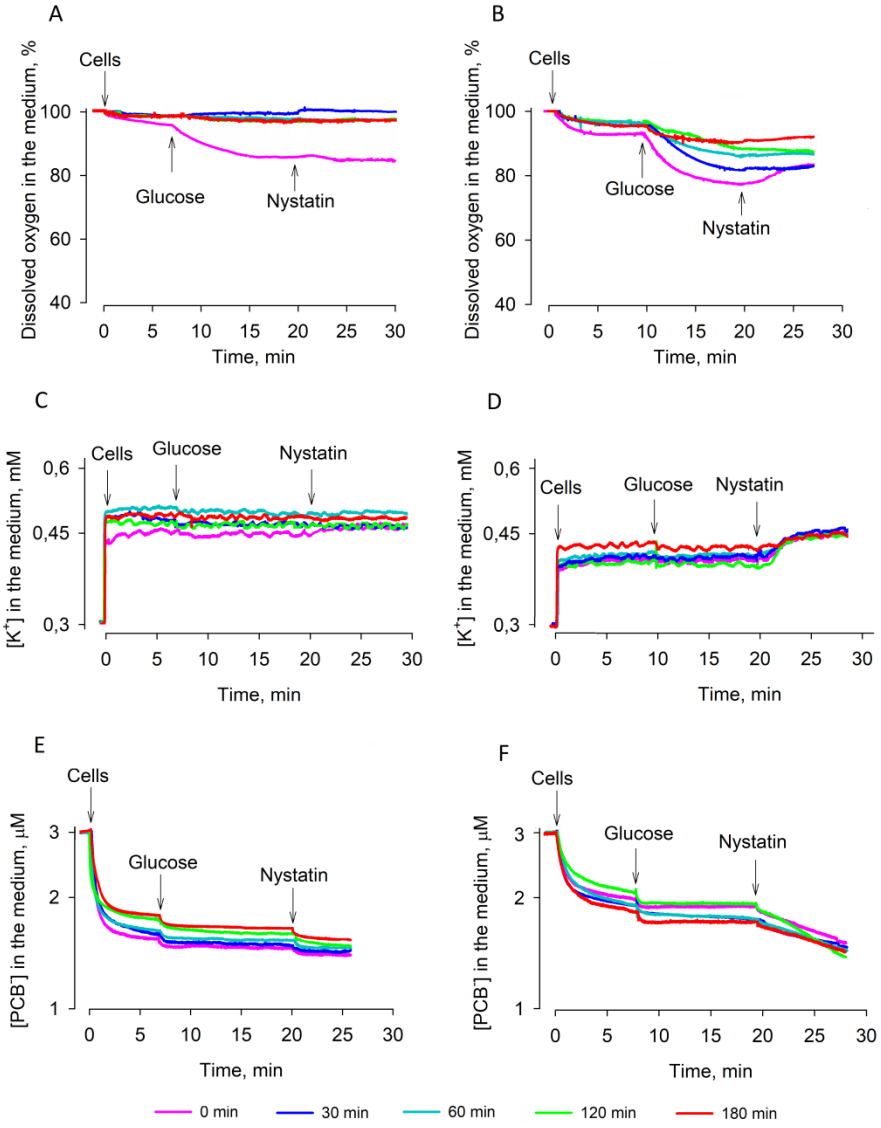

Figure 3. Changes in the energy status of dry *S. cerevisiae* 14 (**A, C, E**) and *S. cerevisiae* 77 (**B, D, F**) cells during the rehydration in deionized water. A and B – activity of the respiration, C and D – capability to accumulate K^+, E and F – binding of PCB^-. The dry cells were rehydrated at room temperature placing them for a definite period into deionized water. After rehydration, $3.4x10^8$ of pelleted cells were added to 5 ml of 0.1 M sodium phosphate, pH 7.0. The measurements were performed in thermostated glass vessels with magnetic stirring. Glucose was added to the final concentration of 0.8% and Nystatin - to 10 µg/ml. Monitoring the dissolved oxygen, dry $Na_2S_2O_4$ was added to the final concentration of ~20 mM.

Measurements of K^+ in the suspensions of dry cells resuspended in the deionized water showed that during desiccation most of this ion leaked out of the cells. Nystatin released almost equal amounts of K^+ from strain 77 cells independently on the duration of preincubation in deionized water. In the case of strain 14 cells, some nystatin-dependent release of K^+ was observed only in the case of non-preincubated cells. After the preincubation of these cells in the deionized water, nystatin was not able to release any K^+ from the cells. The amount of K^+ in the medium indicated that most of the preincubated strain 14 cells have lost their capability to accumulate this ion.

In summary, the results of our experiments indicated that after preincubation in the deionized water strain 14 cells lost their respiration activity and capability to accumulate K^+. However, strain 77 cells after preincubation in the deionized water demonstrated some respiration activity, were

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keeping the nystatin-sensitive barrier to PCB^- and were able to accumulate K^+, if the preincubation did not continue longer than 2.5 h.

During reactivation in the YPD growth medium, the cells of both strains were restoring their physiological activity more efficiently compared to the rehydration in deionized water (Figure 4). Strain 77 cells after 120-180 min of reactivation and addition of glucose were very strongly consuming the dissolved oxygen. At equilibrium conditions, the concentration of the dissolved oxygen was only ~40% of the initial level. However, strain 14 cells considerably weaker consumed the dissolved oxygen and at the equilibrium conditions concentration of the dissolved oxygen in the medium decreased only to 85-80%. Such low activity of the respiration of these cells was observed regardless of duration of the reactivation period.

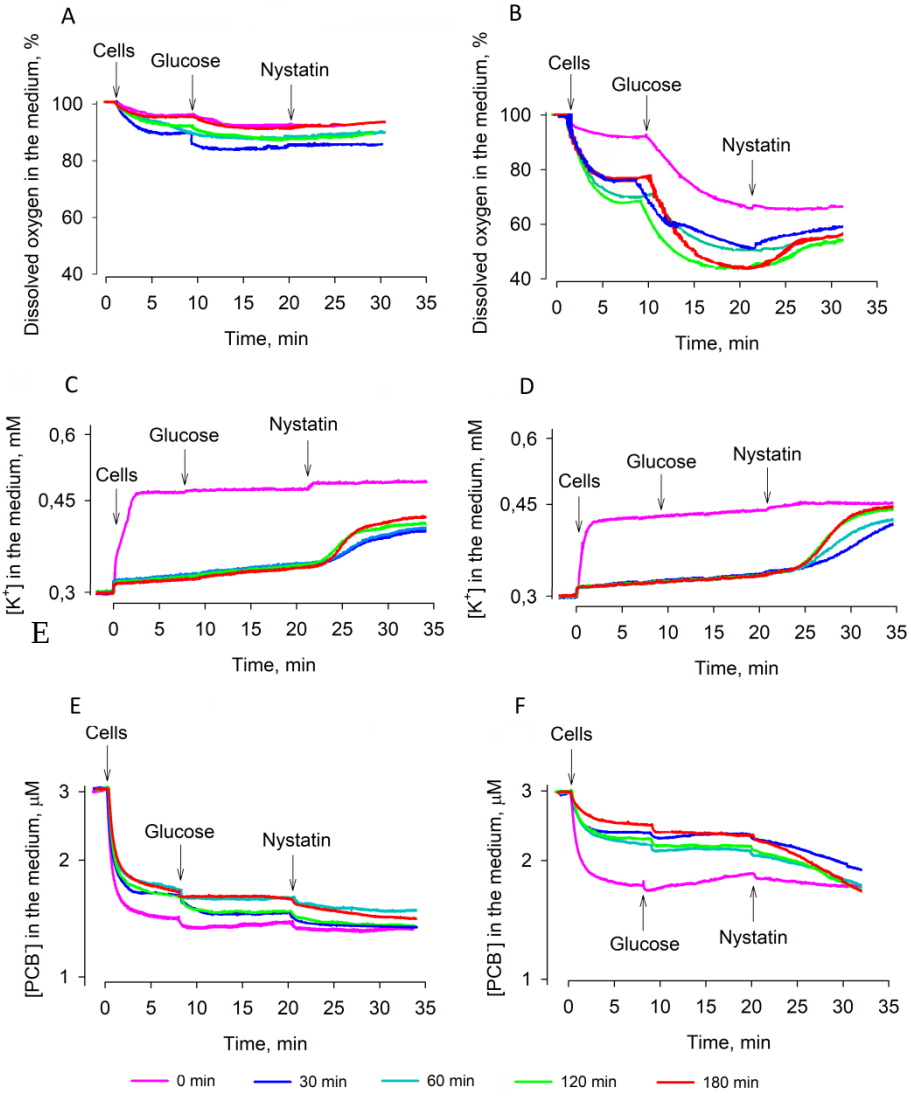

Figure 4. Changes in the energy status of dry *S. cerevisiae* 14 (**A, C, E**) and *S. cerevisiae* 77 (**B, D, F**) cells during the reactivation in YPD medium. A and B – activity of the respiration, C and D – capability to accumulate K^+, E and F – binding of PCB^-. Dry cells were reactivated at room temperature placing them for a definite period into YPD growth medium. After reactivation, $3.4x10^8$ of pelleted cells were added to 5 ml of 0.1 M sodium phosphate, pH 7.0. Measurements were performed in the magnetically stirred and thermostated glass vessels. For K^+ measurements the reactivated cells were twice washed with 0.1 M sodium phosphate, pH 7.0. Glucose was added to the final concentration of 0.8% and Nystatin - to 10 µg/ml. Monitoring the dissolved oxygen concentration, dry $Na_2S_2O_4$ was added to the final concentration of ~20 mM.

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Measurements of K^+ (Figure 4, C and D) also showed different capabilities of the strains to restore the gradient of this cation on the plasma membrane of cells. After addition to the incubation medium, cells of both strains were slowly releasing K^+, but the main amount of this cation was released after nystatin addition to the cell suspensions. Strain 77 cells released more K^+ than strain 14 ones and the accumulated amount of this ion inside the cells was dependent on the duration of reactivation. It is worth to mention that in both cases nystatin-induced release of the intracellular K^+ proceeded after a considerable lag period.

Accumulation of PCB^- by strain 77 cells gradually decreased during the reactivation (Figure 4, E and F) and after 30 min of preincubation in YPD medium these cells bound considerably less of this indicator. However, preincubation in the growth medium had a considerably weaker effect on the strain 14 dry cells, as they bound the higher amount of PCB^- regardless of the duration of reactivation.

3.4. ATP content of the cells during dehydration and rehydration

Samples for luciferin-luciferase luminometry of ATP content were taken from the vessels for electrochemical measurements after the transfer of cells from tubes for drying into 100 mM sodium phosphate buffer and incubation for 10 min.

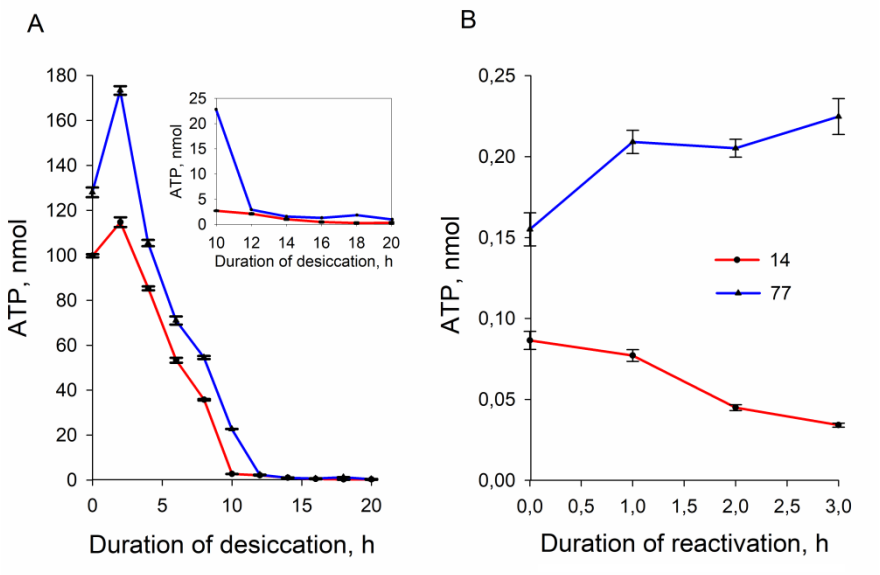

Figure 5. Changes in ATP content of *S. cerevisiae* 14 and 77 cells during their desiccation (**A**) and reactivation in the YPD medium (**B**). The amount of ATP was determined using a luciferin-luciferase bioluminescence assay, taking 5 µl glucose containing samples ($3.4x10^5$ cells) from vessels. Data are presented as Mean ± S.D. (n=3, three independent experiments).

The initial amount of ATP in strain 77 cells was approximately 20% higher compared to strain 14 ones (Figure 5A). During the first 2.5 hours of desiccation, the amount of ATP in yeast cells increased by 35% in the case of strain 77 and by ~15% in strain 14 cells. However, after 5 h of desiccation the ATP content decreased in both cells, in strain 77 being ~15% higher. After 10 h of drying in the case of strain 14, and after 12 hours - in strain 77 cells, the intracellular ATP content dropped to less than 1% of the initial level and stayed rather stable during the next 8-10 h of desiccation. During the first 3 h of reactivation in the YPD growth medium, the intracellular ATP content of 77 strain cells slowly increased, but it was slowly decreasing in the case of strain 14 cells.

4. Discussion

The main goal of this study was to determine factors, important for the survival of yeast cells during their transition into the state of anhydrobiosis and out of it, with the aim to analyze the

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possibilities to have highly active biological systems after reactivation of the dry cells. It is important to enrich our knowledge about the main biochemical mechanisms working during the process of transition. Such findings would be the control key to improve methods for yeast preservation, to understand the mechanisms of anhydrobiosis. Considering that yeasts are an optimal model of eukaryotic cells and are widely used for various studies in the fields of molecular biology, translational medicine, and pharmaceutics, they have more general importance, including treatment of pathological states of the human body.

During desiccation, yeast cells face mechanical, structural, and oxidative challenges, including intracellular crowding, plasma membrane lysis, and permeabilization. To survive these stresses, the yeasts have developed many endogenous protective mechanisms, including a unique elastic cell wall, the accumulation of intracellular glycerol and trehalose, the induction of stress proteins and biochemical antioxidative systems [2,20].

It is still not clear why strain 77 cells are more resistant to desiccation the strain 14 ones. Most probably, there is a complex of reasons. In addition, resistant to desiccation depends on the growth phase. For experiments we used cells from the stationary growth phase. Such cells show better adaptation to the environment [3,5]. Results of the monitoring of respiration activities indicated the differences in behavior of the studied *S. cerevisiae* cells during dehydration: very resistant to dehydration strain 77 cells maintained higher glucose-induced respiration activity than semi-resistant strain 14 ones. Answering to the addition of glucose, *S. cerevisiae* 14 cells not only demonstrated weaker respiration activity, but also carried out more intense acidification of the incubation medium.

It is known that energy-deprived yeasts acidify their cytoplasm. This feature is related to dormancy state of cells [21,22]. The increase in acidity of the cytosol during dormancy causes many proteins to interact with each other and form large clumps or filament structures that result in the cytoplasm becoming stiffer. Mobility of the organelles decreases, and mechanical stability of the cells increases. Results of our experiments indicate that strain 14 cells almost four times more efficiently acidify the medium compared to strain 77 cells. After 10 h of desiccation the medium acidification considerably decreased, but strain 14 cells still twice more intensively extruded acid. It is possible, that lower extrusion of acidic products, but more intense respiration (stronger extrusion of H^+ from mitochondria to cytosol) helps strain 77 cells to reach the dormancy state more efficiently than strain 14 cells do.

Our results revealed the decrease of ATP content in cells of both strain during dehydration. It, probably, indicates the usage of ATP for metabolic processes in the cells for intracellular protection of cell structure and maintenance of viability. While losing the water content, yeasts synthesize the endogenous compounds, such as glycerol and trehalose [2]. A rapid change in the cellular energy metabolism reflects the higher energy demands required for surviving solutes synthesis against osmotic shock [19,20]. After ATP depletion, Marini et al [21] observed significant cytosolic compaction and extensive cytoplasmic reorganization, as well as the emergence of distinct membrane-bound and membraneless organelles. The levels of ATP in both cells decreased with similar kinetics, but very resistant to dehydration 77 cells contained 10-20% more ATP than semi-resistant 14 ones. The higher amount of ATP in strain 77 cells before dehydration can also support a higher resistance of these cells to desiccation.

During the reactivation, strain 77 cells more efficiently recovered intracellular ATP than strain 14 ones: ATP level in strain 77 cells was slowly increasing, when ATP content of strain 14 cells was decreasing. These results support the ideas about higher stability and activity of mitochondria in 77 cells, giving them the capability of more efficient repair of their structure and functions. However, before decreasing, the amount of ATP increased by 35% in the case of strain 77 and by ~15% in strain 14 cells during the first 2.5 hours of desiccation (Fig.5). The increase of the ATP level could be a result of a temporal stimulation of synthesis or a consequence of sharp ceasing of ATP-consuming process inside the cells. To our mind, the latter process would be more probable. If desiccation initially

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decreases the ATP consumption, at conditions of good aeration the yeast cells in thin pellet, formed after centrifugation, at a high rate continue the oxidative phosphorylation.

Plasma membrane barrier to PCB^- (Figure 4) and ATP content of 77 cells (Figure 5B) were growing rather slowly. This could be due to very active consumption of ATP by the processes of cell recovery. The efficiency of ATP hydrolysis depends on Mg^{2+} ions, and these cations from the YPD medium could make ATP bioavailable [19] for many antioxidative systems to abrogate the resulting damage [2]. This could be one of the reasons why the cell reactivation in the YPD medium is more efficient than rehydration in water.

Erkut and colleagues [23] studied entry into the state of anhydrobiosis of two eukaryotic organisms - *S. cerevisiae* and roundworm *Caenorhabditis elegans*. Both organisms entered the state of anhydrobiosis during the cellular survival under the conditions of starvation. They discovered that glyoxylate shunt is the metabolic switch in both organisms, which helps to survive the desiccation by enabling or promoting gluconeogenesis for biosynthesis of trehalose. They compared the energetic/metabolic states and suggested, that the hypoaerobic lifestyle could be the feature of desiccation tolerance. In our case mid-stationary phase *S. cerevisiae* cells were not starving and were studied at aerobic conditions. Strain 14 cells started to demonstrate the damaged respiration at rather early stages of dehydration: after 14 h of drying these cells ceased the consumption of oxygen and did not demonstrate any reaction to the additions of glucose. However, this substrate was able to energize strain 77 cells even after 22 h of dehydration (Figure 1B).

Intracellular K^+ helps to keep the osmotic balance across the plasma membrane and stabilizes the turgor pressure. During the dehydration of strain 14 cells, reduction of K^+ gradient on the plasma membrane proceeded more slowly and after 8 h of desiccation these cells contained more K^+ than 77 ones. On the other hand, the addition of nystatin revealed that during 8-20 h of desiccation strain 77 cells were at some "low, but stable K^+" state. Strain 14 cells were accumulating K^+ till ~14^{th} h of desiccation, but they did not get to such state: starting from the 16^{th} h nystatin did not induce any leakage of accumulated K^+. It is possible, that nystatin was releasing K^+ accumulated in mitochondria of strain 77 cells. The activity of K^+ uptake systems in *Saccharomyces* (i.e., Trk uniporters) and/or the corresponding gene expression can vary among the strains, and the cells can have different membrane potential, which is a driving force for K^+ uptake. After reduction of the driving force, the release of accumulated K^+ can occur via transporters of this ion in the plasma membrane [15,16]. Our results suggest that the different modes of accumulation or the ability to keep the amount of intracellular K^+ in *S. cerevisiae* cells could affect the osmotolerance of the cells and be one of the factors determining the higher viability of strain 77 cells after rehydration.

PCB^- binding measurements demonstrated an efficient barrier of the plasma membrane to lipophilic anions in strain 77 cell. Binding of PCB^- to desiccated strain 77 cells decreased gradually, but already after 30 min of reactivation in the YPD medium these cells were binding considerably lower amount of this compound. Restoration of the barrier properties of semi-resistant strain 14 cells was less efficient, but the reactivation worked much better than rehydration in the deionized water. These results indicate that nutrients are needed for active recovery of the cells. As already mentioned before, the remaining K^+ in the cells could help strain 77 yeasts to resist the hyperosmotic stress. K^+ transporters in the plasma membrane depend on the stability of the electrical potential difference and integrity of this membrane [17]. Accumulation of ergosterol in the plasma membrane leads to higher stability of *S. cerevisiae* thermotolerant strains to dehydration compared to mesophilic strains [8,18]. Our results support the idea that higher stability of the cell membranes in the presence of ergosterol could help yeasts to resist desiccation and it could be related to better maintenance of K^+ in mitochondria.

Summarizing results of our study, we suppose that at least two interconnected factors are responsible for the resistance of *S. cerevisiae* strain 17 cells to dehydration-rehydration: stability of mitochondria and intracellular content of ATP. Higher viability of *S. cerevisiae* 77 cells after reactivation could be linked to better maintenance of mitochondria in the dehydrated strain 77 cells and, correspondingly, to more efficient ATP synthesis by oxidative phosphorylation during reactivation. This means, that special, stability of the mitochondria increasing biotechnological

approaches or pre-treatments of yeasts could improve the resistance of cells to dehydration-rehydration. This hypothesis, of course, should be verified in the future experiments.

Author Contributions:

RD, NK and AR designed the study, NK, GK, and RZ performed the experiments, NK, RD, AR analyzed the data and drafted the manuscript, C-RC provided guidance and suggestions for experimental design, RD, AR, and C-RC acquired the funding. All authors read and approved the final manuscript.

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References

[1] Beker, M. J.; Rapoport, A. Conservation of yeasts by dehydration. In *Biotechnology Methods*; Springer, **1987**; pp. 127–171.

[2] Dupont, S.; Rapoport, A.; Gervais, P.; Beney, L. Survival kit of Saccharomyces cerevisiae for anhydrobiosis. *Appl. Microbiol. Biotechnol.* **2014**, *Volume* 98, pp. 8821–8834.

[3] Rapoport, A.; Golovina, E. A.; Gervais, P.; Dupont, S.; Beney, L. Anhydrobiosis: Inside yeast cells. *Biotechnol. Adv.* **2019**, *Volume 37*, no. 1, pp. 51–67.

[4] Rapoport, A.; Turchetti, B.; Buzzini, P. Application of anhydrobiosis and dehydration of yeasts for non-conventional biotechnological goals. *World J. Microbiol. Biotechnol.* **2016**,*Volume 32*, no. 6, pp. 1–10.

[5] Rapoport, A. Anhydrobiosis and dehydration of yeasts. In *Biotechnology of yeasts and filamentous fungi*; Springer, **2017**, Sibirny; pp. 87–116.

[6] Rapoport, A.; Khrustaleva, G.; Chamanis, G.; Beker, M. E. Yeast anhydrobiosis: permeability of the cytoplasmic membrane. *Mikrobiol.* **1995**, *Volume 64*, no. 2, pp. 275–278.

[7] Rapoport, A. Anhydrobiosis in Non-conventional Yeasts. In *Non-conventional Yeasts: from Basic Research to Application*; Springer, **2019**; Sibirny, A.; pp. 341–359.

[8] Rapoport, A.; Rusakova, A.; Khroustalyova,G.; Walker, G. Thermotolerance in Saccharomyces cerevisiae is linked to resistance to anhydrobiosis. *Process. Biochem.* **2014**, *Volume 49*, no. 11, pp. 1889–1892

[9] Khroustalyova, G.; Giovannitti, G.; Severini, D.; Scherbaka, R.; Turchetti, B.; Buzzini, P.; Rapoport, A. Anhydrobiosis in yeasts: Psychrotolerant yeasts are highly resistant to dehydration. *Yeast* **2019**, *Volume 36*, pp. 375–379.

[10] Daugelavicius,R.; Gaidelyte, A.; Cvirkaite-Krupovic, V.; Bamford, D. H. On-line monitoring of changes in host cell physiology during the one-step growth cycle of Bacillus phage Bam35.*J. Microbiol. Methods* **2007**, *Volume 69*, no. 1, p. 174—179.

[11] Flewelling, R. F.; Hubbell, W. L. The membrane dipole potential in a total applications to hydrophobic ion interactions with membranes. *Biophys. Jour.* **1986**, , *Volume 49*, no. 2, pp. 541–552.

[12] Daugelavicius, R.; Bakiene, E.; Berzinskiene, J.; Bamford, D. H. Binding of lipophilic anions to microbial cells. *Bioelectrochem. Bioenerg.* **1997**, *Volume 42*, no. 2, pp. 263–274.

[13] Daugelavicius, R.; Bakiene, E.; Berzinskiene, J.; Bamford, D. H. Use of lipophilic anions for estimation of

biomass and cell viability. *Biotechnol. Bioeng.* **2000**, *Volume 71*, no. 3, pp. 208–216.

[14] Mookerjee, S.A.; Brand, M.D. Measurements and analysis of extracellular acid production to determine glycolytic rate. *J Vis Exp*. **2015**, *Volume 106,* pp. 53464. doi: 10.3791/53464

[15] Ariño, J.; Ramos, J.; Sychrová, H. Alkali metal cation transport and homeostasis in yeasts.*Microbiol. Mol. Biol. Rev.* **2010**, vol. 74, no. 1, pp. 95–120, 2010.

[16] Ramos, J.; Ariño, J.; Sychrová, H. Alkali–metal–cation influx and efflux systems in nonconventional yeast species.*FEMS Microbiol. Lett.* **2011**,*Volume* 317, no. 1, pp. 1–8.

[17] Ariño, J.; Ramos, J.; Sychrova, H. Monovalent cation transporters at the plasma membrane in yeasts. *Yeast*. **2019**,*Volume 36*, no. 4, pp. 177–193.

[18] Dupont, S.; Lemetais, G.; Ferreira, T.; Cayot, P.; Gervais, P.; Beney, L. Ergosterol biosynthesis: a fungal pathway for life on land? *Evolution.* **2012**, *Volume 66*, pp: 2961-2968. doi:10.1111/j.1558-5646.2012.01667.x

[19] Roca-Domènech, G.; Poblet, M.; Rozès, N.; Cordero-Otero R. Magnesium enhances dehydration tolerance in *Schizosaccharomyces pombe* by promoting intracellular 5′-methylthioadenosine accumulation. *Yeast* **2019**,*Volume 36*, no. 7, pp. 449–461.

[20] Camara, A. A. Jr; Sant'Ana, A.S. Advances in yeast preservation: physiological aspects for cell perpetuation. *Curr. Opinion Food Sci*. **2021**, 38:62–70. https://doi.org/10.1016/j.cofs.2020.10.019

[21] Marini, G., Nuske, E., Leng, W., Alberti, S., Pigino, G. Reorganization of budding yeast cytoplasm upon energy depletion. *Mol. Biol. Cell* **2020**, *Volume 31*, pp. 1232-1245.

[22] Munder, M.C., Midtvedt, D., Franzmann, T., Nüske, E., Otto, O., Herbig, M., Ulbricht, E., Muller, P., Taubenberger, A., Maharana, S. *et al*. A pH-driven transition of the cytoplasm from a fluid- to a solid-like state promotes entry into dormancy. *eLife* **2016**, 5:e09347. doi: 10.7554/eLife.09347

[23] Erkut, C., Gade, V.R., Laxman, S., Kurzchalia, T.V. The glyoxylate shunt is essential for desiccation tolerance in *C. elegans* and budding yeast. *eLife* **2016,** 5:e13614. doi: 10.7554/eLife.13614.