UNIVERSITY OF LATVIA
FACULTY OF BIOLOGY

DIANA BOROVIKOVA

ANHYDROBIOSIS OF YEAST: CHANGES OF CELL SURFACE STRUCTURES AND NON-CONVENTIONAL APPLICATIONS OF ANHYDROBIOSIS

DOCTORAL THESIS

Submitted for the degree of Doctor of Biology

Subfield of Microbiology

Supervisor: Prof., Dr.habil.biol.

ALEKSANDRS RAPOPORTS

Riga 2014

This work has been supported by the European Social Fund within the project «Support for Doctoral Studies at University of Latvia».

CONTENT

ANNOTATION

Laboratory of Cell Biology, Institute of Microbiology and Biotechnology, University of Latvia takes the leading place in the world in investigations of yeast organisms` transfer into the state of anhydrobiosis. The main structural and functional changes of yeast cell wall, plasma membrane, mitochondria, vacuoles, lysosomal apparatus and nuclei were revealed. The aim of this thesis was to continue these investigations of anhydrobiosis and to find new theoretical facts about changes linked with cell surface (cell wall and plasma membrane). Taking into account acquired information about yeast cell changes during dehydration/rehydration, it was decided also to find new non-conventional applications of anhydrobiosis in biotechnological processes.

As a result of this work for the first time the significant role of the definite cell wall proteins in the maintenance of yeast viability during anhydrobiosis was found. Continuing the investigations about changes occurring in plasma membrane, the main aim of the work was connected with protein components of the membrane. The changes of the certain proteins (transport systems) in plasma membrane during the dehydration of the yeast cells and after cell reactivation from the state of anhydrobiosis were determined as a strain specific. It was shown that the first changes in the proteins of the transport systems already can occur during the evaporation of 'free' water. At the same time it was concluded that in the case of extremely resistant to dehydration treatment cells there is no any change in the physiological activity of Agt1 transporter. The significance of the certain transport systems in plasma membrane in determination of cell resistance to dehydration was shown. The physiological role of potassium ions transporter Trk2 and its importance for the stability of *Saccharomyces cerevisiae* to dehydration/rehydration stress was found.

Taking into account the findings about changes in the structures of the cell surface, a new method for microorganisms' immobilization was developed where the stage of cell dehydration was included. The possibilities of efficient application of immobilized yeast preparations were shown in such different biotechnological processes as ethanol production and wastewater treatment. Besides, high stability of yeast preparations immobilized by new method was established. It was observed, that immobilization significantly improves cell resistance to dehydration, and this approach can be recommended as the way to maintain the biotechnologically important strains.

Based on the changes in the cell resistance to dehydration, a new test-system was developed for the analysis of possible influence of various chemical compounds and biological substances upon eukaryotic cell. This approach has many important advantages: it is cheap, fast and informative.

The main results of this work are published in seven research papers (2007-2014); six of them were published in international peer-reviewed journals. The results of the studies have been presented at eight international conferences. Two Latvian patents were received.

The promotion research was carried out at the Laboratory of Cell Biology, Institute of Microbiology and Biotechnology, University of Latvia.

INTRODUCTION

Anabiosis is a unique natural phenomenon of temporary reversible delay of metabolism by organisms in unfavorable conditions of the environment. It was discovered in 1701 by great Dutch naturalist Antonie van Leeuwenhoek. Despite the fact that anabiotic state of live organisms was found more than 300 years ago only during last 40 years the main mechanisms of organisms' survival and of viability maintenance were demonstrated. Different types of anabiosis exist and one of them is anhydrobiosis - the state which can be reached as a result of the significant losses of water through evaporation. Laboratory of Cell Biology, Institute of Microbiology and Biotechnology, University of Latvia during many years investigates the mechanisms of yeast organisms` transfer into the state of anhydrobiosis, and it takes world leading position in these studies.

The conventional application of the induced anhydrobiosis in microoganisms is connected with the conservation of microorganisms` strains for culture collections, production of active dry baker's yeasts, and production of dry vaccines e.t.c.

Investigations which have been performed till the moment showed that during yeast cells dehydration essential structural and functional changes took place practically in all organelles and other cell components. The plasma membrane has one of the most important roles in the maitenance of cell viability during dehydration treatment. The possible increase of plasma membrane permeability can lead to big losses of cell components during its reactivation and can lead to the death of the cell. It was discovered that such changes in plasma membrane could be connected with structural changes of its lipids. All information which was received till the moment about structural and functional changes of plasma membrane was linked only with the changes of lipid components of membranes.

The goal of this work was to continue the investigations of anhydrobiosis and to find new theoretical facts about changes linked with cell surface (cell wall and plasma membrane). Taking into account acquired information about yeast cell changes during dehydration/rehydration, it was decided also to find new non-conventional applications of anhydrobiosis in biotechnological processes.

The following **tasks** were set up:

1. to evaluate possible role and changes of proteins of cell wall and plasma membrane at yeast cells dehydration:

- to study possible changes of one of maltose transport systems in plasma membrane during dehydration/rehydration,
- to evaluate possible role of potassium transport systems in the maintenance of cell viability during dehydration/rehydration,

- to investigate possible role of some cell wall proteins in the maintenance of cell viability in the state of anhydrobiosis;

2. to develop a new method for cell immobilization based on the knowledge of cell wall changes at dehydration;
3. to investigate the influence of immobilization on physiological and biotechnological characteristics of yeast cells;
4. to develop a new test-system for evaluation of influence of various compounds upon eucaryotic cell.

To achieve the tasks of the work the following methods were used:

- classical methods for investigation of microorganisms` physiology,
- scanning electron microscopy,
- spectrophotometry,
- emission atom microscopy,
- high performance liquid chromatography,
- enzymatic biochemical methods.

This work has been supported by the European Social Fund within the project «Support for Doctoral Studies at University of Latvia».

The scientific novelty and significance of promotion study

1. For the first time the functional changes of maltose transport system Agt1 during dehydration process in different *Saccharomyces cerevisiae* strains were demonstrated.
2. For the first time the role of potassium transport system Trk2 in the maintenance of *Saccharomyces cerevisiae* cells viability during dehydration process was revealed.
3. For the first time it was shown the role of some cell wall proteins in the maintenance of yeasts viability during dehydration/rehydration process.
4. New method for microorganisms' immobilization was developed, and possibility of its effective application for biotechnological processes was shown.
5. A new test-system based on yeast cells reaction upon extreme treatments was developed for the evaluation of various chemical compounds and biological substances upon eukaryotic cell.

THESES FOR DEFENSE

1. Certain plasma membrane proteins – transporters of various compounds have significant role in the maintenance of yeast cell viability during yeast transfer into the state of anhydrobiosis.
2. Changes of plasma membrane proteins which take place at yeast transfer into the state of anhydrobiosis are yeast strain specific.
3. The acquired results of cell wall changes during dehydration gave the possibility to develop a new method for microorganisms' immobilization.
4. The preparations of yeast immobilized by new method are effective in various biotechnological processes.
5. A new test-system based on yeast cells reactions to dehydration treatment can show the influence of the various compounds upon the eukaryotic cell.

APPROVAL OF THE PROMOTION RESEARCH

The results of the promotion research have been reported at 13 presentations at 8 international conferences.

1. A.Rapoport, N.Vedernikov, I.Kruma, M.Puke, D.Borovikova, L.Rozenfelde, G.Khroustalyova, N.Matyuskova. Waste-less bioethanol and other valuable substances production from hardwood. - In: FEEM 2013. 2013 International Conference on Future Energy, Environment, and Materials. Abstracts. Hong Kong, 2013, p.8 (oral presentation).
2. D.Borovikova, G.Kizane, P.Mekss, I.Muiznieks, A.Rapoport. Development of yeast anhydrobiosis based test system for the screening of candidate substances for cosmetics and health care compositions. – In: FEMS 2013 5th Congress of European Microbiologists. Advancing Knowledge on Microbes. Leipzig, Germany, 2013, p. 3209 (oral and poster presentations).
3. D. Borovikova, E. Dauss, T. Alamae, A. Rapoport. Anhydrobiosis of yeast: influence of yeast dehydration upon AGT1 permease activity. – In: Proceedings 30th ISSY Cell Surface & Organelles in Yeasts. ISSN 1336-4839, Stara Lesna, Slovakia, 2013, p.64 (poster presentation).
4. D. Borovikova, P. Herynkova, A. Rapoport, H. Sychrova. Potassium uptake systems are crucial for the yeast cell viability dyring anhydrobiosis. - In: Proceedings 30th ISSY Cell Surface & Organelles in Yeasts. ISSN 1336-4839, Stara Lesna, Slovakia, 2013, p.65 (poster presentation).
5. A.Rapoport, G.Khroustalyova, L.Rozenfelde, D.Borovikova, E.Kolomiets. Anhydrobiosis in microorganisms and new biotechnological solutions. – International Conference “Microbial Biotechnologies: Fundamental and Applied Issues”. Minsk, 2013 (oral presentation).
6. A.Rapoport, G.Khroustalyova, L.Rozenfelde, D.Borovikova. Anhydrobyosis in yeasts: mechanisms and applications. – In: 1st Congress of Baltic Microbiologists. Book of Abstracts. Riga, Latvia, 2012, p. I 16 (oral presentation).
7. D. Borovikova, A. Patmalnieks, A. Rapoport. New approach for yeast immobilization. – In: 1st Congress of Baltic Microbiologists. Book of Abstracts. Riga, Latvia, 2012, p. O 24 (poster presentation).
8. D. Borovikova, R. Scherbaka, A. Katashev, Yu. Dekhtyar, A. Patmalnieks, A. Rapoport. Physiological characteristics of yeast, immobilized on hydrogenated hydroxyapatite. – In: 1st Congress of Baltic Microbiologists. Book of Abstracts. Riga, Latvia, 2012, p. P 16 (poster presentation).

9. D. Borovikova, A. Patmalnieks, A. Rapoport. New approach for yeast immobilization. 1st Congress of Baltic Microbiologists. Riga, Latvia, 2012 (oral presentation).
10. N.Vedernikov, M.Puke, I.Kruma, L.Rozendelde, G.Khroustalyova, D.Borovikova, A.Patmalnieks, N.Matyushkova, A.Katashev, Yu.Dekhtyar, A.Rapoport. New approaches for waste-less bioethanol production from hardood. – In: 1st Congress of Baltic Microbiologists. Book of Abstracts. Riga, Latvia, 2012, p. P 40 (poster presentation).
11. Yu.Dekhtyar, N.Polyak, D.Borovikova, A.Rapoport. Immobilization of yeast cells on the surface of hydroxyapatite ceramics. - In: Electroceramics XI. Abstracts and CD proceedings. Manchester, 2008, p. M-004-O (oral presentation).
12. Yu.Dekhtyar, D.Borovikova, V.Bystrov, S.Filippov, A.Karlov, A.Katasshev, C.Meissner, G.Mezinskis, E.Paramonova, I.Pavlovska, N.Polyaka, A.Sapronova, A.Rapoport. Nanostructured hydroxyapatite ceramics as new carrier for the immobilization of yeast cells. - In: Ņanotech Northern Europe 2007. Abstracts. Helsinki, 2007, p. 88-89 (oral presentation).
13. A.Rapoport, D.Borovikova, A.Katashev, A.Kokina, G.Khroustalyova, A.Patmalnieks, L.Saulite, Y.Trofimova, Y.Dekhtyar. Brewers' yeast: some physiological and biotechnological characteristics. - In: From Alcoholic Beverages to Bioethanol for Transportation: a New Challenge for Fermenting Yeast. ISSY 26. Book of Abstracts. Sorrento, 2007, p. 85 (oral presentation).

The results of the promotion research are summarized and published in 2 Latvian patents and 7 research papers, 6 of them were published in international peer-reviewed journals:

1. Rapoports A., Dehtjars J., Ambroziak W., Borovikova D., Kokina A., Kriegel D., Diowksz A., Kordialik-Bogacka E., Berlowska J., Koziol G. Method of microorganisms' immobilization on the carrier. – Latvian Patent, 2007, LVL13632 B.
2. Rapoports A., Dehtjars J., Ambroziak W., Borovikova D., Kokina A., Kriegel D., Diowksz A., Kordialik-Bogacka E., Berlowska J., Koziol G. Method of microorganisms' immobilization on the carrier. – Latvian Patent, 2007, LVL13633 B.
3. Borovikova D., Cifansky S., Dekhtyar Y., Fedotova V., Jakushevich V., Katashev A., Patmalnieks A., Rapoport A. Atomic Force Microsopy Study of Yeast Cells Influenced by High Voltage Electrical Discharge. – IFMBE Proceedings, 2008, 20: 602-605.
4. Rapoport A., Borovikova D., Kokina A., Patmalnieks A., Polyak N., Pavlovska I., Mezinskis G., Dehtyar Y. Immobilization of yeast cells on the surface of

hydroxyapatite ceramics. – Process Biochemistry, 2011, 46: 655-670; DOI 10.1016/j.procbio.2010.11.009.

5. Rapoport A., Khroustalyova G., Rozenfelde L., Borovikova D., Kolomiets E. Anhydrobiosis in microorganisms and new biotechnological solutions. - Microbial Biotechnologies: Fundamental and Applied Issues. Minsk, Belaruskaya navuka, 2013, 5: 141-154 (in Russian).
6. Borovikova D., HerynkovaP., Rapoport A., Sychrova H. Potassium uptake system Trk2 is crucial for yeast cell viability during anhydrobiosis. – FEMS Microbiol Lett, 2014, 350: 28-33; DOI 10.1111/1574-6968.12344.
7. Borovikova D., Scherbaka R., Patmalnieks A., Rapoport A. Effects of yeast immobilisation on bioethanol production. – Biotechnology and Applied Biochemistry, 2014, 61: 33-39; DOI: 10.1002/bab.1158.
8. Rapoport A., Vedernikov N., Kruma I., Puke M., Borovikova D., Rozenfelde L., Khroustalyova G., Matyuskova N. Waste-less bioethanol and other valuable substances production from hardwood. – WIT Transactions on Engineering Sciences. 2014, 88: 311-317.
9. Borovikova D., Rozenfelde L., Pavlovska I., Rapoport A. Immobilisation increases yeast cells' resistance to dehydration-rehydration treatment. – Journal of Biotechnology, 2014, 184: 169-171; DOI: 10.1016/j.jbiotec.2014.05.017.

The promotion research has been performed at the Laboratory of Cell Biology, Institute of Microbiology and Biotechnology, University of Latvia.

1. THE LITERATURE REVIEW

1.1. Characterization of yeast cell

Yeasts are eukaryotic microorganisms classified in the kingdom Fungi, with 1.500 species currently described (Kurtzman and Fell, 2006). Yeasts are widespread in the natural environments and can be isolated from terrestrial, aquatic and aerial environments. The most preferred habitats are plant tissues especially surface of grapes. A few species are found in commensal or parasitic relationships with animals and people: non-pathogenic yeasts can be found in the intestinal tract and on the skin of warm-blooded animals but some species of yeasts are pathogenic towards animals and people. Soil can serve for many types of yeast as a reservoir for long-term survival, rather than a habitat for free growth. For example, agricultural soils may have as many as 40000 viable yeasts per gram. Yeasts are widely distributed in both fresh water and seawater. Air currents can distribute yeasts above soil surfaces in amount about a few viable yeast cells per m^3 of air. In addition to natural habitats, some yeast can be found in man-made environments. As examples for such habitats can be mentioned different surfaces in wineries or in hospitals (Walker, 1998). Extreme habitats can be mentioned too, but unfortunately studies on yeast occurrence in extreme environments are scarce. Available reports deal with psychrophiles, deep-sea environments, and hydrothermal vents (Beker and Rapoport, 1987; Walker, 1998; Gadanho et al., 2006).

Yeasts are strictly chemoorganotrophic organisms which require fixed, organic forms of carbon for growth. Diversity in sources of carbon for yeast metabolism is wide: simple sugars, sugar alcohols, organic and fatty acids, aliphatic alcohols, hydrocarbons, various heterocyclic and polymeric compounds (Walker, 1998).

Yeasts are of major economic, social and health significance in human culture. Yeasts have been used to produce alcoholic beverages and leaven bread dough for millennia. Consider that the brewing of beer was the world's first biotechnology (Leskosek-Cukalovic and Nedovic, 2005). Yeasts have numerous applications:

1. fundamental biological research – cell biology, genetics, biochemistry, molecular biology;
2. biomedical research – for example, studies of cancer and AIDS, drug metabolism, genotoxicity screens, human genetic disorders;
3. environmental technologies – bioremediation, waste utilization, crop protection, biosorption of metals;
4. fermentation industries – brewing, wine, ethanol, novel processes and fermentation products;

5. food/chemical industries – savoury flavours, enzymes, baking, pigments, food acidulants, chemical reductions;
6. health-care industries – pharmaceuticals, vaccines, probiotics, vitamins, hormones, blood factors (Walker, 1998; Schneiter, 2004).

Yeast cells exhibit great diversity in cell size, shape and color. Yeast cell size can vary widely: some yeasts may be only 2-3 µm in length, other species may attain lengths of 20-50 µm. Cell width appears less variable, between 1-10 µm. Yeasts can be ellipsoidal/ovoid, cylindrical with hemi-spherical ends, apiculate/lemon-shaped, ogival, flask-shaped, triangular, curved, filamentous, stalked or elongated. Several yeasts are pigmented and different colors may be distinguished: cream, white, black, pink, red, orange and yellow. *S. cerevisiae* cells are generally ellipsoidal in shape with a large diameter ranging from 5-10 µm and *S. cerevisiae* have cream-colored colonies (Walker, 1998).

Yeasts are unicellular organisms that divide by budding or fission. Cell division, where a daughter cell is adjacent to the mother cell which does not change in shape during the reproduction process, is probably the major unifying characteristic of budding yeast species. The need to segregate cellular content from the mother compartment into the bud in every cell division made *S. cerevisiae* also an important model for studying asymmetric cell division. Yeasts have evolved mechanisms that allow a carefully monitoring the cellular content that should – or should not – be segregated into the bud. Many, but not all yeast species undergo a major morphogenic transition: the dimorphic transition to pseudohyphal or hyphal/filamentous growth. The transition from unicellular growth to pseudohyphae or true septate hyphae (filaments) is typically triggered by adverse environmental conditions. It has been demonstrated that the dimorphic transition can be gradual and able to generate a broad range of cell shapes, as a response to an environmental parameter setting (Knop, 2011). In *S. cerevisiae* the pseudohyphal transition involves a switch from a bipolar to a unipolar budding pattern. This generates, together with the maintenance of weak cell-cell contacts after cytokinesis, an asymmetric polarized cell colony that 'permits otherwise sessile cells to forage for nutrients and substrate at a distance from their initial colonization site' (Knop, 2011). The morphogenic transition that leads to a sporogenic form starts with either of the two vegetative dimorphic variants, budding cells or pseudohyphae/hyphae. Spore formation is preceded by meiosis, and thus leads to the formation of meiospores. Spore formation occurs by endobudding, whereby multiple nuclei that are present in a single cytoplasm at the end of meiosis become separated from the common cytoplasm. The cellular decisions to undergo morphogenic transitions that are related to the sexual life cycle, namely mating and meiosis, require an integration of external factors (available nutrients in the surrounding medium) with

the internal status (e.g. haploid versus diploid, amount of available stored nutrients). Cells commit to these transitions in the G1 phase of the cell cycle, as only nuclei at the G1 stage can undergo the necessary transformations, e.g. in mating or meiosis (Fig. 1).

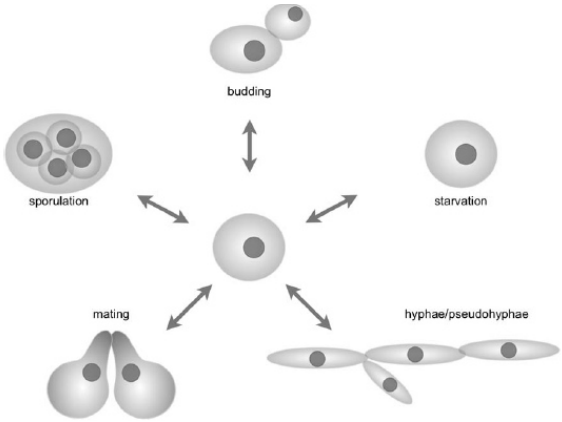

Fig. 1. Developmental decisions in yeasts, exemplified by *S. cerevisiae* (Knop, 2011).

Mating denotes the formation of an asexual diploid cell from two sexual haploid cells of opposite mating type (MATa and MATα). This involves the fusion of two cellular cytoplasms, and the process is concluded with the fusion of the two nuclei (karyogamy). A mutual communication is required in order to execute mating. *S. cerevisiae* haploid cells mate spontaneously, and it is not exactly known to what extent stress or starvation conditions will interfere with the mating process. Yeast mating involves the secretion of a soluble pheromone, the α-factor, from cells of the mating type MATα. Secretion of the α-factor occurs via the secretory pathway. Cells with the mating type MATa on the other hand use as a mating signal a fatty acid modified and therefore highly lipophylic peptide, the a-factor, which is cytoplasmically produced and excreted via an ABC-transporter (Knop, 2011).

Yeasts are eukaryotic organisms which have numerous membrane bound organelles, including a nucleus, mitochondria, Golgi apparatus, secretory vesicles, endoplasmic reticulum, vacuoles, and the peroxisome. Fig. 2 provides a view of an idealized yeast cell.

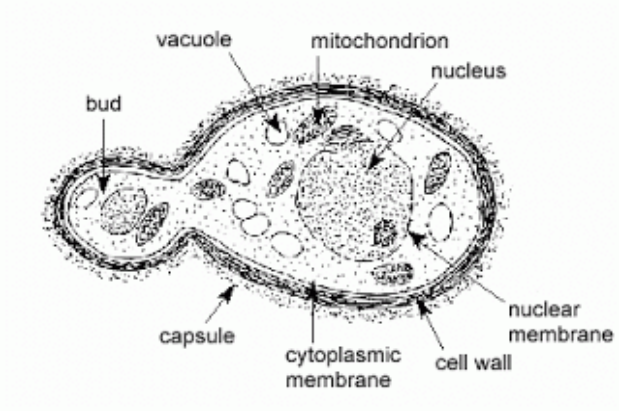

Fig. 2. Scheme of a dividing yeast cell (Feldmann, 2012).

Yeast cell is surrounded by yeast cell envelope represented by the cell plasma membrane, the periplasmic space, and the cell wall. It occupies about 15% of the total cell volume and plays a major role in controlling of osmotic and permeability properties of the cell (Walker, 1998).

The cell wall determines the shape and integrity of the yeast cell during growth and cell division, physical protection, osmotic stability, permeability barriers, enzyme support, cation binding, cel-cell recognition, cell-cell adhesion (Aguilar-Uscanga and Francois, 2003; Klis et al., 2006). The cell wall is a dynamic structure that can adapt to physiological (i.e. from logarithmic to stationary phase) and morphological changes (mating, conjugation, sporulation or pseudohyphal growth) (Cid et al., 1995; Orlean, 1997; Molina et al., 2000; Cabib et al., 2001; Smits et al., 2001). Moreover, a cell wall compensatory mechanism is activated in response to cell wall perturbing agents or cell wall mutations, which allows remodelling of the cell wall to combat cell lysis (Klis et al., 2002). One of the major outcomes of this mechanism is a strong increase of chitin that can reach up to 20% of the cell wall dry mass (Popolo et al.,1997; Dallies et al., 1998; Lagorce et al., 2002).

S. cerevisiae cell wall is a rigid structure about 100–200 nm thick constructed from three main groups of polysaccharides: polymers of mannose (mannoproteins, *ca* 40% of the cell wall dry mass), polymers of glucose (β-glucan, *ca* 60% of the cell wall dry mass) and polymers of N-acetylglucosamine (chitin, *ca* 2% of the cell wall dry mass). β-glucan can be divided into two subtypes following the mode of glucose linkages: long chains of *ca* 1500 β-1,3-glucose units which represents *ca* 85% of total cell wall β-glucan, and short chain of *ca* 150 β-1,6-glucose units that accounts for *ca* 15% of the β-glucan (Klis et al., 2002). According to the study of Kopecka (2013) on solubilization and reaggregation of yeasts cell wall polysaccharides, there are 3 types of yeast ß-glucan: alkali-insoluble branched β-1,3-D-glucan, acid-soluble branched β-1,6-D-glucan and alkali-soluble branched β-1,3-D-glucan. ß-1,3-D-glucan molecules were found to consist of about 1.500 glucose monomers. This polymer chains form a single- or triple-helix structure stabilized by interchain hydrogen bonds. ß-1,6-D-glucan is a low-molecular weight polymer, consisting on average 130–150 glucose moieties and strongly branched via ß-1,3-linked monomers (Lipke and Ovalle, 1998; Bzducha-Wrobel et al., 2013). While a ß-1,3-glucan–chitin complex constitutes the inner rigid structure, mannoproteins form the outer layer of the cell wall, and ß-1,6-glucan interconnects all cell wall components (Sestak et al., 2004). Aguilar-Uscanga and Francois (2003) confirmed that content of ß–glucans and mannan in cell wall of unicellular fungi is strongly linked with growth conditions. Biosynthesis of polysaccharides of yeast cell wall is influenced by the kind of carbon and nitrogen source, pH of an environment, temperature,

degree of medium oxygenation, as well as the phase of cell growth, and cultivation parameters. The cell wall can be considered as a vital extracellular organelle that has to withstand turgor pressures greater than $15*10^5$ Pa. The extraordinary stability of the cell wall against tension due to high internal hydrostatic pressure can be explained by the fact that the various components of the wall are interlinked and form a highly branched meshwork consisting of covalently linked ß-1,3-D- and ß-1,6-D-glucan, chitin, and mannoproteins, whereby a stable phosphodiester linkage has been proposed to connect the mannoproteins to the rest of the meshwork (Mrsa et al., 1999).

In general, yeast cell wall is considered porous to molecules around 300 Da but it will retain molecules greater than around 700 Da. Maximum porosity is observed during bud growth when the cell wall is in a more plastic, expanded state compared with stationary phase cells. In the influence of heat-shock the yeast cell wall may become weakened leading to plasma membrane stretching (Walker, 1998).

The plasma membrane of yeasts is about 7.5 nm thick with occasional invaginations protruding into the cytoplasm. It harbors lipid components comprised mainly of phospholipids (phosphatidylcholine, phosphatidylethanolamine, and minor proportions of phosphatidylinositol, phosphatidylserine, and phosphatidylglycerol) as well as sterols (principally ergosterol and zymosterol). The lipid components of cellular membranes both maintain the required physical properties of the membrane and store and release many bioactive lipids that act as signaling molecules. In addition to their role as docks for proteins, bioactive lipids could change membrane fluidity, curvature, and domain architecture, which also have an impact on activation and/or localization of signaling components (Bitew et al., 2010). The protein components include different classes which involved in: solute transport (ATPase, permeases, channels); cell wall biosynthesis (glucan and chitin synthases); transmembrane signal transduction and cytoskeletal anchoring (Walker, 1998). The plasma membrane can change structurally and functionally depending on the conditions of yeast growth. For example, lipid composition, particularly the unsaturated fatty acid constitution, can alter quite dramatically with changing growth rates, temperature and oxygen availability (Walker, 1998).

Different models of the plasma membrane structure were developed. In 1935, H. Davson and J. Danielli proposed a sandwich model in which the phospholipid bilayer lies between two layers of globular proteins. This model allowed for selective permeability of the membrane (Danielli and Davson, 1935). In 1972, S.J. Singer and G. Nicolson presented the Fluid Mosaic Model of the plasma membrane structure (Singer and Nicolson, 1972). It was proposed that lipid bilayer was as a single 2D fluid in which embedded membrane proteins

are able to diffuse freely in the lateral dimension (Owen and Gaus, 2013). Later it was shown that lipids and membrane proteins are not homogeneously distributed in the plasma membrane. In 1997 Simons and Ikonen proposed the lipid raft hypothesis which said that the phase behavior of different lipid species is exploited to create lateral heterogenecity in the plasma membrane (Simons and Ikonen, 1997; Owen and Gaus, 2013). The liquid-disordered phase, presented mainly by unsaturated phospholipids, would coexist in the plasma membrane with a liquid-ordered phases formed from saturated phospholipids and sphingolipids in the presence of cholesterol in eucaryotic organisms which amount in the plasma membrane is about 30 mole percent. In the ordered phase, a higher degree of conformational order is imposed on the acyl tails of lipids by the rigid ring structure of cholesterol (ergosterol in yeasts). This results in an increase in the thickness of the lipid bilayer and tighter lipid packing although unlike the gel phase (consisting of saturated lipids in the absence of cholesterol), liquid-ordered bilayer lipids remain laterally mobile. In this model therefore, the plasma membrane is viewed as a 'sea' of disordered phase lipids containing stable, ordered phase 'islands' or 'rafts' enriched in saturated lipids, sphingolipids and cholesterol (Owen and Gaus, 2013). The lack of direct visualization resulted in interpretation of domains with limited size as a 'molecular complex in the membrane that consists of at least 3 molecules that includes a molecule with a saturated alkyl chain or a cholesterol molecule that plays a critical role in the formation of the complex itself' (Kusumi et al., 2004). Later definition characterized the dynamic nature of the domains defining rafts as 'small (10–200 nm), heterogeneous, highly dynamic, sterol and sphingolipids-enriched domains that compartmentalize cellular processes. Small rafts can sometimes be stabilized to form larger platforms through protein–protein or protein–lipid interactions' (Pike, 2006). The membrane must be treated as a 'lipid–protein composite' in which a very high density of transmembrane domains may impose order on nearby lipids complimenting lipid domains organizing proteins (Jacobson et al., 2007).

The main functions of plasma membrane are physical protection of the cell, control of osmotic stability, control of cell wall biosynthesis, anchor for cytoskeletal compounds, location of the components of signal transduction pathways, sites of cell–cell recognition and cell–cell adhesion, transport-related functions in endocytosis and exocytosis and selective permeability barrier controlling compounds that enter or that leave the cell. There are four basic mechanisms whereby nutrients are taken up and metabolites exported across yeast membranes: free diffusion, facilitated diffusion, diffusion channels and active transport (Walker, 1998):

- free diffusion is the simplest and slowest mode of nutrient transport in yeast which involves passive penetration of lipid-soluble solutes through the plasma membrane, from a high extracellular concentration to a lower intracellular concentration; this kind of transport can be used for uptake of undissociated organic acids, short-chain alkanes and long-chain fatty acids into the cell and the export of ethanol and gaseous compounds from the yeast cell;
- facilitated diffusion is faster than simple diffusion since solutes are translocated down a transmembrane concentration gradient in an enzyme-mediated fashion referred to as a permease, or carrier, or facilitator, which traverses the membrane and exhibits stereospecificity for the transported solute; certain sugars are transported in this matter, notably glucose;
- diffusion channels for certain ions exist in yeast as voltage-dependent 'gates' to move transiently ions down concentration gradients; such channels are normally closed at the negative membrane potential of resting yeast cells and opened when the membrane potential becomes positive; for example, K^+-efflux channel which is the predominant ion channel in yeast, is activated by membrane depolarization; plasma membrane diffusion pores exist to permit the passage of water and sugar alcohols; in addition, under hypoosmotic stress cells facilitate glycerol permeation through a membrane channel, the FPS1 facilitator protein;
- active transport is a concentrative, energy-dependent mechanism based on chemiosmotic principles which is responsible for the uptake of the majority of nutrients into yeast cells; the driving force for nutrients against a concentration gradient during active transport is the membrane potential and the transmembrane electrochemical proton gradient generated by the plasma membrane H^+-ATPase; concentrative sugar uptake occurs by proton symport (Walker, 1998).

The plasma membrane is especially significant when considering the physiology of industrial yeasts, for example, the ability of brewing yeast strains to produce and tolerate ethanol. The cell membranes enriched in linoleyl fatty acid residues and with ergosterol exhibit a greatly enhanced tolerance to ethanol. In this case the role of oxygen is efficient in dictating the fatty acids/sterol make-up of brewing yeast membranes – oxygen is absolutely required for the synthesis of unsaturated fatty acids and sterols (Walker, 1998).

The Periplasm is a thin (35–45A), cell wall-associated region external to the plasma membrane. It comprises mainly secreted proteins that are unable to permeate the cell wall, such as invertase and phosphatase. Invertase and acid phosphatase catalyse the hydrolysis of substrates which do not cross the plasma membrane (Walker, 1998).

The Cytoplasm is an aqueous, slightly acidic (pH 5.2) colloidal fluid that contains low- and intermediate molecular-weight compounds, such as proteins, glycogen, and other soluble macromolecules. Larger macromolecular entities like ribosomes, proteasomes, or lipid particles are suspended in the cytoplasm. The cytosolic (non-organellar) enzymes include the glycolytic enzymes, the fatty acid synthase complex, and the components and enzymes for protein biosynthesis. It is the site for many cellular activities and the space for intracellular traffic (Walker, 1998).

Intense research of **the Cytoskeleton** have shown that the three most prominent actin structures (patches, cables, and rings) are all highly dynamic, with patches and cables assembling and turning over in less than 60 seconds. Numerous genetic, two-hybrid, and biochemical studies have brought close to having a complete list of actin-associated proteins involved in these processes (Moseley and Goode, 2006). It is involved in several aspects of yeast physiology: mitosis and meiosis, organelle motility, and septation. Recently it was discovered the functional role of actin during endocytosis – the process through which the plasma membrane becomes invaginated into the cell and is then pinched off, resulting in the production of a vesicle (Aghamohammadzadeh and Ayscough, 2010).

The Nucleus is a nearly round organelle of about 1.5 μm in diameter located in the center of the cell or slightly excentrically. The nucleoplasm is surrounded by a double membrane bilayer (inner and outer nuclear membrane), thus separating the nucleus from the cytoplasm. Nuclear pore complexes of about 50–100 nm in diameter form the natural channels for exchange of components between the nucleus and cytosol, whereby export and import pathways can be distinguished. The outer nuclear membrane is largely contiguous with the membrane of the Endoplasmic Reticulum. Unlike most eukaryotic cells, the yeast nuclear membrane is not resolved during mitosis, while is breaks down in higher eukaryotic cells. On its outside, the nucleus carries a spindle pole body that serves as an anchor for continuous and discontinuous microtubules across the nucleus as well as for cytosolic microtubules. During mitosis, the spindle pole body will be duplicated and this apparatus effects the movement of the duplicated chromosomes into mother and daughter cell before cell separation. The cell nucleus harbors and expresses essential genetic blueprint, ensures the proper expression, duplication, repair and segregation of chromosomes while ensuring proper processing and export of messenger and ribosomal RNA (rRNA). Chromosomes, and the nucleosomal fibers within them, can be thought of as basic structural elements of the nucleus. The budding yeast carries its genome of nearly 6000 genes in 12 megabase pairs of DNA on 16 linear chromosomes in the nucleus (Taddei and Gasser, 2012).

The Nucleolus is a subnuclear compartment, a crescent-shaped structure occupying approximately one-third of the nucelar volume. The nucleolus locates the rRNA genes, and is the site for the synthesis and processing of rRNA. It is also involved in the assembly of the ribosomal subunits and in pre-mRNA processing (Taddei et al., 2010).

The Endoplasmic Reticulum is an organized into an extended system of branching tubules surrounded by a lipid double-layer membrane, which is intimately connected to the nuclear membrane. The cisternal space of the endoplasmic reticulum may make up to 10% of the cell's volume. It is the cellular site for the production of all transmembrane proteins and lipids of most of the cell's organelles; a key organelle for all processes controlling the stability, modification, and transport of proteins (Fehrenbacher et al., 2002).

Morphology **of the Golgi apparatus** varies among yeasts species. In the case of budding yeasts it exists as a system of dispersed cisternae, whereas in *Pichia pastoris* it is organized into stacks. The main functions of the Golgi apparatus are to modify and sort newly synthesized proteins (it is called by secretory cargo) (Papanikou and Glick, 2009).

Vacuole is a lysosome-like compartment, forms an integral component of the Endoplasmic Reticulum–Golgi–vesicle route. Vacuoles are dynamic structures that may exist in cells as a single large compartment or as several smaller compartments, called 'prevacuolar compartments', 'prevacuolar endosomes', or 'late endosomes'. They are bounded by a single membrane, which has a phospholipid, unsaturated fatty acid, and sterol content different from the plasma membrane. Vacuole is a key organelle involved in intracellular protein trafficking and nonspecific intracellular proteolysis. The degradative processes are catalyzed by the activities of the more than 40 different intravacuolar hydrolases: endopeptidases, aminopeptidases, and carboxypeptidases and nucleases, glycosidases, lipases, phospholipases, and phosphatases. Vacuoles serve as storage compartments for basic amino acids, polyphosphates, and certain metal cations, participate in osmoregulation and the homeostatic regulation of cytosolic ion concentration and pH (Walker, 1998; Li and Kane, 2009).

Mitochondria are the organelles of energy transduction, producing adenosine triphosphate (ATP) through the process of oxidative phosphorylation (Taanman, 1998). The shape, size and number of mitochondria are regulated during the yeast cell growth. In anaerobic conditions promitochondria are forming; in aerobic conditions – enlarged and elongated, positioned at the cell periphery – near the point of entry of oxygen and to facilitate the rapid conduction of ATP through the cell. During the logaritmic growth phase from 1 to 10 elongated mitochondria can be found; stationary growth phase – many small round mitochondria (Jensen et al., 2000). Mitochondria have a double membrane. The outer mitochondrial membrane separates the mitochnodrion from the cytosol but the inner

mitochondrial membrane – the intermediate space from the matrix (Schenkel and Bakovic, 2014). The composition of the mitochondrial membranes is similar to that of other membranes, and is presented by phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, and phosphatidic acid, as to plasma membrane; and phosphatidylglycerol and cardiolipin, are exclusively components of mitochondrial membrane (Schenkel and Bakovic, 2014). The outer mitochondrial membrane contains enzymes involved in lipid metabolism and the inner mitochondrial membrane contains (i) cytochromes for the respiratory chain, (ii) the ATP synthase coupled to the respiratory chain, and (iii) a number of transport proteins for the exchange of low-molecular- weight components (Walker, 1998). The matrix is the site for the citric acid cycle and contains the mitochondrial DNA, together with the protein synthesizing machinery including mitochondrial ribosomes. One of the most important features of the setup comprising all compartments of the mitochondria are the systems for the internalization and processing of proteins that are manufactured on cytosolic ribosomes and imported into the mitochondria. Mitochondria determine the fate of the cell, drives the processes like apoptosis, and aging (Walker, 1998).

The number and morphology of **peroxisomes** in yeast cells is largely determined by the growth conditions. Organelles are bounded by a single double-layer lipid membrane, often they are of spherical appeareance and do not contain DNA or any protein synthetic pathway. They are called 'Enzyme bags'. Peroxisomes contain hydrogen peroxide-producing oxidase and a detoxifying catalase, an enzyme that is capable of metabolizing hydrogen peroxide. In addition to the oxidase/catalase system, they harbor a complete system for ß-oxidation of fatty acids (Aitchison et al., 1992).

1.2. Yeast as a model

S. cerevisiae was adopted as a model system for laboratory study in the 1930s, as investigators developed genetic tools to understand its life cycle and differentiation. It provided an important tool to understand recombination and the transmission of genetic material, and launched into greater prominence with the molecular era in the 70s (Forsburg, 2005). Yeasts are ideal platform for discovery research and for developing of testing methods due their prominent properties:

- single celled with a typical eukaryotic organisation and with conserved processes of replication, cell division, protein folding and itracellular transport (Forsburg, 2005; Mattiazzi et al., 2012);
- the richness of the species landscape (Forsburg, 2005);

- rapid and cost-efficient reproduction under a wide variety of conditions and the absence of requirements for elaborate sterile techniques or expensive media (Mattiazzi et al., 2012);
- lack of the ethical problems associated to the animal models (Pereira et al., 2012);
- exist in both haploid and diploid state and it is easy to mate haploid cells and sporulate diploid cells (Mattiazzi et al., 2012);
- yeast genome for which the complete sequence was reported and the best described eukaryotic organism which genes and proteins are extensively annotated in several databases (Goffeau et al., 1996; Mattiazzi et al., 2012; Pereira et al., 2012);
- facile genetics and highly conserved cell cycle machinery and metabolic pathways with clear human orthologs (Bjornsti, 2002; Eisenberg et al., 2007);
- easy genetics manipulations for experimental testing of hypotheses - a high endogenous rate of homologous recombination, and a host of extrachromosomal DNA elements can stably transform yeast cells, thus the screening and segregation analysis and cloning of genes in this organism is significantly easier than in more complex eukaryotes; yeasts are free of endotoxins and they are classified as GRAS (generally recognised as safe) (Nasser et al., 2003; Mattiazzi et al., 2012);
- useful as a host cell in genetic engineering because it folds and glycosylates heterologous eukaryotic proteins, has a potential to display other eukaryotic proteins, and can display different kinds of protein on the same cell surface, called ‘co-display’ (Shibasaki et al., 2009).

During the last decades yeasts are frequently used as an efficient model in the studies of various processes in higher eukaryotic organisms including:

- investigation of main pathways of cellular metabolism, DNA replication, recombination and repair, RNA transcription and translation, intracellular trafficking etc. (Walker, 1998; Barrientos, 2003; Pray, 2008),
- cancer research and to establishing molecular mechanisms of human diseases (Barrientos, 2003; Pray, 2008; Pereira et al., 2012),
- testing possible toxicity of new drugs (OECD, 1986),
- screening for the antioxidant activity (Zyracka et al., 2005; Slatnar et al., 2012),
- studying oxidative modifications of proteins (Luschak, 2006),
- in ageing and apoptosis research (Breitenbach et al., 2003; Carmona-Gutierrez et al., 2010);
- neurodegeneration research (Pereira et al., 2012),

- for the understanding the mechanisms of drugs activities (Bjornsti, 2002),
- different types of biotechnology – food production, ethanol production, waste purification (Walker, 1998).

1.3. Anabiosis

1.3.1. Phenomenon of anabiosis

Anabiosis is a unique natural phenomenon of temporary reversible delay of metabolism by live organisms upon untoward conditions. It was discovered in 1701 by great Dutch naturalist Antonie van Leeuwenhoek. Despite the fact that anabiotic occurance of organisms was found more than 300 years ago only during last 40 years the main mechanisms of organisms' capabilities to survive and to maintain viability during disadvantaged conditions were demonstrated. It was discovered that sometimes microorganisms can survive even for thousands years in anabiotic state (Beker and Rapoport, 1987).

Different types of anabiosis can be distinguished depending on the ways of live organisms' transition into anabiotic state:

- anhydrobiosis – a state, caused by significant losses of water through evaporation;
- cryobiosis – takes place as the result of live organisms freezing;
- osmobiosis – a state, brought about by extraction of water from the organisms by various solutions with a high osmotic pressure;
- anoxybiosis – caused by a decrease of oxygen concentration in the gas phase below critical limits for aerobic metabolism (Beker and Rapoport, 1987).

Anabiosis is very widely spread in nature, it is a form of adaptation, way of survival (crucial for population maintenance) and preservation of organized matter; many different organisms represent this phenomenon: bacteria, fungi, several species of rotifers, tardigrades, nematodes, larvae of some insects, eggs of some crustaceans, plant seeds (Beker and Rapoport, 1987; Wharton and Barclay, 1993; Capriolia et al., 2004; Uzunova-Doneva and Donev, 2004-2005; Potts et al., 2005; Calahan et al., 2011; Welch et al., 2013). Black microcolonial fungi and black yeasts together with lichens and cyanobacteria are among the most stress-tolerant organisms on the Earth (Zakharova et al., 2013).

At the state of anhydrobiosis organisms maintain different amount of water inside the cells. For example, in yeasts residual amount of water is about 8-10%, in bacteria – 1-2% (Beker and Rapoport, 1987). Resistance against drying is a specific characteristic of microorganisms and depends on taxonomical and physiological properties determining the chemical composition of the cells from various species or strains (Beker and Rapoport, 1987; Calahan et al., 2011; Welch et al., 2013). Yeasts are recognized as one of most resistant microorganisms at dehydration. Although, various yeast strains exhibit different resistance

against dehydration. As it was shown the same yeast strain grown in different media can respond to dehydration differently. For example, viability of *S. cerevisiae*, grown on a rich molasses medium may reach 90% while that of the same strain grown on a synthetic medium is only 20-40% (Beker and Rapoport, 1987). Yeasts sampled at the exponential growth phase are less resistant against drying than those of the stationary growth phase (Beker and Rapoport, 1987; Calahan et al., 2011). It was shown that resistance of yeasts against drying can be significantly enhanced by increasing of the trehalose content in cells. The synthesis of trehalose in yeast can be stimulated by elevated temperature and decreased aeration at the final growth stage. Additionally the amount of trehalose in *S. cerevisiae* up to 15-16% can be obtained at increased osmotic pressures or cell starvation of 2-3 h prior to termination of the batch (Beker and Rapoport, 1987).

The preservation of microorganisms has been a challenge since the beginning of mankind. Some strains of beneficial microorganisms are involved in the production of dairy, bakery, spirits, alcohol, vaccines, antibiotics, enzymes, silage, vinegar, and others (Uzunova-Doneva and Donev, 2004-2005). There are several methods for the preservation of industrial microorganisms, for example, subcultivation, and use of mineral oils, water–salt solutions, cryogenic conservation, and drying (Uzunova-Doneva and Donev, 2004-2005). Among these methods, the preservation of microorganisms by desiccation has been the preferred method for long-term storage (Morgan et al., 2006). Nowadays, there are big culture collections that depend on drying methods for preserving their microorganisms (Uzunova-Doneva and Donev, 2004-2005).

Concerning the industrial importance of anhydrobiosis, the worldwide market in the stabilization of cells and cell products is approximately $500 billion (Potts et al., 2005; Garcia, 2011). Yeast industry produces huge amounts of active dry baker's yeast of very high quality. Moreover, induced anhydrobiosis is applied in the preservation of reference strains used in the quality control of pharmaceutical and food industries, the conservation of reference strains from culture collections, and the generation of biotechnological products in the emerging fields of probiotics and biocontrol (Morgan et al., 2006). In the fields of probiotics and biocontrol, an intense amount of work has been performed during the last decade using the technologies of freeze-drying and spray-drying. These technologies show relevant results in the use of bacterial dehydration as a powerful tool for future applications in agriculture, food industry, and medicine (Garcia, 2011).

1.3.2. Intracellular stresses at the influence of dehydration

It is well-known that cell water represents up to 80% of cell`s mass and volume. There are two types of water: 'free' and 'bounded'. The main role of 'free' water is to provide the

liquid media for processes of metabolism. The 'bounded' water has the structural role and it is related to its ability to interact with the various groups of macromolecules. It plays the important role in the structural organization of proteins, in molecular flexibility and the activity of enzymes, in interactions of protein–protein, protein–DNA and protein–ligand, in the structure of nucleic acids, and finally water is responsible for the structure of cell membranes (Potts et al., 2005). Water evaporation during dehydration causes the increasing concentrations of intracellular macromolecules and ions potentially generating cellular toxicity through aggregation of biopolymers leading to hypersalinity and hyperosmotic stress (Calahan et al., 2011). In addition, intracellular viscosity is increased causing low rates of diffusion. Protein denaturation causes a loss of its biological activity (Franca et al., 2007).

During desication process there is an increase of production of reactive oxygen species (ROS) which influences lipid peroxidation and phospholipids deesterification which may in their turn damage DNA, proteins, or membranes (Franca et al., 2005; Calahan et al., 2011; Welch, 2013). ROS can cause denaturation of proteins, their aggregation and activity loss (Prestrelski et al., 1993). Increased package of polar groups of phospholipids and formation of endovesicules can lead to cell lysis during rehydration (Simonin et al., 2007).

1.3.3. Cell structural changes at the influence of dehydration

Investigations which have been realised till the moment showed that during yeast cells dehydration very essential structural and functional changes took place practically in all organelles and other cell components.

Cell wall. Electron microscopical investigations revealed essential changes of yeast cell shape and of cell wall structure as the result of their dehydration. Branched mannoprotein fibrils were found on the surface of the cell (Rapoport et al., 1983; Ventina et al., 1984; Beker and Rapoport, 1987). The significant reversible increase of cell surface negative charge was shown and was explained as the result of the increase in the number of free anionic groups of the mannoprotein component of the cell wall (apparently carboxyl and phosphate groups) (Rapoport and Beker, 1985). Data on the changes of geometrical characteristics of the cell during its drying were received and the corresponding conclusions on the changes of glucan component of cell wall were made (Rapoport et al., 1986c).

Plasma membrane. It was shown the appearance of strong invaginations of plasma membrane which form folds network on its surface (Rapoport and Kostrikina, 1973; Biryuzova and Rapoport, 1978). This phenomenon could be considered as a protective intracellular reaction which promotes to maintain membrane integrity in the conditions of essential diminishing of cell volume and correspondingly of its surface area. In these studies it was proposed also that functional significance of plasma membrane folds (which were found

a little earlier) are linked with the necessity to provide the possibility for the increase and decrease of cell volume and surface area in different osmotic conditions (Biryuzova and Rapoport, 1978). It is shown that structural reconstructions of plasma membrane are accompanied with significant increase of its permeability (Novichkova and Rapoport, 1984; Beker and Rapoport, 1987). On the one hand this effect is rather dangerous for the maintenance of single cell viability but at the same time leakage of some cells supplies the remaining viable part of the population with a lot of very important for them intracellular compounds which are absent in the initial media and can significantly improve physiological characteristics of culture (Rapoport et al., 1986b). It was established that permeability of dried yeasts sharply increases during rehydration and the total losses can reach up to 20-30% of cell dry weight. Free amino acids, potassium and magnesium ions, protein compounds can be leaked from the cell during rehydration (Beker and Rapoport, 1987).

Mitochondria. It was revealed that mitochondria are the organelle which underwent the fewest changes that could be found at the ultrastructural level. They are the first structures which restore their activity and repeatedly multiply by division already at the initial stages of cell reactivation process from the dehydrated state (Rapoport and Kostrikina, 1973). Undoubtedly it provides the generation of energy necessary for the realization of various reparative processes in the cell. At the same time high structural stability of mitochondria could be linked with the presence of two membranes which surround these organelles.

Vacuoles. The decrease of big vacuoles' size or their fragmentation as well as change in the form of a number of smaller vacuoles during yeast cell dehydration was revealed. The conclusion was made on the influence of this appearance for the maintenance or changes of cell shape at dehydration (Beker and Rapoport, 1987). In some cases redistribution of proteins on the surface of vacuolar membrane was established (Rapoport et al., 1986a). The increase of lysosomal activity which in yeast cells is linked with vacuolar system was shown during yeast dehydration and subsequent reactivation (Rapoport and Beker, 1978).

Ribosomes. Biochemical experiments showed significant (up to 40%) degradation of ribosomal RNA especially during initial stages of yeast dehydration process (Rapoport and Beker, 1986). The conclusion was made on the destruction of definite part of ribosomes. At the same time it was revealed that this effect does not influence the viability of dehydrated organisms and the remaining amount of ribosomes is enough for the cells for the start of protein synthesis during reactivation stage.

Lipid granules. It was shown that intracellular lipid granules also underwent certain changes during yeast dehydration. Most often in these conditions takes place their merging. After cells' reactivation from dehydrated state sometimes the existence of contacts between

lipid granules and vacuoles was revealed as well as intrusion of merged lipid granules into the vacuole. It was also proposed the participation of lipid granules in the repair processes of damaged areas of intracellular membranes (Ventina et al., 1986).

Nucleus. The changes of nucleus shape in dehydrated yeast cells were revealed. Besides it serious damages of nuclear membrane were found as a notable widening of their pores. Lipid granules or mitochondria were usually localized near these 'opened' areas of nuclear membrane (Rapoport and Kostrikina, 1973; Rapoport et al., 1973; Biryuzova and Rapoport, 1978; Ventina et al., 1986). The presence of these structures in damaged regions undoubtedly facilitates membranes' reparation supplying them with reserve lipid material and necessary energy. We suppose such changes are linked with some damage of membrane lipid components. At the same time in nuclei of viable cell chromatin condensation was revealed. The conclusion that chromatin condensation is one of the most important intracellular protective reactions was made (Rapoport et al., 1973)

Other important intracellular changes. It was revealed that during dehydration-rehydration there take place in yeast cells an increase in the ratio of unsaturated and saturated fatty acids in total lipids and phospholipids (Zikmanis et al., 1982, 1983). The most significant changes in these fatty acids ratios were observed in yeasts which were the less resistant to drying and subsequent rehydration (Zikmanis et al., 1982, 1983; Beker and Rapoport, 1987). It was supposed that the loss of water causes disorientation of phospholipids molecules in membranes. Under these conditions membrane lipids may change their organization in membrane because of the transition from lamellar to hexagonal phase II (Beker and Rapoport, 1987). Up to current understanding in anhydrobiotic (viable) cells this situation may take place only at some small rather limited sites of membranes. Usually during water removal, the distance between the phospholipids headgroups decreases, leading to increased van der Waals' interactions between the acyl chains (Crowe et al., 1992). The phospholipid bilayer in such conditions can pass into the gel phase undergoing phase transitions (Crowe et al., 1992; Laroche et al., 2005). The restoration of membranes during cells reactivation from dry state is linked with back phase transitions of lipids from gel to lamellar phase. In some cases this phase transition temperature (T_m) of phospholipids can be rather high. So, when such 'dry' phospholipids are placed in water, they have to undergo a reverse phase transition, from gel to the lamellar phase. Phospholipid bilayers are known to become transiently leaky during such phase transitions. Because of defects in the bilayer at the interphases of gel and liquid crystalline domains during the phase transition, the membranes become permeable to solutes and it is generally supposed to be one of the main reasons of cell death during dehydration (Tetteroo et al., 1996; Laroche and Gervais, 2003).

Since T_m reaches minimal values at water contents in the cells of about 20–25%, preliminary rehydration of dry cells over water vapors would be expected to lead to the decrease of plasma membrane permeability because of this back phase transition takes place not in the bulk of water. That is indeed what is seen, with dry cells (Rapoport et al., 2009). By the usage of lipidic fluorescent probes targeted to the plasma membrane it was demonstrated by Soubeyrand et al., 2005 that in the first 15 minutes of rehydration a great mobilization of lipids occurs. During rehydration, a fast mobilization of lipid storage within the cells was indeed previously observed by electron microscopy (Saulite et al., 1986). This fast lipid mobilization allows yeast cells to recover quickly functional cellular membranes. It was discovered that during the first part of rehydration process the active dry yeast can incorporate exogenously provided specific natural yeast sterols by assimilating them under the form of micelles and increasing by this way cells' viability (Soubeyrand et al., 2005).

1.3.4. Protection strategies of the cells during dehydration for the maintenance of their viability

On the basis of the results of realised investigations the conclusion about the most important factors favouring the preservation of cell viability was made. These factors are the following:

a) level of stability of the intracellular nucleic acids;

b) chromatin condensation in nuclei which has to take place at the early stages of yeast dehydration;

c) preservation of molecular organization of different intracellular membranes;

d) accumulation of significant amounts of intracellular protective substances (trehalose, glycerol, polyols);

e) possibility for the cell to initiate the adaptation reaction complex.

During the initial phase of dehydration the intracellular protective mechanisms involved in osmoregulation are activated. The high osmolarity glycerol (HOG) pathway is initiated by the changes in the tensile force of the plasma membrane and as the result the intracellular glycerol concentration increases (Schaber et al., 2010). The high concentration of glycerol inside the cell is also effected by the closure of the aquaglyceroporin Fps1 - an osmolarity-regulated channel, and by the avoiding the leakage of glycerol (Tamas et al., 1999).

Numerous studies have demonstrated the stabilizing effects of intracellular non-reducing disaccharides (trehalose and sucrose) on proteins and membranes during dehydration. Two mechanisms have been proposed to explain this protective role. The first is based on the water replacement hypothesis (Clegg et al., 1982) where the sugars form hydrogen bonds with macromolecules instead of water hydroxyls during dehydration, thereby preserving their

native structures. These interactions prevent increases in the membrane gel to fluid phase transition temperature (T_m). As a consequence, dry membranes remain in a fluid state at physiological temperatures and they avoid a phase transition during rehydration (Crowe et al., 1984; Crowe et al., 1992). According to the recently modified water replacement hypothesis, trehalose stabilizes dry membranes by preventing decreases in the spacing between membrane lipids during dehydration. Trehalose causes a concentration-dependent increase in the area per lipid as well as fluidizing the bilayer core (Golovina et al., 2009, 2010). The second mechanism proposed to explain the protective role of trehalose depends on the ability of sugars to form glasses at low water levels. The inclusion of the cell contents in an amorphous matrix with very high viscosity can avoid crystallization destructuring effects and significantly reduce the mobility and reactivity of molecules. It has also been hypothesized that glass formation in the cytosol prevents membrane fusion between adjacent phospholipid bilayers. Stationary phase *S. cerevisiae* cells synthesize high amounts of trehalose compared with exponential phase cells (Gadd et al., 1987). These cells also contain trehalose transporters, which are fully active but they are repressed by glucose during the exponential phase (Crowe et al., 1991). These observations may help to explain the relationship between the growth phase and dehydration resistance in cells. Indeed, yeasts are highly resistant in the stationary growth phase whereas they are more sensitive during the exponential phase (Beker and Rapoport, 1987; Calahan et al., 2011; Welch et al., 2013). It was also shown that the genes involved in trehalose biosynthesis are up-regulated during hyperosmotic stress (Posas et al., 2000).

Other sugar alcohols, such as sorbitol and xylitol, have similar protective effects to trehalose in yeast cells (Rapoport et al., 1988; Krallish et al., 1997). Similar to trehalose (Leslie et al., 1994), the synthesis and accumulation of sugar alcohols in *S. cerevisiae* cells before their dehydration lead to a significant decrease in the temperature of the membrane lipid phase transition in anhydrobiotic cells (Rapoport et al., 2009). The protective effect of sugar alcohols on yeast growth was also observed under oxygen limitation conditions. Following growth in these conditions, yeast was unable to resist desiccation but pretreatments with sugar alcohols, specifically xylitol, increased resistance to dehydration (Rozenfelde and Rapoport, 2014).

The preservation of macromolecule structures during dehydration can be facilitated by proteins – hydrophilins which the beneficial effect on the viability of yeast during dehydration may be linked to the protection of cellular molecules and macromolecules either via direct interaction or by acting as molecular shields (Tunnacliffe and Wise, 2007).

The use of chemical oxidants can help to elucidate numerous mechanisms of resistance

to oxidative stress in yeast. Superoxide dismutase (SOD) is involved in the conversion of the superoxide anion (O_2^-) to dioxygen and hydrogen peroxide. Thioredoxin is considered to be involved in the resistance of yeast to dehydration because of its role in the prevention of oxidative damage - can be a reductant for thioredoxin peroxidase and ribonucleotide reductase (Jamieson 1998).

The lipid composition is crucial for the oxidation of phospholipids in membranes, where polyunsaturated fatty acids are particularly sensitive to oxidation. The membrane of *S. cerevisiae* mainly comprises saturated and monounsaturated phospholipids (Steels et al., 1994). Modifications of this composition to yield a higher content of polyunsaturated phospholipids lead to hypersensitivity to oxidative stress in yeast (Steels et al., 1994; Howlett and Avery, 1997). Thus, the very low content of polyunsaturated fatty acids in *S. cerevisiae* confers constitutive protection against oxidative stress during desiccation. Another mechanism that protects yeast phospholipids from oxidation during dehydration is the presence of a high proportion of ergosterol in the plasma membrane and it was also shown that the structural specificity of ergosterol is essential for the resistance of yeast to the oxidative stress induced by dehydration/rehydration cycles (Dupont et al., 2012).

1.3.5. Laboratory-scale preservation methods

To improve the properties of cell culture to survive during the state of anhydrobiosis different strategies were developed and applied in the laboratory and biotechnological scale:

- cultivation method, temperature regime, composition and ph of the nutrient medium, aeration which plays one of essential roles for preservation of cells ensuring the formation of system scavenging ROS (Uzunova-Doneva and Donev, 2004-2005);
- the properties of cell culture: age, physiological condition and culture concentration at the moment of conservation (Uzunova-Doneva and Donev, 2004-2005);
- the use of protectants which can be added during microorganism growth, or just before the drying process: skimmed milk, serum, trehalose, glycerol, betaine, adonytol, sucrose, glucose, lactose, and polymers such as dextrane and polyethylene glycol (Morgan et al., 2006);
- rehydration is one of the most important stages once the microbial cells are subjected to the desiccation process: type of rehydration solution, rehydration temperature, speed of rehydration. For example, it has been observed that a protective solution used before the drying is a good rehydration solution (Potts et al., 2005; Garcia 2011). In addition, a complex medium generally has higher

osmotic pressure, which diminishes the negative effect of osmotic shock in anhydrobiotic cells. The rehydration temperature is another variable to be considered, mainly because rehydration at a temperature below Tm, generally leads to phase transition with the subsequent leakage of intracellular material and lethal damages (Potts et al., 2005; Garcia 2011);

- selection of resistant strains through training through multiple exposures to stress (Potts et al., 2005) and genetic engineering (López-Martínez et al., 2013).

1.4. Immobilization of cells

1.4.1. Definition of immobilization

Attachment of microorganisms to different kinds of surfaces is an essential and common ecological process in nature. Surface adhesion and biofilm development was discovered as one of survival strategies refined over millions of years (Nikolajeva at al., 2012). Based on naturally occurred attachment process definition of immobilization appeared. The cell immobilization emerged as an alternative for enzyme immobilization and it was defined as the physical localization of intact cell to a certain region of support with preservation of some desired catalytic activity (Kourkoutas et al., 2004).

Different methods of cell immobilization have been developed which can be roughly divided in two groups:

1. ‘passive’ or based on physical interactions - using the natural tendency of microorganisms to attach to surface by the weak interactions between support and cell membrane;
2. ‘active’ or based on chemical interactions – where covalent bonds are formed using different flocculant agents, chemical attachment and gel encapsulation (Stolarzewicz et al., 2011; Martins et al., 2013) (Fig. 3).

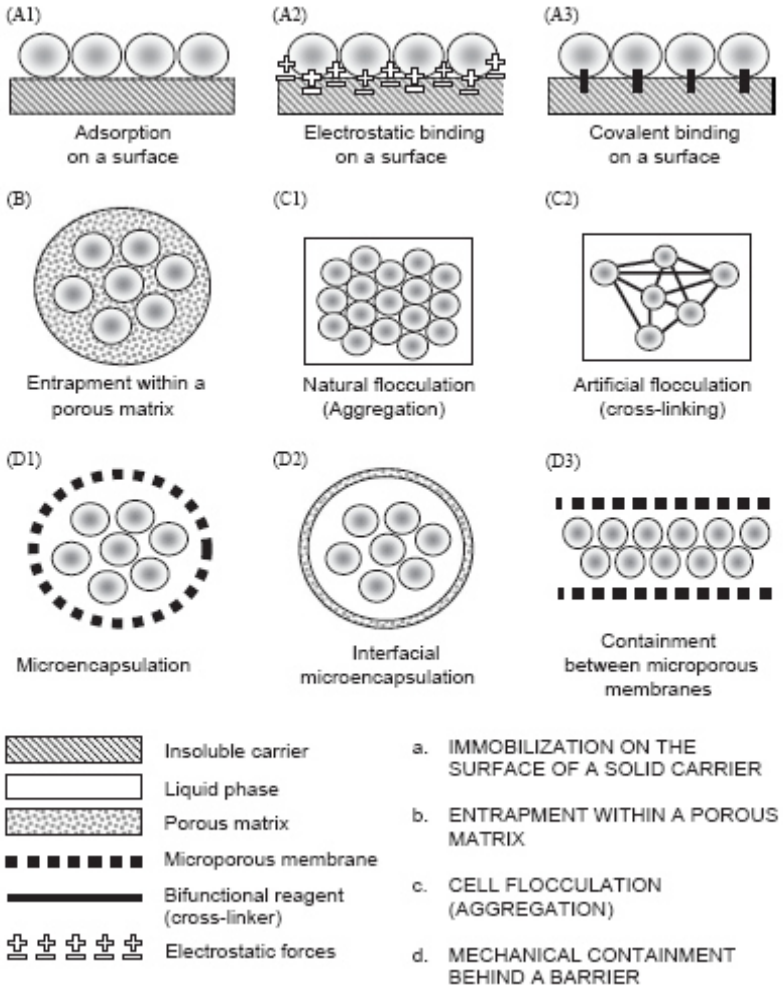

Fig. 3. Methods of cell immobilization (Kourkoutas et al., 2004).

The physical methods comprise:

- physical or ionic adsorption on a water-insoluble matrix,
- inclusion or gel entrapment,
- microencapsulation within a solid or liquid membranes,
- containment of an enzyme or whole cells within a membrane.

The chemical imobilization methods include:

- covalent attachement to a water-insoluble matrix,
- cross-linking with the use of multifunctional, low-molecular mass reagent,
- co-cross-linking with other neutral substances (Stolarzewicz et al., 2011).

1.4.2. Characterization of immobilization methods

Covalent bonding/Cross linking - the mechanism involved in this method is based on covalent bond formation between activated inorganic support and cell in the presence of a binding (crosslinking) agent. For covalent linking, chemical modification of the surface is necessary. Covalent attachment and cross-linking are effective and durable to enzymes, but it is rarely applied for immobilization of cells. It is caused mainly by the fact that agents used

for covalent bonds formation are usually cytotoxic and it is difficult to find conditions when cells can be immobilized without any damage (Ramakrishna and Prakasham, 1999).

Entrapment - is an irreversible method, where immobilized cells are entrapped in a support matrix or inside fibers. This technique creates a protective barrier around the immobilized microbes, ensuring their prolonged viability during not only processing but also storage in polymers (Gorecka and Jastrzebska, 2011). The matrices used for this method are: agar, alginate, carrageenan, cellulose and its derivatives, collagen, gelatin, epoxy resin, photo cross-linkable resins, polyacrylamide, polyester, polystyrene and polyurethane (Lopez et al., 1997; Ramakrishna and Prakasham, 1999). Entrapment of the microorganisms in porous polymer carrier was often used to capture the microorganisms from suspended solution and then obtain the immobilized microorganisms. The polymer matrix used for this method has to be of porous structure, and thus the pollutant and various metabolic products could easily diffuse through into the matrix. In this method, a lot of porous polymers can entrap microorganisms under ambient conditions (Verma et al., 2006). As a rule, the entrapment methods are based on the inclusion of cells within a rigid network to prevent the cells from diffusing into surrounding medium while still allowing penetration of substrate. Entrapment of cells in alginate gel is popular because of the requirement for mild conditions and the simplicity of the used procedure. Several reports are available employing alginate gel (Kierstan and Bucke, 1977). Entrapment allows high mechanical strength, but contains some disadvantages, such as, cell leakage, costs of immobilization, diffusion limitations, and deactivation during immobilization and abrasion of support material during usage. Another disadvantage is low loading capacity as biocatalysts have to be incorporated into the support matrix (Stolarzewicz et al., 2011).

Encapsulation - is another irreversible immobilization method, similar to entrapment. In this process, biocatalysts are restricted by the membrane walls (usually in a form of a capsule), but free-floating within the core space (Gorecka and Jastrzebska, 2011). The membrane itself is semi-permeable, allowing for free flow of substrates and nutrients (when cells are used as a biocatalyst), yet keeping the biocatalyst inside. The factor determining this phenomenon is the proper pore size of the membrane, attuned to the size of core material. This limited access to the microcapsule interior is one of the main advantages of microencapsulation, because it protects the biocatalyst from the harsh environmental conditions. As most immobilization method, it prevents biocatalyst leakage, increasing the process efficiency as a result (Park and Chang, 2000). The encapsulation method was used to enclose the microorganisms in a polymer gel by Jen et al. (1996) and is one of the most frequently used in laboratory experiment up to now (Lozinsky and Plieva, 1998). However,

even though in encapsulation, high cell loading can be achieved, but the capsules are still very weak. The diffusion limitation is one of the inevitable drawbacks associated with encapsulation method (Lozinsky and Plieva, 1998).

Adsorption (adhesion) - the passive immobilization or natural adsorption of microorganisms onto porous and inert support materials is similar to the adsorption of colloid particles (Araujo et al., 2010). Apparently, it is the first example of cell immobilization and probably is the simplest method of reversible immobilization. This technique is based on the physical interaction between the microorganism and the carrier surfaces, while it is frequently reversible, simple, cheap and effective. Cell immobilization through adsorption provides a direct contact between nutrients and the immobilized cells. This cell immobilization technique involves the transport of the cells from the bulk phase to the surface of support (porous and inert support materials), followed by the adhesion of cells, and subsequent colonization of the support surface (Kilonzo and Bergougnou, 2012).

The adhesion of yeast to a surface is the first step in immobilization and results from complex physicochemical interactions between the cell, the surface and the liquid phase. It depends on physicochemical properties of the support and cells (surface charge, hydrophobicity, functional groups, electron donor-electron acceptor properties and support porosity and roughness) and environmental conditions (ionic strength, temperature and contact time). Initial interactions between microbes and biomaterials may be explained by the extended DLVO (Derjaguin, Landau, Verwey, Overbeek) theory, which considers the four fundamental, non-covalent interactions: Lifshitz-van der Waals, electrostatic, Lewis acid-base and Brownian motion forces. The first two are usually termed long-range forces i.e. initially involved in bringing the two surfaces together, while Lewis acid-base depends on short range specific interactions between molecular groups (Gorecka and Jastrzebska, 2011; White and Walker, 2011).

Many carriers can be used for immobilization of microorganisms by adsorption method, like porous glass, polyurethane foam, ceramics - hydroxylapatite (HAP) and chamotte (Kregiel et al., 2012). HAP has numerous biomedical applications, it can bond directly to living bone. Its affinity for proteins is exploited in high performance liquid chromatography systems and its metal adsorption capacity is used for removal of metals. It is used widely in medicine as bioactive ceramic materials for bone and dental implants (White and Walker, 2011). Different organic supports are used too, for example, wood chips and straw. These fibrous matrices provide adequate supporting surfaces for cell adsorption due to their high specific surface area, void volume, mechanical characteristics and permeability, low pressure drop, diffusion problems and toxicity, maximum loading, biodegradability and durability and

low cost and high availability. Their natural configuration also allows them to trap more cells than other materials.

There are many factors that influence the sorption of microbial cells. The age of the cells or the growth phase may have a strong impact on the attachment properties of the cells. In the experiments carried out by Bowen et al. (2001), cell adhesion of *S. cerevisiae* brewing strain was significantly higher for the cells from the stationary growth phase compared to those from the exponential phase. The cells from the exponential phase are usually characterized by greater sensitivity to changing environmental factors and lower resistance to stress (Kregiel et al., 2012). Strain specificity should be taken in consideration immobilizing a new strain by adsorption method and the optimal conditions for yeast adhesion should be determined individually for each strain (Kregiel et al., 2012).

The limitation of the medium components is also one of the factors responsible for the synthesis of proteins classified as adhesins (Verstrepen and Klis, 2006). The mechanism of adhesion of *S. cerevisiae* on glass and polymer carriers was identified by Guillemot et al. (2006) as substratum-dependent. In experiments of Kregiel et al. (2012) the nutrients limitation for *S. cerevisiae* TT and *D. occidentalis* Y 500/5 had a positive effect on the efficiency of adhesion.

Adhesion of yeast to a surface depends also on complex physicochemical interactions of cell-surface-liquid phase. It was observed that localized positive charges of yeast cells could be very important in cell adhesion. Microbial adhesion is also often associated with the overall surface hydrophobicity of microorganisms. It was claimed that increased cell-surface hydrophobicity favored cell adhesion. Yeast cells are predominantly characterized by the negative charge due to the presence of carboxyl, phosphoryl and hydroxyl groups. However, it was indicated that the overall cell wall charge was not the principal determinant in cell adhesion (White and Walker, 2011). It was proved that cell surface hydrophobicity, as well as cell surface charge, differ significantly among all strains, indicating that these properties and their variations are characteristic for a particular strain. The composition of the medium, its pH, and environmental conditions considerably influence the adsorption of cells by changing their electrokinetic potential (Kilonzo and Bergougnou, 2012).

The surface properties of support also affect the process of cell immobilization. The degree of cell immobilization depends on the structure and the size of adsorbent pores. On porous carriers cells accumulate mainly due to steric retention, the prevailing interactions between cells and nonporous supports are considered to have physicochemical character (electrostatic, hydrophobic) (Branyik et al., 2004). The nature of adsorbents is also important. Organic adsorbents are chemically stable and show a great variety of surface properties and

pore structures, whereas inorganic adsorbents are resistant to biological degradation and can be easily regenerated. The disadvantage of inorganic adsorbents is that they are soluble in alkaline solutions (Samonin and Elikova, 2004).

The principal factors affecting the microbial cell adsorption are summarized in Table 1.

Table 1. Factors affecting cell immobilization by adsorbtion

Immobilization support	Immobilization medium	Cells
Structure	Flow velocity	Age and physiological state
Topography	pH	Surface structure
Nature	Temperature	Hydrophobicity
Porosity	Cations	Charge
Functional groups	Antimicrobial agents	

1.4.3. Effect of immobilization on microbial cells and advantages of immobilized cells over free cell systems

It has been shown that free and immobilized yeast cells can differ in physiological activity (Verbelen et al., 2006). This term could describe various important parameters: growth or reproduction abilities, aging, stress tolerance and fermentation potential (Berlowska et al., 2013). Various reviews discussed the reasons for the changes in metabolic behaviour of immobilized cells (Junter et al., 2002; Kourkutas et al., 2004; Berlowska et al., 2013). It has been generally observed that it is difficult to predict the type and magnitude of metabolic changes possible through immobilization. A number of parameters have been considered responsible for these alterations, such as mass transfer limitations by diffusion, disturbances in the growth pattern, surface tension and osmotic pressure effects, reduced water activity, cell-to-cell communication, changes in the cell morphology, altered membrane permeability and media components availability. In general comparative studies on immobilized and free cells reported effects on activation of yeast energetic metabolism, increase in storage polysaccharides, altered growth rates, increased substrate uptake and product yield, lower yield of fermentation by-products, higher intracellular pH values, increased tolerance against toxic and inhibitory compounds (Kourkutas et al., 2004).

Effects on growth and physiology. Differences in the morphology and shape between free and immobilized in alginates *S. cerevisiae* cells were shown. It was explained by insufficient space for growth in the support. Immobilized cells, in comparison with free cells, had a higher content of glycogen, trehalose, structural polysaccharides (glucans and mannans), fatty acids and DNA. Immobilization also caused changes in the proteome of a cell, in the level of gene expression, and has a significant impact on the quantitative

composition and organization of the plasma membrane and cell wall structures (Branyik et al. 2008). In the fermentation of wort by *S. cerevisiae* cells immobilized in calcium alginate gel beads, in packed- and fluidized-bed bioreactor configurations, the cells exhibited altered growth behaviour compared to free cells, where the striking feature was the decrease of growth rate when the proportion of yeast increased into the gel beads. The intrinsic growth rate of the yeast cells immobilized by adsorption to various solid surfaces was either increased or decreased. It was reported that cells of *Candida tropicalis* and *S. cerevisiae* immobilized in calcium alginate showed insignificant morphological alterations although their metabolic activity was affected. Comparing different results it was suggested that it is the microenvironment inside the beads that affects physiology and metabolic behaviour and not the nature of the gel matrix (Kourkutas et al., 2004).

Effects on metabolic activity. Many studies have reported an increase in metabolic activity (increased rate of sugar uptake and productivity of selected metabolites) in immobilized cells. Adsorption of *S. carlsbergensis* onto porous glass beads increased the yield of ethanol on glucose and decreased the CO_2 yield. Similar observations were reported with immobilized *S. cerevisiae* cells on ceramics (Kourkoutas et al., 2004). It was observed that optimum pH for fermentation using free *S. cerevisiae* cells was 4.0, while fermentative activity for immobilized cells in alginates was independent of pH. Intracellular pH in alginate immobilized *S. cerevisiae* cells was lower than in free cells. The reduced intracellular pH value in the immobilized cells resulted to an increased enzyme activity and therefore productivity. The reduced intracellular pH was attributed to increased permeability of plasma membrane to protons, which led to higher consumption of ATP causing increased glucolytic activity and glucose uptake. In yeast entrapped in alginate matrices, a slight decrease was noticed in intracellular pH due to increased enzymatic activity. This promotes the permeability of membranes, which in turn leads to an increase in proton transport and ATP use, stimulating glycolysis processes (Galazzo and Bailey, 1990). Higher efficiency in the pentose phosphate pathway and of glycolytic flux may also be explained by the increased activity of alcohol dehydrogenase and by more efficient regeneration of the NADH and NADPH cofactors (Branyik et al., 2008).

Effects on stress tolerance. It was shown that immobilized yeast cells demonstrate the increased ethanol tolerance and this phenomenon can be attributed to cell encapsulation by a protective layer of gel material or to modified fatty acid concentration in cell membranes due to oxygen diffusion limitations. Several reports highlighted increased productivity in immobilized thermotolerant *K. marxianus* IMB3. Osmotic stress caused by the immobilization techniques was found to lead to an intracellular production of pressure

regulating compounds such as polyols, which lead to decreased water activity and consequently higher tolerance to toxic or inhibitory compounds. Cells immobilized in various polymer matrices showed enhanced viability and thermal stability in freezing and freeze-drying conditions compared to free cells (Kourkoutas et al., 2004; Martins et al., 2013).

Effects on flavour formation. Yeast metabolic activity, especially amino acid metabolism, has a crucial contribution to flavour, because it is linked to the production of compounds such as vicinal diketones, esters, higher alcohols, aldehydes, fatty acids and sulphur compounds. Increased ester and decreased alcohol formation observed in immobilized cells fermentations, as well as the ratio of esters to alcohols have the greatest impact on beverage technology. The use of immobilization for the removal of diacetyl and therefore controlling flavour and reducing maturation time and production costs in brewing has been reported as a promising technological application (Kourkoutas et al., 2004).

1.4.4. Aplication of immobilized yeasts

Immobilized yeast finds application in many life areas: alcohol-distilling industry, winemaking, brewing, baking, biotechnological fuel production, pharmaceutical and chemical industries, agriculture, electronics (biocells), medicine (biosensors) (Stolarzewicz et al., 2011), waste water treatment and the bioremediation of contamination from numerous toxic chemicals and metals (Majdik et al., 2010), biosynthesis of vitamins or aminoacids (Gorecka and Jastrzebska, 2011), ethanol production for fuel needs from different renewable resourses as one of the most advanced liquid fuels because it is environmental friendly (Neelakandan and Usharani, 2009), biosynthesis of numerous enzymes useful in biotechnological processes (Johnson and Echavarri-Erasun, 2011). Cell immobilization for wine-making is a rapidly expanding research area, although applications of this technology at industrial scale are limited. The purpose for using such technique is to improve alcohol productivity and overall product aroma, taste and quality (Kourkoutas et al., 2004).

2. MATERIALS AND METHODS

2.1. Strains and growth conditions

Different yeast strains were used to achieve the aim and to fulfil the tasks of the work. Yeast strains used according to the tasks of the research are summarized in the Table 2. Their growth conditions are mentioned too.

Table 2. Yeast strains used in the study

Yeast strains	Tasks	Yeast growth conditions
***S. cerevisiae* 14** (deposited in the Microbial Strain Collection of Latvia as *S.cerevisiae* MSCL984) ***S. cerevisiae* L-77** (Microbial Strain Collection of Latvia) ***S. cerevisiae* Evolution canyon N6** (isolated in Israel and obtained from Prof. Andrey Sibirny, Institute of Cell Biology, National Academy of Sciences of Ukraine)	to study possible changes of certain maltose transport systems in the plasma membrane during dehydration/rehydration	Yeasts were grown in YPM medium (1% extract, 2% peptone, 2% maltose) on an orbital shaker at 160 rpm at 30 °C.
***S. cerevisiae* BY4741** and its derivatives (Table 3)	to evaluate the possible role of potassium transport systems in the maintenance of cell viability during dehydration/rehydration	Yeasts were grown in YPD medium (1% extract, 2% peptone, 2% glucose) supplemented with 50 mM KCl in an orbital shaker at 160 rpm at 30 °C.
***S. cerevisiae* SEY6210** and its derivatives (Table 4)	to investigate the possible role of cell wall certain proteins in the maintenance of cell viability in the state of anhydrobiosis	Yeasts were grown in YPD medium (1% extract, 2% peptone, and 2% glucose) on an orbital shaker at 160 rpm at 30 °C.
***S. cerevisiae* 14**	to develop a new method for cell immobilization based on the knowledge of cell wall changes at dehydration to investigate the influence of immobilization on physiological and biotechnological characteristics of yeast cells	Yeast was grown in the medium that contained (in g l^{-1}): molasses –43 (final glucose concentration – 20 g l^{-1}), $MgSO_4$ – 0.7; NaCl – 0.5; $(NH_4)_2SO_4$ – 3.7; KH_2PO_4 – 1.0 and K_2HPO_4 – 0.13. The pH value of the nutrient medium was adjusted to pH 5.0 using H_2SO_4. Yeast was cultivated on an orbital shaker at 160 rpm at 30 °C or in static state under anaerobic conditions. Yeasts were grown in medium (1% extract, 2% peptone, 5% ethanol) on an

		orbital shaker at 160 rpm at 30 °C.
***S. cerevisiae* L-77** ***S. cerevisiae* L-73** (Microbial Strain Collection of Latvia)	to investigate the influence of immobilization on physiological and biotechnological characteristics of yeast cells	Yeasts were grown in YPG medium (1% extract, 2% peptone, 2% glucose) on an orbital shaker at 160 rpm at 30 °C.
***S. cerevisiae* 14** recombinant yeast strains ***Pichia pastoris* GS115** *his4* and ***S. cerevisiae* AH22** *MATa leu2 his4* (Freivalds et al., 2006)	to investigate the influence of immobilization on physiological and biotechnological characteristics of yeast cells	Yeasts were grown in YPG medium (1% extract, 2% peptone, 2% glucose) on an orbital shaker at 160 rpm at 30 °C.
***S. cerevisiae* 14**	to develop a new test-system for evaluation of influence of various chemical compounds upon eucaryotic cell	Yeasts were grown in YPM medium (1% extract, 2% peptone, 2% maltose) on an orbital shaker at 160 rpm at 30 °C.

The *S. cerevisiae* BY4741 strain (MATa his3Δ1 leu2Δ met15Δ ura3Δ; EUROSCARF) and its derivatives were used to evaluate the possible role of potassium transport systems in the maintenance of cell viability during dehydration/rehydration (Table 3).

Table 3. Derivatives of *S. cerevisiae* BY4741 used in the study

Strain	Genotype	Reference
BYT345	BY4741 tok1Δ::loxP nha1Δ::lox P ena1-5Δ::loxP	Zahradka and Sychrova, 2012
BYT45	BY4741 nha1Δ::loxP ena 1-5Δ::kanMX	This study
BYT12	BY4741 trk1Δ::loxP trk2Δ::loxP	Petrezselyova et al., 2010
BYT1	BY4741 trk1Δ::loxP	Petrezselyova et al., 2011
BYT2	BY4741 trk2Δ::loxP	Petrezselyova et al., 2011

The *S. cerevisiae* SEY6210 (MATα ura3-52 leu2-3,112 his3-Δ200 trp1-Δ901 lys2-810 suc2-Δ9 GAL) and its derivatives were used to investigate the possible role of cell wall certain proteins in the maintenance of cell viability in the state of anhydrobiosis (Table 4).

Table 4. Derivatives of *S. cerevisiae* SEY6210 used in the study

Strain	Genotype	Reference
VMY5678	SEY6210 ccw5::(kanMX) ccw6::(kanMX) ccw7::(kanMX) ccw8::URA3	Mrša and Tanner, 1999
MSP02G	SEY6210 scw4::LEU2 scw10::HIS3	Cappellaro et al., 1998
MSP03G	SEY6210 scw4::LEU2 scw10::HIS3 scw11::TPR1	Cappellaro et al., 1998
MEY12A	SEY6210 ccw12::URA3	Mrša et al., 1999
MEY5	SEY6210 ccw12::URA3 ccw13::TPR1 ccw14::HIS3 tip1::LEU2 cwp1::(kanMX)	Cappellaro et al., 1998

2.2. Dehydration and rehydration procedures

Yeast cells were grown to the stationary phase, harvested by centrifugation, washed and dehydrated by convective drying at 30 °C. Dehydrated biomass was rehydrated using fast

and/or slow rehydration. Fast rehydration was performed in distilled water (or in 50 mM KCl) for 10 min at room temperature. Slow rehydration was performed in water vapor chamber at 37 °C for 2 h.

2.3. Determination of cell viability

Cell viability was estimated using the fluorochrome primulin and fluorescence microscopy (Rapoport and Meysel, 1985).

2.4. Determination of cell humidity and the plasma membrane permeability

The humidity of dehydrated cells was determined by the drying of yeast biomass at 105°C till constant weight. The difference of the yeast sample weight before and after drying was evaluated.

The changes of the permeability of the cell plasma membrane during the rehydration were quantified by the losses of cells dry substance as measured by the difference of dry weight of the yeast samples before and after rehydration.

2.5. Investigation of possible changes of Agt1 in the plasma membrane at the influence of dehydration/rehydration

Yeast cell suspension was prepared and dry weight of 1 ml was determined by the drying of at 105 °C till constant weight. 20 µl of yeast cell suspension were suspended in 160 µl of potassium phosphate buffer (pH 5.0) for 5 min at 30 °C, and then 20 µl of *p*-nitrophenyl-α-glucoside (pNPaG) (40 mM in water) was added. After timed intervals aliquots were removed and immediately placed in a thermoblock at 100 ^{o}C for 3 min. After samples were cooled on ice, then 1 ml of 2.0 M $NaHCO_3$, (pH 10.0) were added to the samples, the cells were removed by centrifugation, and the p-nitrophenol presented in the supernatant fluid was determined at 400 nm. Controls using previously boiled cells were used. Calibration curve for p-nitrophenol was made to recalculate the optical values to concentrations (Stambuk 2000). The amount of produced p-nitrophenol was recalculated to yeast dry weight (p-nitrophenol, mM/mg y.d.w.).

2.6. Investigation of maltase activity in yeast cell extracts

Cells for the preparation of extracts were suspended in 100 mM K-phosphate buffer (pH 6.5) containing 0.1 mM EDTA (maltase buffer). Yeast cells were disintegrated using glass beads. Supernatant obtained after the centrifugation (20 min at 14 000 rpm at 4 °C) was used as crude cell extract. Maltase activity in the cell extract was measured in maltase buffer using pNPaG as a substrate (Liiv et al., 2001).

2.7. Estimation of internal potassium content

The internal potassium (K^+) content in cells from the stationary growth phase was estimated as described earlier (Kinclova et al., 2001). Briefly, cells (three aliquots per strain) were collected on Millipore membrane filters (0.8 μm pore diameter) and quickly washed with 20 mM $MgCl_2$. The cells were then extracted with HCl and analyzed with a flame atomic absorption spectrophotometer.

2.8. Immobilization procedure

Two ceramic supports were used: hydroxyapatite (HAP) and chamotte.

The immobilization of yeast on ceramics was performed using conventional approaches. In one case, the tablets of ceramics were added into the flasks with the nutrient medium before the start of yeast cultivation. In another case of the incubation of yeast cells together with ceramics, these supports were added to 100 ml of yeast suspension, which consisted of sterile distilled water (pH 6.5) with 3 g of yeast taken from the stationary growth phase of the culture as well as from the same yeast in different buffer solutions: phosphate buffer (pH 4.0, 6.0 or 7.0), acetate buffer (pH 3.0, 4.0 or 5.0), or citrate-phosphate buffer (pH 6.0 or 7.2). Incubation was performed in a rotary shaker (160 rpm) at 30 °C for 3, 12, and 24 h.

The new immobilization method consisted of: (1) the short joint incubation of yeast (stationary growth phase) with ceramics using the rotary shaker (160 rpm; 1 h at 30 °C); (2) the short (10 min) sedimentation of yeast on the tablets (without stirring the suspension); and (3) the dehydration of preparations at 30; 45, or 105 °C.

The amount of immobilized cells was counted by dry weight: dry weight of cell suspension before immobilization minus dry weight of cell suspension after immobilization.

To compare the efficiency of yeast cell immobilization on different supports, the amount of immobilized cells in dry weight was recalculated on the support surface area.

2.9. Preparation of samples for scanning electron microscope

The immobilized samples were observed using a scanning electron microscope (SEM) (S-4800; Hitachi, Japan) at 10-20 kV. Preparations for scanning electron microscopy were made by using the following method: (1) supports with sedimented yeast cells and immobilized preparations received by 'classical' methods were subjected to dehydration at 30°C at least for 1 h and then were covered by thin layer of gold in an ion coater (JB-3; Eiko, Japan); (2) immobilized preparations received by the 'new' method were directly covered by gold in an ion coater.

2.10. Yeast cell stability after immobilization

Stability of immobilized cells was checked after the incubation of ceramics tablets with yeast immobilized on them for different time periods in water on the rotary shaker (160 rpm)

at 30 °C or in the biotechnological experiments in the solutions containing chromium ($K_2Cr_2O_7$). After the incubation of the tablets, the amount of immobilized cells was examined using scanning electron microscope as described above. In each experiment, at least 10 tablets of ceramics were evaluated.

2.11. Immobilized yeast viability determination

Immobilized preparations were kept in humid chamber at 37 °C and then placed in water and incubated on the rotary shaker during 1 h at 100 rpm. To collect the cells the suspension was then centrifuged for 5 min at 3000 g. Viability of cells was determined by the method described above. To have the correct control similar procedure was performed also with free dehydrated yeast cells.

2.12. Chromium sorption experiments

Biotechnological experiments on chromium sorption by immobilized or free dried yeast were undertaken in 50 ml tubes. Definite amounts (0.1 g dw) of immobilized or free dried yeast were incubated in 10 ml of chromium ($K_2Cr_2O_7$) solution (10 mg ml^{-1}) on the rotary shaker (180 rpm) at 30 °C for 24 h. The remaining chromium concentration in the supernatants was determined by reaction with 1.5-diphenylcarbazide. The corresponding absorbance at 540 nm was measured using a spectrophotometer (Ultrospec 2100 pro; Amersham Biosciences).

2.13. Experiments of ethanol production

The fermentation medium consisted of 100 g l^{-1} glucose, 5 g l^{-1} peptone, 5 g l^{-1} yeast extract, 2 g l^{-1} KH_2PO_4, 1 g l^{-1} $(NH_4)Cl$, and 0.3 g l^{-1} mg $MgSO_4 \cdot 7H_2O$. The pH was adjusted to 5.0. Fermentations were performed in 50 ml tubes at 30 °C under static conditions. Immobilized, free, and dehydrated cells (0.2 mg dw/ml) were inoculated in 10 ml of fermentation medium. For glucose and ethanol analysis, samples from each culture were collected after 24, 36, 40, and 48 h of the fermentation experiments.

Repeated-batch fermentations were performed in 100 ml flasks containing 40 ml of fermentation medium. After 48 h, all fermented medium was drained, supports with immobilized cells were washed with sterile distilled water, and the same amount of fresh sterile fermentation medium was added to the flask to initiate the start of the next fermentation cycle. Six subsequent cycles were performed.

2.14. Determination of glucose and ethanol concentrations

Concentrations of glucose and ethanol were measured using an Agilent 1100 high-performance liquid chromatography system with RI Detector RID G1362A. A Shodex Asahipak SH1011column (8.0 mm internal diameter and 300 mm long) and a front column SH-G (6 mm internal diameter and 50 mm long) were used. The temperature was set to 50 °C.

The flow of the mobile phase (0.01 N H_2SO_4) was 0.6 ml min^{-1}, and the amount of injected sample was 5 μL.

2.15. Determination of yeast cell chemical composition

Determination of yeast cell chemical composition was done with Fourier Transform Infrared spectroscopy (FT-IR) absorption spectra which were registered on a microplate reader HTS-XT (Bruker, Germany) and the data processed with OPUS 6.5 software. Quantitative analysis of carbohydrates, nucleic acids, proteins and lipids, was carried out as in Grube et al. (2002).

2.16. Preparation of peloid samples

Peloid obtained from Rider-Ta Ltd, Latvia was heat sterilized before the use at 121 ^oC for 20 min. Humidity of peloid was determined by drying till constant weight at 105 °C. To prepare the experimental extracts for further studies 11 g of fresh peloid were mixed with 40 ml of distilled water. This suspension was stirred during 24 h using the rotary shaker (180 rpm) followed by centrifugation for 20 min at 3200 g. The upper phase was diluted at 1:50; 1:25, 1:5 with distilled water and used in the further incubation procedure.

2.17. Incubation of the yeasts with peloid water extracts

3 g of fresh yeast biomass was incubated in 40 ml of peloid water extracts (PWE) using rotary shaker for 25 min or 3 h. Thereafter the yeast biomass was harvested by centrifugation during 10 min at 2600 g at room temperature for its further exposure to dehydration. The control samples were incubated in water.

2.18. High-voltage electrical discharge (HVD) treatment

At Riga Technical University Institute of Mechanics HVD device was used to treat yeast cells with electromagnetic radiation. The treatment was applied on the cells in water environment.

2.19. Statistical analysis

Experiments were done at least in triplicate. Results were expressed as the mean ± standard deviation (SD). A Student's t-test was used for comparisons between two groups. $P<0.05$ was defined as the threshold of statistical significance.

3. RESULTS AND DISCUSSION

3.1. Structural and functional changes of yeast cell surface at the influence of dehydration and the role of their particular components in the maintenance of cell viability

3.1.1. The possible changes of maltose transport systems at the influence of dehydration

This part of work was linked with the studies of possible changes of protein components of yeast plasma membrane during the transfer of yeast into the state of anhydrobiosis and at the following cell reactivation. Changes of various intracellular membranes and especially of plasma membrane during such treatments are very interesting and important for the understanding of main mechanisms which provide the maintenance of cell integrity (Rapoport et al., 1994). Transfer of yeast cells into the state of anhydrobiosis results in a very significant decrease of cell volume (up to 60%). Such huge decrease of cell volume is accompanied by formation of big invaginations of the plasma membrane inside the cytosol (Rapoport and Kostrikina, 1973; Biryuzova and Rapoport, 1978; Beker et al., 1984). Previous research was devoted mainly to important role of lipids in plasma membrane and their changes during dehydration/rehydration treatment. In this work it was decided to start the studies of possible changes of protein components of plasma membrane. The proposal was made that they also can be subjected to serious changes during cells' dehydration. Previously it was established that changes of various enzymes activities in dried yeast cells can occur. Some hydrogenases tend to increase their activity as the result of drying. It was supposed that changes in enzyme activity, taking place during drying of yeast cells, may be connected with changes in protein conformation (Beker and Rapoport, 1987). Later it was shown using Fourier-transform infrared spectroscopy that dehydration of 'pure' proteins may result in rather strong conformational changes. For some of studied proteins such conformational changes were irreversible (Prestrelski et al., 1993).

For the beginning of this investigation one of the plasma membrane proteins - Agt1, one of maltose transporters, was selected. Maltose is transported into *S. cerevisiae* yeast by several transporters, including Agt1 (alpha-glucoside transporter), Mphx, Mtt1, and several versions of Malx1 (Vidgren et al., 2014). Experiments have been started with application of transport assay for the native yeast cells taken from exponential and stationary growth phases of *S. cerevisiae* 14 cultures (Fig. 4).

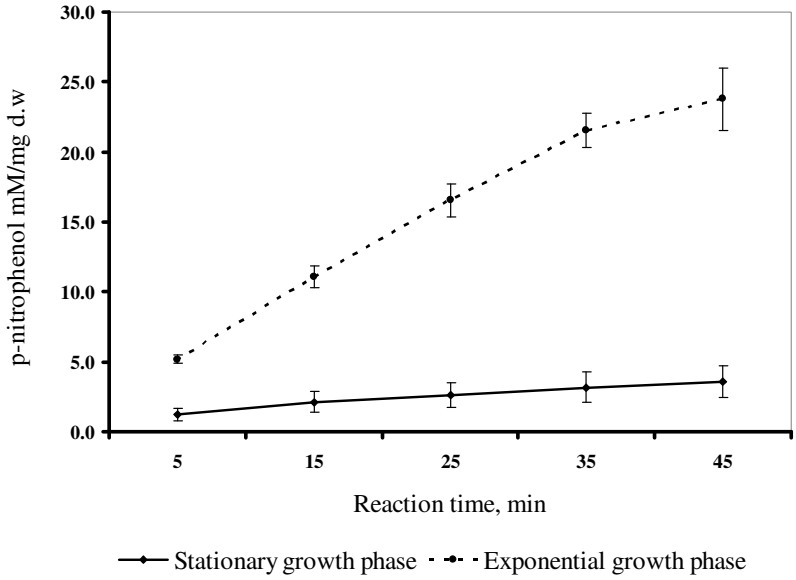

Fig. 4. The amount of p-nitrophenol produced by *S. cerevisiae* 14 live cells taken from stationary and exponential growth phases. SD is shown as error bars.

As it can be seen from Fig. 4 p-nitrophenol concentrations released by yeast cells from exponential growth phase were significantly higher during the whole time of reaction than p-nitrophenol concentrations released by cells from stationary growth phase. Already in the first 5 min of reaction p-nitrophenol concentration released by yeast cells from exponential growth phase was approximately four times higher than by cells from stationary growth phase. It can be explained by the fact that during cell active growth and division in exponential growth phase expression of necessary transporters in plasma membrane is higher than in cells of stationary growth phase. At the same time cells from stationary growth phase are used usually for dehydration experiments because they are significantly more resistant to dessication comparing with the cells of exponential growth phase (Plesset et al., 1987; Beker and Rapoport 1987; Callahan et al., 2011). Results of these experiments showed that cells from stationary growth phase have rather big Agt1 transporter activity and correspondingly can be used for investigation of Agt1 transporter possible changes at the influence of dehydration. Therefore in further experiments *S. cerevisiae* 14 cells from stationary growth phase were subjected to dehydration to reach the state of anhydrobiosis. This strain was used in the main amount of the laboratory studies of anhydrobiosis and can be characterised as a semi-resistant against dehydration treatment. Viability of dehydrated cells after fast rehydration usually is at the level of 50 ± 5%. The permeability of plasma membrane was checked and obtained results showed that during reactivation of dehydrated cells they can loose about 19 ± 3% of their total dry weight. In the first series of experiments the activity of Agt1 transporter was studied after

dehydration-rehydration of the cells. Cells were rehydrated using fast and slow (gradual rehydration in water vapours) methods of rehydration. After fast rehydration viability of the cells was at the level of 50% but after slow rehydration, which can improve molecular organization of the plasma membrane, viability of cells increased till 70%. Acquired results of p-nitrophenol production by the cells at the influence of dehydration and rehydration are shown in Fig. 5.

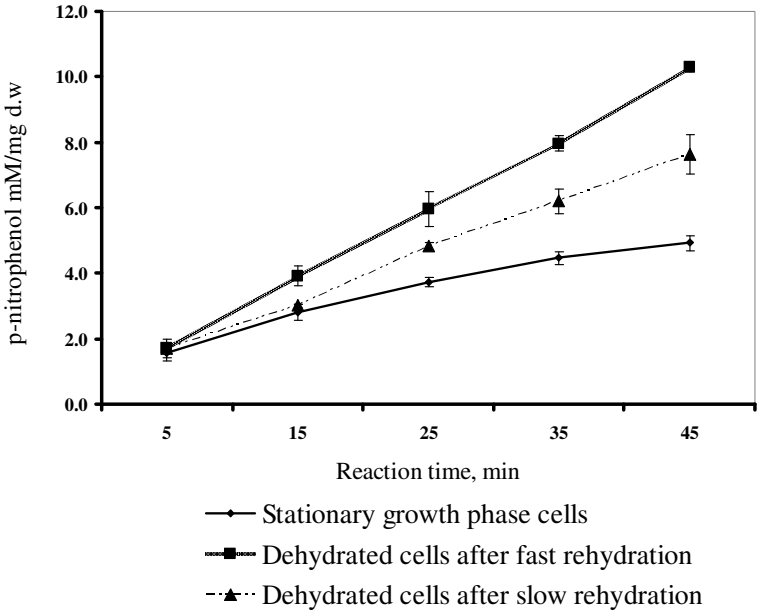

Fig. 5. The amount of p-nitrophenol produced by *S. cerevisiae* 14 native and dehydrated cells. Viability of dehydrated cells after fast rehydration was approximately 50%. Viability of dehydrated cells after slow rehydration was approximately 70%.

Our results showed that after fast rehydration of dehydrated cells production of p-nitrophenol was significantly higher than production of p-nitrophenol by native cells (Fig. 5). In the first 5 min of reaction p-nitrophenol concentration produced by dehydrated cells was the same as in the case of native cells. But at other time points of reaction dehydrated cells released significantly higher concentrations of p-nitrophenol than native cells. The production of p-nitrophenol by dehydrated cells after slow rehydration differed from that released by native cells after 25 min of reaction but the amounts of released p-nitrophenol were less than in the case of fast rehydration of dehydrated cells.

This activation of p-nitrophenol production by dehydrated cells could be connected with: 1) possible changes in Agt1 transporter as the result of dehydration treatment influence, or 2) with possible changes in maltase activity inside the cells, or 3) with increased permeability of plasma membrane for maltose similar substrate - p-nitrophenyl-α-D-glucopyranoside.

To understand which of these options was in our case, a number of additional experiments were performed. First, dehydration of yeast cells was prolonged with the goal to obtain low viability of cells. Second, extracts of yeast cells were prepared to analyze the intracellular activity of maltase without involvement of transport system into reaction.

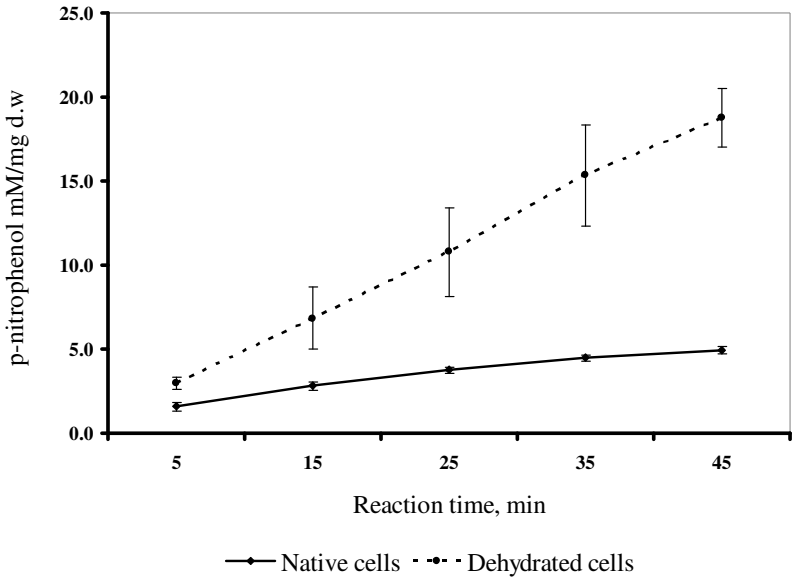

Fig. 6. The amount of p-nitrophenol produced by *S. cerevisiae* 14 native and strongly dehydrated cells. Viability of dehydrated cells after fast rehydration was at the level of 20%.

Results of p-nitrophenol production by cells after long dehydration in comparison with native cells are presented at Fig. 6. Viability of cells after long dehydration was low at the level of 20% but in spite of that, concentrations of p-nitrophenol were significantly higher than concentrations of p-nitrophenol produced by native cells and approximately 1.8 times higher than in the case of cells subjected to 'usual' dehydration time (12 hours) (Fig. 5). It is necessary to mention here that in the same experiment it was determined also total losses of dry weight in the case of both these samples. The obtained results showed that permeability of plasma membrane in dehydrated cells after long dehydration significantly increased (about 1.5 times) in comparison with cells which were dehydrated for 12 h (Table 5).

Table 5. Permeability of plasma membrane of dehydrated *S.cerevisiae* 14 cells at the influence of dehydration time

Permeability of plasma membrane evaluated by losses of yeast dry weight during rehydration, %	
Duration of dehydration 12h	Long dehydration
19.0 ± 3.0	28.0 ± 2.0

So, one of the reasons of increase of p-nitrophenol release from cells could be linked with the serious changes of yeast cell plasma membrane permeability.

Second series of experiments was directed to the studies of possible changes of intracellular activity of maltase as the result of dehydration-rehydration treatment. The results which were obtained are presented at Fig. 7.

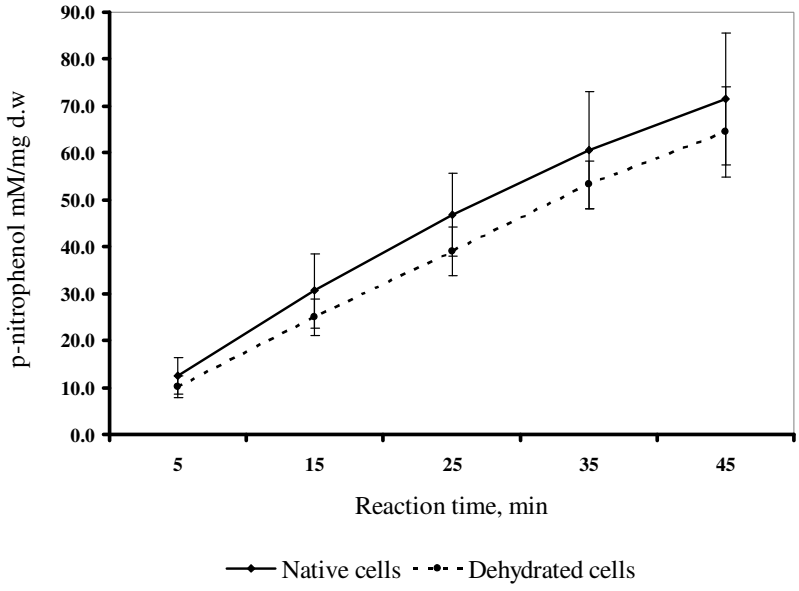

Fig. 7. Activity of maltase in extracts of *S. cerevisiae* 14 native and dehydrated cells.

Acquired results indicate that after dehydration of *S. cerevisiae* 14 cells activity of maltase remains practically the same as in the native yeast cells (Fig. 7).

So, all these results gave the possibility to suppose that the increase of p-nitrophenol production by dehydrated cells could be connected with the changes of permeability of plasma membrane. It seems that most probably transport of substrate inside the dehydrated cells could occur by non-specific uptake just because of strongly increased permeability of plasma membrane. It would lead to reaching the maltase by the substrate significantly faster than in the case of native cells where transport of substrate occurs only with the help of Agt1 transporter. It can be seen that during prolonged dehydration plasma membrane permeability increased significantly and it influenced the entrance inside the cells of additional amounts of substrate (Fig. 6).

To understand at which point of cells dehydration the changes in the plasma membrane take place, yeast biomass in the next series of experiments was gradually dried and the samples for the investigations were taken during the process of dehydration. They were checked for Agt1 activity, viability, remaining humidity of dehydrated cells and plasma membrane permeability. Acquired results are presented in Table 6.

Table 6. The influence of duration of yeast *S. cerevisiae* 14 cells dehydration upon their production of p-nitrophenol

Viability of cells, %	**100**	**97** ±1.2	**93** ±1.4	**92** ±0.7	**89** ±0.2	**73** ±0.7	**69** ±1.4	**58** ±0.7	**41** ±0.7	**23** ±0.7
Humidity of cells, %	**70.0** ±2	**58.0** ±1.0	**47.0** ±0.7	**41.0** ±0.7	**28.0** ±1.5	**21.0** ±2.8	**17.0** ±2.1	**11.0** ±0.7	**9.5** ±0.7	**7.0** ±0.1
Permeability of the plasma membrane, %	**0**	**3.0** ±0.7	**4.5** ±0.7	**7.0** ±1.4	**8.0** ±0.9	**10.0** ±1.4	**13.0** ±1.4	**19.0** ±3.5	**22.0** ±1.4	**26.0** ±1.1
Reaction time, min	p-nitrophenol concentration, mM/mg y.d.w.									
5	**1.5** ±0.5	**1.5** ±0.5	**1.4** ±0.1	**1.3** ±0.2	**1.1** ±0.5	**1.6** ±0.1	**1.6** ±0.2	**2.0** ±0.1	**2.0** ±0.0	**3.5** ±0.9
15	**2.7** ±0.2	**2.6** ±0.2	**2.5** ±0.1	**2.1** ±0.1	**2.5** ±0.5	**2.6** ±0.0	**3.2** ±0.7	**4.6** ±0.6	**4.5** ±0.4	**7.0** ±1.1
25	**3.7** ±0.3	**4.2** ±0.3	**3.3** ±0.2	**2.9** ±0.1	**3.2** ±1.1	**4.3** ±0.1	**5.1** ±0.6	**6.7** ±1.0	**7.5** ±0.3	**10.0** ±1.5
35	**4.7** ±0.1	**4.7** ±0.1	**3.9** ±0.0	**3.7** ±0.1	**3.7** ±1.4	**5.3** ±0.2	**6.1** ±0.7	**10.3** ±2.3	**11.0** ±0.0	**16.0** ±1.7
45	**5.5** ±0.4	**5.0** ±0.1	**4.6** ±0.1	**4.5** ±0.1	**4.3** ±1.1	**6.8** ±0.1	**8.8** ±1.0	**12.3** ±0.3	**14** ±0.6	**20.1** ±2.0

It can be seen from Table 6 the first changes in the p-nitrofenol production were revealed at the humidity of the cells at the level of 47%. At this dehydration stage there is some decrease of p-nitrophenol production which can indicate the decrease of Agt1 transporter activity. It is very important to mention that such decrease takes place at rather high viability of dehydrated culture (93%) when there still is rather high content of 'free' water. Earlier it was shown that the removal from the cells of part of 'bound' water starts approximately at the level of remaining humidity about 25-30% (Beker and Rapoport, 1987). Therefore the results shown in Table 6 are the first information that possible changes of membrane proteins which lead to some decrease of their activities can take place not after losses by cells of some amounts of 'bound' water as it was hypothesized earlier but already at the stage of partial losses by cells of 'free' water. It seems very important that these first changes of membrane proteins do not increase during subsequent period of cell dehydration till the moment when there starts the elimination of part of 'bound' water. Such next changes were revealed at the level of relative humidity between 28 and 20%. Somewhere at this level of remaining humidity there was revealed rather significant increase of p-nitrophenol production by dehydrated cells which progressively continued to increase during further cells dehydration and corresponding decrease of cells remaining humidity. Most probably at this stage of dehydration rather serious structural changes can occur in the plasma membrane

molecular organization and most probably they lead to the significant increase of plasma membrane permeability. It can be mentioned also that at the dehydration stage when the remaining humidity of cells is between 28 and 20% the results of p-nitrophenol production are similar to those which were observed in dehydrated yeasts cells after their slow rehydration approximately to the same levels of relative humidity.

Taken into account that it was shown in the previous experiments made in laboratory incubation of sensitive to dehydration yeasts in 1 M xylitol before dehydration treatment improved their resistance to dehydration, it was decided to investigate if incubation of *S. cerevisiae* 14 in 1M xylitol solution before dehydration can influence the changes of Agt1 transporter activity. In these experiments yeast samples after their incubation in 1M xylitol solution again were taken for the studies during the process of dehydration. The obtained results were compared with those which were received for 'native' dehydrated biomass and described above (Table 3.2.). Results of these experiments are presented in the Table 7.

Table 7. The influence of duration of *S. cerevisiae* 14 yeast cells dehydration after their incubation in 1M xylitol solution upon their production of p-nitrophenol

Viability of cells, %	**100**	**96** ±1.0	**94** ±1.4	**80** ±2.5	**75** ±1.4	**69** ±2.4	**71** ±1.0
Humidity of cells, %	**70.0** ±2	**55.0** ±1.5	**41.0** ±0.7	**21.0** ±0.7	**18.0** ±1.4	**16.0** ±1.4	**10.0** ±0.1
Permeability of the plasma membrane, %	**0**	**2.0** ±0.4	**6.0** ±0.7	**8.0** ±0.2	**8.5** ±0.7	**11.0** ±1.4	**14.0** ±1.4
Reaction time, min	p-nitrophenol concentration, mM/mg y.d.w.						
5	**1.7** ±0.5	**1.3** ±0.4	**1.1** ±0.1	**1.6** ±0.2	**1.9** ±0.3	**2.2** ±0.5	**1.9** ±0.2
15	**2.3** ±0.4	**2.5** ±0.2	**2.3** ±0.1	**2.9** ±0.1	**2.4** ±0.3	**3.9** ±0.4	**3.7** ±0.5
25	**3.5** ±0.4	**2.9** ±0.5	**2.8** ±0.2	**3.8** ±0.9	**4.0** ±0.0	**5.6** ±0.8	**5.7** ±0.3
35	**4.1** ±0.3	**3.3** ±0.9	**3.4** ±0.0	**5.4** ±0.1	**5.5** ±1.3	**7.0** ±0.4	**7.2** ±0.7
45	**4.4** ±0.2	**4.4** ±0.1	**4.1** ±0.1	**5.5** ±0.1	**5.6** ±0.8	**8.2** ±0.6	**8.5** ±1.0

As it can be seen from Table 7 viability of dehydrated cells increased after incubation in xylitol solution and it reached 70% after fast rehydration. Production of p-nitrophenol in these cells was increased in the comparison with native cells but was significantly lower than in dehydrated cells which were not incubated in xylitol solution. Comparing the results in Table 6 and Table 7 it can be concluded that also after incubation of yeast biomass in xylitol during subsequent dehydration of cells approximately at the same level of remaining humidity (of

41%) similar decrease of activity of Agt1 transporter takes place. Such result should indicate that incubation in xylitol solution does not protect Agt1 transporter from changes at the stage of elimination from the cells of part of 'free' water. It can be revealed also that at the level of humidity of approximately 21% there takes place increase of the permeability of plasma membrane for the substrate (p-nitrophenyl-α-D-glucopyranoside). Such effect is also similar to that which was found for the cells without incubation in xylitol. At the same time after incubation in xylitol further progressive increase of plasma membrane permeability for the substrate could be not revealed. So, these experiments showed that xylitol protects the state of plasma membrane but does not protect the state of membrane protein - Agt1 transporter. To determine if incubation in xylitol solution can influence the chemical composition of the cells we investigated them using Fourier-transform infrared spectroscopy. Results showed that no changes occur in the chemical composition of yeast cells after their incubation in xylitol solution. So, it can be concluded that most probably xylitol is protecting membranes by the way predicted by water replacement hypothesis (exchanging hydroxyl groups of water) or its current modifications (by preventing the decrease of spacing between membrane lipids under dehydration causing a concentration-dependent increase of the area per lipid (APL) accompanied by fluidizing the bilayer core) (Golovina et al., 2009, 2010).

To reveal if the results which were described above are typical for any yeast strain or they are strain-specific in the next series of experiments the same study was performed for more resistant to dehydration yeast strains. In the first series of experiments the possible changes in Agt1 transporter activity were investigated in the strain *S. cerevisiae* Evolution canyon N6 (Table 8).

Table 8. The influence of duration of dehydration of yeast cells of *S.cerevisiae* Evolution canyon N6 strain upon their production of p-nitrophenol

Viability of cells, %	**100**	**99** ±0.6	**95** ±0.7	**91** ±1.4	**84** ±0.2	**70** ±0.0	**60** ±1.4	**51** ±0.7
Humidity of cells, %	**70.0** ±2	**46.0** ±2.1	**39** ±5.7	**29.0** ±0.7	**27.0** ±1.0	**17.0** ±2.8	**14.0** ±1.0	**13.0** ±0.7
Permeability of the plasma membrane, %	**0**	**0**	**3.0** ±0.0	**3.5** ±0.7	**6.0** ±0.1	**12.0** ±1.4	**12.0** ±2.4	**13.0** ±4.3
Reaction time, min	p-nitrophenol concentration, mM/mg y.d.w.							
5	**1.0** ±0.1	**1.0** ±0.3	**0.4** ±0.0	**0.6** ±0.2	**0.7** ±0.3	**0.8** ±0.2	**1.6** ±0.2	**1.5** ±0.3
15	**1.4** ±0.2	**1.4** ±0.2	**0.4** ±0.1	**0.7** ±0.3	**1.0** ±0.2	**1.2** ±0.3	**1.7** ±0.3	**2.3** ±0.5
25	**1.6** ±0.3	**1.5** ±0.2	**0.5** ±0.1	**0.8** ±0.4	**1.0** ±0.1	**1.4** ±0.4	**1.9** ±0.5	**3.5** ±1.1
35	**1.8** ±0.3	**1.6** ±0.1	**0.6** ±0.2	**0.8** ±0.5	**1.0** ±0.4	**1.5** ±0.2	**2.6** ±0.3	**4.4** ±1.3
45	**1.9** ±0.3	**1.7** ±0.1	**0.7** ±0.2	**1.0** ±0.3	**1.4** ±0.3	**1.6** ±0.2	**2.7** ±0.3	**5.6** ±1.4

It can be seen that the activity of Agt1 transporter in the native cells of this strain is significantly lower than in the previous strain *S. cerevisiae* 14. At the same time also in this case it can be revealed the decrease of Agt1 transporter activity during cells dehydration at the level of remaining cells' humidity of about 40%. Then, there is also increase of production of p-nitrophenol during further dehydration of the cells at the beginning of 'bound' water elimination but this increase is significantly smaller comparing with *S. cerevisiae* 14 (Table 6). It is necessary to mention here also that the permeability of this strain dry cells plasma membrane at rehydration procedures was significantly lower than in the case of *S. cerevisiae* 14 cells and it was about 13% after fast rehydration and about 10% after slow gradual rehydration in water vapors. Determination of maltase activity showed that in this strain it is less resistant at dehydration than in the strain *S. cerevisiae* 14 and during drying procedure its activity decreased almost two times comparing with native cells of the same strain (Fig. 8).

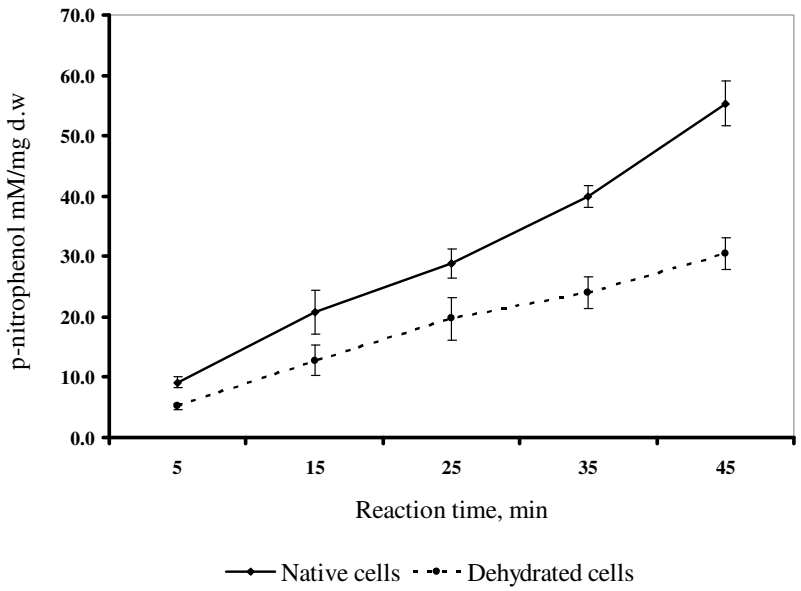

Fig. 8. Activity of maltase in extracts of *S .cerevisiae* Evolution canyon N6 native and dehydrated cells.

Summarizing all these results it can be concluded that because of the changes of plasma membrane permeability at dehydration of *S. cerevisiae* Evolution canyon N6 cells are significantly lower than for the cells of *S. cerevisiae* 14, the substrate used for the determination of Agt1 transporter activities also can not so actively penetrate inside the cells. Therefore results obtained in all these experiments can give already some first indications on the possible changes of Agt1 transporter activity at dehydration treatment. It can be supposed that first of all changes of this membrane protein are reversible. There is no activation of Agt1 transporter as the result of its possible conformational changes but there are no also significant losses of its activity.

In the next experiments the possible influence of this strain cells incubation in xylitol on the changes linked with Agt1 transporter were determined (Table 9).

Table 9. The influence of duration of *S. cerevisiae* Evolution canyon N6 yeast cells dehydration after their incubation in 1M xylitol solution upon their production of p-nitrophenol

Viability of cells, %	**100**	**99** ±0.0	**99** ±0.0	**97** ±0.0	**90** ±7.1	**70** ±8.7
Humidity of cells, %	**70.0** ±2	**46.0** ±7.8	**38** ±1.4	**30.0** ±0.0	**22.0** ±3.5	**14.0** ±4.9
Permeability of the plasma membrane, %	**0**	**0**	**0**	**1.0** ±0.0	**3.0** ±1.0	**8.0** ±2.4
Reaction time, min	p-nitrophenol concentration, mM/mg y.d.w.					
5	**1.0** ±0.1	**1.0** ±0.1	**0.7** ±0.0	**0.8** ±0.1	**0.7** ±0.1	**0.9** ±0.5
15	**1.2** ±0.3	**1.3** ±0.1	**0.9** ±0.6	**1.0** ±0.2	**1.0** ±0.2	**1.2** ±0.6
25	**1.3** ±0.2	**1.4** ±0.1	**1.1** ±0.5	**1.1** ±0.3	**1.2** ±0.2	**1.5** ±0.6
35	**1.5** ±0.5	**1.4** ±0.0	**1.1** ±0.4	**1.4** ±0.4	**1.5** ±0.2	**2.0** ±0.5
45	**1.5** ±0.5	**1.6** ±0.1	**1.2** ±0.3	**1.3** ±0.4	**1.3** ±0.0	**2.3** ±0.8

In this case for the first time in this study there was not found any ‘visible’ decrease of Agt1 activity at the decrease of the amount of free water (in the area of remaining humidity about 40%). It means that in more resistant strains xylitol besides the protection of molecular organization of plasma membrane can increase also the resistance to dehydration of at least one of membrane proteins – Agt1 transporter. In this case there was not revealed also any change of this transporter activity in dry cells as well as any decrease of maltase activity (Fig. 9) as the result of dehydration treatment. So, it is additional indication of the conclusion that in the resistant to dehydration treatment cells xylitol incubation has multiple effects – protection of plasma membrane molecular organization, stabilization of membrane proteins as well as of also some internal proteins (such as enzyme maltase).

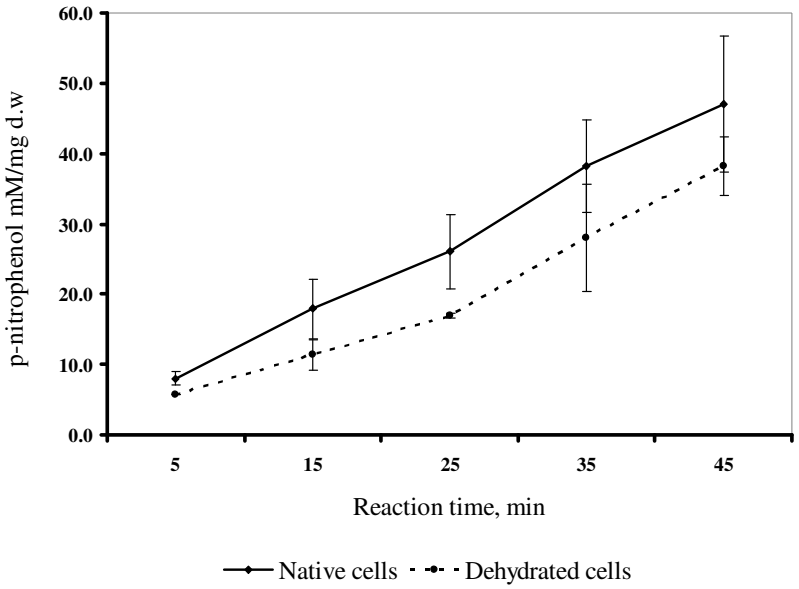

Fig. 9. Activity of maltase in extracts of *S. cerevisiae* Evolution canyon N6 live and dehydrated cells (incubation in 1M xylitol).

To confirm all these conclusions the experiments were performed with one more resistant to dehydration yeast strain - *S. cerevisiae* L-77. The viability of dry cells of this strain after fast rehydration was higher than 80% but after slow gradual rehydration in vapors of water – above 90% (for the cells of *S. cerevisiae*14 and *S. cerevisiae* Evolution canyon N6 as it was mentioned earlier these figures were correspondingly: 40-50 – 70% and 50-60 – 80%). The plasma membrane permeability of dry cells of the strain *S. cerevisiae* L-77 at fast rehydration determined as the total losses of dry weight was about 10 %, but after slow rehydration – about only 7 %. These results were significantly lower than for the strain *S. cerevisiae* 14 (about 22 and 17%) and for the strain *S. cerevisiae* Evolution canyon N6 (about 13 and 10%). It means that the stability of molecular organization of plasma membrane of *S. cerevisiae* L-77 cells was significantly higher than of other two strains studied in this work. All previous experiments were repeated with this strain cells. The results which were received are presented in the Table 10 (for the cells without pre-incubation in xylitol) and Table 11 (with pre-incubation in xylitol solution) as well as at the Fig. 10 (maltase activity).

Table 10. The influence of duration of dehydration of yeast cells of *S. cerevisiae* L-77 strain upon their production of p-nitrophenol

Viability of cells, %	**100**	**99** ±0.0	**99** ±0.0	**97** ±0.0	**96** ±0.0	**94** ±0.5	**82** ±3
Humidity of cells, %	**70.0** ±2	**58** ±1.0	**39.3** ±1.5	**26.0** ±2.8	**14.3** ±3.5	**11.0** ±4.9	**9.0** ±1.0
Permeability of the plasma membrane, %	**0**	**0**	**0**	**0.6** ±0.0	**2.5** ±1.0	**6.3** ±2.4	**10.5** ±0.1
Reaction time, min	p-nitrophenol concentration, mM/mg y.d.w.						
5	**1.1** ±0.6	**1.0** ±0.1	**0.7** ±0.0	**0.7** ±0.3	**0.8** ±0.2	**0.9** ±0.0	**1.0** ±0.2
15	**1.5** ±0.3	**1.2** ±0.1	**1.0** ±0.6	**1.4** ±0.2	**1.4** ±0.2	**1.6** ±0.3	**1.4** ±0.3
25	**1.9** ±0.5	**1.7** ±0.1	**1.4** ±0.5	**1.5** ±0.1	**2.0** ±0.2	**2.0** ±0.4	**2.3** ±0.4
35	**2.3** ±0.4	**2.2** ±0.0	**1.6** ±0.5	**1.8** ±0.3	**2.2** ±0.2	**2.2** ±0.3	**2.5** ±0.4
45	**2.5** ±0.6	**2.4** ±0.1	**2.0** ±0.2	**2.1** ±0.3	**2.3** ±0.0	**2.4** ±0.2	**3.0** ±0.1

Table 11. The influence of duration of *S. cerevisiae* L-77 yeast cells dehydration after their incubation in 1 M xylitol solution upon their production of p-nitrophenol

Viability of cells, %	**100**	**99** ±0.0	**97** ±0.0	**94** ±0.0	**93** ±0.1	**91** ±0.2	**95** ±0.0
Humidity of cells, %	**70.0** ±2	**44** ±4.2	**26** ±2.0	**14.5** ±0.0	**14.5** ±1.0	**11.5** ±1.0	**10.5** ±0.5
Permeability of the plasma membrane, %	**0**	**0**	**0**	**2.8** ±1.0	**4.1** ±1.2	**6.9** ±0.9	**10.5** ±0.2
Reaction time, min	p-nitrophenol concentration, mM/mg y.d.w.						
5	**1.3** ±0.5	**1.0** ±0.2	**0.9** ±0.2	**1.0** ±0.2	**1.0** ±0.1	**1.0** ±0.1	**1.1** ±0.2
15	**1.8** ±0.3	**1.3** ±0.4	**1.2** ±0.5	**1.4** ±0.2	**1.5** ±0.3	**1.5** ±0.0	**1.9** ±0.5
25	**2.1** ±0.5	**1.8** ±0.3	**1.8** ±0.2	**1.5** ±0.5	**1.7** ±0.2	**2.1** ±0.2	**2.1** ±0.1
35	**2.3** ±0.9	**2.3** ±0.5	**2.1** ±0.2	**1.9** ±0.1	**2.2** ±0.2	**2.3** ±0.1	**2.3** ±0.7
45	**2.4** ±0.5	**2.5** ±0.4	**2.2** ±0.3	**2.0** ±0.1	**2.2** ±0.1	**2.4** ±0.3	**2.9** ±0.2

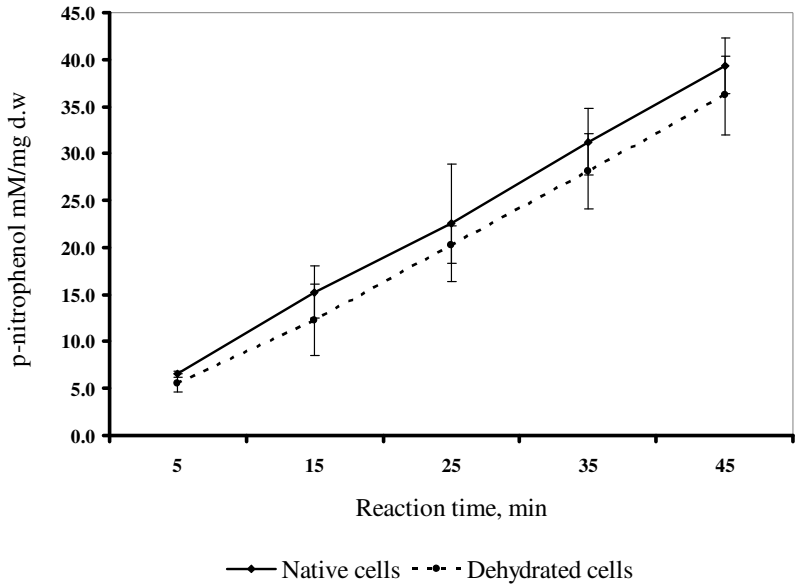

Fig. 10. Activity of maltase in extracts of *S. cerevisiae* L-77 native and dehydrated cells (incubation in 1 M xylitol).

As it can be seen from acquired data (Table 10) the high stability of molecular organization of plasma membrane of *S. cerevisiae* L-77 cells led to the absence of practically any changes in the activity of Agt1 transporter during dehydration treatment. Pre-incubation of these cells in xylitol solution additionally increased their resistance to dehydration and the viability of this strain dry cells reached almost maximum - 95%. Also for these cells any changes of Agt1 activity during dehydration of these cells were not revealed (Table 11). Activity of maltase has not been changed (Fig. 10).

Thus, this study for the first time in the research of yeast anhydrobiosis showed that in the case of extremely resistant to dehydration treatment cells there is no any changes in the physiological activity of at least one of membrane proteins – Agt1 transporter. It was observed that if initially cell plasma membrane is stable enough pre-incubation of cells in the xylitol solution besides additional protection of membrane molecular organization can increase also the resistance of membrane proteins as well as of intracellular proteins too. If the 'initial' resistance of cells to dehydration is at the level of 50% the xylitol pre-treatment can increase only the stability of molecular organization of plasma membrane (most probably it takes place in the accordance with water replacement hypothesis or its current modification) but for the proteins other protective mechanisms are necessary. At the same time also in this case the changes linked with this plasma membrane protein (some decrease of its activity) are not significant and in all cases these small changes are reversible.

3.1.2. The role of potassium transport system during yeast dehydration/rehydration

In this part of work another plasma membrane proteins were studied and we tried to understand the possible role of potassium transporters in cell resistance to dehydration/rehydration treatment.

It is known that the plasma membrane of *S. cerevisiae* possesses at least seven transport systems with different substrate specificities and diverse mechanisms to maintain optimal cytosolic K^+ concentration (c. 200–300 mM). Five main potassium transporters have been extensively studied in *S. cerevisiae* cells (Arino et al., 2010). K^+ uptake is mainly mediated by the plasma membrane Trk1 and Trk2 uniporters. K^+ accumulation in the cytosol via these systems is driven by the electrochemical H^+ gradient across the plasma membrane generated by H^+-ATPase Pma1 (Serrano et al., 1986). Trk1 is the primary high-affinity K^+ transport system (Km 25 mM) (Rodriguez-Navarro and Ramos, 1984; Caber et al., 1988). The activity of Trk1 has been described to be important for K^+ and pH homeostasis (Madrid et al., 1998; Yenush et al., 2002), turgor (Merchan et al., 2004) and plasma membrane potential (Madrid et al., 1998; Mulet et al., 1999).

To export surplus potassium, *S. cerevisiae* cells use three types of exporters: Tok1 channel, Nha1 Na^+ (K^+)/H^+- ATPase un Ena Na^+ (K^+)-ATPase (Arino et al., 2010).

To study the role of the five main *S. cerevisiae* potassium transporters during transition of cells into anhydrobiosis, a set of isogenic strains lacking one or more genes encoding the plasma membrane K^+ transporters in the BY4741 genetic background were used and the ability of mutant cells to survive desiccation and the subsequent rehydration processes was studied. The experimental conditions were set to achieve the survival level of approximately 70% for the parental BY4741 strain. Fig. 11 shows that the absence of potassium exporting systems (BYT45 and BYT345 cells) did not significantly change the ability of cells to survive dehydration/rehydration treatment.

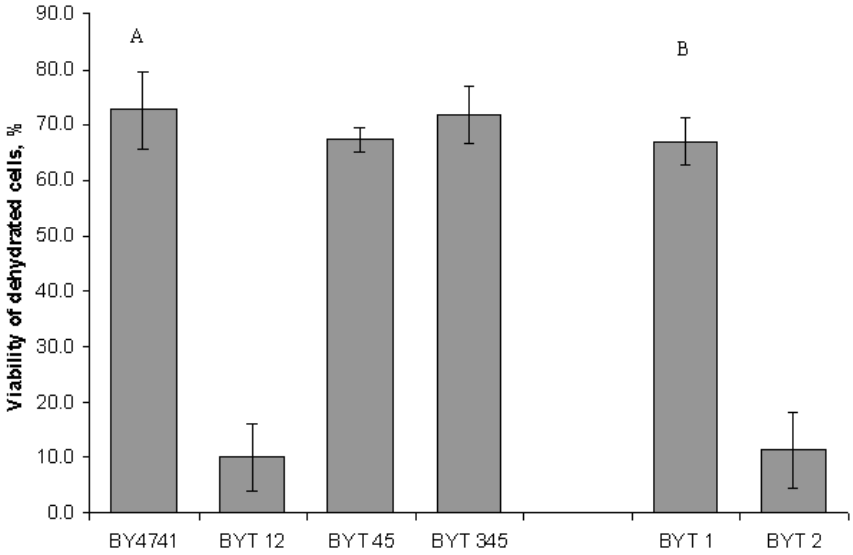

Fig. 11. Viability of dehydrated cells. (A) Parental strain with all the transporters (BY4741), BYT12 (trk1Δ trk2Δ) strain lacking the main two potassium uptake systems, BYT45 without active potassium efflux (nha1Δ ena1-5Δ), and BYT345 (tok1Δ nha1Δ ena1- 5Δ). (B) Single mutants lacking either TRK1 (BYT1) or TRK2 (BYT2).

As it can be seen from our results about 65–70% of cells lacking potassium exporters were able to survive the desiccation and rehydration processes. On the other hand, the absence of potassium uptake systems (BYT12, trk1Δ trk2Δ) brought about a dramatic decrease in the survival rate - only about 8% of cells were viable after dehydration/rehydration treatment. This result suggested the importance of potassium uptake for anhydrobiosis. To distinguish which of the two Trk transporters' absence causes the observed phenotype, the same experiment was repeated with single mutants lacking either the Trk1 (BYT1) or Trk2 (BYT2) transporter. It was the absence of Trk2 that diminished the ability of cells to survive desiccation stress (Fig. 11).

Since the deletion of the TRK2 gene has almost no phenotype in exponential cells harboring an intact copy of TRK1 (Petrezselyova et al., 2011), it was known that there was a non-specific mutation that could occur during the construction of the BYT2 mutant, e.g. upon electroporation. To be sure that the observed phenotype is related to the absence of the TRK2 gene and not to an additional non-specific mutation, the survival of two independently prepared BYT1 (trk1Δ) and three BYT2 (trk2Δ) mutants were tested (Fig. 12).

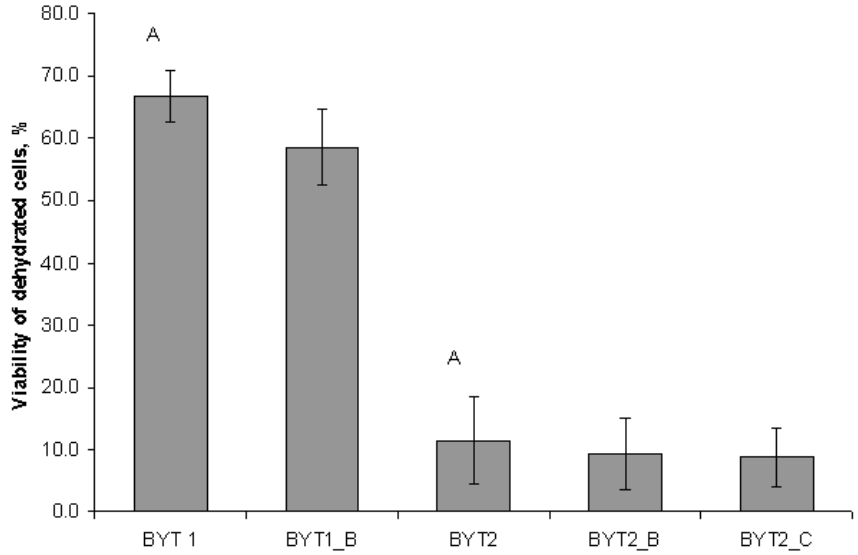

Fig. 12. Viability of dehydrated cells of BYT1 (trk1Δ) and BYT2 (trk2Δ).

Whereas about 55–65% of cells lacking TRK1were able to survive in the experiment, all three trk2Δ mutants exhibited very low viability after the treatment, with only about 10% survival. This result showed unambiguously that the role of Trk2 in the cell survival of dehydration stress is much more important than that of the Trk1 transporter. One of the reasons for the decreased viability could be the need for the active uptake of potassium during the rehydration process. As mentioned above, dehydration is accompanied by a substantial decrease in cell volume. Such a decrease in cell volume may be not only related to a loss of water but may be accompanied by a loss of ions to preserve sustainable intracellular osmotic conditions. After obtaining the initial results, it was hypothesized that a substantial amount of intracellular potassium content may be lost during desiccation, and it is the Trk2 (and not Trk1) transporter that mediates the reuptake of required potassium during the rehydration procedure. To confirm this hypothesis, the survival of dehydrated cells was determnined rehydrating them in either water or 50 mM KCl. If the regeneration of internal potassium content during rehydration were crucial, the increased availability of potassium in the rehydration solution would enhance the survival of cells.

Table 12. Influence of rehydration solution on viability of cells

	Viability (%)	
Strain	**Water**	**50 mM KCl**
BY4741	73.2 ± 9.3	72.1 ± 11.9
BYT45	74.9 ± 7.3	62.4 ± 15.4
BYT345	65.3 ± 5.5	61.1 ± 2.7
BYT1	68.9 ± 7.2	74.5 ± 3.5
BYT2	10.1 ± 3.5	8.4 ± 2.5
BYT12	8.3 ± 2.4	10.2 ± 3.0

As shown in Table 12, the presence of KCl during the rehydration of cells had no significant effect. Viability of wild-type BY4741 cells was almost the same under both sets of conditions; viability of cells lacking potassium exporters (BYT345 and BYT45) was slightly decreased in the presence of KCl, probably due to the impaired ability of potassium flux and membrane potential regulation (Zahradka and Sychrova, 2012). Vaibility of BYT1 cells (trk1Δ) was not changed upon the addition of potassium, and the same was found for cells lacking either Trk2 alone (BYT2) or in combination with the trk1 mutation (BYT12, trk1Δ trk2Δ).

These results showed clearly that the uptake or efflux of potassium by cells during the rehydration process is not crucial for their dehydration survival.

To verify the possibility of the effect of the absence of TRK2 on stationary cells, the potassium content was measured in cells from the stationary phase of growth harvested for dehydration.

Table 13. Potassium content in native cells

Strain	**$K+_{in}$ (mg g^{-1} dry weight)**
BY4741	18.6 ± 1.0
BYT45	21.4 ± 1.8
BYT345	19.3 ± 1.7
BYT1	17.8 ± 4.2
BYT2	12.7 ± 3.1
BYT12	14.6 ± 1.5

As shown in Table 13, cells lacking the Trk2 transporter contained a significantly lower amount of potassium, which confirmed the presumption that Trk1 was not very active in the stationary cells. Measurements of K^+ content in cells lacking the active exporters Nha1 and Ena1 (BYT45) revealed slightly higher potassium content compared with the parental BY4741 strain, and this higher content diminished upon the deletion of TOK1 (BYT345), which depolarizes the plasma membrane potential and thus slightly diminishes potassium uptake (Zahradka and Sychrova, 2012). The measurements of potassium content revealed a lower level of potassium in BYT2 (trk2Δ) and BYT12 (trk1Δ trk2Δ) stationary cells, and these lower amounts correlated with decreased resistance of cells to dehydration/rehydration stress.

Acquired results confirmed the importance of Trk2 (whose role in *S. cerevisiae* physiology was not clear till this moment) activity for the potassium homeostasis and dehydration survival of stationary cells. It was concluded that Trk2 to be the major potassium uptake system in stationary cells, and potassium content to be a crucial for yeast cells to successfully survive anhydrobiosis.

3.1.3. Possible role of certain cell wall proteins in yeast transfer into the state of anhydrobiosis

The yeast cell wall has four major functions: stabilization of internal osmotic conditions, protection against physical stress, maintenance of cell shape and it is a scaffold for proteins (Teparic et al., 2004; Klis et al., 2006). The cell wall of *S. cerevisiae* consists of β-1,3-glucan, β-1,6-glucan, chitin and various kinds of mannoproteins, which are interconnected to form a macromolecular complex. Cell wall proteins play an important role, both as structural components and as enzymes involved in cell wall assembly.

Depending on their linkage to the cell wall, they are divided into two classes:

- non-covalently entrapped in or S-S-linked to the cell wall, named soluble cell wall proteins (Scwp), which can be extracted with sodium dodecyl sulfate (SDS) and Sulfhydryls (SH)-reagents;
- covalently linked to the glucan framework proteins (Ccwp) can be grouped into two subclasses depending on their linkage to glucan: PIR (proteins with internal repeats)-Ccwps are bound directly to β-1,3-glucan through an unidentified linkage and can be released from the cell wall by β-1,3-glucanase or by mild alkali extraction, and the glycosylphosphatidylinositol (GPI)-Ccwps which are bound to β-1,6-glucan through a processed form of the GPI anchor (Hagen et al., 2004). In yeast, these GPI-anchored proteins are the major components of the cell wall. They are critical for maintenance

of the normal cellular morphology and they have essential properties in cells including transmembrane signaling, cell surface protection, cell adhesion, cell wall synthesis, sexual agglutination, cell surface hydrophobicity, flocculation and resistance towards lytic enzymes in stationary phase cells (Hagen et al., 2004).

In this part of work the possible role of certain cell wall proteins was studied at yeast transfer into the state of anhydrobiosis. For this purpose a set of strains lacking one or more genes encoding different mannoproteins in *S. cerevisiae* SEY6210 genetic background were used (Table 4). The importance of deleted mannoproteins was discribed earlier. It was shown that the ccw12Δ mutant (MEY12A) had no significant difference in the phenotype compared with wild type but had increased generation time and also had a mating defect. In addition this mutant had significantly weakened cell wall indicated by the increased sensitivity to Calcofluor white and Congo red which interfer with the cell wall biogenesis (Mrsa et al., 1999). The mutant with deletions of five genes *CCW12, CCW13, CCW14, CWP1 and TIP1* named by ΔGPI (MEY5) (Mrsa et al., 1999; Hagen et al., 2004) had not exacerbated the phenotype of the single ccw12Δ mutant. To the contrast to ccw12Δ mutant the strong hypersensitivity to Calcofluor white was decreased and the doubling time was the same as for wild type (Hagen et al., 2004). Mutant VMY5678 lacking Pir proteins (ccw5 ccw6 ccw7 ccw8) had an increased sensitivity to zymolyase (Teparic et al., 2007). This mutant grew more slowly, had larger and irregularly shaped cells, and it showed pronounced susceptibility to cell wall synthesis inhibitors like Calcofluor white and Congo red. It had a decreased mating ability (Mrsa and Tanner, 1999). When compared to wild-type yeast, scw4scw10Δ mutant cells displayed slower growth rates, morphological abnormalities. Moreover, scw4scw10Δ mutant cells showed higher resistance to zymolyase compared to wild-type indicated that the cell-wall composition was changed (Sestak et al., 2004). These observations led to a thought to study the resistance of these mutants to dehydration and results of their viability can be observed in Fig. 13.

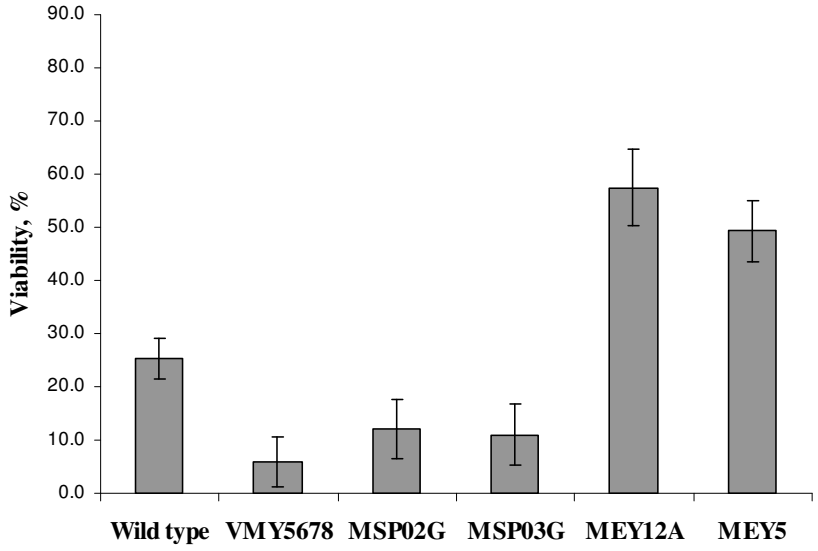

Fig. 13. Viability of *S. cerevisiae* SEY6210 isogenic strains after dehydration/rehydration.

As it can be seen wild type *S. cerevisiae* SEY6210 was sensitive to dehydration, viability of cells was only about 25%. It was found that viability of yeast strains MEY12A and MEY5 after dehydration was significantly higher than viability of wild type and it was 58 ± 7 and 49 ± 6% correspondingly. Other mutant strains had significantly lower viability after dehydration-rehydration than wild type (Fig. 13).

For interpretation of obtained results previous published investigations were used. It was reported that deletion of *Ccw12* caused the increase of chitin content in 2.5 times comparing it with wild type and probably it is linked with the activation of known cell wall compensation mechanisms such as the PKC1 pathway (Hagen et al., 2004). The overall cell wall composition of the ΔGPI mutant (MEY5) seemed to be fairly similar to that of wild-type cells, but electron microscopy revealed pronounced morphological differences in the comparison with wild type. The inner glucan layer was considerably thicker and most probably less compact in the mutant with ΔGPI (180–250 nm compared with 100–140 nm for the wild type), whereas the length of the mannan brush-like fibers was reduced and less regular (Hagen et al., 2004). Taking into consideration these data it was supposed that this compensation mechanism increasing the chitin content and the thicker glucan layer can be a reason for increase of resistance of cells to dehydration in these experiments with the strains MEY12A and MEY5.

To reveal if these changes can be found as surface changes all these yeast strains were studied using scanning electron microscopy (SEM). Two different approaches were used in this study. Using different supports for cell dehydration more soft or more severe drying conditions were achieved.

Typical view of morphology of cell surfaces of all strains is shown at Fig. 14 and 16.

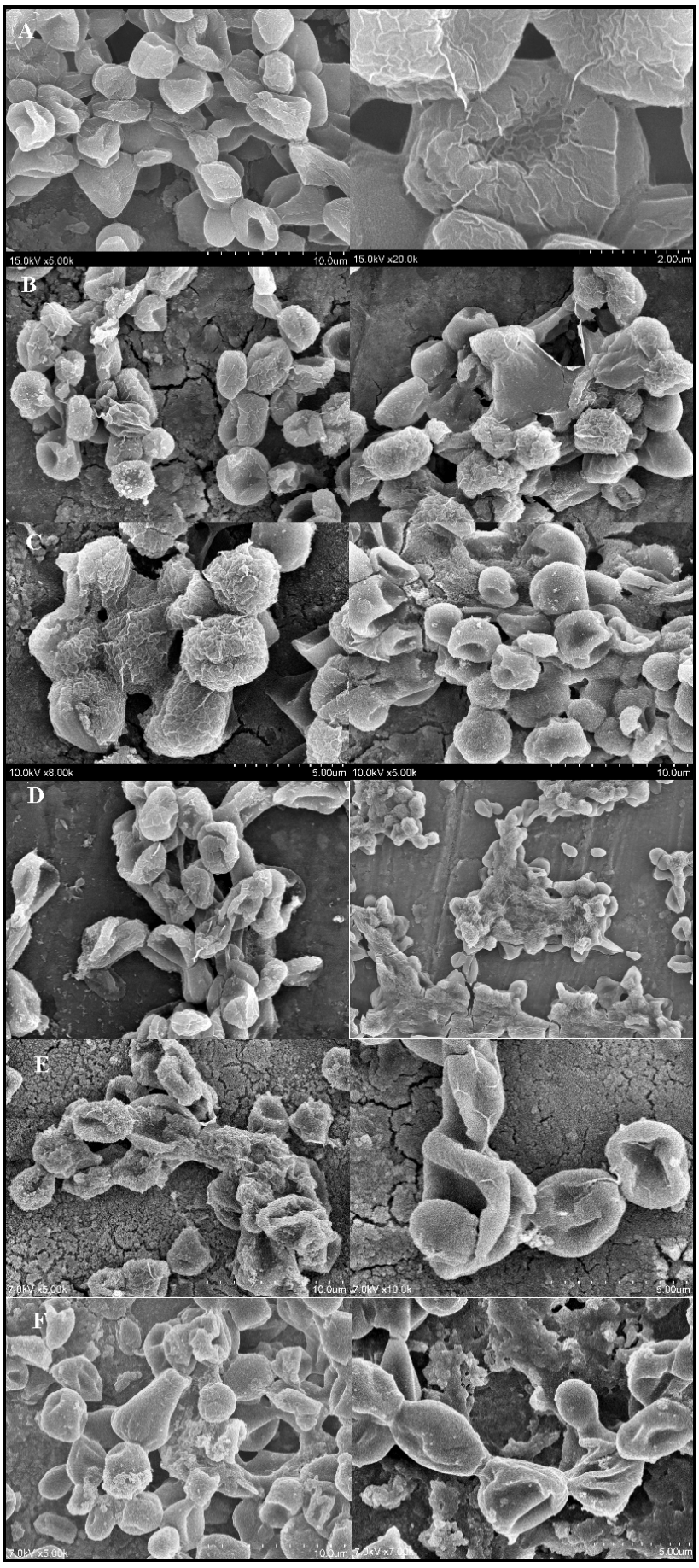

Fig. 14. Morphology of cell surface after more soft dehydration. Abbreviations: A – Wild type, B – MEY12A, C – MEY5, D – VMY5678, E – MSP2G, F – MSP3G.

Usually after more soft dehydration cells of all strains had some fibers or some film similar formations. These fibers were not different from each other in various strains and could be observed in all samples. Sometimes surface of the cells had some particles in addition to fibers (Fig. 15). At the same time we have not detected any essential variations in cells' surfaces as the result of some mannoproteins deletions.

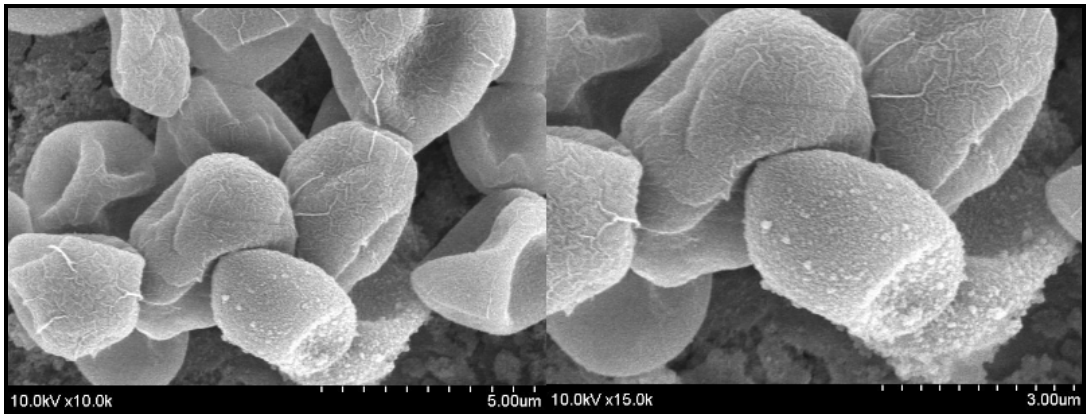

Fig. 15. Morphology of cell surface after more soft dehydration (MEY12A).

Usually the surface of cells after more severe dehydration was smooth without some specific morphology (Fig. 16).

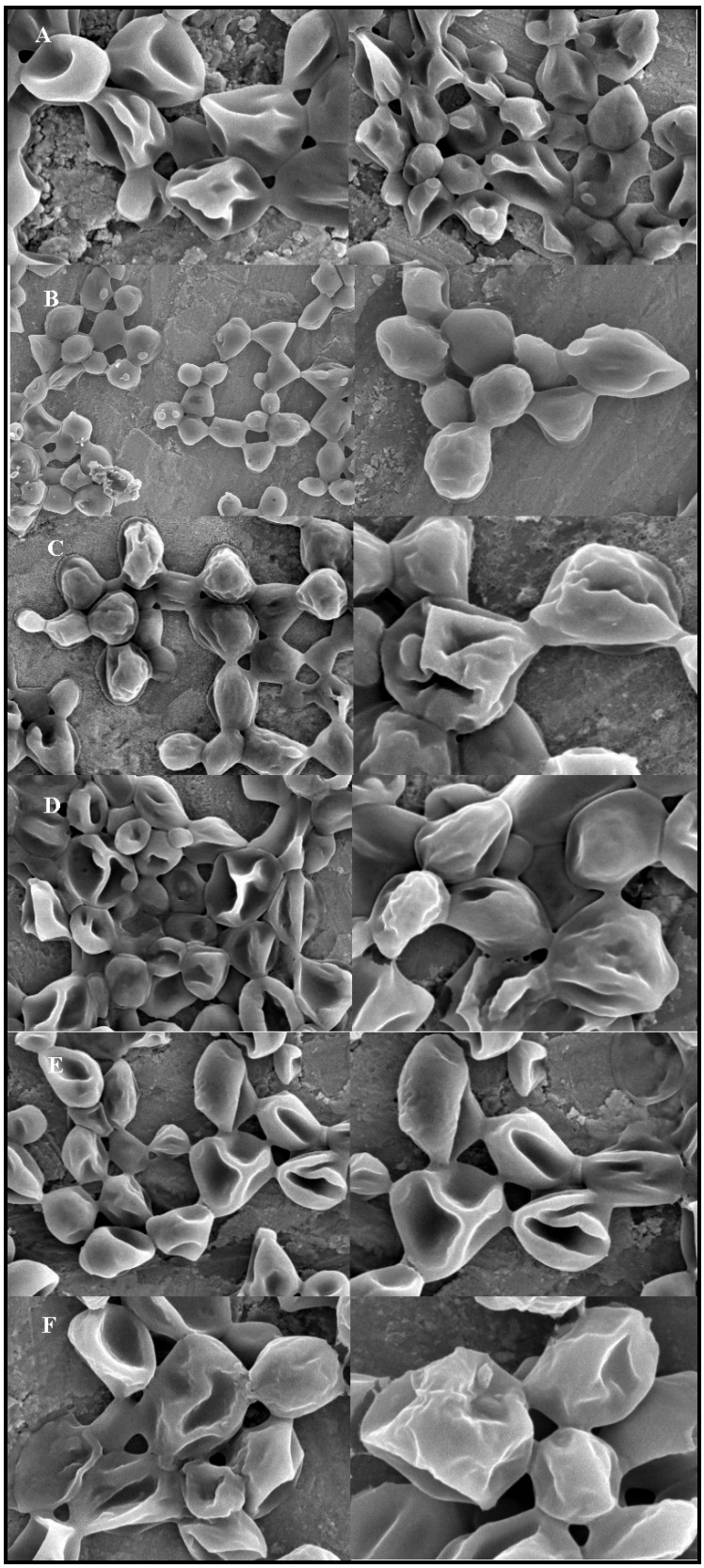

Fig. 16. Morphology of cell surface after more severe dehydration. Abbreviations: A – Wild type, B – MEY12A, C – MEY5, D – VMY5678, E – MSP2G, F – MSP3G.

So, the way of yeast cells dehydration can lead to the variations in the morphology of cell surface of the cells which belong to the same mutant strain. It was observed at more

severe dehydration that cells of yeast strains MEY12A and MEY5 better maintained their shape than cells of wild type strain and of other mutants. The amount of round-shaped cells was higher in these strains and especially in the case of the strain MEY12A. Usually in the laboratory studies of yeast cells shape changes during dehydration treatments folded shape of the cells is explained by the existence in the native cells (before dehydration treatment) of big vacuoles which are rather characteristic for yeast cells in stationary growth phase. One of the functions of these vacuoles is to maintain the turgor pressure inside the cells. At the same time these organelles contain big amounts of water which is lost by them during dehydration of the cells. So, usually these losses of water lead to the essential decrease in the sizes of vacuoles which is accompanied with the changes of yeast cells shape - they become folded. Taking into account these ideas it was decided to compare the vacuoles in the cells of our wild strain and mutant strains before the dehydration. The results of phase contrast microscopy have not revealed any differences in the sizes of vacuoles in native cells of all strains.

Therefore on the basis of the results on the maintenance of round shape of the cells of two mutant strains after their dehydration, the hypothesis can be proposed that deletion of definite proteins in these cells lead to some compensatory changes in them and probably to increased amount of hitin. So, as the result of these changes cell wall of these cells most probably becomes much more resistant to the dehydration treatment and maintains the shape of the cells in dehydrated state.

Summarizing all results received in this study some conclusions can be done. First of all, the most unexpected phenomenon which was revealed is linked with the results that the deletion of some mannoproteins which exist in the cell wall of wild strain can be favorable for cells' viability after their dehydration. Simultaneously it leads to the higher resistance of cell wall at this treatment which promotes the better maintenance of cells' shape in dry state. It is supposed that it can be linked with switching on of some compensatory mechanisms which lead to increased amount of chitin in cell walls. Finally, for the first time it was shown that cell wall can also be considered as an important factor determining the viability of the cells at dehydration-rehydration treatments.

3.2. Non-conventional application of investigation results of yeast anhydrobiosis in biotechnologies

3.2.1. A new method for yeast immobilization

3.2.1.1. Development of new method of immobilization

This part of work was linked with the attempts to use the information which was accumulated during studies of yeast anhydrobiosis for their 'non-conventional' application in biotechnology.

The idea was proposed that one of such non-conventional approaches can be linked with the development of a new method for cell immobilization. It is known that usually cell immobilization on the surface of support is beneficial process, but acquired preparations can be rather non-stable. At the same time entrapment of cells into matrix of support produces stable immobilized preparations but this process is economically rather expensive. In this work it was attempted to combine the positive sides of both immobilization approaches using for immobilization cheap supports – nanostructured ceramics from hydroxylapatite (HAP) and chamotte. In the beginning of experiments conventional methods for cell immobilization were used: namely joint cultivation - when support was added to the cultivation medium simultaneously with its inoculation with yeast, or joint incubation - when support was added for some hours to the suspension of the yeasts taken from the stationary growth phase of the culture in water or in different buffer solutions (incubation under non-growing conditions). Biofilms of yeast on the ceramics were observed using SEM (Fig. 17).

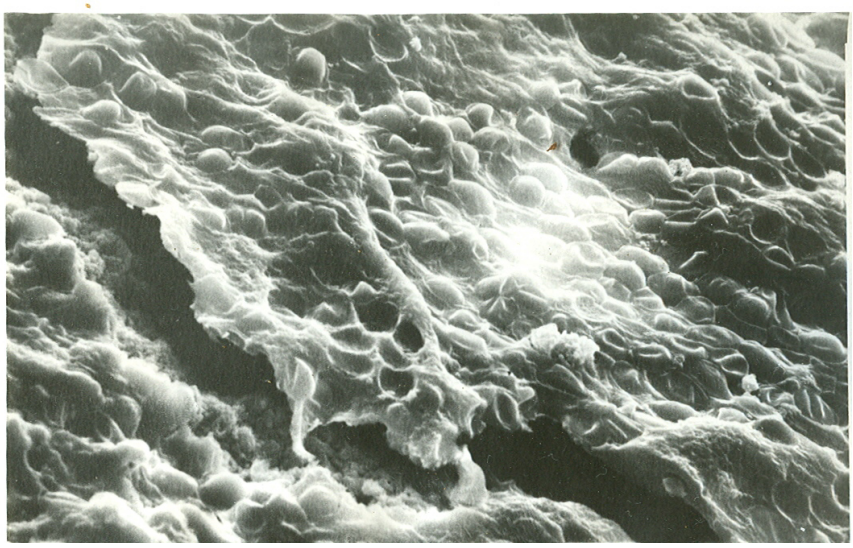

Fig. 17. Biofilm formed by yeast cells on HAP surface.

However, 5 min of rinsing of these preparations with water led to the full elimination of this biofilm from the surface of the ceramics. The possible influence of pH value of the

solutions during the incubation procedure upon immobilization and its stability was checked. It was revealed that the sedimentation of yeast cells on the ceramics depends on suspension pH value. The best results were received using 0.01 M acetate buffer with pH 4.0 and the worse when 0.1 M phosphate buffer (pH 6.0 or 7.0) was used. However, in all cases the adhesion of the cells on the ceramics was unstable and all cells were removed from the ceramics after 5 min of rinsing with water.

Because the immobilization of the cells (or at least its first stages) is determined by the surface characteristics of both the cells and the carrier, it would be necessary to change the characteristics of the surfaces of the cells and/or ceramics to reach a stable yeast immobilization. The next experiments were performed with modification of the surfaces of the cells and ceramics. To achieve the first goal (to change the characteristics of the surfaces of the cells) the knowledge of the yeast anhydrobiosis was used. These investigations showed that the desiccation of cells in the conditions similar to natural ones is accompanied by rather serious changes of cell surface structures including the cell wall. These changes also include alterations of mannoproteins of the cell wall, changes in the cell surface charge, and the formation of big cell conglomerates (Rapoport et al., 1983; Ventina et al., 1984; Rapoport and Beker, 1985; Rapoport et al., 1986).

It was decided to use these observations in our experiments. For this goal special procedure was worked out: the incubation of ceramics in yeast cell suspension, cells sedimentation on the surface of ceramics and the drying of ceramics with yeast cells. The results confirmed the initial idea. In these conditions a stable immobilization of yeast cells on ceramics was achieved. By incubating immobilized preparations in water for 4 days it was possible to maintain significant amounts of cells on the surface of the ceramics.

At the same time, electron microscopy showed that various samples differed significantly (sometimes up to 5–10 times) in the amount of cells initially (before the dehydration stage) attached to them. The surface charge of the ceramics surface is another important factor contributing to the attachment of the microorganisms. Therefore, it was assumed that the differences in the amount of attached cells are linked with variations in the physical characteristics of the ceramics surface, particularly because of its charge. To check this hypothesis at the next stage of the investigation, the surfaces of the support were provided with different electrical charges. Then, the immobilization procedure was made and the extent of immobilization was determined.

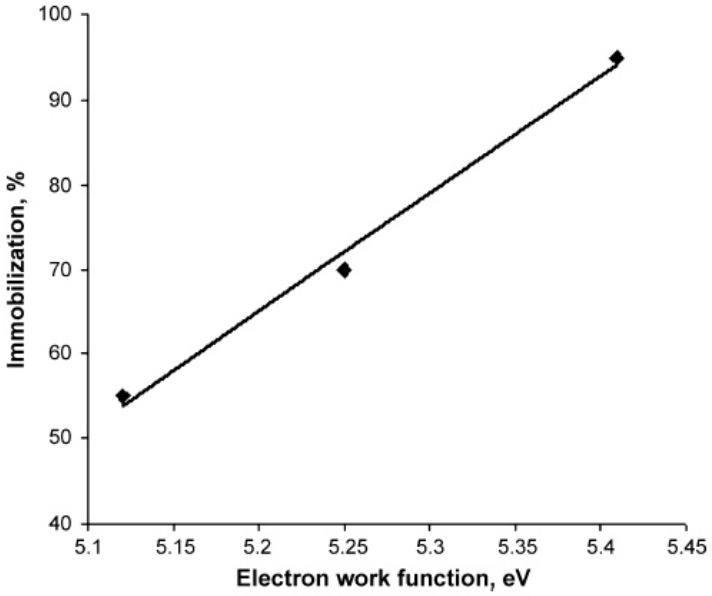

Fig. 18. The correlation of electron work function values with the amount of yeast cells immobilized on the surface of HAP ceramics.

Fig. 18 shows that the larger amounts of yeast cells were immobilized on the surface with higher negative charges (higher values of electron work function (φ)). The stability of this immobilization did not depend on the negative charge of the HAP ceramic surface. Therefore, it can be concluded that the value of the ceramics surface charge is important for the first stages of the immobilization process – the cell sedimentation/adhesion on the ceramics surface.

Results showed that an increase in negative surface charge leads to an increase in the adhesion of yeast cells to ceramics. The dehydration procedure, in its turn, strongly and stably fixes immobilized cells on the ceramics surface. Such a strong fixation can be linked to the formation of fibrils of mannoproteins and their aggregates on the surfaces of dehydrated cells as well as with the increased synthesis of adhesins in response to dehydration stress as discussed above.

3.2.1.2. Yeast immobilization as approach for improvement of cell resistance to dehydration

Taking into account that dehydration of yeast cells usually leads to the death of some part of microbial population it was necessary to check how much of cells immobilized by the new immobilization method which is linked also with dehydration stage maintain their viability. The results of experiments which were performed with the yeast strain *S. serevisiae* 14 gave rather unexpected results. In each of these experiments the viabilities of

dehydrated free and immobilized (on hydroxylapatite) yeast cells (from one and the same batch culture) were determined. The conditions of their dehydration were similar and corresponded to the conditions which are applied at yeast immobilization by our new developed method. In the case of free yeast cells their viability was 69 ± 3%, but in the case of immobilized cells it was 86 ± 5%. It means that yeast cells attached to the carrier are more resistant to dehydration than free yeast cells.

To check these results a series of additional experiments were performed with immobilization of cells which were very sensitive to dehydration-rehydration. For this study different yeast species and strains were used. Some of them were subjected to growth in various conditions. One of the strains was *S. cerevisiae* 14 from stationary growth phase which is usually used in experiments on dehydration-rehydration for anhydrobiosis studies. Cultures of this strain were grown in two different conditions. In one series of experiments ethanol-containing medium was used for yeast cultivation. Viability of cells grown in these conditions after dehydration is usually around 30%. In the second series, yeast cells were grown in a molasses-containing medium under conditions of severe oxygen limitation. Usual viability of dehydrated cells grown in anaerobic conditions never exceeds 1–2% (Rozenfelde and Rapoport, 2014). Besides these experiments with the cells of the *S. cerevisiae* 14 strain two recombinant yeast strains – *S. cerevisiae* AH22 and *P. pastoris* GS115 with the expression of bacteriophage Qß coat protein gene were also checked. It was known that these recombinant strains also are very sensitive to dehydration/rehydration treatment. The results obtained are presented in Table 14.

Table 14. Viability (%) of dehydrated and immobilized yeast cells

Yeast strains	Dehydrated/ rehydrated cells	Immobilized cells
S. cerevisiae 14 grown in ethanol-containing medium	27 ± 4	58 ± 3
S. cerevisiae 14 grown in anaerobic conditions	1 ± 1	61 ± 5
S. cerevisiae AH22	6 ± 3	43 ± 3
P. pastoris GS115	45 ± 5	75 ± 5

It can be seen that immobilization definitely increases the resistance of yeast cells to dehydration. To obtain additional information scanning electron microscopy was applied to reveal if the structural changes of cells' shape, which take place during the immobilization stage, remain after placement of these preparations into the water medium. The results obtained in this investigation are shown at Figure 19.

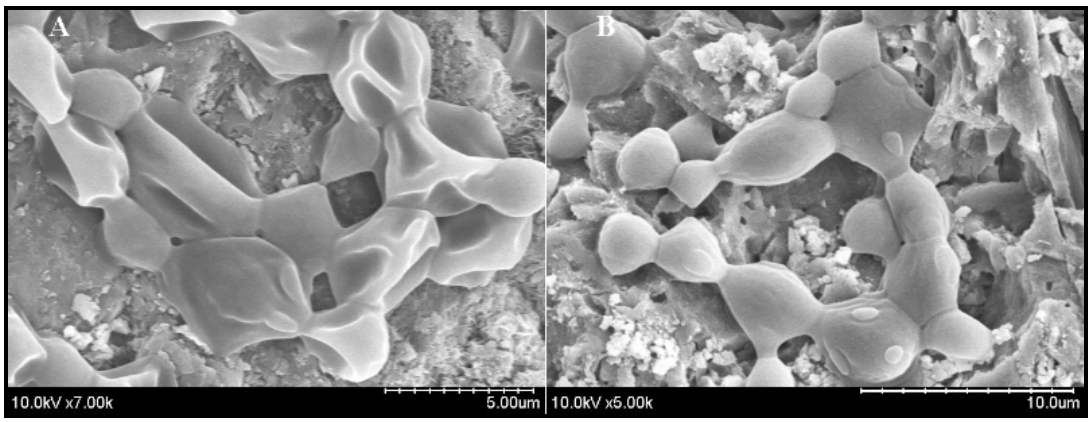

Fig. 19. Structural changes of immobilized cells' shape after rehydration. A – Immobilized cells. B – Immobilized cells after rehydration.

It was observed that after rehydration, which would take place when dry immobilized preparations are used in technological processes, yeast cells completely restore their normal shape. At the same time they remain attached to the carrier.

The acquired results showed that immobilization of cells definetely increased their survival after subsequent dehydration. It is important that this effect was shown for all kinds of yeasts – for aerobically and anaerobically grown organisms as well as for wild and recombinant strains. It is necessary to underline that such positive effects were obtained also for yeast cells grown in conditions of severe oxygen limitation and all experiments on the dehydration-rehydration of these yeasts until the last moment were unsuccessful; their viability after dehydration never exceeded 1–2%. Only recently success in such experiments was achived after the cells were previously incubated in solutions of glycerol or xylitol (Rozenfelde and Rapoport, 2014). Additionally, positive results after immobilization of anaerobically grown cells shows that cells sensitive to desiccation may be transferred into the state of anhydrobiosis. It is interesting to note that the viability rate of the cells in these current experiments (61 ± 5%) corresponds to the best results obtained previously, for cells subjected to pre-incubation in glycerol solutions (Rozenfelde and Rapoport, 2014). Of course, it is necessary in future experiments to reveal if the mechanisms of the increase of cell resistance to dehydration are similar or differ in both cases (after pre-incubation with polyols and immobilization). It is important to mention here that in spite of the probable different reasons for high sensitivity of various yeast cells used in the study, immobilization in all cases increases their resistance. A similar phenomenon was shown by Chinese researchers (Qun et al., 2002), who described that immobilized cells had an increased resistance to organic solvents. Mechanisms of such chemical tolerance were not studied. In these studies, the increase in resistance to a physical factor was related. Earlier a number of intracellular protective reactions, responsible for the maintenance of cells' viability at their transfer into

the state of anhydrobiosis, were revealed. They are linked with chromatin condensation, separation of a damaged cell's parts, decrease of a cell's nucleus volume, and synthesis of trehalose, polyols, antioxidants, heat shock proteins etc. (Beker and Rapoport, 1987; De Souza Espindola et al., 2003; Guzhova et al., 2008). Now it can be also general protective reactions at the population level. Such reactions undoubtedly have been developed by live organisms during the long evolution period. Therefore their mechanisms are of special interest for researchers studying the cells' response and possible protection mechanisms at extreme environmental conditions. Of course, it is important to reveal in further studies which intracellular protective reactions take place simultaneously with such population protection in each of the cases studied in this work. At the same time, it seems that these results are of interest primarily for the further application of the new immobilization method for various goals. For example, it is clear now that this method does not lead to the significant loss of yeast cell viability after stress treatment by dehydration-rehydration. As it was shown above the viability of yeast cells after their immobilization on hydroxylapatite carrier was very high, at the level of 86 ± 5%. So, there is no risk of cells losing biotechnological activities for preparations immobilized by this method. It is now clear that such an approach can be used for the production of dry active yeast preparations for industry, for strains which earlier were not produced because of their very high sensitivity to dehydration; these can now be used in an immobilized form. Potentially, they could be used for such classical biotechnologies as the production of beer, wine and ethanol. The development of efficient methods for the storage of recombinant yeast strains may now be possible, which until this point, have been maintained predominantly by the application of low temperatures, which often results in a loss of activity. Finally, this information about the importance of cell immobilization for increasing resistance to extreme environmental conditions is essential for furthering understanding of cellular protective mechanisms. It means that comparison of reactions of the same organisms to the same treatment in free and immobilized states can help to understand their potential, which usually is not realised in normal conditions. Such comparison is a new model system which can give a lot of new knowledge about how to increase the resistance of eukaryotic cells in unfavourable conditions. It may even be possible to extrapolate this knowledge to higher organisms for the goals of medicine.

3.2.1.3. Immobilized yeast application for chromium sorption

In this work the efficiency of immobilized yeast preparations was studied in biotechnological processes: sorption of heavy metal and ethanol production. To evaluate the possibility of yeast cells to be used as biosorbents for the purification of wastewaters from

heavy metals chromium (Cr (VI)) solution was used. The biosorption of chromium by yeast preparations immobilized at 30 °C and 105 °C was compared with the biosorption of chromium by non-immobilized dry yeast preparations (the dehydration of yeast cells was realized at the same temperatures: 30 °C and 105 °C). It is necessary to mention that previous studies had shown that the preparations of dry yeast have a higher chromium sorption activity compared with the sorption activity of intact yeast cells (which were not subjected to dehydration) (Rapoport and Muter, 1995; Muter et al., 2001).

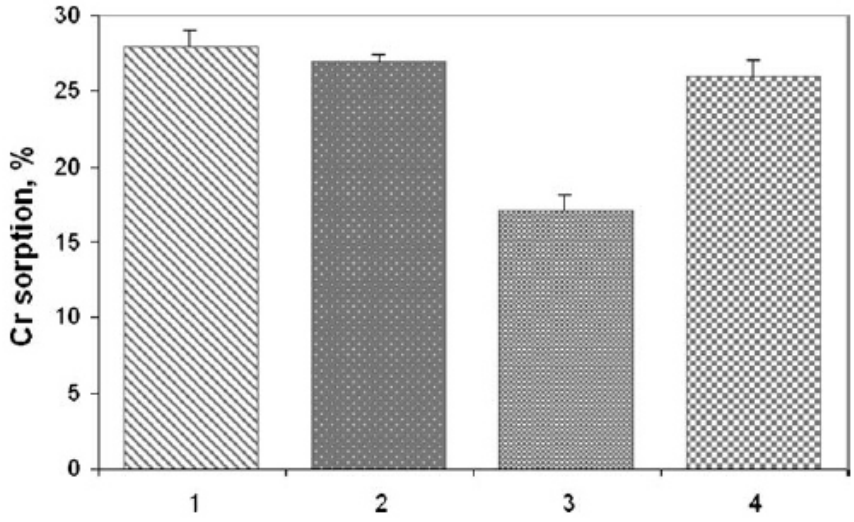

Fig. 20. Sorption of chromium by immobilized (1, 2) or free dried (3, 4) yeast cells. The immobilization of yeast cells included a dehydration procedure at 30 °C (1) or 105 °C (2). Free dried cells were dehydrated at 30 °C (3) or 105 °C (4).

As it can be seen from Fig. 20 yeast cells that were immobilized at different temperatures have similar sorption activities. Their sorption activities notably exceeded the activities of 'free' (non-immobilized) yeast cells dehydrated at similar temperatures. Therefore, it was supposed that the immobilization of yeast cells on the surface of HAP ceramics by this new method significantly improved their sorption characteristics compared with 'free' cells. This conclusion is important for the further biotechnological development of wastewater purification processes.

The simplest technological scheme for the purification of wastewaters from heavy metals is connected with the development of the system based on the use of a column in which yeast cells serve as a biofilter. The use in this case of the 'free' yeast biomass leads quickly to the sedimentation of yeast cells and the formation of a dense yeast layer on the filter, which becomes poorly penetrateable for new portions of wastewaters. The development of this yeast biofilter on the basis of the cells immobilized on the surface of porous particles

or granules of HAP ceramics might solve this technological problem because such an 'impermeable' layer cannot be formed.

3.2.1.4. Application of immobilized yeasts for ethanol production

To acquire additional information about possibilities to use immobilized yeast preparations in other biotechnological processes, it was decided to investigate the activity of immiobilized yeast cells in ethanol production. In this case two ceramics supports were used: hydroxylapatite and chamotte. First, SEM was used to compare the surface topography of both these carriers. Hydroxylapatite was shown to have a smoother surface compared to chamotte. The greater surface area of chamotte may provide an advantage over hydroxylapatite, enabling a greater amount of immobilized yeast biomass. To compare the efficiency of yeast cell immobilization on hydroxylapatite and chamotte, two strains of *S. cerevisiae* (*S. cerevisiae* L-77 and *S. cerevisiae* L-73) were used. Evaluation of immobilization efficiency in the current study was performed by calculating the weight of yeast biomass immobilized on the ceramic carrier. The results are presented in Fig. 21.

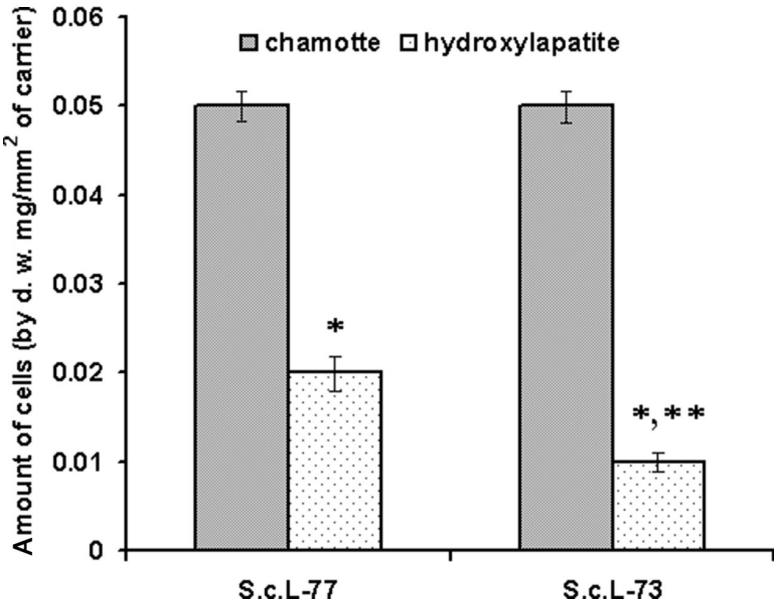

Fig. 21. Results of yeast immobilization on hydroxylapatite and chamotte. *P < 0.05 indicates a statistically significant difference between results of yeast immobilization on different carriers: chamotte and hydroxylapatite. **P < 0.05 indicates a statistically significant difference between results of *S. cerevisiae* L-77 and *S. cerevisiae* L-73 immobilization on hydroxylapatite.

As shown at Fig. 22 immobilization of significantly greater yeast biomass was achieved with the use of a chamotte carrier compared to hydroxylapatite, likely due to the greater surface area demonstrated by SEM.

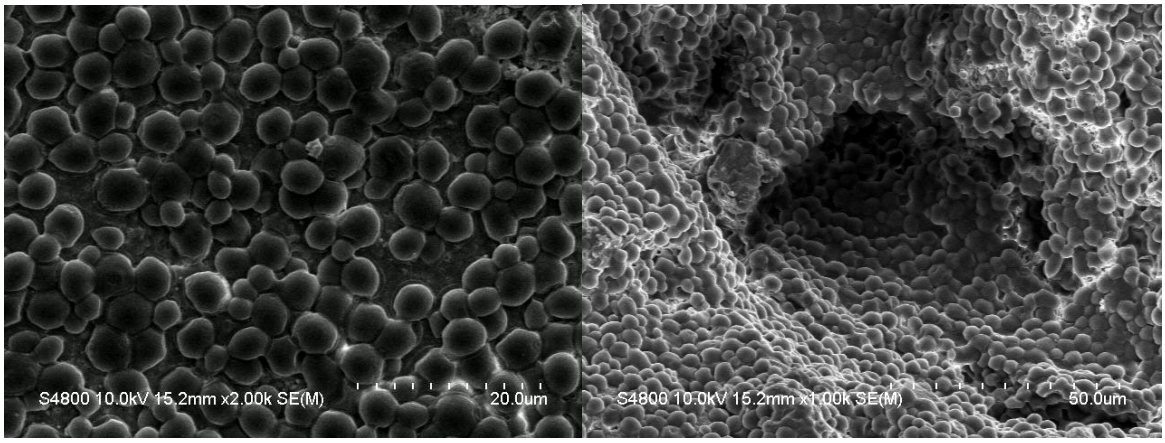

Fig. 22. *S. cerevisiae* L-77 immobilization on hydroxylapatite (A) and chamotte (B).

Acquired results are consistent with those reported by Kregiel et al., (2012) who investigated the influence of the carrier surface structure on attachment of cells and the possibilities to immobilize yeast cells on this new (for immobilization) support - chamotte.

The amount of immobilized cells on a chamotte support was the same for both strains of yeast tested (Fig. 21). Conversely, hydroxylapatite immobilization of the two tested yeast strains differed significantly. Specifically, based on dry weight per square millimeter of carrier surface, the amount of *S. cerevisiae* L-77 was higher than *S. cerevisiae* L-73.

The ethanol fermentation capacity of immobilized cells and free yeast cells in a batch system was compared. In addition to free cells, dried yeast cells were also used. Preliminary experiments showed similar ethanol concentrations produced by both yeast strains. As such, all subsequent experiments were done using immobilized and free *S. cerevisiae* L-77, which also showed superior immobilization on a hydroxylapatite support. During the fermentation process, glucose consumption and ethanol production were monitored. Fig. 23 illustrates measured ethanol production (mg/ml) during different process time points.

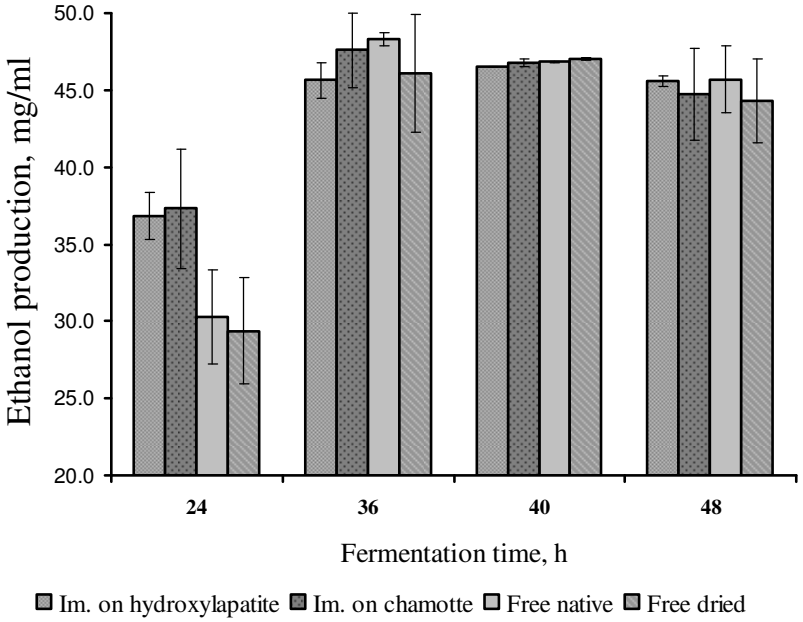

Fig. 23. Ethanol production (mg/ml) by *S. cerevisiae* L-77 during the fermentation process. Data are expressed as mean with SD.

At 24 h, yeast biomass immobilized on hydroxylapatite and chamotte produced similar amounts of ethanol. However, a significantly lower amount of ethanol was produced by free cells (native or dried) compared to cells immobilized on hydroxylapatite or chamotte, indicating that immobilization may accelerate ethanol production in the early stages of the process. Peak production (48 ± 0.5 mg/ml) was observed at 36 h, at which point both immobilized and free cells produced equal amounts of ethanol. It is necessary to mention here that at this point of fermentation processes there was not more detected any amount of glucose in the fermentation medium in any of six tested repeated ethanol production cycles.

A major advantage of immobilized systems is the ability to potentially reuse them in biotechnological processes. As such, it is crucial to ensure that immobilized cells are strongly attached to the carrier for high stability of the immobilized system. To evaluate the strength of yeast cell immobilization to the ceramic carriers, biotechnological activity (in the case—bioethanol production) of the same immobilized cells was monitored over multiple fermentation cycles. For this goal, the same preparations of immobilized cells were repeatedly used in six fermentation cycles until the hydroxylapatite carrier started to crumble. Comparison of chamotte and hydroxylapatite showed that the latter was a comparatively soft material, which can start to be degraded at pH values lower than 5, i.e., at values that frequently occur in ethanol fermentation processes. Each fermentation cycle was terminated at 48 h. Results are illustrated in Fig. 24, where it can be seen that cells immobilized on hydroxylapatite produced the same amount of ethanol during all six fermentation cycles.

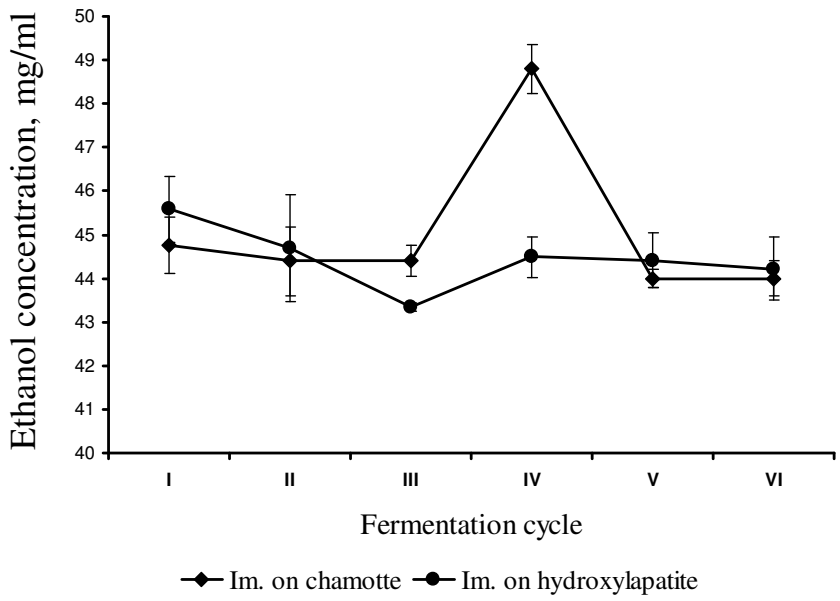

Fig. 24. Repeated-batch fermentation of immobilized *S. cerevisiae* L-77 for ethanol concentrations. Data are expressed as mean with SD.

An increase in ethanol production was observed at the fourth fermentation cycle of chamotte-immobilized cells, which is consistent with previous reports used other supports (Mariam et al., 2009).

Current methods of immobilization, such as entrapment into gel matrices or attachment to a carrier surface, offer great potential but also possess serious disadvantages. For instance, cell entrapment into a gel matrix leads to highly stable immobilized cell preparations, but the method uses costly raw materials and specialized equipment (Branyik et al., 2004; Vasconcelos et al., 2004; Escobar et al., 2012). Conversely, attachment of cells to a carrier surface is less expensive due to simplicity of the technique and cheap carriers, which are often used for this purpose, but at the same time it provides unstable immobilization of cells that can be easily detached from the carrier during industrial processes (Branyik et al., 2004; Stolarzewicz et al., 2011; Montealegre et al., 2012).

Our research showed that high levels of stable cell immobilization using our new relatively simple and inexpensive method. The peak amount of ethanol produced (48 mg/ml) did not differ from free yeast cells. This amount is equal to or higher than the values obtained in other studies performed with preparations obtained by the attachment method with various carriers — indicating that the newer immobilization method does not reduce the ability of yeast cells to produce ethanol. As it was shown ethanol production was greater during the early stages of the process compared to free cells, suggesting stimulation of cellular metabolism by this method of immobilization. These results indicate that the efficiency of

yeast cell immobilization depends both on carrier material and yeast strain, which is in accordance with previous studies.

It was revealed that cheap ceramic carrier chamotte can be successfully used for obtaining efficient (in ethanol fermentations) preparations of immobilized yeast cells. Additional 'cost advantages' of this carrier can be obtained if for immobilization of cells waste particles that remain in chamotte industrial production would be used. In such a case, this carrier definitely can compete in cost with those cheap supports that usually are proposed for immobilization by attachment - raw materials obtained from agroindustrial waste.

Another important finding from the current study was confirmation that the same immobilized yeast preparations could be reused in multiple cycles of ethanol production - for up to six continuous batch fermentations without any reduction in ethanol production.

3.2.1.5. Application of immobilized yeasts for protein production

In this work the production of bacteriophage Qß coat protein by free and immobilized yeast *P. pastoris* GS115 was compared. Previously it was shown that this strain is sensitive to dehydration treament but in immobilized state its resistance to dehydration was significantly increased. According to received data about enhanced sorption activity and ethanol production by immobilized yeasts it was suppossed that production of bacteriophage Qß coat protein by recombinant *P. pastoris* can be increased at the influence of immobilization. For these experiments different yeast samples were used: native biomass of *P. pastoris*, dehydrated cells, dehydrated cells prior incubated in 1 M xylitol solution and cell immobilized on HAP and chamotte. To produce Qß coat protein by yeast cells YPD media with addition of 1% methanol was used. Determination of protein production efficiency was done according to method described by Freivalds et al. (2006). Obtained results are illustrated in Fig. 25.

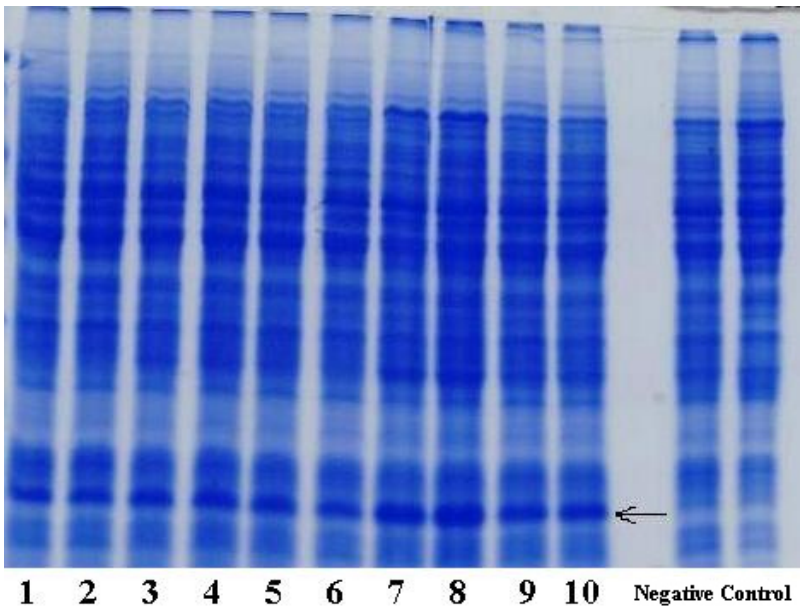

Fig. 25. Polyacrylamide gel electrophoresis of *P. pastoris*. Abbreviations: 1, 2 – native biomass; 3, 4 – dehydrated cells (viability 19%); 5, 6 – dehydrated cells prior incubated in 1 M xylitol (cell viability 70%); 7, 8 – cells immobilized on HAP (viability 83%); 9, 10 - cells immobilized on chamotte (viability 85%).

As it can be observed in Fig. 25 production of protein was determined in all samples. Cells immobilized on hydroxylapatite produced bigger amount of protein than 'free' cells.

To prove the acquired data additional experiments should be done. These preliminary results clear suggest that immobilization of yeast cells by method including dehydration step has positive effect on the stimulation of cellular metabolism.

3.2.2. Development of new test-system

In this work it was attempted to develop a new yeast test-system for evaluation of possible influence of various compounds upon physiological state of eucaryotic cells. Yeasts were already proposed as a perfect eukaryotic cell model for different scientific investigations (Barrientos, 2003; Pray, 2008; Mattiazzi et al., 2012; Pereira et al., 2012). The results acquired in another our study showed the possibility to use *S. cerevisiae* 14 cells for investigation of the influence of the electromagnetic radiation upon cells` morphology and physiology (Borovikova et al., 2008). Previously in our laboratory works it was shown that cell resistance against dehydration/rehydration stress is an integral characteristic of the physiology of eukaryotic cells. This postulate has been used for the development of a new effective test-system to evaluate the possible influence of various compounds upon the cell. Based on the fact that stability of cell plasma membrane is one of the main factors which determine viability of cells during dehydration/rehydration stress, next experiments were made with idea to investigate the possible influence of peloid water extracts (PWE) on the

improvement of cell plasma membrane stability and yeast cell resistance in total. For these purposes yeast cells of stationary growth phase were incubated in PWE for 25 min and for 3 h. After the incubation yeast samples were subjected to dehydration/rehydration stress. The results presented at the Fig. 26 indicate that viability of the PWE treated cells was increased by 16-23% in comparison with control sample. It is shown that incubation of the yeasts in PWE during 25 min gave practically the same positive effect on the improvement of cell resistance to dehydration as the incubation of yeasts in PWE during 3 h.

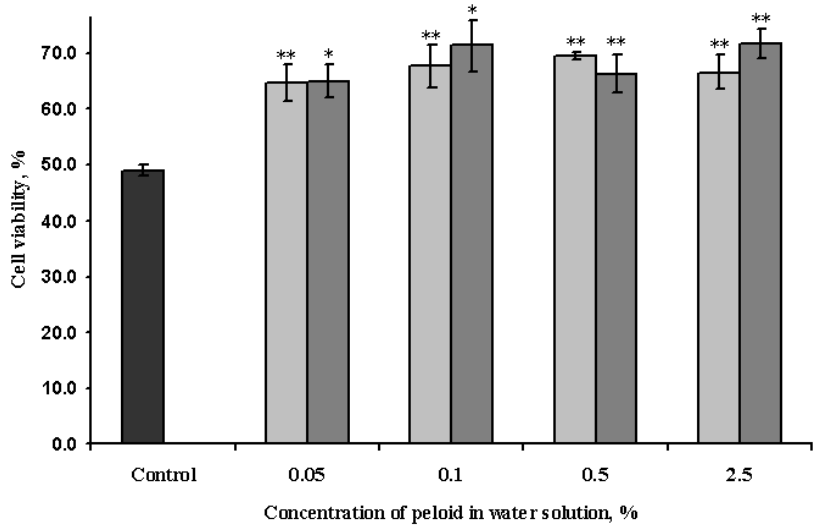

Fig. 26. Influence of incubation of yeast cells in PWE with different concentrations upon their viability after dehydration. ☐ Yeast incubation in PWE for 25 min. ■ Yeast incubation in PWE for 3 h. SD is indicated by error bars. The differences in comparison to the control at significance level $p<0.05$ are marked by (*), at $p<0.01$ are marked by (**).

Obtained results didn`t show the concentration dependent effect of PWE on the increased cell resistance to dehydration treatment. The increase of peloid concentrations from 0.05% to 2.5% was not accompanied with pronounced increase of the observed positive effects.

Further experiments were done to investigate the effects of PWE on cell resistance to dehydration using different conditions of drying: more soft and more severe drying procedures. The cells that have undergone more severe drying conditions (viability was only 38%) exhibited stronger peloid protective effect and their viability was increased by 30–40%. At the same time the effect of PWE on yeast cells dehydrated in soft conditions (viability was 60%) was lower and resistance of cells was increased only by 10-15% (Table 15).

Table 15. Influence of incubation of yeast cells (which differ in their resistance to dehydration/rehydration) in the solutions with various concentrations of peloid water extracts upon their viability after dehydration. The differences in comparison to the control at significance level $p<0.05$ are marked by (*), at $p<0.01$ are marked by (**)

	Viability of cells after dehydration, %		
	Control	**Incubation in peloid water extracts with various concentrations, %**	
		0.5	**2.5**
Severe drying conditions	38 ± 4	68 ± 6.2 **	76 ± 5.1 **
Soft drying conditions	60 ± 4	72 ± 6.2 *	70 ± 3.4 *

In general, the pre-treatment of the yeast cells with PWE in all cases increases their resistance to dehydration/rehydration stress until some high resistance level (viability at 68–76%) is reached but not above that.

To understand the mechanisms of this positive influence of the PWE upon yeast viability after dehydration/rehydration stress the possible changes of plasma membrane permeability were examined. As it was already mentioned above the plasma membrane is crucial element for the maintenance of cell homeostasis. Usually various pathological processes as well as just 'weakening' of different higher organisms correlates with changes of functional characteristics of cellular and intracellular membranes (Bansal et al., 2003; Andrews, 2005; Callahan et al., 2009; Howard et al., 2011; Zhang et al., 2012). Previous studies have shown that stabilization of cell plasma membrane is one of the most important properties for the maintenance of cell viability at dehydration/rehydration stress (Rapoport et al., 1995; Rapoport et al., 2009). The correlation of the membrane permeability with the increased viability of PWE treated cells was addressed to clarify, if the loss of substances through the destabilized cell membranes may be one of the reasons of decreased viability of the cells after dehydration/rehydration stress, what may be counteracted by the PWE treatment. Experiments showed that the PWE treatment before the dehydration decreases the permeability of plasma membrane of yeast cells after dehydration/rehydration stress and prevent the efflux of the cells' internal substances (Table 16).

Table 16. Influence of yeast cells incubation in peloid water extrracts upon the permeability of their plasma membrane evaluated by the total losses of dry substance after yeast dehydration/rehydration. The differences in comparison to the control at significance level at $p<0.01$ are marked by (*), at $p<0.001$ are marked by (**).

Yeast samples	Total losses of substances in dry weight, %	
Control	**24 ± 0.5**	
Concentration of peloid in water extracts, %	Incubation of samples in PWE for 25 min	Incubation of samples in PWE for 3 h
0.05	**22 ± 0.09** *	**22 ± 0.8** *
0.1	**21 ± 0.4** *	**21 ± 0.1** *
0.5	**20 ± 0.1****	**20 ± 0.9** *
2.5	**20 ± 0.6** *	**20 ± 0.6** *

Already the incubation with PWE for 25 min at low, 0.05% - 0.10% concentrations of the extract led to the significant increase of cells' resistance and to the stabilisation of their plasma membrane. Within the used range of the peloid dilutions the effect was not concentration dependent, the increase of peloid concentrations from 0.05% to 2.5% was not accompanied with additional increase of the observed positive effects.

It was concluded that the increase of the yeast viability after dehydration/rehydration stress and concomitant decrease of the efflux of substances from the cell through the plasma membrane may be used as a sensitive and fast assay to assess the anti-stress effect of any tested substance and in this case of natural peloid and their components. The peloid may contain biologically active compounds, which can increase the resistance of eukaryotic cells to dehydration/rehydration stress. Of course, data provides just phenomenology of the anti-stress activity. To elucidate the exact molecular mechanism of the observed effect additional studies are necessary.

The observation that the PWE treatment creates more pronounced stabilizing effect with the cells, which have lower viability due to the suboptimal drying conditions, is of special importance for their eventual use in cosmetic recipes. Skin ageing is associated with the losses of water from the skin cells and concomitant effects of dehydration. Acquired data on the recovery of the survival rate of the more vulnerable dehydrated cells by PWE treatment can be considered in context of revitalization of senescent dehydrated skin cells. PWE samples are prospective candidates to be included and further studied as eventual components

for the skin anti-ageing compositions, using, i.e., the yeast dehydration/rehydration stress and membrane integrity models.

Summarizing the results of this study it is necessary to mention that it gave important information for two different issues. First, it is interesting data on the prospects of further detailed investigations of components of PWE for new cosmetic skin anti-ageing creams. Secondly, it was concluded that this new yeast test-system which is linked with determination of features of cells physiological state: increase in viability of cells and decrease in the losses of dry weight after dehydration/rehydration treatment, can be used as efective, sensitive and fast approach for the evaluation of possible effects of various chemical compounds and biological substances.

Concluding remarks

This work was dedicated to the investigations of unique phenomenon of live nature – the anhydrobiosis. The goal of this work was to continue the investigations of anhydrobiosis and to find new theoretical facts about changes linked with cell surface (cell wall and plasma membrane). Taking into account acquired information about yeast cell changes during dehydration/rehydration, it was decided also to find new non-conventional applications of anhydrobiosis in biotechnological processes.

This work is dealing with the questions about possible changes of protein components in plasma membrane during dehydration, with their unclear role in the maintenance of cell viability during dehydration or in determination of cell resistance to dehydration. Searching the answers on these questions the new insights were made. First of all, functional changes of plasma membrane proteins were characterized during the dehydration process of yeast strains differed in their resistance to dehydration. The obtained information for the first time showed that the reaction of plasma membrane proteins to dehydration treatment is strain specific. Using gradual dehydration of yeasts for the first time the critical points of changes in the activity of proteins were determined and they were directly connected with residue amount of ‘free’ and ‘bounded’ water inside the cells.

Secondly, for the first time the crucial role of one of the plasma membrane proteins - certain potassium transporter (Trk2) in the maintenance of yeast viability during dehydration was discovered. It was shown that its presence is required for the maintenance of essential level of potassium inside the cells during the dehydration.

For the first time it was observed that deletion of some mannoproteins in the cell wall can significanty improve cells resistance to dehydration. It is supposed that such unexpected

improvement of cell properties may be connected with some compensatory mechanisms in cell wall.

A new method for yeasts immobilization which includes dehydration step was developed. It was shown that immobilized preparations obtained by this method have many benefits in the improvement of different biotechnological processes like distillery or bioremediation. For the first time it was shown that immobilization increases resistance of yeast cells to dehydration-rehydration treatment, and this approach can be used for preservation of sensitive yeast cells as well as for the improvement of their physiological properties for different biotechnological purposses.

Based on the determination of possible changes of cells resistance to dehydration, a new test-system was developed for the evaluation of influence of various chemical compounds and biological substances upon eukaryotic cells. This new approach has many important advantages: it is cheap, fast and informative.

The acquired data show new interesting and important directions for further studies of this unique live nature phenomenon - anhydrobiosis.

CONCLUSIONS

1. For the first time it was shown that the stability of plasma membrane protein - maltose transporter Agt1 during dehydration-rehydration treatment is strain-dependent. For the first time it was revealed that changes of plasma membrane proteins during cell dehydration can start already at the stage of 'free' water elimination. For the first time it was shown that sugar alcohols (for example, xylitol) can protect also the state of proteins.
2. Physiological role of potassium transporter Trk2 and its crucial role in the resistance of *Saccharomyces cerevisiae* against dehydration/rehydration stress were revealed. It was concluded that potassium content is one of those factors which are critical for yeast cells to survive desiccation.
3. It was discovered that yeast *Saccharomyces cerevisiae* with deletion of certain cell wall mannoproteins (for example, Ccw 12) can be more resistant to dehydration/rehydration.
4. It was shown that application of dehydration stage in the process of cell immobilization can significantly improve quality of immobilized yeast preparations.
5. It was shown that both studied new ceramic supports (hydroxylapatite and chamotte) are suitable for yeast cells immobilization. It was concluded that among them chamotte is potentially superior support for cells immobilization.
6. It was shown that yeasts immobilized by the use of new method have improved physiological activities in biotechnological processes comparing them with free cells. These properties of cells were proved in such different biotechnological processes as chromium sorption and ethanol production. It was concluded that yeast preparations prepared by new immobilization method are stable during at least six fermentation cycles.
7. It was shown that immobilization significantly increases cell resistance to dehydration and this approach can be used especially in the case of sensitive yeast cultures.
8. It was shown that the changes in yeast resistance to dehydration can be used for determination of effects of various compounds on the physiological state of eucaryotic cells.

ACKNOWLEDGEMENTS

Foremost I am grateful to my supervisior, Professor Alexander Rapoport. I was very lucky to be involved in such exciting field of science – investigation of the state of anabiosis; so I thank my supervisor for putting me in the track of this research. My supervisior`s immense knowledge; patience and encouragement were key motivations through my PhD work. I have been extremely lucky to have a supervisor who cared so much about my work, and who responded to my questions and queries so promptly.

I would like to thank all the colleaques from the Laboratory of Cell Biology and Institute of Microbiology and Biotechnology: Aloizijs Patmalnieks, Galina Khroustalyova, Edgars Dauss, Linda Rozenfelde, Agnese Kokina, Janis Liepins. Individually I want to express my gratitude to Aloizijs Patmalnieks for helping and teaching me in work using scanning microscopy. I am grateful to Edgars Dauss for great work done together. I want to thank FTIR spectroscopy research group: Mara Grube and Karlis Shvirksts; and Rita Scherbaka in helping me in data analysis using FT-IR and HPLC.

Specially I would like to thank Donats Erts, Institute of Chemical Physics, University of Latvia, for providing me a possibility to use scanning microscope.

It is my pleasure to thank all people with whom I have collaborated during my PhD research: Research group of Department of Membrane Transport, Institute of Physiology, Prague and especially Head of Group, Dr. Hana Sychrova; Prof. Dr. Vladimir Mrsa Laboratory of Biochemistry, Faculty of Food Technology and Biotechnology University of Zagreb; Dr. Janis Freivalds Latvian Biomedical Research and study Centre; Prof. Yuri Dekhtyar and Prof. Alexei Katashev Biomedical Engineering and Nanotechnologies Institute, Riga Technical University; Dr. Semion Cifansky and Vladimir Jakushevich Riga Technical University; Associate Prof. Ida Jakobsone Department of Food Chemistry, Faculty of Chemistry, University of Latvia; Prof. Tiina Alamae Institute of Molecular and Cell Biology, University of Tartu.

This work has been supported by the European Social Fund within the project «Support for Doctoral Studies at University of Latvia». Additionally I was granted from Federation of European Microbiological Societies.

These acknowledgemnts would not be complete without thanking my family for their constant support and care. Specially I would like to mention a person who is very important in my life, my husband. I thank him for everything!

REFERENCES

1. Aghamohammadzadeh S. & Ayscough K.R. (2010) The yeast actin cytoskeleton and its function in endocytosis. *Fungal Biology Reviews*, 24: 37–46.
2. Aguilar-Uscanga B. & Francois J.M. (2003) A study of the yeast cell wall composition and structure in response to growth conditions and mode of cultivation. *Lett Appl Microbiol*., 37: 268-74.
3. Aitchison J.D., Nuttley W.M., Szilard R.K., Brade A.M., Glover J.R. & Rachubinski R.A. (1992) Peroxisome biogenesis in yeast. *Molecular Microbiology*, 6: 3455-3460.
4. Andrews N.W. (2005) Membrane repair and immunological danger (Review). *EMBO Reports*, 6: 826-830.
5. Araujo E.A., Andrade N.J., Carvalho A.F., Ramos A.M. & Silva C.A.S. (2010) Colloidal aspects of bacterial adhesion. *Quím. Nova*., 33: 1940-1948.
6. Arino J., Ramos J. & Sychrova H. (2010) Alkali metal cation transport and homeostasis in yeasts. *Microbiol Mol Biol Rev*, 74: 95–120.
7. Bansal D., Miyake K., Vogel S.S., Groh S., Chen C.-C., Williamson R., McNeil P.L. & Campbell K.P. (2003) Defective membrane repair in dysferlin deficient muscular dystrophy. *Nature*, 23: 168-172.
8. Barrientos A. (2003) Yeast models of human mitochondrial diseases, *IUBMB Life*, 55: 83-95.
9. Beker M.E., Blumbergs J.E., Ventina E.J. & Rapoport A.I. (1984) Characteristics of Cellular Membranes at Rehydration of Dehydrated Yeast *Saccharomyces cerevisiae*. *Appl. Microbiol. Biotechnol*., 19: 347–352.
10. Beker M. J. & Rapoport A.I. (1987) Conservation of Yeast by dehydration. *Advances in Biochemical Engineering/Biotechnology*, 35: 127 – 171.
11. Berlowska J., Kregiel D. & Ambroziak W. (2013) Enhancing adhesion of yeast brewery strains to chamotte carriers through aminosilane surface modification. *World J Microbiol Biotechnol*, 29: 1307–1316.
12. Biryuzova V.I. & Rapoport A.I. (1978) Cryofractographic Investigation of the Structure of Yeast Cells in an Anabiotic State. *Microbiol*., 47: 245–251.
13. Bitew T., Sveen C.E., Heyne B. & Zaremberg V. (2010) Vitamin E Prevents Lipid Raft Modifications Induced by an Anti-cancer Lysophospholipid and Abolishes a Yap1-mediated Stress Response in Yeast. *THE JOURNAL OF BIOLOGICAL CHEMISTRY*, 285: 25731–25742.
14. Bjornsti M.A. (2002) Cancer therapeutics in yeast. *Cancer Cell,* 2: 267-273.

15. Borovikova D., Cifansky S., Dekhtyar Y., Fedotova V., Jakushevich V., Katashev A., Patmalnieks A., Rapoport A. Atomic Force Microsopy Study of Yeast Cells Influenced by High Voltage Electrical Discharge. – IFMBE Proceedings, 2008, 20: 602-605.
16. Bowen W.R., Lovitt R.W. & Wright C.J. (2001) Atomic force microscopy study of the adhesion of *Saccharomyces cerevisiae*. *J Colloid Interface Sci*., 237: 54–61.
17. Branyik T., Vicente A., Oliveira R. & Teixeira J. (2004) Physicochemical surface properties of brewing yeast influencing their immobilization onto spent grains in a continuous reactor. *Biotechnol. Bioeng*., 88: 84–93.
18. Breitenbach M., Madeo F., Laun P., Heeren G., Jarolim S., Frohlich K-U., Wissing S. & Pichlova A. (2003) Yeast as a model for aging and apoptosis research. Model systems in aging. Springer, Berlin/Heidelberg, pp. 61-97.
19. Bzducha-Wrobel A., Kieliszek M. & Blazejak S. (2013) Chemical composition of the cell wall of probiotic and brewer's yeast in response to cultivation medium with glycerol as a carbon source. *Eur Food Res Technol*, 237: 489–499.
20. Caber R.F., Styles C.A. & Fink G.R. (1988) TRKl encodes a plasma membrane protein required for high-affinity potassium transport in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.*, 8: 2848-2859.
21. Cabib E., Roh D-H., Schmidt M., Crotti L.B. & Varma A. (2001) The Yeast Cell Wall and Septum as Paradigms of Cell Growth and Morphogenesis. *J. Biol. Chem*., 276: 19679-19682.
22. Calahan D., Dunham M., DeSevo C. & Koshland D.E. (2011) Genetic Analysis of Desiccation Tolerance in *Saccharomyces cerevisiae. Genetics*, 189: 507–519.
23. Callahan J.W., Bagshaw R.D. & Mahuran D.J. (2009) The integral membrane of lysosomes: Its proteins and their roles in disease (Review). *Journal of Proteomics*, 72: 23-33.
24. Cappellaro C., Mrsa V. & Tanner W. (1998) New Potential Cell Wall Glucanases of *Saccharomyces cerevisiae* and Their Involvement in Mating. *Journal of Bacteriology*, 180: 5030–5037.
25. Capriolia M., Katholmb A.K., Melonea G., Ramlbvb H., Ricci C. & Santoc N. (2004) Trehalose in desiccated rotifers: a comparison between a bdelloid and a monogonont species. *Comparative Biochemistry and Physiology, Part A*, 139: 527 – 532.
26. Carmona-Gutierrez D., Eisenberg T., Buttner S., Meisinger C., Kroemer G. & Madeo F. (2010) Apoptosis in yeast: triggers, pathways, subroutines. *Cell Death and Differentiation*, 17: 763–773.

27. Cid V. J., Duran A., del Rey F., Snyder M.P., Nombela C. & Sanchez M. (1995) Molecular Basis of Cell Integrity and Morphogenesis in *Saccharomyces cerevisiae*. *Microbiological Reviews,* 59: 345–386.
28. Clegg J.S., Seitz P., Seitz W. & Hazlewood C.F. (1982) Cellular responses to extreme water loss: the water-replacement hypothesis. *Cryobiology*, 19: 306-316.
29. Crowe J.H., Crowe L.M. & Chapman D. (1984). Preservation of membranes in anhydrobiotic organisms: the role of trehalose. *Science*, 223: 701–703.
30. Crowe J.H., Hoekstra F.A. & Crowe LM (1992) Anhydrobiosis. *Annu Rev Physiol*, 54: 570-599.
31. Crowe J.H., Panek A.D., Crowe L.M., Panek A.C. & de Araujo P.S. (1991) Trehalose transport in yeast cells. *Biochem. Int*., 24: 721–730.
32. Dallies N., Francois J. Paquet V. (1998) A new method for quantitative determination of polysaccharides in the yeast cell wall. Application to the cell wall defective mutants of *Saccharomyces cerevisiae*. *Yeast*, 14: 1297-306.
33. Danielli J.F. & Davson H. (1935) A contribution to the theory of permeability of thin films. *Journal of Cellular and Comparative Physiology*, 5: 495-508.
34. De Souza Espindola A., Silva Gomez D., Panek A.D. & Araujo E.C. (2003) The role of glutathione in yeast dehydration tolerance. *Cryobiology*, 47: 236–241.
35. Dupont S., Lemetais G., Ferreira T., Cayot P., Gervais P. & Beney L. (2012) Ergosterol biosynthesis: a fungal pathway for life on land? *Evolution*, 66: 2961-2968.
36. Eisenberg T., Buttner S., Kroemer G. & Madeo F. (2007) The mitochondrial pathway in yeast apoptosis. *Apoptosis*, 12: 1011-1023.
37. Escobar L. M. A., Alvarez U. S. & Penuela M. (2012) Continuous production of ethanol in packed bed-bioreactors with immobilized yeast cells on lignocellulosic waste. *Dyna*, 174: 107–113.
38. Fehrenbacher K. L., Davis D., Wu M., Boldogh I. & Pon L.A. (2002) Endoplasmic Reticulum Dynamics, Inheritance, and Cytoskeletal Interactions in Budding Yeast. *Molecular Biology of the Cell*, 13: 854-865.
39. Feldmann H. (2012) Yeast: Molecular and Cell Biology. *Wiley-Blackwell*, ISBN: 978-3-527-33252-6, pp. 464.
40. Freivalds J., Dislers A., Ose V., Skrastina D., Cielens I., Pumpens P., Sasnauskas K. & Kazaks A. (2006) Assembly of bacteriophage Qß virus-like particles in yeast Saccharomyces cerevisiae and Pichia pastoris. *Journal of Biotechnology*, 123: 297–303.

41. Forsburg S.L. (2005) The yeasts Saccharomyces cerevisiae and Schizosaccharomyces pombe: models for cell biology research. *Gravit Space Biol Bull*.,18: 3-9.
42. Franca M. B., Panek A. D. & Eleutherio E. C. A. (2005) The role of cytoplasmic catalase in dehydration tolerance of *Saccharomyces cerevisiae*. *Cell Stress & Chaperones*, 10: 167 – 170.
43. Franca M.B., Panek A.D. & Eleutherio E.C. (2007) Oxidative stress and its effects during dehydration. *Comp. Biochem. and Phys. Part A*, 146: 621–631.
44. Gaber R.F., Styles C.A. & Fink G.R. (1988) TRK1 Encodes a Plasma Membrane Protein Required for High-Affinity Potassium Transport in *Saccharomyces cerevisiae*. *Mol. Cell. Biol*., 8: 2848-2859.
45. Gadanho M., Libkind D. & Sampaio J.P. (2006) Yeast Diversity in the Extreme Acidic Environments of the Iberian Pyrite Belt. *Microbial Ecology*, 52: 552-563.
46. Gadd G.M., Chalmers K. & Reed R.H. (1987) The role of trehalose in dehydration resistance of Saccharomyces cerevisiae. *FEMS Microb. Letts*., 48: 249–254.
47. Galazzo J.L.& Bailey J.E. (1990) Growing *Saccharomyces eerevisiae* in calcium-alginate beads induces cell alterations which accelerate glucose conversion to ethanol. *Biotechnol Bioeng*, 36: 417-426.
48. Garcia A.H. (2011) Anhydrobiosis in bacteria: from physiology to applications. *J Biosci*., 36: 939-950.
49. Goffeau A., Barrell B.G., Bussey H., Davis R.W., Dujon B., Feldmann H., Galibert F., Hoheisel J.D., Jacq C., Johnston M., Louis E.J., Mewes H.W., Murakami Y., Philippsen P., Tettelin H. & Oliver S.G. (1996) Life with 6000 genes. *Science*, 274: 546, 563-567.
50. Golovina E.A., Golovin A., Hoekstra F.A. & Faller R. (2009) Water Replacement Hypothesis in Atomic Detail-Factors Determining the Structure of Dehydrated Bilayer Stacks. *Biophysical Journal*, 97: 490-499.
51. Golovina E.A., Golovin A., Hoekstra F.A. & Faller R. (2010) Water Replacement Hypothesis in Atomic Details: Effect of Trehalose on the Structure of Single Dehydrated POPC Bilayers. *Langmuir*, 26: 11118–11126.
52. Gorecka E. & Jastrzebska M. (2011) Immobilization techniques and biopolymer carriers. *Biotechnol. Food Sci*., 75: 65-86.
53. Grube M., Bekers M., Upite D. & Kaminska E. (2002) IR-spectroscopic study of Zymomonas mobilis levan fermentation. *Vibrational Spectroscopy*, 28: 277-285.

54. Guillemot G., Vaca-Medina G., Martin-Yken H., Vernhet A., Schmitz P. & Mercier-Bonin M. (2006) Shear-flow induced detachment of Saccharomyces cerevisiae from stainless steel: influence of yeast and solid surface properties. *Colloids Surf B Biointerfaces*, 1: 126-35.
55. Guzhova I., Krallish I., Khroustalyova G., Margulis B. & Rapoport A. (2008) Dehydration of yeast: changes in the intracellular content of Hsp70 family proteins. *Process Biochemistry*, 43: 1138 – 1141.
56. Hagen I., Ecker M., Lagorce A., Francois J.M., Sestak S., Rachel R., Grossmann G., Hauser N.C., Hoheisel J.D., Tannerand W. & Strahl S. (2004) Sed1p and Srl1p are required to compensate for cell wall instability in Saccharomyces cerevisiae mutants defective in multiple GPI-anchored mannoproteins. *Molecular Microbiology*, 52: 1413–1425.
57. Howard A.C., McNeil A.K. & McNeil P.L. (2011) Promotion of plasma membrane repair by vitamin E. *Nature Communications*, 2: 1-8.
58. Howlett N.G & Avery S.V. (1997) Induction of Lipid Peroxidation during Heavy Metal Stress in *Saccharomyces cerevisiae* and Influence of Plasma Membrane Fatty Acid Unsaturation. *APPLIED AND ENVIRONMENTAL MICROBIOLOGY*, 63: 2971–2976.
59. Jacobson K., Mouritsen O.G. & Anderson R.G.W. (2007) Lipid rafts: at across road between cell biology and physics. *Nat.Cell Biol.*, 9: 7–14.
60. Jamieson D.J. (1998) Oxidative stress responses of the yeast *Saccharomyces cerevisiae*. *Yeast*.,14: 1511–1527.
61. Jen A.C., Wake M.C. & Mikos A.G. (1996) Review: Hydrogels for cell immobilization. *Biotechnol. Bioeng*., 50: 357-364.
62. Jensen R.E., Aiken Hobbs A.E., Cerveny K.L. & Sesaki H. (2000) Yeast mitochondrial dynamics: Fusion, division, segregation, and shape. *Microscopy Research and Technique*, 51: 573-583.
63. Johnson E.A. & Echavarri-Erasun R. (2011)Yeast biotechnology. In: Kurtzman SP, Fell JW, Boekhout T, editors. The yeast, a taxonomic study. London: Elsevier BV: 21–44.
64. Junter G-A., Coquet L., Vilain S. & Jouenne T. (2002) Immobilized-cell physiology: current data and the potentialities of proteomics. *Enzyme and Microbial Technology*, 31: 201–212
65. Kierstan M. & Bucke C. (1977). The immobilization of microbial cells, subcellular organelles and enzimes in calcium alginates gel. *Biotechnol. Bioeng*., 29: 387-397.

66. Kilonzo P. & Bergougnou M. (2012) Surface Modifications for Controlled and Optimized Cell Immobilization by Adsorption: Applications in Fibrous Bed Bioreactors Containing Recombinant Cells. *J Microb Biochem Technol*, S8:001. doi:10.4172/1948-5948.S8-001.
67. Kinclova O., Ramos J.A., Potier S. & Sychrova H. (2001) Functional study of the *Saccharomyces cerevisiae* Nha1p C-terminus. *Molecular Microbiology*, 40: 1-14.
68. Klis F.M., Boorsma A., Piet W. J. & De Groot P.W.J. (2006) Cell wall construction in *Saccharomyces cerevisiae. Yeast*, 23: 185–202.
69. Klis F.M., Mol P., Hellingwerf K. & Brul S. (2002) Dynamics of cell wall structure in *Saccharomyces cerevisiae*. *FEMS Microbiol Rev.*, 26: 239-56.
70. Knop M. (2011) Yeast cell morphology and sexual reproduction – A short overview and some considerations. *C. R. Biologies*, 334: 599–606.
71. Kopecka M. (2013) Yeast and fungal cell-wall polysaccharides can self-assemble in vitro into an ultrastructure resembling in vivo yeast cell walls. *Microscopy,* 62: 327–339.
72. Kourkoutas Y., Bekatorou A., Banatb I.M., Marchant R. & Koutinas A.A. (2004) Immobilization technologies and support materials suitable inalcohol beverages production: a review. *Food Microbiology*, 2: 377-397.
73. Krallish I., Jeppsson H. & Rapoport A. (1997) Effect of xylitol and trehalose on dry resistance of yeasts. *Appl Microbiol Biotechnol,* 47: 447–451.
74. Kregiel D., Berlowska J. & Ambroziak W. (2012) Adhesion of yeast cells to different porous supports, stability of cell-carrier systems and formation of volatile by-products. *World J Microbiol Biotechnol*., 28: 3399–3408.
75. Kregiel D., Berlowska J. & Ambroziak W. (2013) Growth and metabolic activity of conventional and non-conventional yeasts immobilized in foamed alginate. *Enzyme Microb Technol.*, 53: 229-234.
76. Kurtzman C.P. & Fell J.W. (2006) Yeast Systematics and Phylogeny - Implications of Molecular Identification Methods for Studies in Ecology. Biodiversity and Ecophysiology of Yeasts. *The Yeast Handbook*: 11-30.
77. Kusumi A., Koyama-Honda I. & Suzuki K. (2004) Molecular Dynamics and Interactions for Creation of Stimulation-Induced Stabilized Rafts from Small Unstable Steady-State Rafts. *Traffic*, 5: 213-230.
78. Lagorce A., Le Berre-Anton V., Aguilar-Uscanga B., Martin-Yken H., Dagkessamanskaia A. & Francois J. (2002) Involvement of GFA1, which encodes

glutamine-fructose-6-phosphate amidotransferase, in the activation of the chitin synthesis pathway in response to cell-wall defects in *Saccharomyces cerevisiae*. Eur J Biochem, 269: 1697-707.

79. Laroche C. & Gervais P. (2003) Achievement of rapid osmotic dehydration at specific temperatures could maintain high *Saccharomyces cerevisiae* viability. *Appl Microbiol Biotechnol*, 60: 743–747.
80. Laroche C., Simonin H., Beney L. & Gervais P. (2005) Phase transition as a function of osmotic pressure in S. cerevisiae whole cells, membrane extracts and phospholipid mixtures. *Biochim Biophys Acta*, 1669: 8–16.
81. Leskosek-Cukalovic I.J. & Nedovic V.A. (2005) Immobilized cell technology in beer brewing – current experience and results. *Proc.Nat.Sci., Mtica Srpska Novi Sad*, 109: 129-141.
82. Leslie S.B., Teter S.A., Crowe L.M. & Crowe J.H. (1994) Trehalose lowers membrane phase transitions in dry yeast cells. *Biochemical and Biophysical Acta*, M1192: 7-13.
83. Li S.C. & Kane P.M. (2009) The Yeast Lysosome-like Vacuole: Endpoint and Crossroads. *Biochim Biophys Acta*., 1793: 650–663.
84. Liiv L., Parn P. & Alamae T. (2001) Cloning of maltase gene from a methylotrophic yeast *Hansenula polymorpha*. *Gene*, 26: 77–85.
85. Lipke P.N. & Ovalle J.R. (1998) Cell Wall Architecture in Yeast: New Structure and New Challenges. *Bacteriol*., 180: 3735–3740.
86. Lopez A., Lazaro N. & Marques A.M. (1997) The interphase technique: a simple method of cell immobilization in gel-beads. *J. Microbiol. Methods*, 30: 231-234.
87. Lopez-Martinez G., Rodriguez-Porrata B., Margalef-Catala M. & Cordero-Otero R. (2012) The STF2p hydrophilin from *Saccharomyces cerevisiae* is required for dehydration stress tolerance. *PLoS One*, 7: e33324.
88. Lozinsky V.I. & Plieva F.M. (1998) Poly (vinyl alcohol) cryogels employed as matrices for cell immobilization. Overview of recent research and developments. *Enzyme Microb. Technol.*, 23: 227-242.
89. Lushchak V.I. (2006) Budding yeast *Saccharomyces cerevisiae* as a model to study oxidative modification of proteins in eukaryotes. *Acta Biochimica Polonica* 53(4): 679-684.
90. Madrid R., Gomez M.J., Ramos J. & Rodrıguez-Navarro A. (1998) Ectopic potassium uptake in trk1 trk2 mutants of *Saccharomyces cerevisiae* correlates with a highly hyperpolarized membrane potential. *Journal of Biological Chemistry*, 273: 14838–14844.

91. Majdik C., Burca S., Indolean C., Maicaneanu A., Stanca M., Tonk S. & Mezey P. (2010) Suspended and immobilized brewery waste biomass and commercial yeast as biosorbents for Cd (II) removal. A thermodynamic study. *Rev. Roum. Chim*., 55: 871-877.

92. Mariam I., Manzoor K., Ali S. & Ikram-Ul-Haq (2009) Enhanced production of ethanol from free and Immobilized Saccharomyces cerevisiae under stationary culture. *Pak. J. Bot*., 41: 821–833.

93. Martins S.C.S., Martins C.M., Oliveira, Fiúza L.M.C.G. & Santaella S.T. (2012). Selection of culturable environmental microbial strains for cellular immobilization: association of phenotypic adhesive characteristics and quantitative cellular retention. *Afr. J. Biotechnol.,* 58: 12206-12212.

94. Mattiazzi M., Petrovic U. & Krizaj I. (2012) Yeast as a model eukaryote in toxinology: a functional genomics approach to studying the molecular basis of action of pharmacologically active molecules. *Toxicon,* 60: 558-571.

95. Merchan S., Bernal D., Serrano R. &Yenush L. (2004) Response of the *Saccharomyces cerevisiae* Mpk1 mitogen-activated protein kinase pathway to increases in internal turgor pressure caused by loss of Ppz protein phosphatases. *Eukaryot Cell,* 3: 100–107.

96. Molina M., Gil C., Pla J., Arroyo J. & Nombela C. (2000) Protein localisation approaches for understanding yeast cell wall biogenesis. *Microsc Res Tech.,* 51: 601-12.

97. Montealegre C. M., Dionisio E. R., Sumera L. V., Adolacion J. R. T. & De Leon R. L. (2012) A Comparison between the Performance of *S. cerevisiae* Cells Immobilized in Nata de Coco Biocellulose and Calcium Alginate during Continuous Bioethanol Production. *Int. J. Chem. Eng. Appl*., 3: 237–242.

98. Morgan C.A., Herman N., White P.A. & Vesey G. (2006) Preservation of micro-organisms by drying; a review. *J Microbiol Meth*, 66: 183–193.

99. Moseley J.B. & Goode B.L. (2006) The Yeast Actin Cytoskeleton: from Cellular Function to Biochemical Mechanism. *Microbiol. Mol. Biol. Rev*., 70: 605–645.

100. Mrsa V., Ecker M., Strahl-Bolsinger S., Nimtz M., Lehle L. & Tanner W. (1999) Deletion of New Covalently Linked Cell Wall Glycoproteins Alters the Electrophoretic Mobility of Phosphorylated Wall Components of *Saccharomyces cerevisiae. J. Bacteriol*., 181: 3076–3086.

101. Mrsa V. & Tanner W. (1999). Role of NaOH-extractable cell wall proteins Ccw5p, Ccw6p, Ccw7p and Ccw8p (members of the Pir protein family) in stability of the *Saccharomyces cerevisiae* cell wall. *Yeast*, 15: 813–820.
102. Mulet J.M., Leube M.P., Kron S.J., Rios G., Fink G.R. & Serrano R. (1999) A novel mechanism of ion homeostasis and salt tolerance in yeast: the Hal4 and Hal5 protein kinases modulate the Trk1-Trk2 potassium transporter. *Mol Cell Biol*, 19: 3328–3337.
103. Muter O., Patmalnieks A. & Rapoport A. (2001) Interrelations of the yeast Candida utilis and Cr (VI): metal reduction and its distribution in the cell and medium. *Process Biochem*, 36: 963–970.
104. Nasser M.W., Pogoja V., Abdin M.Z. & Jain S.K. (2003) Evaluation of Yeast as an Expression System. *Indian Journal of Biotechnology*, 2: 477-493.
105. Neelakandan T. & Usharani G. (2009) Optimization and Production of Bioethanol from Cashew Apple Juice Using Immobilized Yeast Cells by *Saccharomyces cerevisiae*. *American-Eurasian Journal of Scientific Research*, 4: 85-88.
106. Nikolajeva V., Griba T. Petrina Z. (2012) Factors influencing adhesion of *Pseudomonas putida* on porous clay ceramic granules. *Environmental and Experimental Biology*, 10: 77–80.
107. Novichkova A.T. & Rapoport A.I. (1984) State of the intracellular pool of free amino acids in dehydrated yeast organisms. *Microbiology*, 53: 1-4.
108. OECD Guidelines for the testing of chemicals (1986) Test No. 480: genetic toxicology:*Saccharomyces cerevisiae*, gene mutation assay, section 4: 1-5.
109. OECD Guidelines for the testing of chemicals (1986) Test No. 481: genetic toxicology: *Saccharomyces cerevisiae*, mitotic recombination assay, section 4: 1-6.
110. Orlean P. (1997) Biogenesis of yeast wall and surface components. *The Molecular Biology of the Yeast Saccharomyces,* 3: 229-362.
111. Owen D.M. & Gaus K. (2013) Imaging lipid domains in cell membranes: the advent of super-resolution fluorescence microscopy. *Frontiers in Plant Science*, 4: 1-9.
112. Park J.K. & Chang H.N. (2000). Microencapsulation of microbial cells. *Biotechnol. Adv.*, 18: 303-319.
113. Papanikou E. & Glick B.S. (2009) The yeast Golgi apparatus: Insights and mysteries. *FEBS Letters*, 583: 3746–3751.
114. Pereira C., Bessa C., Soares J., Leao M. & Saraiva L. (2012) Contribution of yeast models to neurodegeneration research. *J. Biomed. Biotechnol.*, 2012: 1-12.

115. Petrezselyova S., Ramos J. & Sychrova H. (2011) Trk2 transporter is a relevant player in K+ supply and plasma-membrane potential control in *Saccharomyces cerevisiae*. *Folia Microbiol*, 56: 23–28.
116. Petrezselyova S., Zahradka J. & Sychrova H. (2010) *Saccharomyces cerevisiae* BY4741 and W303-1A laboratory strains differ in salt tolerance. *Fungal Biol*, 114: 144–150.
117. Pike L. J. (2006). Rafts defined: a report on the Keystone symposium on lipid rafts and cell function. *J. Lipid Res.*, 47: 1597–1598.
118. Plesset J., Ludwig J.R. Cox B.C. & McLaughlin C.S. (1987) Effect of Cell Cycle Position on Thermotolerance in *Saccharomyces cerevisiae*. *JOURNAL OF BACTERIOLOGY*, 169: 779-784.
119. Popolo L., Gilardelli D., Bonfante P. & Vai M. (1997) Increase in chitin as an essential response to defects in assembly of cell wall polymers in the ggp1delta mutant of *Saccharomyces cerevisiae*. *J Bacteriol*, 179: 463-9.
120. Posas F., Chambers J.R., Heyman J.A., Hoeffler J.P., de Nadal E. & Arino J. (2000) The transcriptional response of yeast to saline stress. *J Biol Chem.*, 275: 17249-17255.
121. Potts M., Slaughter S.M., Hunneke F., Garst J.F. & Helm R.F. (2005) Desiccation Tolerance of Prokaryotes: Application of Principles to Human Cells. *Integr. Comp. Biol.*, 45: 800–809.
122. Pray L. (2008) L.H. Hartwell`s Yeast: A Model Organisms for Studying Somatic Mutations and Cancer. *Nature Education,* 1: 183.
123. Prestrelski S.J., Tedeschi N., Arakawa T. & Carpentert J.F. (1993) Dehydration-induced Conformational Transitions in Proteins and Their Inhibition by Stabilizers. *Biophysical Journal*, 65: 661-671.
124. Qun J., Shanjing Y. & Lehe M. (2002) Tolerance of immobilized baker's yeast in organic solvents. *Enzyme and Microbial Technology*, 30: 721–725.
125. Ramakrishna S.V. & Prakasha R.S. (1999). Microbial fermentations with immobilized cells. *Curr Sci.*, 77: 87-100.
126. Rapoport A.I. & Beker M.E. (1978) Increased intracellular activity of acid phosphatase on desiccation of the yeast, *Saccharomyces cerevisiae*. *Microbiology*, 47: 136-137.
127. Rapoport A.I. & Beker M.E. (1983) Effect of Sucrose and Lactose on Resistance of the Yeast *Saccharomyces cerevisiae* to Dehydration. *Microbiol.*, 52: 556–559.

128. Rapoport A.I. & Beker M.E. (1985) Changes in the surface charge of yeast cells during their dehydration and rehydration. *Microbiology*, 54: 362–365.
129. Rapoport A.I. & Beker M.E. (1986) Ribonucleic acid degradation during dehydration of yeast cells. *Microbiology*, 55: 689-691.
130. Rapoport A.I., Berestennikova N.D., Yanovsky K.A. & Beker M.J. (1986a) Changes in the shape and size of yeast cells upon their dehydration followed by reactivation. *Microbiology*, 55: 881–882.
131. Rapoport A.I., Birjusova V.I. & Meissel M.N. (1973) The structure of the yeast cell nucleus in an anabiotic state. *Reports of the Academy of Science of the USSR*, 213: 708-710 (In Russian).
132. Rapoport A.I., Biryuzova V.I. & Beker M.E. (1983) Effect of dehydration on the surface structure of the yeast cell wall. *Microbiology*, 52: 204–207.
133. Rapoport A.I., Biryusova V.I. & Svetlichnaya T.P. (1986b) Freeze-fracture study of changes in vacuoles during dehydration of Saccharomyces cerevisiae cells. - *Microbiology*, 55: 835-839.
134. Rapoport A.I., Dreimane M.A. & Beker M.E. (1986c) Influence of the dehydration process on Endomycopsis fibuligera yeasts. *Microbiology*, 55: 542-545.
135. Rapoport A.I. & Meysel M.N. (1985) Survival rates of yeast organisms after dehydration as determined by fluorescence microscopy. *Microbiology*, 54: 53–55.
136. Rapoport A.I., Khrustaleva G., Chamanis Ya. & Beker M.E. (1994) Yeast Anhydrobiosis: Permeability of the Plasma Membrane. *Microbiol*, 64: 229-232.
137. Rapoport A.I., Khroustalyova G.M., Chamanis G.J. & Beker M.E. (1995) Yeast Anhydrobiosis: Permeability of the Plasma Membrane. *Microbiol*., 64: 229–232.
138. Rapoport A.I., Khroustalyova G.M., Crowe L.M. & Crowe J.H. (2009) Anhydrobiosis in yeast: stabilization by exogenous lactose. *Microbiology*, 78: 624-629.
139. Rapoport A.I. & Kostrikina N.A. (1973) Cytological investigation of an anabiosis state of yeast organisms. *Proceed. Acad. Sci. USSR. Biol*., 5: 770–773 (in Russian).
140. Rapoport A. & Muter O. (1995) Biosorption of hexavalent chromium by yeasts. *Process Biochem*, 30: 145–149.
141. Rapoport A.I., Puzyrevskaya O.M. & Saubenova M.G. (1988) Polyols and resistance of yeasts to dehydration. *Microbiology*, 57: 269–271.
142. Rodriguez-Navarro A. & Ramos J. (1984) Dual system for potassium transport in *Saccharomyces cerevisiae*. *J. Bacteriol.*, 159: 940-945.

143. Rozenfelde L. & Rapoport A. (2014) Anhydrobiosis in yeast: is it possible to reachanhydrobiosis for yeast grown in conditions with severe oxygen limitation? *Antonie Van Leeuwenhoek*, 106: 211-217.
144. Samonin V.V. & Elikova E.E. (2004) A study of the adsorption of bacterial cells on porous materiAls. *Microbiology*, 73: 696-701.
145. Saulite V.A., Rapoport A.I. & Beker M.E. (1986) Lipid inclusions in cells and changes in them during dehydration and reactivation of yeasts. *Microbiology*, 55: 99-104.
146. Schaber J., Adrover M.A., Eriksson E., Pelet S., Petelenz-Kurdziel E., Klein D., Posas F., Goksör M., Peter M., Hohmann S. & Klipp E. (2010) Biophysical properties of *Saccharomyces cerevisiae* and their relationship with HOG pathway activation. *Eur Biophys J*., 39: 1547-1556.
147. Schneiter R. (2004) Genetics, molecular and cell biology of yeast. *Schweiz*, *Universität Freiburg Schweiz*: 4 – 18.
148. Schenkel L.C. & Bakovic M. (2014) Formation and Regulation of Mitochondrial Membranes. *International Journal of Cell Biology*, 2014: http://dx.doi.org/10.1155/2014/709828.
149. Serrano R., Kielland-Brandt M.C. & Fink G.R. (1986) Yeast plasma membrane ATPase is essential for growth and has homology with (Na+ + K+), K+- and Ca^{2+}-ATPases. *Nature*, 319: 689–693.
150. Sestak S., Hagen I., Tanner W. & Strahl S. (2004) Scw10p, a cell-wall glucanase/transglucosidase important for cell-wall stability in *Saccharomyces cerevisiae*. *Microbiology*, 150: 3197–3208.
151. Shibasaki S., Maeda H. & Ueda M. (2009) Molecular Display Technology Using Yeast —Arming Technology. *ANALYTICAL SCIENCES*, 25: 41-49.
152. Simonin H., Beney L. & Gervais P. (2007) Seguence of occuring damages in yeast plasma membrane during dehydration and rehydration: Mechanisms of cell death. *Biochimica et Biophysica Acta*, 1768: 1600-1610.
153. Simons K. & Ikonen E. (1997) Functional rafts in cell membranes. *Nature*, 5: 569-572.
154. Singer S.J. & Nicolson G.L. (1972) The fluid mosaic model of the structure of cell membranes. *Science,* 175: 720-731.
155. Slatnar A., Jakopic J., Stampar F., Veberic R. & Jamnik P. (2012) The Effect of Bioactive Compounds on *In Vitro* and *In Vivo* Antioxidant Activity of Different Berry Juices. *PLOS ONE*, 7: DOI:10.1371/journal.pone.0047880.

156. Smits G.J., van den Ende H. & Klis F.M. (2001) Differential regulation of cell wall biogenesis during growth and development in yeast. *Microbiology*, 147: 781–794.

157. Soubeyrand V., Luparia V., Williams P., Doco T., Vernhet A., Ortiz-Julien A. & Salmon J.M. (2005) Improvement of the fermenting capacity of active dry yeast sterols during rehydration. *J. Agric. Food Sci*., 53: 8025-8032.

158. Stambuk B. U. (2000) A simple laboratory excercise illustrating active transport in yeast cells. *Biochemistry and Molecular Biology Education*, 28: 313 – 317.

159. Steels E.L., Learmonth R.P. & Watson K. (1994) Stress tolerance and membrane lipid unsaturation in Saccharomyces cerevisiae grown aerobically or anaerobically. *Microbiology*, 140: 569–576.

160. Stolarzewicz I., Bialecka-Florjanczyk E., Majewska E. & Krzyczkowska J. (2011) Immobilization of Yeast on Polymeric Supports. *Chem. Biochem. Eng*., 25: 135–144.

161. Taanman J-W. (1999) The mitochondrial genome: structure, transcription, translation and Replication. *Biochimica et Biophysica Acta*, 1410: 103-123.

162. Taddei A. & Gasser S.M. (2012) Structure and Function in the Budding Yeast Nucleus. *Genetics*, 192: 107–129.

163. Taddei A., Schober H. & Gasser S.M. (2010) The Budding Yeast Nucleus. *Cold Spring Harb Perspect Biol*; doi: 10.1101/cshperspect.a000612.

164. Tamas M.J., Luyten K., Sutherland F.C., Hernandez A., Albertyn J., Valadi H., Li H., Prior B.A., Kilian S.G., Ramos J., Gustafsson L., Thevelein J.M. & Hohmann S. (1999) Fps1p controls the accumulation and release of the compatible solute glycerol in yeast osmoregulation. *Mol Microbiol*., 31: 1087-1104.

165. Teparic R., Stuparevic I. & Mrsa V. (2004) Increased mortality of Saccharomyces cerevisiae cell wall protein mutants. *Microbiology* 150: 3145–3150.

166. Teparic R., Stuparevic I. & Mrsa V. (2007) Binding assay for incorporation of alkali-extractable proteins in the *Saccharomyces cerevisiae* cell wall. *Yeast*, 24: 259–266.

167. Tetteroo F.A.A., de Bruijn A.Y., Henselmans R.N.M., Wolkers W.F., van Aelst A.C., & Hoekstra F.A. (1996) Characterization of Membrane Properties in Desiccation-Tolerant and Intolerant Carrot Somatic Embryos. *Plant Physiol*., 11: 403-412.

168. Tunnacliffe A. &Wise M.J. (2007) The continuing conundrum of the LEA proteins. *Naturwissenschaften*, 94: 791-812.

169. Uzunova-Doneva T. & Donev T. (2004-2005) Anabiosis and conservation of microorganisms. *Journal of culture collections*, 4: 17-28.
170. Vasconcelos J. N., Lopes C. E. & Franca F. P. (2004) Continuous ethanol production using yeast immobilized on sugar-cane stalks. *Braz. J. Chem. Eng*., 21: 357–365.
171. Ventina E.J., Saulite L.A., Rapoport A.I. & Beker M.E. (1984) Electron-microscopic study of yeasts in a state of anabiosis and reactivated from this state. *Microbiology*, 53: 536–541.
172. Ventina E.J., Saulite L.A., Rapoport A.I. & Beker M.E. (1986) Lipid inclusions in yeast cells and their changes during dehydration and reactivation of yeast organisms. *Mikrobiologia*, 55: 116–119.
173. Verbelen P.J., Schutter D.P.D., Delvaux F., Verstrepen K.J. & Delvaux F.R. (2006) Immobilized yeast cell systems for continuous fermentation applications. *Biotechnol Lett*. 28: 1515–25.
174. Verma M., Brar S.K., Blais J.F., Tyagi R.D. & Surampalli R.Y. (2006) Aerobic biofiltration processes-advances in wastewater treatment. *Pract. Period. Hazard Toxic Radioact. Waste Manage*, 10: 264-276.
175. Verstrepen K.J. & Klis F.M. (2006) Flocculation, adhesion and biofilm formation in yeasts. *Mol Microbiol*., 60: 5-15.
176. Vidgren V., Viljanen K., Mattinen L., Rautio J. & Londesborough J. (2014) Three Agt1 transporters from brewer's yeasts exhibit different temperature dependencies for maltose transport over the range of brewery temperatures (0–20 °C). *FEMS Yeast Research*, 14: 601-613.
177. Walker G.M. (1998) Yeast Physiology and Biotechnology. *Wiley*, ISBN-13: 9780471964469. 362 pp.
178. Welch A.Z., Gibney P.A., Botstein D. & Koshland D. E. (2013) TOR and RAS pathways regulate desiccation tolerance in Saccharomyces cerevisiae. *Molecular Biology of the Cell*, 24: 115-128.
179. Wharton D.A. & Barclay S. (1993) Anhydrobiosis in the free-living antarctic nematode *Panagrolaimus davidi* (Nematoda: Rhabditida). *Fundam. appl. Nematol*., 16: 17-22.
180. White J.S. & Walker G.M. (2011) Influence of cell surface characteristics on adhesion of *Saccharomyces cerevisiae* to the biomaterial hydroxylapatite. *Antonie Van Leeuwenhoek*, 99: 201–209.

181. Yenush L., Mulet J.M., Arino J. & Serrano R. (2002) The Ppz protein phosphatases are key regulators of K+ and pH homeostasis: implications for salt tolerance, cell wall integrity and cell cycle progression. *EMBO J*., 21: 920–929.
182. Zahradka J. & Sychrova H. (2012) Plasma-membrane hyperpolarization diminishes the cation efflux via Nha1 antiporter and Ena ATPase under potassium-limiting conditions. *FEMS Yeast Res*, 12: 439–446.
183. Zakharova K., Tesei D., Marzban G., Dijksterhuis J., Wyatt T. & Sterflinger K. (2013) Microcolonial Fungi on Rocks: A Life in Constant Drought? *Mycopathologia*, 175: 537–547.
184. Zikmanis P.B., Auzina L.P., Auzane S.I. & Beker M.J. (1982) Relationship between the fatty acid composition of lipids and the viability of dried yeast Saccharomyces cerevisiae. *European Journal of Applied Microbiology and Biotechnology*, 15: 100-103.
185. Zikmanis P.B., Auzane S.I., Kruce R.V., Auziņa L.P. & Beker M.J. (1983) Interrelationships between the fatty acid composition and metabolic pathways upon dehydration-rehydration of the yeast Saccharomyces cerevisiae. *European Journal of Applied Microbiology and Biotechnology*, 18: 298-302.
186. Zhang D., Iyer L.M., He F. & Aravind L. (2012) Discovery of novel DENN proteins: Implications for the evolution of eukaryotic intracellular membrane structures and human disease. *Frontiers in Genetics*, 3: 1-10.
187. Zyracka E., Zadrąg R., Koziol S., Krzepilko A., Bartosz G. & Bilinski T. (2005) Yeast as a biosensor for antioxidants: simple growth tests employing a *Saccharomyces cerevisiae* mutant defective in superoxide dismutase. *Acta Biochimica Polonica*, 52: 679–684.