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Brno, 2020 Ing. Dana Byrtusová

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PRODUCTION OF BETA-GLUCANS AND OTHER POLYSACCHARIDES BY YEAST AND MICROALGAE
STUDIUM PRODUKCE BETA-GLUKANŮ A DALŠÍCH POLYSACHARIDŮ POMOCÍ KVASINEK A MIKROŘAS

DOCTORAL THESIS
DIZERTAČNÍ PRÁCE

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PRODUCTION OF BETA–GLUCANS AND OTHER POLYSACCHARIDES BY YEAST AND MICROALGAE

Doctoral Thesis:

The aim of presented thesis was to explore possibility of production of the β–glucan from carotenogenic yeast and some microalgae as unexplored sources.
The experimental part solves following goals:
1) Metabolic screening of yeast and microalgal strains (β–glucans, extracellular polymeric substances, intracellular lipids and pigments)
2) Optimization of culture conditions for β–glucans and other high–valuable compounds
3) Comparison of β–glucans production within the heterotrophs and autotrophs
4) Analysis of beta–glucans in commertial sources – higher fungi and cereals
5) Discussion

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Dean

ABSTRACT

Beta **(**β)–glucans are polysaccharides composed of D-glucose monomers. Nowadays, β-glucans are gaining attention due to their attractive immunomodulatory biological activities, which can be utilized in pharmaceutical or food supplementation industries. Nowadays, the only biotechnological yeast source is Saccharomyces cerevisiae. However, some carotenogenic *Basidiomycetes* yeasts, previously explored for lipid and carotenoids co-production, could potentially co-produce a significant amount of β–glucan.

In the present Doctoral Thesis, the culture conditions were optimized for the production of β-glucans and other microbial polysaccharides at carotenogenic yeast and microalgae. The genera of *Rhodotorula*, *Sporobolomyces*, *Cystofilobasidium*, and *Dioshegia* were tested. From non-carotenogenic yeast, the *Metschnikowia* genera and some ascomycetes yeast were selected together with some green and red algal strains. The experimental part is focused also on the possibility of co-production of other metabolites, like lipids, pigments, and extracellular polymers.

In the first part of the experiment, the effect of four C/N ratios (10:1, 40:1, 70:1 and 100:1) on the production of biomass, β-glucans, carotenoids, and lipids was evaluated et yeast strains. From all strains, the *S. cerevisiae* CCY 21-4-102, *C. infirmominiatum* CCY 17-18-4, *P. rhodozyma* CCY 77-1-1 and *R. kratochvilovae* CCY 20-2-26 exhibited the highest β-glucans production and were chosen for next analysis, mainly salt stress, temperature, and nitrogen sources in the culture medium. Additionally, *R. kratochvilovae* CCY 20-2-26 produces extracellular glycolipids and *S. pararoseus* CCY 19-9-6 extracellular polysaccharides. Next, the diversity of β-glucan content among the other twelve *Saccharomyces* strains and polysaccharides production at microalgae was assessed.

KEY WORDS:

β-glucan, polysaccharides, yeast, microalgae, lipids, carotenoids

ABSTRAKT

Beta-glukany jsou polysacharidy složeny z monomerů D-glukózy. V dnešní době se β-glukany těší zvýšené pozornosti zejména kvůli imunomodulační aktivitě a využitelnosti ve farmaceutickém a potravinařském průmyslu. *Saccharomyces cerevisiae* je dodnes jediným kvasinkovým zdrojem požívaným v biotechnologické produkci. Avšak některé kvasinky z oddělení *Basidiomycetes*, které jsou schopny produkce lipidů a karotenoidů, mohou být využity rovněž jako alternativní zdroj β-glukanů.

Dizertační práce se zabývá možností a optimalizací produkce β-glukanů a dalších mikrobiálních sacharidů u karotenogenních kvasinek a mikrořas. Testovány byli zástupci rodů *Rhodotorula, Sporobolomyces, Cystofilobasidium* a *Dioshegia*. Z nekarotenogenních kvasinek byly do screeningu zařazeny kvasinky rodu *Metschnikowia*, askomycetní kvasinky a z mikrořas zástupci zelených a červených řas. Experimentální část cílí rovněž na možnosti koprodukce dalších metabolitů, jako jsou lipidy, pigmenty a extracelulární polymery.

První část experimentu se zabývá vlivem čtyř C/N poměrů (10:1, 40:1, 70:1 a 100:1) na produkci biomasy, β-glukanů, karotenoidů a lipidů. Ze všech testovaných kmenů, *S. cerevisiae* CCY 21-4-102, *C. infirmominiatum* CCY 17-18-4, *P. rhodozyma* CCY 77-1-1 a *R. kratochvilovae* CCY 20-2-26 vykazovaly nejvyšší produkci β-glukanů a byly proto vybrány k podrobnější optimalizaci, zejména osmotického stresu, teploty a zdroje dusíku v kultivačním médiu. Dodatečně, kmen *R. kratochvilovae* CCY 20-2-26 je schopný produkce extracelulárních glykolipidů a *S. pararoseus* CCY 19-9-6 extracelulárních polysacharidů. Následně bylo stanoveno množství β-glukanů u dalších dvanácti kmenů *S. cerevisiae* a rovněž možnost produkce polysacharidů u mikrořas.

KLÍČOVÁ SLOVA

β-glukan, polysacharidy, kvasinky, mikrořasy, lipidy, karotenoidy

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DECLARATION

I declare that the doctoral thesis has been worked out by myself and that all the quotations from the used literary sources are accurate and complete. The content of the doctoral thesis is the property of the Faculty of Chemistry of Brno University of Technology and all commercial ure are allowed only if approved by both the supervisor and the dean of the Faculty of Chemistry, BUT.

…..……
student's signature

Poděkování:
Na tomto místě bych chtěla poděkovat hlavně vedoucí me dizertační práce, prof. RNDr. Ivaně Márové, CSc., za odborné vedení a cenné rady v průběhu celého doktorského studia. Stejně tak děkuji doc. Volze Shapaval za veškerou její ochotu a pomoc a i výzkumné skupině Biospektroskopie z Norwegian University of Life Sciences za vytvoření příjemného pracovního prostředí.
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1 INTRODUCTION

Yeast played an important role in human society as early as ancient Egypt and ancient Greece when they were an important component in the production of bread, or in alcoholic beverages, where they laid the foundation for the later production of wine and beer. Although fermentation processes have remained popular in today's society, these organisms are gaining in importance mainly in the pharmaceutical and molecular biology industries, which focus on research into metabolic pathways, gene expression, and genetic modification.

Occurrence is a ubiquitous organism, found mainly on sugary foods, such as fruit, and in the air or soil. Certain colonial representations are present in the human body, where diseases can lead to diseases (such as candidiasis).

"Red" yeast play an important role in this group concerning the presence of a metabolic pathway for lipophilic red-orange pigments, so-called carotenoids. These substances have strong antioxidant properties and, therefore they are used in cosmetics, pharmacy, or the food industry for food fortification. Other abundant red yeast metabolites are lipids and their possible use in the production of third-generation fuels. In this way, these organisms are also slowly but surely entering the field of environmental research.

Autophototrophic organisms are a very attractive group of microorganisms in terms of producing a wide range of metabolites only from light energy, carbon dioxide, water, and salts. In this way, the cost of the required culture medium is lower. The opposite of this positive is the low production of biomass, the need for lighting, and the need for special cultivation equipment. These requirements ultimately make the whole cultivation process more expensive.

However, important and known autotrophic organisms appear to be, their production of polysaccharides is still very little studied. In the efford of the largest possible accumulation of photosynthetic pigments or lipids, one of the most important metabolites of cellular metabolism has therefore been somehow “forgotten”.

2 THEORETICAL PART

Microscopic organisms, also known as microorganisms or microbes, occur all around us, and even in our bodies. This extensive category includes a wide range of organisms such as bacteria, fungi, viruses, algae, archea, and protozoa [1].

Classification according to metabolic abilities is not a method respecting the evolutionary relationships between individual microorganisms, but it provides us with information to understand the acquisition of energy and metabolite flows, which plays an important role especially in phototrophic organisms. The word *trof* comes from the Greek language and means feeding. Thus, autotrophs are "self-feeding" organisms that are using carbon dioxide as a carbon source, while heterotrophs receive it from organic compounds [1].

Microorganisms are classified into six categories: archea, bacteria, protozoa, algae, fungi, and some microscopic animals. Viruses are generally regarded as not living organisms. Each type has a characteristic cellular composition, morphology and reproduction, and have ability to produce a broad spectrum of high-value metabolites [1].

2.1 Fotoautotrophic microorganisms

2.1.1 Cyanobacteria

The precambrium is sometimes reffered to as the „age of green-blue algae". Schopf and Walter (1982) called the proterozoic (2.5 to 0.54 million years ago) the age of cyanobacteria [2]. At that time, the atmosplhere changed from anoxygenic to oxygenic as a result of photosynthesis which was provided by cyanobacteria [2].

The term of cyanobacteria refers to all prokaryotic microorganisms capable of oxygenic photosynthesis. Phylogenetically (based on the small subunit of the ribosomal 16S rRNA), it is a group belonging to the realm of gram-negative bacteria. The cyanobacterial pedigree also includes the chloroplast of eukaryotic cells and plants. According to Bergey's Manual of Systematic Bacteriology, classification into so-called subsections was introduced [2, 3]. Cyanobacteria from subsection I multiply by binary division, while representatives of subsection II are characterized by multiple division and creating a large number of small daughter cells. Filamentous cyanobacteria are classified in subsections III, IV and V. Subsections IV and V contain species that show cell differentiation, which is within prokaryotic organisms a rare phenomenon and also all representatives are able to fix nitrogen. They generally lack the possibility of sexual reproduction [2, 3].

Cyanobacteria are an important component in the nitrogen cycle as the main group of prokaryotes capable of binding air oxygen. Nitrogenase, the enzyme complex responsible for reducing nitrogen molecules to ammonium ion is irreversibly inactivated by molecular oxygen, a product of photosynthesis. Cyanobacteria have developed several strategies for this problem. The first is the separation of nitrogen fixation processes from oxygen photosynthesis; the second strategy is temporary separation - oxygen production by day and nitrogen fixation at the night [2].

Nitrogenase is localised in heterocysts, cells that have lost the ability of oxygen photosynthesis and carbon dioxide fixation but have retained photosystem I to supply the energy necessary for the nitrogen fixation process. There is also respiration and oxidative phosphorylation, so that fixation of nitrogen is not dependent on light. Heterocysts occur in subsections IV and V [2].

The color of cyanobacteria depends on the presence of whide range of photosynthetic pigments. The main dye in the cell is chlorophyll a, which occurs in the reaction centers of photosystem I and

II. Carotenoids (echinenone, myxoxanthophyll and others) are also present. With only a few exceptions, phycobilisomes are attached to thylakoid membranes - macromolecular complexes made up of phycobiliproteins, forming up to 40% of total soluble cellular proteins [2]. Allophycocyanine (absorption maximum 650 nm) is the core of the structure of phycobilisome, which is directly associated with the photosynthetic reaction center. Blue phycocyanin (620 nm), red phycoerythrin (565 nm) and rarely occurring phycoerythrocyanine (570 nm) fixed at the corners of the core. Some cyanobacteria may change their color depending on the spectral properties of light. This phenomenon is known as "complementary chromatic adaptation". The reason is a change in the production of individual phycobiliproteins. During cultivation in red light, blue phycocyanine is synthesized preferably, while in green light it is red phycoerythrin. The reason is better capture and use of light energy [2, 4, 5].

Cyanobacteria are aerobic microorganisms. However, some types (especially from subsection III) may live without oxygen. The cells have developed anoxygenic photosynthesis in this case, where the electron acceptor is a sulfide. Anaerobic lifestyles reflects also lipid metabolism, where there is a lack of polyunsaturated fatty acids whose biosynthesis requires oxygen [2].

Cyanobacteria have the ability to assimilate and store key nutrients and metabolites for later use. In addition, cytoplasmic inclusions such as granules of glycogen, lipids, cyanophycin (a non-ribosomal protein serving as a nitrogen source), storage polyphosphates and carboxysomes were found. Many of these substances are used in industry, such as polyhydroxyalkanoates, generated with increased availability of carbon substrate and nitrogen limitation. They can be used for the production of bioplastics or as biocompatible materials in biomedicine and biopharmacy. Polyphosphates are used in the production of pesticides, detergents and / or antibiotics [2, 6, 7].

In addition to the Calvin-Benson-Basham cycle, an alternative pathway for carbon dioxide assimilation in cyanobacteria is catalyzed by phosphoenolpyruvate carboxylase (PEP) [2]. The presence of two parallel carboxylation systems (RuBisCo and PEP) is another sector of research into a technology application for reducing CO2 in the atmosphere [2].

2.1.2 Algae

The term “algae” is not a formal taxonomic designation. It is a group of polyphyletic, oxygen-evolving photosynthetic organisms. They occur in microscopic form (0.2 - 2.0 μm, microalgae) up to 60 m (macroalgae) with a diverse structure ranging from unicellular to multicellular organisms that show similarity to higher plants, where different morphological cells can play different physiological roles [9]. Generally, algae as eukaryotic organisms belong to three kingdoms, Plants, Chromist, and Protozoa. Except the aquatic environment, they can also inhabit the mainland. They are present in unexpected places such as trees, fur coats, snow, hot lakes or they can be overgrown on desert stones. It forms a symbiosis with fungi, known as lichens [8, 9].

Algae have a wide range of reproductive processes. They undergo different types of division, from simple yeast-like cell division (binary division) to the production of four to thousands of daughter cells in a single cell cycle. For green algae, this is done by multiple divisions. In some algae species, both binary and multiple divisions are observed under different growth conditions [8].

Algal biomass (mainly marine algae) consisting of large content of polysaccharides which mainly serve as structural (e.g., cellulose, hemicellulose), storage molecules (e.g., agar, fucoidan, carrageenan, glucan), and mucopolysaccharides (e.g., porphyran).

2.1.2.1 *Chlorophyta*

Green algae are the most diverse group of algae (about 17 000 known species) range in the size from microscopic unicellular to large colonies and extensive filamentous growths. The green coloration is caused by presence of chlorophyll-*a* and chlorophyll-*b*. Another accessory pigments are *β*-carotene, lutein, antheraxanthin, violaxanthin, and neoxanthin [8, 9]. Beside these major pigments, small amounts of other carotenoids can be detected: *α*-carotene, cryptoxanthin, loroxanthin, pyrenoxanthin, lycopene and siphonein. Another shared characteristic is presence of starch as the main reserve polysaccharide deposited in the plastid [8].

Most of the algae have many metabolic pathways in common with plants. They are beneficial not only by converting large amounts of atmospheric CO_2 into O_2, they are also very valuable source of nutrients. They contain many antioxidants, in addition to the carotenoids and phenolic substances, some strains contain sulfo-derivatives of polysaccharides as well [10, 11]

For the cultivation of photoautotrophic organisms, such as microalgae, light regime is essential. Photosynthesis is a source of biomass, oxygen and energy for metabolism and growth of these organisms. However, the transmission of light through the cell suspension depends not only on the intensity of the light, but also on the intensity of mixing and cell density. The efficiency of photosynthesis is usually highest at lower irradiation [11, 12].

It is also proven that microalgae can be cultivated even under heterotrophic conditions, when they are able to produce many times more biomass and accumulate more carbohydrates (in phototrophic cultures accumulate more lipids) [13, 14]. However, passive diffusion through the plasma membrane is for hexoses sterically impossible. Hexoses thus penetrate the cell due to inducible active transport using a membrane protein [15, 16].

Currently, only a few microalgal strains are grown commertially. These strains are characterised by high growth rates, adaptability to unfavourable conditions and production of high amounts of the desired substances. These are, for example, genera like *Chlorella*, *Arthrospira*, *Dunaliella*, *Haematococcus* and *Nannochloropsis*. By far however, centrifugation is the most commonly used method for algal harvesting. Flocculation is also part of down-stream, when algal aggregates formed in the presence of aluminum or iron salts, chitosan, or by changing the pH. For use in the food industry, lyophilization follows (or another gentle drying method) [12, 17].

2.1.2.2 *Red algae (Rhodophyta)*

Most of the red algae consist of seaweed, but there are also free-living unicellular microalga within the classes Porphyridiophyceae, Rhodellophyceae, Stylonematophyceae, and extremophilic Cyanidiophyceae. Some unique features are specific for this phylum, like lack of a flagellated stage, the lack of centrioles, the presence of phycobiliproteins and the chlorophyll *a* is the only chlorophyll [18, 19]. Unicellular members of the Porphyridiales posses a high-molecular sulfated mucilaginous sheat as a cell wall, which consists mainly of xylose, glucose, and galactose and with another minor saccharide as mannose, methylated galactose, and pentose. The sulfated groups are being located on the glucose and galactose moieties. The external part of the polysaccharide dissolves continuously into the medium during cultivation and is therefore designated the “soluble polysaccharide”. Under sulfate starvation, cysteine is assimilating for incorporation sulfur into the cell-wall polysaccharide. Habitants of hot and/or acidic environments (Cyanidiophyceae) differ by the presence of a thick, highly proteinaceous cell wall. The storage molecule is the floridean starch, an α-1,4-glucan, deposited in the cytoplasm in contrast with starch grains produced in the Chlorophyta, which lie inside the chloroplast [20, 21].

The (exo)-polysaccharide production and biomass production is affected by environmental conditions such as the sulfate level in the culture medium. In industry, red seaweeds are used to produce hydrocolloids (alginate, agar and carrageenan), which can be used as thickening and gelling agents. Carrageenan is important product for cosmetics, food industry. The group of red algae known as coralline algae are able to secret calcium carbonate onto the surface of their cells. These corallines have been used in bone-replacement therapies [22, 23, 24, 25].

2.1.2.3 *Photosynthetic protozoa*

Euglena gracilis, firstly identified by the Dutch pioneer of microscopy Antoni van Leeuwenhoek in the 1660s, is a flagellated single-cell protozoan belonging to *Euglenozoa* phylum. Storage polymer of *Euglena* is a linear glucan consisting of β-1,3 glycosidic linkage polysaccharide with a helical substructure called paramylon. Paramylon is deposited at extraplastidial granules, which occur in all euglenoids but their number, size, and shape are strain-depended. The specific morphological feature is the presence of pellicle, cell-covering structures of membranes made up from protein which gives *Euglena* its contractility and flexibility [26, 27].

The linear β-glucan biosynthesis begins with the production of primer, followed by the transfer of the primer to a membrane-bound synthase. This enzyme catalyzes the latter repetitive transfer of glucosyl units from activated sugar donor to the primer (acceptor) until the polymerization stops. Many of the steps occurring through the biosynthesis of (1,3)-β-glucans and (1,3;1,6)-β-glucans in protozoa and chromista are nor well explored. A little is known about the regulation of chain production and its length, release of the chain in the cell wall, the primer biosynthesis, or even if the primer is essential for polymerization β-glucan chain. Some suggestions about the presence of two different organelles involved in paramylon synthesis were made and their contributions depend on nutrient conditions [28, 29, 30].

Catabolism of paramylon occurs as a response to environmental changes, such as the presence of ammonium ions, carbon starvation or high oxygen content. Conversely, hypoxic conditions lead to the conversion of paramylon into wax esters [31, 32].

The reason why so much attention is devoted to paramylon is its biological activity. As others β-glucans paramylon belongs to the group of pathogen-associated molecular patterns (PAMPS), molecules recognized by germline-encoded pattern recognition receptors (PRRs) in the cell surface, which is the base of innate immune system [33].

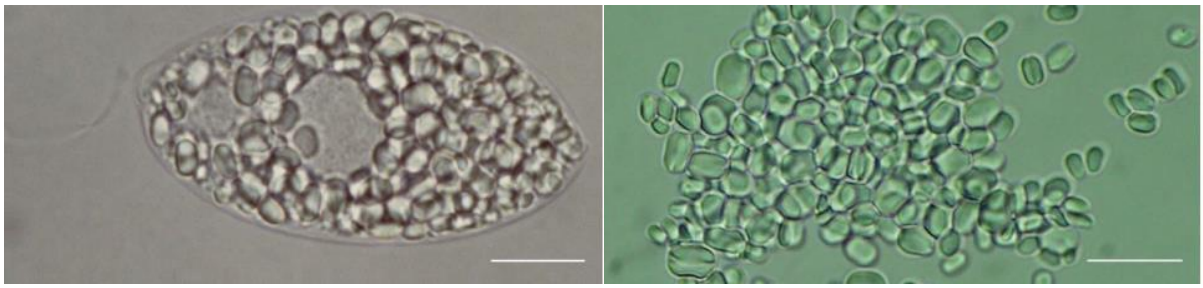


Fig 1: Paramylon granules inside cell Euglena gracilis nonphotosynthetic WSLZ mutant (left); granules dissolving in alkaline solution (right) [9]

2.1.3 Algal and cyanobacterial life strategy

The common sign of algae and cyanobasteria is photosynthesis – the cascade of chemical reactions beginned after the capture of light. Besides, there are other growth modes, presented in Table 1 [34].

Table 1: Growth modes of microalgae [34]

Growth mode	Energy source	Carbon source	Light requirements	Metabolism variability
Photoautotrophic	Light	Inorganic	Obligatory	No switch between sources
Heterotrophic	Organic	Organic	No requirements	Switch between sources
Photoheterotrophic	Light	Organic	Obligatory	Switch between sources
Mixotrophic	Light and organic	Inorganic and organic	No obligatory	Simultaneous utilization

2.1.3.1 The light dependent photosynthetic pathway

Photosynthesis is a biological process in which solar energy is captured and stored by a series of processes that transform the pure energy of light into the energy that drives life. Only a part of the emitted solar radiation is visible to our eye, it is the representation of wavelengths in the range from 400 to 700 nm. This area is known as photosynthetically active radiation (PAR) [35].

In eukaryotic photosynthetic cells, this process occurs in subcellular structures known as chloroplasts. This organelle contains chlorophyll pigments and, in most cases includes all major phases of photosynthetic processes. Chloroplasts contain internal membrane structures called thylakoids. The non-membrane internal environment of the chloroplast is called stroma, which contains soluble enzymes. In prokaryotic photosynthetic organisms, photosynthesis takes place on specialized membranes derived from cellular cytoplasm and the metabolism of carbon compounds is found in the cytoplasm, along with most other metabolic processes [35].

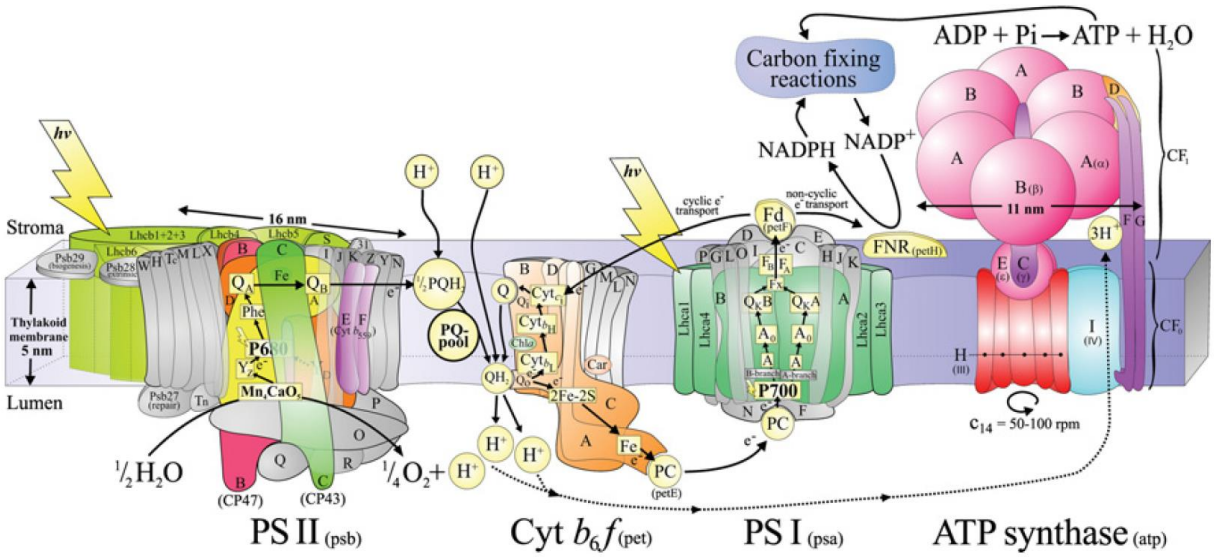


Fig. 2: Scheme of light-depended photosynthesis [9]

The main components of oxygen photosynthesis are two photochemical reaction centers that are working together in the so-called non-cyclic electron transport chain. These centers are referred to as photosystem I and II. Electrons are taken from the water by the photosystem II (Hill reaction), oxygen is produced and released as a "waste" product. Electrons from water are transported by the

quinone and cytochrome b6f complex to the photosystem I and the electron acceptor NADP + is reduced to NADPH upon absorption and transport of the second electron from the light. Protons (a remnant of water digestion) are transported through the membrane into the thylakoid lumen and form a protonmotive force that is used to produce ATP [35, 36].

2.1.3.2 The Calvin cycle

The Calvin cycle consumes the products of the light phase of photosynthesis, ATP, and NADPH and uses them to fix carbon dioxide to form a carbon skeleton. This cycle involves 11 enzymes catalyzing 13 reactions and is initiated by the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) [35, 37].

The whole process occurs in three phases: carboxylation, reduction, and regeneration. The carboxylation step produces 3-phosphoglycerate (PGA). The reduction phase uses NADPH and part of the APT to form 1,3-bisphosphoglycerate (BPG). The next step is the reduction of BPG to glyceraldehyde-3-phosphate (GAP) with NADPH consumption and catalysis by the enzyme NADPH-glyceraldehyde phosphate dehydrogenase. The regenerative phase of the Calvin-Benson cycle is a series of reactions where triose phosphates regenerate to the pentatonic sugar ribulose-5-phosphate (Ru5P). This reaction passes through a number of intermediates such as erythrose-4-phosphate, xylulose-5-phosphate, ribose-5-phosphate, fructose-1,6-bisphosphate, fructose-6-phosphate, sedoheptulose-1,6-bisphosphate and sedoheptulose -7-phosphate. The regeneration phase looks relatively complicated, but it is a necessary rearrangement of the carbon skeleton to form the final five-carbon product, which is necessary for later carboxylation. The final step of regeneration is the phosphorylation of ribulose-5-phosphate to ribulose-1,5-bisphosphate [35, 37].

Most reactions occurring in the Calvin cycle are identical to the metabolic reactions of non-photosynthetic cells, including gluconeogenesis and the oxidative pentose cycle. Two unique reactions are carboxylation and the final regeneration of RuBP [35].

2.1.3.3 Algal heterotrophical growth

Microalgae are generally considered as photoautotrophic microorganisms, however some species developed heterotrophic metabolism in the lightless conditions and the presence of external carbon sources. Reasons to why some microalgae are obligate photoautotrophs are the lack of transporters, incomplete pathways or the absence of an enzymatic reaction in the central carbon metabolism. Neverheless, the benefits of heterotrophic life are significant. In general, energy storage molecules, such as lipids and carbohydrates (starch and glycogen) are accumulated under heterotrophic and mixotrophic conditions; therefore, the content in biomass of these compounds is higher than under photo-autotrophic conditions [34, 38].

The utilization of carbon sources (acetate, pyruvate, C6 sugars, C5 monosaccharides, disaccharides, saturated fatty acids and amino acids) is controled by carrier and transduction systems located in the cytoplasmic membrane. Once the molecule enter the cell, it is activated through phosphorylation. The glucose (the most commonly used carbon source for heterotrophic cultivation of microalgae), or any other related carbohydrate, is metabolized through the oxidative pentose phosphate (PPP) and the Embden–Meyerhof–Parnas (EMP) pathways. Without light, glucose is metabolised predominantly via the PPP pathway, while the EMP is the main pathway in the presence of light. These pathways take place in cytosol [38, 39].

The microalgal and cyanobacterial species capable of heterotrophic cultivation are *Chlorella vulgaris*, *Chlorella saccharophila*, *Chlorella sorokiniana*, *Chlorella zofingiensis*, *Crypthecodinium cohnii*, *Euglena gracilis*, *Schizochytrium sp*, *Nitzschia leavis.*, *Galdieria*

sulphuraria, *Haematococcus pluvialis*, *Spirulina platensis*, and *Dunaliella sp* producing lipids (polyunsaturated fatty acids, biodiesel), phycocyanin and carotenoids [38, 39].

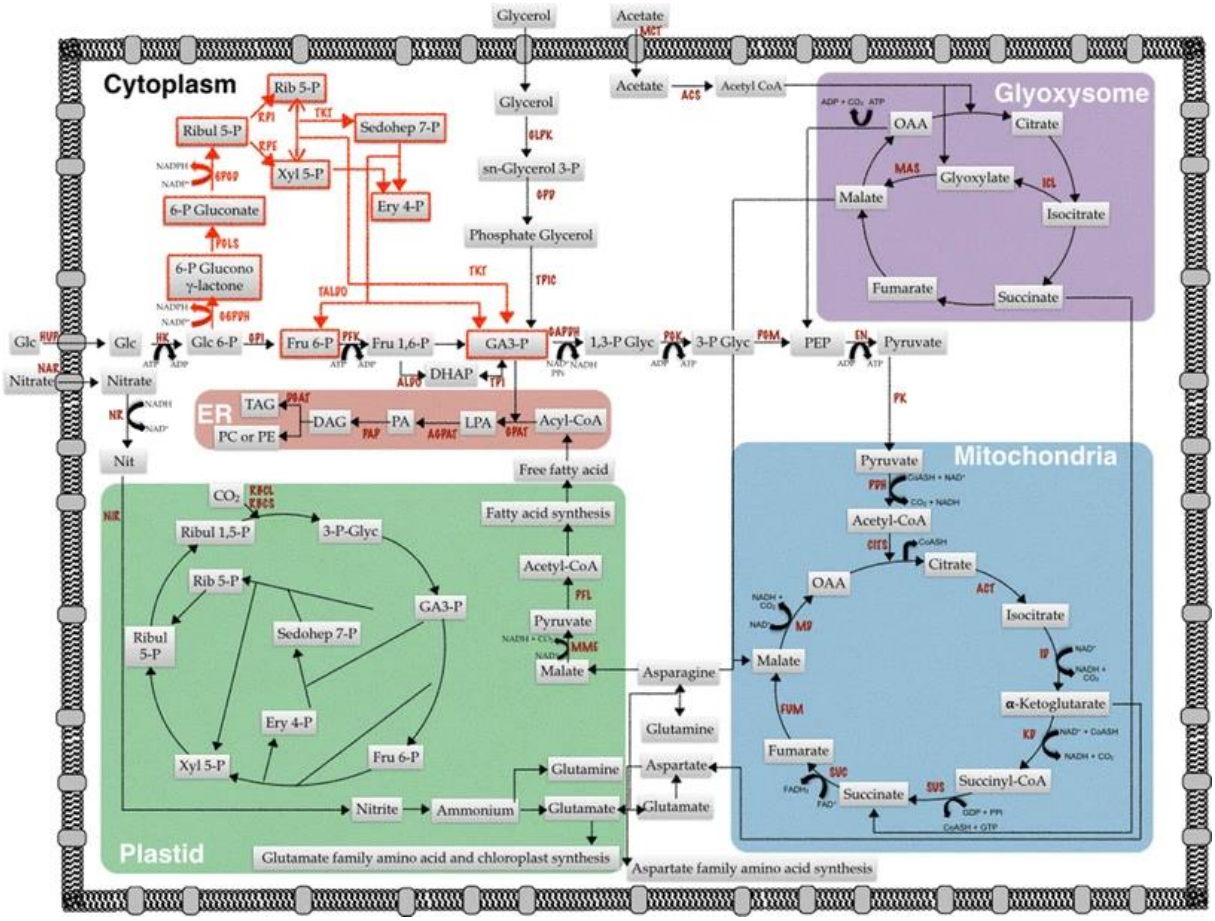


Fig. 3: The metabolic pathways demonstrated to occur in heterotrophic microalgae [38]

2.2 Yeast

Although most of the fungi exist in the form of multicellular organisms, several species have appropriated a unicellular way of life. These organisms are called yeasts [40].

In nature, it is the predominant organism that capable to utilize simple carbohydrates. They also include important industrial organisms, pathogens, and serve as models in the scientific world for understanding the functioning of eukaryotic cells as well. For decades, the *Saccharomyces cerevisiae*, a baker's yeast, has been one of the best characterized microorganisms in the fields of genetics, biochemistry and physiology and it is a leader in the sequencing of eukaryotic genomes. Generally, compared to fungi, several hundreds yeast species have been described so far but they present only a small part of the yeast diversity on our planet [40].

Within the Ascomycota, two yeast lines are well studied. The first is *Saccharomycetes*, which includes *S. cerevisiae*, and the second is *Schizosaccharomycetes*, where *Schizosaccharomyces pombe* takes its place. Several known yeast species are found also in the Basidiomycota division. In addition to vegetative division and budding, yeasts are capable of sexual reproduction by endospores (ascospores) or exospores (basidiospores) [40].

As heterotrophic organisms, they require energy intake from a carbon source, which are mainly carbohydrates (but may also be amino acids, purines and pyrimidines, fatty acid intake has not yet

been fully characterized). For the utilization of monohexoses, mainly glucose and fructose, there are special hexose transporters (HXT). In *S. cerevisiae* this transport is ensured by facilitated diffusion. Yeast also contains proteins to detect substrate levels (Snf3p is a low glucose sensor and Rgt2p is high). Using a given transporter based on substrate concentration detection, various regulatory mechanisms are provided to ensure transcription of the genes for the transporter. Transporters operating at low concentrations and require energy [40, 41].

The sugars must be broken down into smaller parts within the cell in order to convert them into building blocks of other molecules. The major metabolic pathway of carbohydrate oxidation is glycolysis, a series of glucose decomposition reactions up to two pyruvate molecules accompanied by ATP and NADH production without the participation of molecular oxygen. Some yeasts are capable of converting pyruvate to ethanol, the first step being cytosolic decarboxylation to acetaldehyde and CO_2 by the pyruvate decarboxylase enzyme and then reduced to ethanol with the participation of alcohol dehydrogenase. This pathway also occurs in the presence of oxygen, when pyruvate is formed to a high extent and thus switches to anaerobic metabolism. This phenomenon is known as the Crabtree effect [41].

The application of stress conditions to the yeast cell produces specific metabolites. Some species accumulate carotenoids, probably as a protection against intense blue or UV radiation. Some of them accumulate trehalose and glycogen as storage substances in increased amounts. Protection against increased osmotic pressure is provided by a solid cell wall in many yeast species. The biggest advantage of yeast is the utilization of cheap carbon sources and waste materials as well as the short generation time [42].

2.2.1 *Saccharomyces* genera

Despite its low diversity (containing only seven species), the genus Saccharomyces is one of the most technologically important genera [43]. These yeasts are among the best researched microorganisms and are undoubtedly the most economically important MO group used in industry. The amount of yeast produced annually by brewing and distilling technology processes is in the order of millions of tonnes [44]. *S. cerevisiae* is considered as Generally Recognized as Safe (GRAS) microorganism.

The name of the genus, Saccharomyces, was introduced in 1838 by Meyen. *S. cerevisiae* was one of the first yeast discovered. However, it was not until 1870 that Rees defined this genus. *Saccharomyces* is characterised by a vegetative method of reproduction through a multilateral budding. Exceptionally, pseudomycellia are formed, vegetatively growing cells are predominantly diploid or polyploid. Sexual reproduction leading to the formation of ascospores is less common. This genus is fermentative, but also capable of respiration [45]. An interesting phenomenon in this yeast is the ability of diauxia, which is manifested in the presence of glucose in the culture medium. During the growth and consumption of the carbon substrate, ethanol is accumulated, which is then consumed for subsequent growth after glucose depletion. This ability is also observed in other yeasts capable of producing ethanol under aerobic conditions. Species of this genus are able to ferment most sugars. However, they never use lactose as a carbon source or nitrate as a nitrogen source [46].

The most important species of the genus Saccharomyces is the yeast *Saccharomyces cerevisiae* [44]. It is also known as a bakery, distillery or brewery yeast. *Saccharomyces cerevisiae* is a leading industrial yeast mainly used in food production and beverage fermentation [47].

2.2.2 Carotenogenic (red) yeast

Only limited number of non-photosynthetic microorganisms can naturally produce carotenoids, which are protective agents against UV radiation, to block certain cell-damaging wavelengths of light, and oxidative stress. Besides of pigmented bacteria and moulds, a group of naturally red-coloured yeast gained increasing attention due to its outstanding carotenoid and high lipid productivity, and the capability to utilize cheap feedstocks.

They belong to*Basidiomycota* phylum, into division *Erythrobasidium* (subdivision *Pucciniomycotina*), *Sporidiobolales, Cystofilobasidiales,* and *Tremellales* (subdivision *Agaricomycotina*). The most known genera are *Rhodotorula, Sporidiobolus, Sporobolomyces, Rhodosporidium, Phaffia* a *Cystofilobasidium* [48].

"Red" yeasts are known mainly for their ability to accumulate pigments, so-called carotenoids. These include torulene, torularhodin, β-carotene, and astaxanthin, which show cell colonies in various shades from orange to red. Other accumulated metabolites include lipids, ergosterol, coenzymes Q10, and Q9 and others. Carotenogenic yeasts can be found in salt and freshwater, on plants or associated with animals [49, 50].

2.2.2.1 *Rhodotorula* genera

The genus *Rhodotorula* is a strictly aerobic yeast capable of producing glycogen during the exponential phase, and lipids and carotenoids in the stationary phase. This genus falls under the division *Basidiomycota*, subdivision *Pucciniomycotina*. Ellipsoidal, multipolar budding cells of 5 μm in size produce pseudohyphae and may also have sexual reproduction. It occurs in the air, soil, and phyllosphere, additionally in milk and dairy products [52].

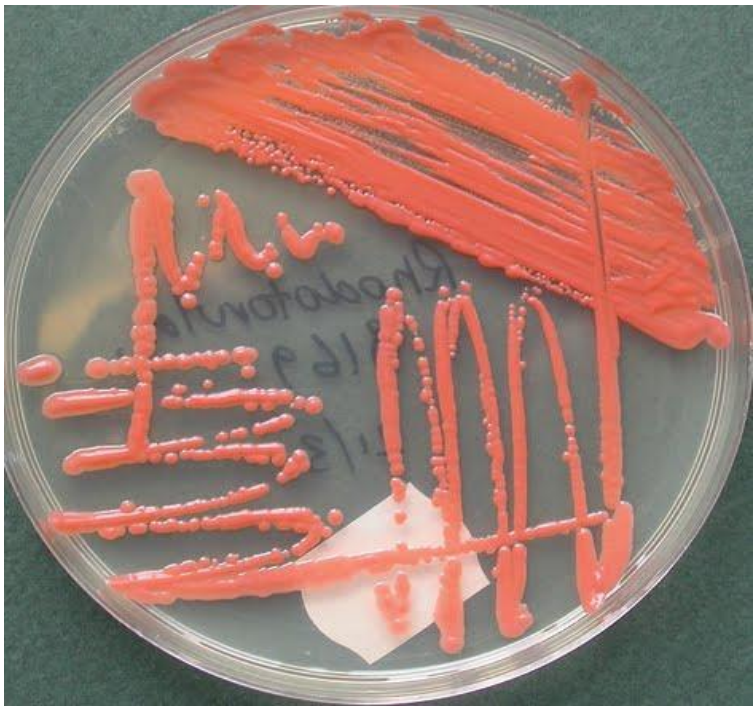


Fig. 4: Yeast *Rhodotorula mucilaginosa* [51]

Rhodotorula produces urease and has no ability to assimilate inositol and ferment sugar. Some of its species are the main producers of carotenoids with a dominant synthesis of β-carotene, torulene, and torularhodine. They are also a great source of protein, lipids, and vitamins. The best-

known representative is *Rhodotorula glutinis*, which is known for its production of lipids in the form of triacylglycerols (TAG). By applying sodium chloride (1-5%) or hydrogen peroxide to the culture, phenolic compounds (gallic acid, benzoic acid, catechin, caffeic acid, ferulic acid) accumulate. Other species of this genus are *R. mucilaginosa*, *R. minuta*, and extremophilic *R. psychrofila*, *R. glacialis* [52, 53].

Some of the *Rhodotorula* species are capable of unusual pseudomycelium forming, which can have a possible association with a gastrointestinal disorder [54].

2.2.2.2 *Cystofilobasidium* genera

The best-known representatives of this genus include *Cystofilobasidium capitatum*, *Cystofilobasidium infirmominiatum*, and *Cystofilobasidium bisporidii*. The cells form colonies with a glossy surface, their shape is rounded to elongate. This genus is unicellular and unseptic, forming the so-called holobasidium. The application of oxidative stress results in the formation of oxidized torulene derivatives as the majority of pigments, namely 16‘-hydroxytorulene in *C. capitatum* and *C. infirmominiatum*, and in the formation of torularhodinaldehyde in other species [55, 56].

2.2.2.3 *Sporobolomyces* genera

This anamorph belongs to the subdivision *Pucciniomycotina*, order *Sporidiobolales*. The cells are strongly elongated, ellipsoidal, producing false and true hyphae and asymmetric ballistacoids. This genus produces an increased amound of coenzymes, namely Q10 and Q(H_2), and is also characterized by strong carotenogenesis. The best-known species of this genus are *Sporobolomyces roseus* and *Sporobolomyces salmonicolor* [57, 58].

2.3 Microbial polysaccharides

Over the last three decades, the microbial polysaccharides have been widely applied in food, chemical and pharmaceutical industries. Althrough the low cost of traditional market-dominant polysaccharides, such as cellulose and cornstarch, microbial-derived PS show the great diversity in structural and functional properties. Besides, microbial sources are favoured due to their fast and high yielding production procedures under controlled conditions. Some of these polysaccharides (e.g. starch, glycogen) are located within the cell and serve as storage compounds. Conversely, exopolysaccharides or capsular polysaccharides are synthetized mainly for protective purposes, either as a general physical barrier, or to prevent dehydratation [59, 60, 61].

Table 2: Examples of microbial polysaccharides for commercial use [59, 60, 61]

Polysaccharide	Producing microorganisms	Applications
Alginate	*Pseudomonas aeruginosa* *Azobacter vinelandii*	Thickening and gelling agend in food industry. Ion-exchange agent. Alginate beads – immobilization of cells and enzymes.
Beta-glucan	*Saccharomyces cerevisiae* *bacterial*	Food supplements.
Curdlan	*Alcaligenes faecalis*	Gelling agent in cooked foods. Immobilization of enzymes.

Polysaccharide	Producing microorganisms	Applications
Dextran	*Leuconostoc mesenteroides* *Acetobacter sp.* *Streptococcus mutans*	Blood plasma expander. Prevention of thrombosis. Wound dressing (as adsorbent) Foodstuff.
Emulsan	*Acinetobacter calcoaceticus* *Arthrobacter sp.*	Enhanced recovery in oil industry. Cleaning of oil spills.
Gellan	*Pseudomonas elodea*	Thickner and solidifying agent in food industry.
Hyaluronan	*Streptococcus equi*	Applications in medicine, cosmetics and speciality foods
Levan	*Halomonas eurihalina* *Zymomonas mobilis*	Cosmetics and food industry.
Pullulan	*Aureobasidium pullulans*	Food coating and packaging.
Scleroglucan	*Sclerotium glucanicum* *S. rolfsii, S. delphinii*	Stabilizing latex paints, printing inks, and drilling muds.
Xanthan	*Xanthomonas campestris*	Food additive for stabilization, gelling and viscosity control. Enhanced oil recovery in oil industry. Preparation of toothpastes, and water-based paints.

2.3.1 Fungal cell wall

The cell wall is the outermost part of the cell that provides both physical protection from its environment and osmotic support. Fungal walls normally make up 10-30% of the biomass and consists of layers of about equal amounts of mannoproteins, glucan and a small part of chitin (1-2 %). The biosynthesis of the cell wall is tight regulated and depends on growth and morphogenesis of the cell [62, 63]. Before of their incorporation to the cell wall, the mannoproteins (40 %) become heavily glycosylated on *N*- and *O*-glycosylation sides when going through the secretory pathway. They can be connected directly to the "spain" of the cell wall, 1,3-β-glucan via alkali-sensitive binding (Pir cell wall proteins) or linked covalently through 1,6-β-glucan to 1,3-β-glucan (the glycosylphosphatidylinositol-dependent cell wall proteins) [63].

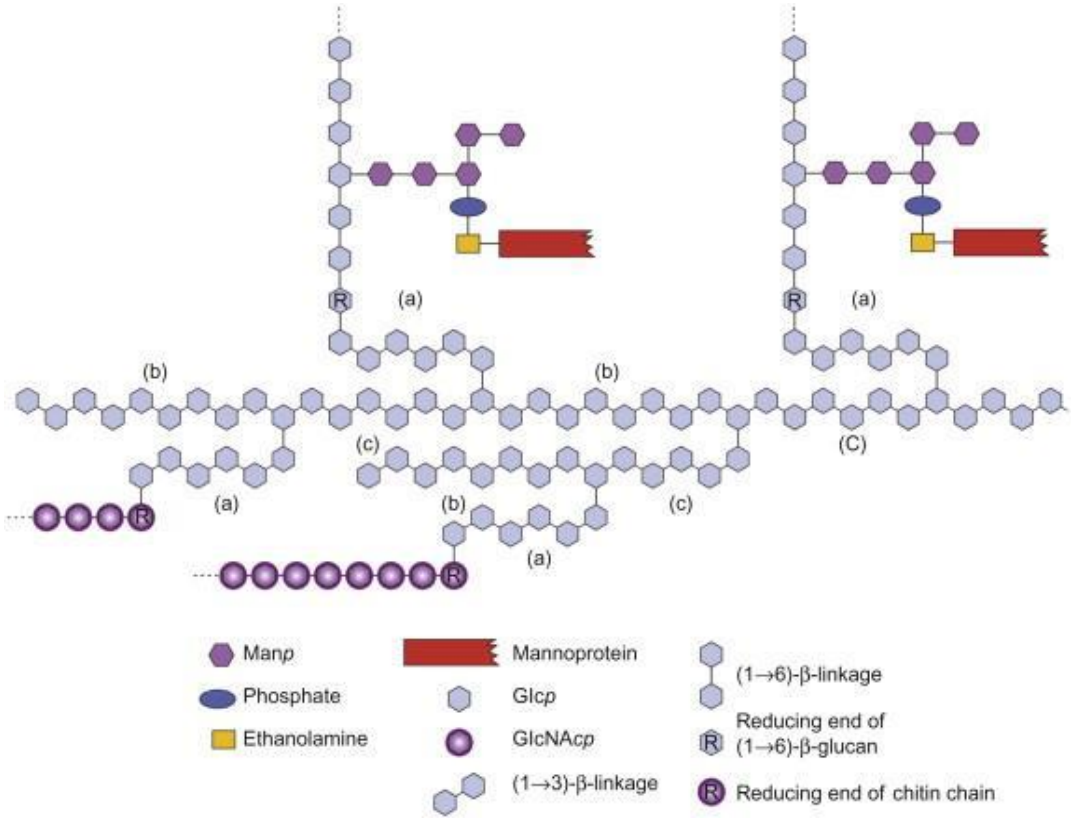


Figure 5: Composition of the cell wall of *S. cerevisiae* [62, 63]

Cell wall precursors are polymerized on the cell surface and then modified and interconnected [62, 63]. Beta-glucan synthase is the key membrane-located enzyme for beta-glucan biosynthesis since it is catalyzing the production of 1,3-β-glucan chain from UDP-glucose as a sugar precursor. This enzyme is composed of two subunits: membrane-bonded catalytic subunit and GTP-bonding regulatory subunit. It is not clear which cell protein represses the catalytic subunit, the most likely it could be Fks1p, Fks2p (catalytic proteins), or Rho1p (regulatory protein from Rho GTPase family) [32]. The enzyme geranylgeranyltransferase I (GGTase I) plays an important role in the regulation of glucan synthase since it connects the geranylgeranyl group to Rho1p in the prenylation process. Thus, prenylated protein can be anchored in the cell membrane [62].

Increased glucan production also occurs during sporulation. Diploid yeast cells enter the meiotic cycle with nutrient limitation and form haploid spores. Each spore is equiped by a special cell wall, which has a protective function against damage from the external environment. The wall contains four layers: two inner layers of β-glucan and mannan (same as for vegetative cells), the outer one composed of chitosan and a dityrosine polymer (both polymers specific for spores) [62].

In vegetative cells (1,6) -β-glucan forms 12% of cell wall polysaccharides. While (1,3) -β-glucan forms the "backbone" of the wall, (1,6) -β-polymer is a branched amorphous structure that acts as an adhesive for other building blocks of the cell wall. Despite efforts to identify the gene responsible for the production of the catalytic enzyme for the synthesis of this polysaccharide, it has not yet been identified yet. The synthesis of (1,6) -β-glucan appears to be found on the plasma membrane, but several endoplasmic reticula and Golgi apparatus proteins also perform a (for now unknown) function [62, 77].

2.3.1.1 Beta-glucan

Beta (β)-glucans represent a widespread group of polysaccharides, consisting of D-glucose monomers linked through β-glycosidic bonds. Beta-glucans of different origin are present in different amounts and structural modifications. For example, beta-glucans of oat and barley are linear unbranched with 1,3 and 1,4-β-linkages [64, 65], beta-glucans of higher fungi and yeasts consist of 1,3-β-linked glucopyranosyl residues with small numbers of 1,6-β-linked branches 1,3- and 1,6-bonded beta-glucans [66], while beta-glucans from bacterial origin consist of cyclic 1,2-β-linkages [67]. Paramylon, callose or curdlan represent the simplest, unbranched linear chains of (1,3)-β-glucans [63]. They are also other bond-types, mostelly in the position C(O)6, and mainly in the polysaccharides like chromistan and fungal laminarin. Cyclic β-glucans occure at *Bradyrhizobium japonicum* [63]. Currently, the commercial sources of beta-glucans are higher fungi (*Rhynchelytrum repens, Lentinus edodes, Grifola frondosa, Tremella mesenterica, Tremella aurantia, Zea may, Agaricus blazei, Phellinus baummi, Pleurotus* spp.) [68], and plants barley and oat, which have been extensively used in food, feed, pharmaceutical or cosmetics industries.

According to a report by BCC Research the global beta-glucan market is expected to reach 576.28 million USD by 2025, growing at a GAGR of 7.3% from 2017 to 2025 [69]. The increasing demand for immunostimulatory polysaccharide requires investigating more yielding new sources applicable in the biotechnological industry. The baker's yeast *Saccharomyces cerevisiae* (SC) is the main microbial biotechnological source of beta-glucan due to its GRAS label. Its content in the cell wall can reach up to 50 %.

2.3.1.2 Biological activity of beta-glucan

Due to the anti-oxidative, antitumor, anti-inflammatory, immunostimulatory and antimicrobial activities, beta-glucans belong to biological response modifiers (BRMs) having beneficial effects on human and animal health [65]. Inclusion of beta-glucans in the diet can regulate blood sugar level that is crucial in the treatment of diabetes and associated with its complications [70, 71]. Besides, they have the potential to be explored as a vaccine adjuvant delivery platform [72].

Molecular weight determines the biological activity of beta-glucans. Short chain molecules (5,000 – 10,000 Da) are not active in general. Conversely, high-molecular glucans are biologically more active. With addition, other properties like the degree of branching, solubility or modification by different functional groups. Furthermore, these properties also affect the degree of branching, solubility and/or modification by various functional groups [62, 63].

Carbohydrates of this type are suitable immunostimulatory biomarkers of the non-specific immune system of vertebrates and invertebrates. Such microbial structures, which are essential for their survival and are therefore invariant, belong to PAMPs (Pathogen Associated Molecular Patterns). Recognition of PAMPs by so-called "Pattern Recognition Receptors" (PRRs), macrophage surface receptors and dendritic cells, elicits an immune response that induces phagocytosis and later cell destruction [62, 63].

At vertebrates, (1,3)-β-glucans are recognized through cell surface receptors. These receptors were first identified in 1980, when many other cells were described, including neutrophils, macrophages, NK-cells (natural killer), and others [63]. These cells recognize (1,3)-β-glucans through their surface receptors, which are mainly lactosylceramide, scavenger receptors, complement receptor 3 (CR3), Dectin-1, and Toll-like receptor (TLR) [63].

Dectin-1 is a lectin that consists of four components, one of which is the extracellular polysaccharides binding domain. This receptor specifically recognizes β- (1,3) - (1,6) -glucans from fungi, plants, and bacteria, which, after binding, produce phagocytosis, ROS production, and

cytokine production. It does not show activity in the presence of α-(1,4)-glucans and α-mannans [63, 73].

The complement receptor 3 is an integrin composed of two non-covalently linked chains, CD11b and CD18. It is present on immune system cells such as monocytes, macrophages, dendritic cells, neutrophils, eosinophils, NK cells, and some T- and B-cells. Whether CR3 binds to beta-glucan directly, was not established. However, its activation triggers cellular signaling, resulting in the induction of various cellular responses, including adhesion, cytotoxicity, and phagocytosis [63, 73, 74].

The role of beta-glucans is also to improve the transport of macrophages to the site of injury, thereby accelerating wound healing. Other positive effects are a reduction in blood cholesterol levels [75, 76, 78].

2.3.2 Intracellular polysaccharides of yeast

Glycogen and trehalose are two glucose storage carbohydrates in a yeast cell. The variation of these two components in their cellular amount depending on environmental changes indicates their metabolic control by a complex regulatory system [79, 80].

The budding yeast *Saccharomyces cerevisiae* accumulates two types of glucose storage carbohydrates, namely glycogen, and trehalose. Glycogen is a high molecular branched polysaccharide formed by a linear α- (1,4) -glucose chain with α- (1,6) branching. Trehalose is a non-reducing disaccharide formed by glucose monomers linked by an α- (1,1) bond. Depending on the growth conditions, there is a different production of these two storage substances [80].

2.3.2.1 Glycogen

As with other high molecular weight biopolymers, glycogen synthesis requires initiation, elongation, and branching steps. The initiation step is provided by a protein called glycogenin, which has autoglycosylation activity and forms short α (1,4) -glucose chains from UDP-glucose (UPD-Glc) covalently attached to tyrosine residues. This protein was "originally" identified as a 38 kDa "contaminant" in the purification of glycogen synthase from muscle [80].

Glp1p differs from Glp2p in size (67 kDa vs. 43 kDa). All eukaryotic glycogenins show a high affinity for UDP-Glc and exist in vivo as oligomers of unknown stoichiometry. Unlike mammalian glycogenins, yeast ones are equipped with multiple Tyr residues that are required for sustained glycogen accumulation and have COOH-terminal domains that interact with yeast glycogen synthase. Truncation of this domain reduces glycogen synthase activity and severely disrupts glycogen accumulation. Surprisingly, increased expression of the GLG gene does not lead to an increase in glycogen in the yeast cell, leading to the hypothesis that the yeast glycogenins are in molar excess relative to glycogen synthase, or that these proteins may be reused. However, this repeated use would require the presence of other enzymes to release glycogenin from the growing glucosyl chain, but such an enzyme has not yet been reported [80].

The group Torija et al. (2005) report in their research that glycogen synthesis is possible even in the absence of glycogenin [42]. No glycogenin-like protein has yet been found, so it was a mystery how glycogen biosynthesis occurs in bacteria. The team of Ugalda et al. demonstrated that the de novo synthesis of this polysaccharide in *Agrobacterium tumefaciensis* is initiated directly by glycogen synthase, which catalyzes both the autoglycosylation and elongation processes. A similar conclusion was reached by Toriji researchers with a mutant strain of *S. cerevisiae* (defective Glg1p and Glg2p) with hyperactive glycogen synthase [80, 81].

Glycogen synthase catalyzes the formation of α (1,4) -glycosidic bonds from UDP-Glc to the non-reducing end of the linear α- (1,4) -chain of glycogen. In the yeast *S. cerevisiae*, there are two isoforms I and II, which are 80% identical and 50% similar to mammals. Glycogen synthase is an allosteric enzyme, which is activated by Glc6P and reversible covalent phosphorylation that occurs at the COOH terminus [80].

The same as in mammalial cells, glycogen degradation occurs in yeast by α-glucosidase catalysis and glucose production as a result.

The second possibility how to degrade glycogen is sequential reactions, which includes phosphorolysis and linearization, where ultimately the release of glucose-1-P and glucose occurs. Yeast glycogen phosphorylase releases glucose-1-P by cleaving α-(1,4)-glycosidic bonds and the branching enzyme transfers maltosyl or maltotriozyl units to the adjacent linear α-(1,4) chain to produce glucose by cleaving the remaining α-(1,6) binding, allowing glycogen phosphorylase to continue its function. This enzyme is sensitive to glucose concentration and is non-competitively inhibited by glucose-6-phosphate [80].

2.3.2.2 Trehalose

Trehalose is synthesised in two steps. In the first of UDP-glucose and glucose-6-P, trehalose-6-P is formed, which is subsequently dephosphorylated to trehalose and phosphate (Pi). This process is catalyzed by a protein complex composed of four different subunits, encoded by TSP1, TSP2, TSL1, and TPS3. The smallest 56-kDa subunit of the protein complex (encoded by the TSP1 gene) is Tre6P synthase. In contrast, loss of TSP2 function in temperature-sensitive strains led to hyperaccumulation of Tre6P, confirming its dephosphorylation function and pointing to the key role of trehalose in cell stress responses and the effect of Tre6P in glycolysis. TSL1 and TPS3 are homologous genes whose resulting proteins (Tsl1p and Tsp3p) do not interact with each other, on the contrary, they interact with Tsl1p and Tsl2p, and these resulting two complexes subsequently combine. Tps3p and Tsl1p also serve as a stabilizer of the resulting protein complex. Also, the increased amount of Tre6P during heat shock suggests that the Tps1p subunit has a complex-independent function and that Tre6P may be dephosphorylated by other (non-specific) phosphatases [80, 82].

The activity of the Tre6P synthase complex shows a high affinity for Glc6P and UDP-Glc, which is 3-10 times higher than the total concentration in the cell. This enzyme is also non-competitively inhibited by Pi, whereas fructose-6-P acts as a potential activator. Unlike other enzymatic systems involved in the metabolism of storage polysaccharides, the trehalose synthase complex does not undergo reversible phosphorylation. This complex is also activated by elevated temperature with an optimum at 42 – 45 ^{o}C [80].

Hydrolysis by neutral (Nth1p, Nth2p) and acidic (Ath1p) trehalase

Most yeasts and fungi produce two types of trehalase with hydrolytic activity. The first, most likely vacuolar, shows optimal function at pH 4.5 - 5.0, the second requires a neutral environment (pH 6.8 - 7.0), which indicates a cytosolic occurrence. The results of the cloning of trehalase genes revealed that only neutral trehalase exhibits an N-terminal extension that contains a phosphorylation regulatory domain. PAK is the only protein kinase that directly phosphorylates and activates Nth1p (unfortunately, no protein phosphatase has been found that dephosphorylates the phosphorylated form of Nth1p) [80].

2.3.2.3 Fuctions and conditions for glycogen and trehalose accumulation

Glycogen and trehalose are typical markers of yeast cell adaptation to environmental changes. Increasing the concentration of ethanol in the medium leads to the accumulation of the substrate UDP-glucose and glucose-6-phosphate, which are necessary for Tre6P synthase, and thus, lead to the overproduction of trehalose. In contrast, sodium chloride (0.3-0.5 M) or hydrogen peroxide (0.4-1.0 mM) result in low or no synthesis of glycogen and trehalose. Elevated temperature (38^{o} C) has a positive effect on the biosynthesis of these metabolites. However, glycogen production is already inhibited above 40 ° C, and trehalose is present to a high extent [80, 83].

Glycogen is a storage polysaccharide that accumulates in the presence of nutrients in the medium during diauxic yeast growth when glucose is still present in the medium and is consumed in the stationary phase when the nutrients are already depleted. Trehalose does not correspond exactly to this concept, as it is synthesized in the second stage of growth in the presence of yeast-produced ethanol and is consumed much later under non-stress conditions [80].

The high concentration of trehalose protects the cell from autolysis and increases its chances of surviving under adverse conditions. Trehalose protects the cell from drying out. Synergies with chaperones are involved in protein assembly, and in addition, when cells are excessively hydrated, it secretes water from the surface of the proteins and, thus, protects them from denaturation. This disaccharide and chaperone Hsp104 have been shown to be essential for refolding proteins in the cytosol as well as for repairing poorly assembled glycoproteins due to heat shock in the lumen of the endoplasmic reticulum [80].

2.3.3 Algal cell wall

Algal cells are covered by extracellular matrix (ECM, cell wall) that consists of diverse neutral and charged polysaccharides and proteins. The major fibrillar component of the cell walls of plants and many algal taxa is cellulose, the 1,4-β-glucan polymer. Cellulose is often synthetized in the plasma membrane and incorporated into the cell wall, creating microfibrils of diverse amount, layers and orientation. Many other algal taxa produce microfibrillar wall from 1,3-β-xylans, 1,4-β-mannans and heteropolymeric fibrillar polysaccharides. Another important component of the cell walls are hemicelluloses (xylans, xyloglucans, mannans) and pectins. The composition of extracellular matrix of diverse algal groups is in the Table 3 [8].

Table 3: Composition of extracellular coverings of algal group [8]

Algal group	Components of extracellular coverings
Chlorophyta	Cellulose, mannans, xyloglucans, xylans, 1,3-β-glucans, mixed linkage glucans, xylogalactorhamnans, rhamnoxylogalactogalacturonans, arabinogalactan proteins
Rhodophyta	Cellulose, xylans, mannans, sulphated mixed linkage glucans, sulphated galactans
Phaeophyta	Cellulose, alginates, fucoidans
Haptophyta	Acidic polysaccharides, proteins, calcite
Dinophyta	Cellulose
Bacillariophyta	Mannose and 1,3- β-glucans, multiple proteins, silica

The cell wall of cyanobacteria, unlike unicellular algae, does not contain cellulose and is thus better digested by humans. It consists of lipoproteins and a solid peptidoglycan (murein). Murein forms a network structure here, just like in the cell walls of bacteria. This network structure is formed by N-acetylglucosamine and acetylmuramic acid, which are linked via (1 → 4) -β glycosidic bonds.

The surface of cells is also often covered with glycocalyx (mucous sheath formed by lipopolysaccharides) [8].

2.3.4 Storage polysaccharides of microalgae

Carbon fixation in the Calvin-Benson cycle is a process of the production of two forms of stored carbon. The first is starch, which is synthesized and stored in the chloroplast. Another form is sucrose, which occurs in the cytoplasm. In both cases, triose phosphate is a necessary starting point for conversion [35].

During the day, starch is synthesized, which forms deposits in the form of starch granules in the chloroplast. Its consumption is initiated at night, where it is used as a carbon source for chloroplast processes, and therefore does not represent a long-term storage product, but rather its intermediate [35, 84, 85].

Starch synthesis is based on triose phosphate, where ribulose-1,5-bisphosphate is regenerated, through fructose-1,6-bisphosphate and fructose-6-phosphate (F6P). Isomerization of F6P produces glucose-6-phosphate (G6P) by the enzyme hexose phosphate isomerase, and G6P is converted to glucose-1-phosphate (G1P) by phosphoglucomutase. G1P is activated by ATP by the enzyme ADP-glucose phosphorylase to form the product ADP-glucose and pyrophosphate. Finally, ADP-glucose forms bonds (1,4) of the growing starch chain by the enzyme synthase [35].

For sucrose synthesis, triose phosphate is transported from the chloroplast using a triose-phosphate/inorganic phosphate translocator. This protein catalyzes the exchange of triose phosphate for inorganic phosphate (Pi). The initial reactions are the same as for starch, with the difference in the occurrence of these enzymes in the cytosol (isoform enzymes). G1P is also activated by uridine triphosphate (UTP) to produce UDP-glucose. This molecule condenses with F6P to form sucrose-6-phosphate and catalyzed by the enzyme sucrose phosphate synthase. The phosphate is then hydrolyzed by phosphatase to produce the final product [35].

Triose phosphate has three possible fates: starch synthesis, sucrose synthesis, and ribulose-1,5-bisphosphate regeneration. The balance between these pathways is controlled by a complex mechanism. If the triose phosphate is formed, it served only to form starch and sucrose. Then the Calvin-Benson cycle intermediate would be depleted and the entire path would collapse [35]. Starch synthesis is regulated by the primary carboxylation product of Rubisco and PGA as well as inorganic phosphate concentrations. The regulation of sucrose synthesis takes place in two steps: in the conversion of fructose-1,6-bisphosphate to fructose-6-phosphate and the condensation of F6P with UDP-glucose. FBPase is inhibited by fructose-2,6-bisphosphate. Sucrose phosphate synthase, in turn, phosphate and G6P, which function as allosteric effectors [35].

Polysaccharides of red algae, the oldest division of lower plants, differed by the polysaccharide composition of their cell walls and the intercellular matrix. Most of these algal contain sulfated galactans, carrageenans and agars, which are produced industrially and can be applied as gelling or/and stabilizing agents. A unique of the red algal monosaccharide components is 3,6-anhydrogalactose. The high-molecular storage product of red algae is known as floridean starch, which is biosynthesized and placed in the cytosol, that real starch is formed and stored in plastids. Enzymatic formation of both glycogen and floridean starch uses the same precursors, UDPG, rather then ADPG, which is the starting material for starch biosynthesis [35].

2.3.4.1 Beta-glucans in algae and cyanobacteria

In research into the production of beta-glucans by microalgae, not much has been published, except for *Euglena gracilis*. Single-cell *Chlamydomonas reinhardtii* is used for the biosynthesis and

analysis of this polysaccharide in microalgae, although its cell wall consists almost exclusively of hydroxyproline-rich glycoproteins. Polysaccharides in photoautotrophic microorganisms are preferably studied in brown seaweed, which produces alginate, agar, or carrageenan and where their products can reach up to 74% dry matter [78, 79].

The best-known representative of beta-glucans in this case is paramylon, produced by the protozoan *Euglena gracilis*, where it forms monomorphic grains in the cytoplasm. It is a linear unbranched β- (1,3) -glucan chain of high molecular weight. Under heterotrophic conditions, this microorganism can synthesize up to 95% of paramylon on dry matter and is thus competitive with *Saccharomyces cerevisiae*. The biosynthetic pathway here is based on the synthesis of an initiation primer on a membrane-bound synthase, and then the attachment of activated glucose (UDP-glucose) results in a chain formation. So far, the pathway is very poorly understood and remains the subject of research [63]

2.3.5 Extracellular polymeric substances

Extracellular polymeric substances are high molecular weight natural polymers that are secreted into the environment by microorganisms. These are different categories of organic molecules, such as polysaccharides, proteins, nucleic acids, (phospho) lipids, and others [86].

2.3.5.1 Exopolysaccharides

Microalgae and cyanobacteria possess a complex carbohydrate metabolic pathway that includes the ability to synthesize intracellular monosaccharides, polymeric glucan storage, and structurally complex exopolysaccharides (EPS). EPS are synthesized in phototrophic organisms according to strictly regulated and energy-intensive processes. They can be adhered to the cell or released into the medium (in the form of mucus). In the case of adhesion, they form specific envelopes and capsules, so-called polysaccharide layers covalently connected to the cell surface [8, 2].

Depending on the properties of the secreted polymers, such as molecular weight, degree of branching, and conformation, they may exhibit unique biological activity. Glucose, galactose, and fucose predominate here, but rhamnose and arabinose can also be found to a lesser extent [8].

The main parameter influencing EPS production in phototrophic organisms is the intensity of illumination. The higher it is, the greater the production of polysaccharides. In algae and cyanobacteria, carbohydrate synthesis occurs in the chloroplast and/or cytosol during the Calvin cycle, consumed by NADPH and ATP, where electrons and protons are generated during the cleavage of water in photosynthetic reactions. In the Calvin cycle, carbon dioxide is assimilated by ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). This enzyme carboxylates 2 molecules of 3-phosphoglycerate. In microalgae, the synthesis of polysaccharides and their sulfation takes place in the Golgi apparatus (GA) [8].

Yeast exopolysaccharides are a very little studied group of metabolites. For the time being, their industrial production is not feasible in terms of low yield. To date, enzymes and biosynthetic pathways, as well as regulatory mechanisms, have not been described [87].

In general, the optimal conditions for the yeast exopolysaccharides biosynthesis are strain depended. In most research paper, the yeast requires strong oxygenation and low temperature of the cultivation. The medium should contain suitable carbon source (sucrose, glucose), inorganic nitrogen source (ammonium sulphate) and the C/N ratio of 15:1. Chemically, the yeast exopolysaccharides are linear mannans, pullulan, glucooligosaccharides, galactooligosaccharides and other heteropolysaccharides containing α-1,2; α-1,3; α-1,6; β-1,3, and β-1,4 bonds [2, 8].

2.3.5.2 Extracellular glycolipids

Biosurfactants represent a broad spectrum of biomolecules containing both, polar and non-polar part. Glycolipids are glycosides of fatty acids containing one or more monosaccharide residues. They can be produced by some yeast, bacteria, and mycelial fungi, where the best known extracellular glycolipids are rhamnolipids, mannosylerythritol lipids, sophorolipids, cellobiose lipids, and trehalose lipids. They differ by structural diversity, biological activities with conection to broad spectrum of application in cosmetology, medicine or agriculture. In comparison to chemical surfactants, biosurfactants possess higher biodegradability and lower toxicity [88, 89].

Among carotenogenic yeast, the genus of Rhodotorula have been reported to be potential of extracellular polyol esters of fatty acids (PEFA) producers (specifically *Rhodotorula babjevae*, *Rhodotorula taiwanensis*, *Rhodotorula aff. paludigena*). *Rhodotorula's* glycolipids consist of hydroxy fatty acids and sugar alcohols (D-arabitol, D-mannitol, (R)-3-hydroxymyristate, (R)-3-hydroxypalmitate, and (R)-3-hydroxystearate) [90]

2.4 Biosynthesis of lipidic metabolites

2.4.1 Biochemistry of lipids accumulation

Fatty acids biosynthesis is one of the primary metabolic pathways and take place in various organelles such endoplazmatic reticulum, Golgi apparatus, mitochondria, peroxisomes or in chloroplasts at autophototrophic organisms. Plastid *de novo* synthesis requires substrate in form of acetyl-coenzyme A, energy (ATP) and reductive cofactors (NADPH) the same as the army of proteins (enzymes) [8].

Acetyl-CoA is the main precursor for fatty acids (FA) synthesis, and, for algae, it is derived in Calvin cycle from glyceraldehyde-3-phosphate catalyzed by PK in the plastid. The addition of acetate accelerates the synthesis of fatty acids and then triacylglycerols (TAG). Another source of carbon can be carbohydrates, in green algae it is mainly starch, which is the reason for the intensive accumulation of TAG (oils) [8].

The synthesis of FA in the plastid stroma is initiated by acetyl-CoA carboxylase requiring acetyl-CoA and bicarbonate. This enzyme is localized in the plastid and requires a cofactor, which is biotin, for its proper function. Acetyl-CoA carboxylase catalyzes the initiation and regulatory step of FA synthesis - the conversion of acetyl-CoA to malonyl-CoA by ATP. Before entering the fatty acid synthase (FAS) complex into the plastid, malonyl-CoA is converted to malonyl-ACP by malonyl-CoA: acyl-carrier protein (ACP). Malonyl-ACP condenses with additional acetyl-CoA, where the dicarbon unit is transferred from malonyl-ACP to the growing acyl chain, forming acyl-ACP. FA synthesis is ensured by stromal FAS type II. Each dicarbon elongation involves four enzymatic steps: condensation, reduction, dehydration, and a second reduction, consuming two molecules of NADPH and releasing one molecule of CO_2 from malonyl-ACP [8, 91, 92].

The condensation reaction is catalyzed by β-ketoacyl-ACP synthase (KAS), which forms C-C bonds by Claisen-type condensation. This intermediate is then reduced with β-ketoacyl-ACP reductase (KAR) with NADPH consumption. The enzyme β-hydroxyacyl-ACP dehydratase (HAD) generates enoyl-ACP, which is subsequently reduced by enoyl-ACP-reductase (ENR) - this enzyme can use NADH and NADPH to form saturated acyl-ACP. The main product of FAS in plastid is C16 and C18 saturated acyl-ACP, 18: 0-ACP is gradually desaturated by stearoyl-ACP desaturase (SAD) in the chloroplast stroma [8].

The synthesis of FA in plastid is terminated by acyl-ACP thioesterase (TE), which produces ACP and free FA from the acyl-ACP complex [8].

Part of FA remains in plastids and is utilized in the prokaryotic pathway for glycerolipid formation, in eukaryotes, they are transported to the ER. The FA carbon chain can be metabolized to various types of products, such as glycerolipids, triacylglycerides, phospholipids, and glycolipids. Triacylglycerols are stored as an energy source in yeasts and algae, while they do not accumulate in prokaryotes (only actinomycetes and a few other bacterial species) [8].

The glycerol-3-phosphate pathway (GPAT) located in the ER serves for the biosynthesis of polar lipids and TAG, consuming glycerol-3-phosphate as a starting material produced outside plastids from dihydroxyacetone phosphate or glycerol by glycerol kinase. The de novo pathway for TAG biosynthesis in the ER, also known as the Kennedy pathway, involves additional steps initiated by glycerol-3-phosphate acyltransferase involving glycerol-3-phosphate and acyl-CoA citation. Microalgae and yeast store TAG in special subcellular organelles, so-called lipid droplets (LD). Their work has not yet been clarified, so far there are several theories and speculations. The most probable mechanism of formation is the accumulation of TAG in the ER bilayer, followed by enlargement of the lipid body and its transport into the cytoplasm [8, 41, 91].

Green algae consist mainly from C16:0 and C18:1 fatty acids. In cyanobacteria, C16 and C18 predominate, from PUFAs it is linoleic acid, α-linolenic acid, and γ-linolenic acid. In yeast, the most common FA are oleic acid (C18:1), palmitoleic acid (C16:1), palmitic acid (C16:0), stearic acid (C18:0) and small amounts of myristic acid (C14:0) [8, 41, 92].

The oleaginous microorganisms are considered as significant lipids producers (more than 20% of DCW). For efficient lipid production two features need to be present, namely acetyl-CoA and NADPH. In the process of nitrogen starvation, protein and nucleic acid syntheses are reduced, but carbon excess continues to be metabolized to lipid. First, the activity of AMP deaminase (AMPD) is upregulated for cleaving the AMP to inosine monophosphate (IMP) and ammonia ions to provide substitute nitrogen source. The declined AMP content in the cell causes the reduction of NAD+ (NADP+)-dependent isocitrate dehydrogenase (ICDH) activity, thus isocitrate (isomerized to citrate by aconitase) accumulates in mitochondria and is transported into the cytoplasm in exchange for malate (citrate/malate translocase, CMT). In the cytoplasm, ATP citrate lyase splits citrate molecule into acetyl-CoA (source for lipid *de novo* biosynthesis) and oxaloacetate, converted by malate dehydrogenase (MDH) and exported to mitochondria *via* CMT. For the synthesis of 1 mol of C18 fatty acid, 16 mol of NADPH is required. These molecules can be produced by transhydrogenase cycle, where malic enzyme (ME) has been proposed as a mojor route for the NADPH production. However, in some yeast species (*Y. lipolityca*), ME is localized in mitochondria and does not have the acces to cytosol-located citrate, and, thus, the expression of this enzyme is not changed upon limitation by N-source. It was founded that another source of NADPH comes from pentose phosphate pathway, generated by glucose-6-phosphate dehydrogenase and 6-phosphogluconate, or adational by cytosolic $NADP^+$-dependent isocitrate dehydrogenase (some eukaryotic organisms) [93, 94, 95, 96].

Not only nitrogen, but phosphorus, iron and sulfur can promote the conversion carbon substrate into lipids. At *R. toruloides* Pi (anorganic phosphorus) limitation facilitates RNA degradation, TAG biosynthesis, and Pi-associated metabolism up-regulation while ribosome biosynthesis id down regulatet, the same as citrate cycle by dephophorylation of adenosine monophosphate. NADPH is then produced by endogenous malic enzyme (the flux through the pentose pathway is limited). Moreover, there are different types of fatty acid synthases. One is sensitive to nitrogen depletion and the second to oxygen [97].

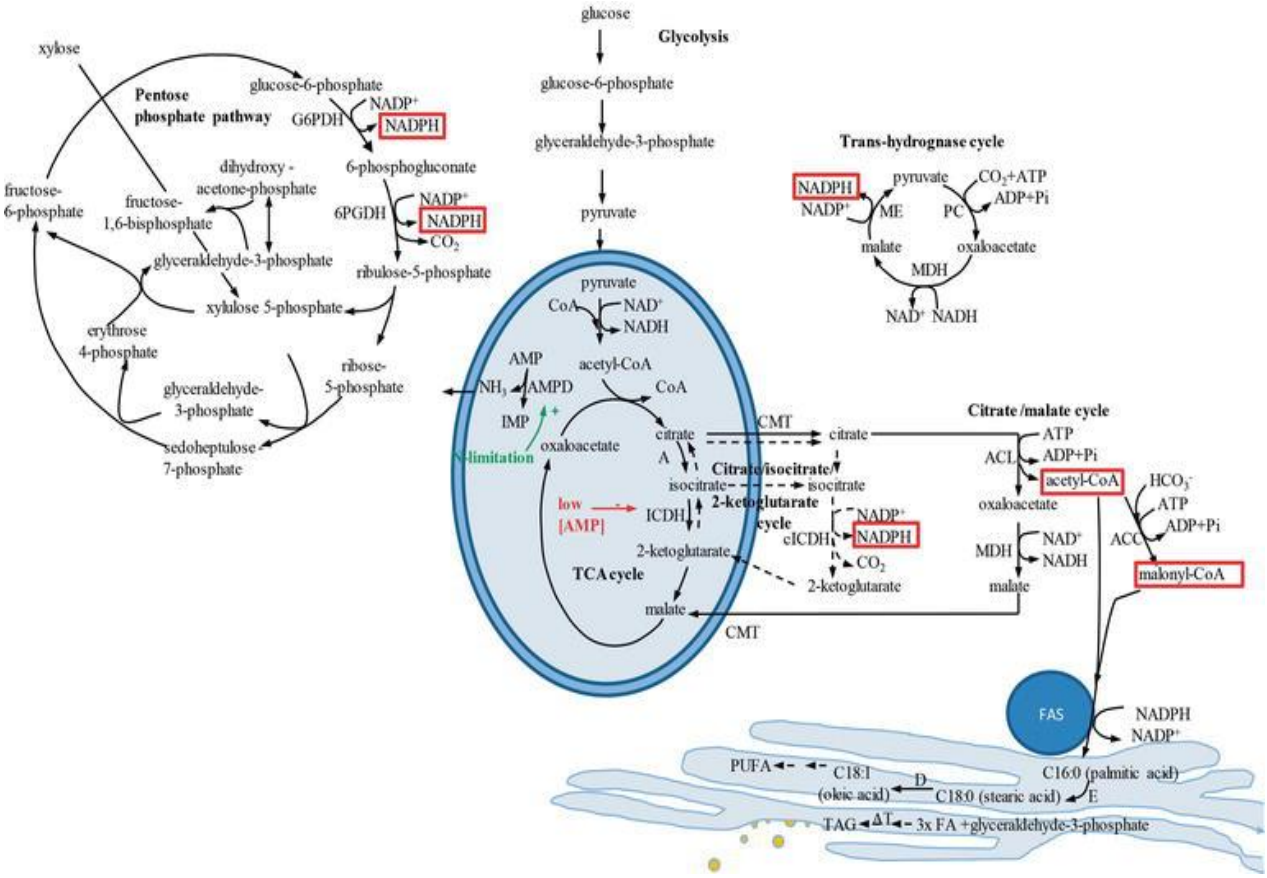


Fig. 6: Pathways of lipids accumulation [98]

2.4.2 Biochemical pathways of isoprenoid compounds

Sterols are the end products of the cyclization of the 30-carbon isoprenoid 2,3-oxidosqualene. Their functions in the cell are structural and signaling. Their biosynthesis is localized in the endoplasmic reticulum and then enriches the plasma membrane, where they increase the permeability of membrane barrier and are therefore important in maintaining membrane potential. On the other hand, steryl esters serve to store metabolic energy in the form of fatty acid esters and are stored in intracellular lipid bodies. In yeast, the main sterols are ergosterol, lanosterol, episterol, zymosterol, and phytosterol. The first step of biosynthesis is catalyzed by acetoacetyl-CoA-thiolase, which condenses two molecules of acetyl-CoA and subsequent reaction with another molecule of acetyl-CoA produces 3-hydroxy-3-methylglutaryl-CoA (MHG-CoA). The latter product is an important regulatory point in yeast, as it is then reduced to mevalonate by HMG-CoA reductase, or this enzyme can be re-inhibited by ergosterol [8, 41].

The subsequent cascade of phosphorylation and decarboxylation of mevalonate results in the formation of isopentenyl pyrophosphate (IPP), which is a precursor for the synthesis of isoprenoids in the so-called mevalonate pathway. Isomerization of IPP to form dimethylallyl pyrophosphate (DMAPP) and condensation reactions lead to the formation of squalene. Squalene undergoes epoxidation and cyclization reactions to form lanosterol. The demethylation, desaturation, and reduction reactions produce zymosterol, which occurs in all eukaryotic cells. Methylation of zymosterol at the C-24 position produces fycosterol, which occurs only in yeast and other fungi. The transfer of double bonds and their subsequent removal produces ergosterol [8, 41].

In algae, sterol biosynthesis produces molecules with 26-31 carbon atoms and with structural variation involving 4,4-dimethyl substituents, 4-methyl substituents, or no methyl group at the C4 position [8].

In the case of cyanobacteria, only a small amount of sterols was always isolated, which gave rise to the theory that these isolates always came from a contaminating microorganism (yeast or other fungi). All other research confirms the inability of cyanobacteria to synthesize sterols, only a few eubacteria can produce this metabolite with an unalkylated side chain [8].

Other metabolites derived from the isoprenoid pathway are carotenoids. These molecules are synthesized by all kinds of photosynthetic bacteria, plants, algae, but also by some fungi. Animals cannot produce them, but they can take them and metabolize them from food [8].

These orange-red pigments consist of a 40-carbon skeleton. Usually, the ends are cycled, but there are also open-ended carotenoids. If only carbon and hydrogen are present in the molecule, it is a group called carotenes. On the contrary, xanthophylls are referred to as carotenoids containing oxygen in their structure [8].

Their synthesis is similar the synthetic pathway to squalene production. The synthesis of isopentenyl diphosphate has long been thought to lead from acetyl-CoA through mevalonate. This pathway occurs in a wide variety of algae but is surpassed by the biosynthetic pathway based on 2-methyl-erythritol phosphate. This mentioned pathway occurs in all studied species of algae (see Fig. 5) [8].

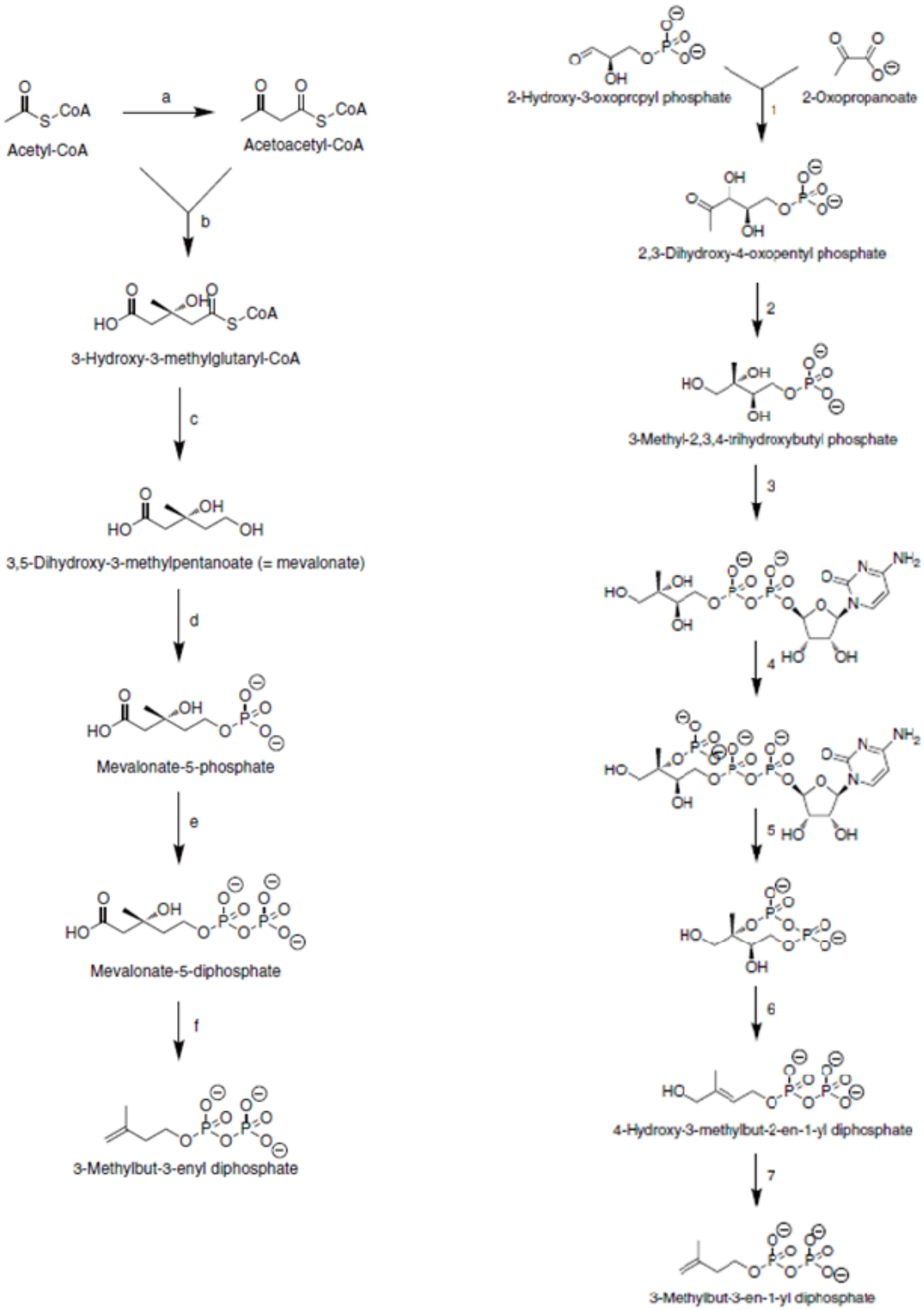


Fig. 7: Synthesis of isopenthenyl diphosphate by mevanolate erythritol phosphate pathway [8]

The isoprenoid unit, isopentenyl diphosphate, isomerized to dimethylallyl diphosphate, followed by condensation of these molecules to form geranyl diphosphate. The addition of another isopentenyl unit forms farnesyl diphosphate and repeating this step leads to the production of geranylgeranyl phosphate. Two molecules of geranylgeranyl diphosphate condense "head to head" to form the first carotenoid, phytoene. This metabolite is isomerized and desaturated to the lycopene, one or both ends of which are then cyclized to give β, β-carotene, β, ε-carotene and ε, ε-

carotene. The synthesis of subsequent secondary carotenoids depends mostly on environmental factors, such as lighting, salinity, or lack of nutrients [8, 50].

In yeast, γ-carotene is the main precursor for the synthesis of β-carotene and torulene. Hydroxylation and oxidation of torulene with bifunctional oxidase produces torularhodine. The biosynthesis of astaxanthin is relatively complicated and leads through several intermediates such as β-zeacarotene, torulene, and astaxanthin [8, 50].

There is a connection between the biosynthesis of carotenoids and lipids, which can be deduced from the common biosynthetic precursor acetyl-CoA. Increased accumulation of carotenoids occurs mainly during glucose deprivation. In the yeast *Rhodotorula glutinis*, beta-carotene is mainly synthesized in the presence of glucose. Glucose depletion at a lower C/N ratio (20: 1, 50: 1) leads to increased accumulation of thorularodine. A higher C/N ratio (70: 1, 100: 1) leads to increased lipid production, but carotenogenesis is suspended. Besides, glucose consumption was linked to a rapid decrease in oleic acid levels in the total intracellular lipids. The kinetic analysis indicated a correlation between oleic acid levels in total lipids and torularhodin accumulation in the cells. The results may suggest that acetyl-CoA formed from oleic acid degradation is metabolized through the mevalonate/isoprenoid/carotenoid pathways directly to torularhodin [8, 41].

The main function of carotenoids in algae is the absorption of light energy and its transfer to chlorophylls and photosynthesis. In connection with light capture, it is also necessary to mention their protective function against high radiation and their antioxidant properties, which protect the cell from reactive singlet oxygen and other radicals [8].

Within microorganisms, it is also possible to distinguish the different proportions of carotenoids produced. In yeast, the dominant carotenoids are β-carotene, torulene, thorularodine, and lycopene. Cyanobacteria are characterized by unique glycosidic carotenoids, oscillatoriaxanthin, and myxoxanthophyl. However, there are also more typical carotenoids, such as beta-carotene, present mainly in low light. Myxoxanthophyll is the dominant form when cultivated under elevated radiation. In green algae, lutein is dominant, together with a minority of β, β-carotene, β, ε-carotene and with a variable proportion of zeaxanthin, antheraxanthin, violaxanthin, and neoxanthine. The main differences in the pigmentation of green and red algae are not carotenoids, but billiproteins (phycoerythrin) [8, 41].

Some species of algae can accumulate large amounts of a specific carotenoid under stress conditions. For the halotolerant alga *Dunaliella salina*, it is beta-carotene, for the alga *Haematococcus pluvialis* it is astaxanthin, where it is largely present in an esterified form with fatty acids. The advantage, in this case, is the yeast *Phaffia rhodozyma* whose astaxanthin produced is not subject to esterification [8]. For the above reasons, photoautotrophic organisms, despite their lower biomass yield, find their important role as a biotechnological producer of carotenoids [8].

2.5 Glucan analysis

Structural characterization of polysaccharides represents a challenging task due to the complexity of the molecules in terms of glycosidic bonds, branching degree, molecular weight and monosaccharide composition. Polysaccharides within the microbial cells are mostly bounded with other cellular molecules by covalent or non-covalent bonds, making the isolation of pure native samples the first crucial step for their further analysis [99].

2.5.1 Structural analysis of glucans

Isolation of glucans from microbial matrices requires some intervention to remove unwanted components such as proteins, lipids, polyphenols as well as other (poly) carbohydrates. The purity and structural characterization of the isolated material are essential components to verify the extraction efficiency. Modern chemical, spectroscopic and separation methods are widely used to solve this problem. The elemental analysis gives us information about the elemental composition of the native sample. Size-exclusion chromatography, laser light scattering, and viscosimetry provide information on homogeneity, molecular weight distribution and branching. The monosaccharide composition is carried out by total hydrolysis of the sample under strongly acidic conditions and optimal temperature, followed by chromatographic separation of the released simple sugars. FTIR and NMR are used for purity analysis. [99, 100].

2.5.1.1 Basic analysis

There are several basic analyzes to assess the purity of a glucan preparation. The amounts of total and/or reducing carbohydrates are mostly used for these cases. In general, reducing saccharides are those with unsubstituted aldo- or keto groups at the C-1 or C-2 terminus. For polysaccharides consisting of aldoses (or the corresponding uronic acid), the unsubstituted C-1 tail is responsible for the "reducing" properties of the polysaccharide. The reducing groups react with various chemical agents to form a precipitate or colour complex that can be quantified. In the case of glucans, total carbohydrates can only be determined after total hydrolysis, when all glucose is reducing. Total and/or reducing sugars can be determined by various methods including titration, gravimetry or photometry: eg total carbohydrates using anthrone or phenol-sulfuric acid; a Fehling reducing carbohydrate, Folin-Wu or Somogyi-Nelson method, or a method using 3,5-dinitrosalicylic acid, p-hydroxybenzoic acid-hydrazine, or 2,2'-bicinchoninate [99, 100].
Starch or amylose-like α-D-glucans, which often occur in soluble fractions, can be determined by a colorimetric iodine test. Chitin and similar parts of the insoluble cell wall material can be determined based on the amount of D-glucosamine as the product of total hydrolysis by a colorimetric or chromatographic method. Uronic acids (UA) may occur to a minor extent in heteroglucans or as part of other polysaccharides in a fungal glucan preparation. Usually, UAs are quantified colorimetrically, using a carbazole or m-hydroxybiphenyl method in concentrated sulfuric acid; the second compound is much more selective for UA than for neutral sugars. Chitin can also be determined by deacetylation and gas-liquid chromatography of free acetic acid. Zamani et al described a novel method for the simultaneous determination of glucosamine and N-acetylglucosamine in fungal cell wall citation preparations. It is based on two-step hydrolysis in concentrated and diluted sulfuric acid at low and high temperatures, followed by degradation by nitrous acid. In this process, chitin and chitosan are converted to anhydromannose (corresponding to the sum of glucosamine and N-acetylglucosamine) and acetic acid (only from N-acetylglucosamine). This method is suitable for the determination of the chitin deacetylation degree in fungal glucan-chitin complexes. Proteins integrated with the polysaccharide cell wall are mostly analyzed by the Kjeldahl method or by colorimetric methods such as the Lowry method, the biuret method and the Bradford method [99, 100, 101].

2.5.1.2 Monosaccharidic composition

The composition of the monosaccharides is mostly analyzed after total hydrolysis under strongly acidic conditions and high temperature, followed by chromatographic separation of the resulting monosaccharides. Total hydrolysis of neutral polysaccharides, including glucans, can be performed

with formic, sulfuric or trifluoroacetic acid (mostly 2 mol/L) at 110 °C for several hours. The hydrolyzate after neutralization and dilution can be directly analyzed by column chromatography (CC), high-performance ion-exchange chromatography (HPAEC). Gas chromatography (GC) reduces the monosaccharides ($NaBH_4$, $AlLiH_4$) and then acetylation (acetic anhydride in pyridine) or silylation (a mixture of N, O-bis (trimethylsilyl) -trifluoroacetamide and trimethylchlorosilane) is applied. The resulting sugars are detected as alditol acetates or methylsilanes [100].

Hydrochloric acid (3-6 mol/L, 100-110 ^oC) is used for the hydrolysis of chitin or chitosan cell wall components followed by quantification of the released D-glucosamine. The N-acetyl group is likely to play a catalytic role in depolymerization, whereas positively charged amino groups may inhibit this process. During acid hydrolysis, depolymerization and deacetylation occur simultaneously [99, 101].

2.5.2 Vibrational spectroscopy

FTIR spectroscopy is a frequently used method for polysaccharide analysis. It is a sensitive method for determining the anomeric configuration and position of glycosidic linkages (Figure 4) [51]. So-called "sugar region" of FTIR spectra (1200-950 cm^{-1}, CO and CC vibration of glycosidic bonds and in pyranoid rings), as well as another "anomeric region" (950-750 cm^{-1}, complex skeletal vibrations), exhibit significant differences between alpha- and beta-D-glucans with different structures [51].

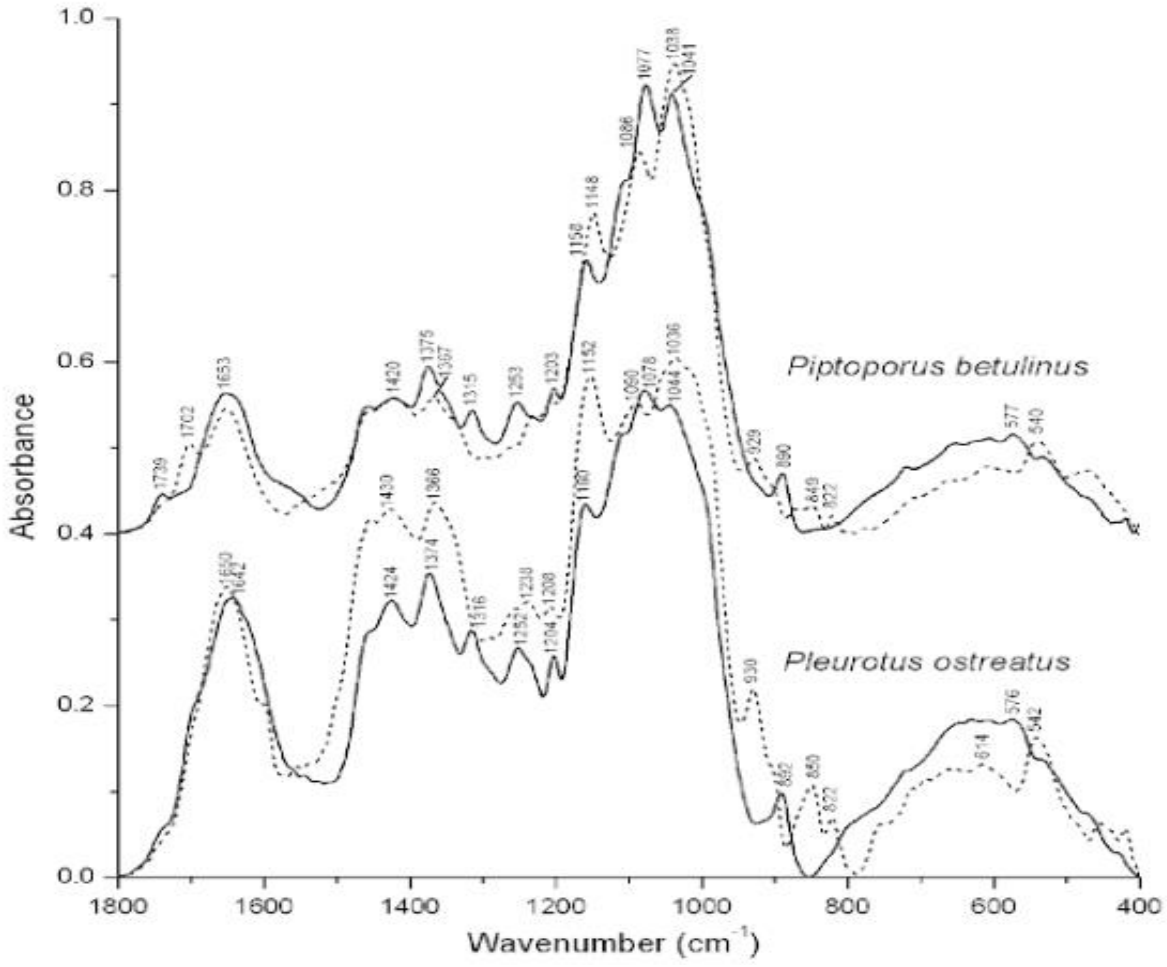


Fig. 8: FTIR spectra of (1,3)-(1,6)-β-D-glukans (line) and (1,3)-α-D-glukan (dots) isolated from higher fungi [99].

The characteristic bands for (1-3)- and/or (1-6)-linked beta-D-glucans can be found near 1160, 1078, 1044 and 890 cm^{-1}, bands for (1-4), (1-6)-linked alpha-D-glucans are located near 1155, 1023, 930, 850 and 765 cm^{-1} IR bands in the 1366, 930 regions , 850, 822, 542 and 420 cm^{-1} indicate (1-3)-alpha-glucan. For relative quantification between alpha- and beta-bonds as well as to verify the purity of the beta-glucan preparation, ratios 1160/1155, 1041/1023, 1160/1023 and 889/930, 889/765 cm^{-1} should be used [99, 102, 103].

Vibrating bands of different functional groups are very intensive in FTIR spectra, so it is possible to determine the purity of the fungal glucan preparation, ie. the presence of ballast compounds such as proteins, aromatic and/or other polysaccharides. The region C = O and C = C by the so-called stretching vibration (1500-1800 cm^{-1}) is sensitive to impurities present in the fungal glucan preparation. Two bands near 1650, 1540 and 1240 cm^{-1} belong to amide I, II and III of the vibration of proteins that are present in an intact material such as a yeast cell. The doublets of 1650 and 1620 cm^{-1} (amide I) and the smaller peak near 1560 cm^{-1} (amide II) are the most prominent IR bands of chitin, a component of the fungal cell wall. In some preparations, such as yeast or fungi, significant spreading may occur in the region of 1630 - 1500 cm^{-1} due to the presence of aromatic compounds [99, 102, 103].

Raman scattering, another technique of vibrational spectroscopy, complementary to IR, has been applied to the structural analysis of plant, animal and microbial glucan polysaccharides - starch, glycogen, cellulose, dextran. Unfortunately, there are few articles dealing with Raman spectra [99, 103].

2.5.3 Enzymatic determination of glucans (commertial kits)

In the enzymatic assay, the first step is the hydrolysis (cell wall) and then the action of specific enzymes. Lyophilized biomass is exposed to sulfuric acid and high temperature. In alkaline medium (mainly KOH) most of the cell wall components are solubilized for further decomposition by exo-(1→3)-β- glucanase and β-glucosidase. Subsequent detection is performed spectrophotometrically, eg using a GOPOD reagent (containing glucose oxidase and peroxidase). The total glucans are thus determined. Determination of α-glucan is performed by incubation of biomass in alkaline medium (no solubilization of β-glucan) and by amyloglucosidase and invertase [102].

2.5.4 Fluorescent glucans determination

(1 → 3) -β-glucans such as calose, kurdlan and others can be specifically detected by staining with a triphenylmethane dye, called aniline blue, at pH 8. Fluorochromes Calcofluor White and Congo Red also exhibit UV-induced fluorescence at binding to these polymers, but this interaction is non-specific and implies other polysaccharides [104, 105].

3 THE AIMS OF THE STUDY

The aim of presented thesis was to explore possibility of production of the β-glucan from carotenogenic yeast as unexplored source together with some algal strains, commertial higher fungi and cereals.

The experimental part solves following goals:

- Metabolic screening of yeast and microalgal strains (β-glucans, extracellular polymeric substances, intracellular lipids and pigments)
- Optimization of culture conditions for β-glucans and other high-valuable compounds
- Compare of β-glucans production within the heterotrophs and autotrophs
- Analysis of commertial higher fungi and cereals

4 EXPERIMENTAL PART

4.1 Materials and chemicals

Chloroform p.a., (Lach-Ner Ltd. Czech Republic)
Methanol p.a., (Lach-Ner Ltd., Czech Republic)
Sulphuric acid 96 %, (Lach-Ner Ltd., Czech Republic)
Ethylacetate for HPLC (Lach-Ner Ltd., Czech Republic)
Acetonitril for HPLC (Lach-Ner Ltd., Czech Republic)
Methanol for HPLC (Lach-Ner Ltd., Czech Republic)
Hexan for HPLC (Lach-Ner Ltd., Czech Republic)
Heptadecanoid acid (Sigma-Aldrich, Germany)
Carotenoids standard (Sigma-Aldrich, Německo)
Ergosterol (Sigma-Aldrich, Germany)
Chlorophyll a (Sigma-Aldrich, Germany)
Chlorophyll b (Sigma-Aldrich, Germany)
Sodium nitrate p.a., (Lach-Ner Ltd., Czech Republic)
Potassium nitrate p.a., (Lach-Ner Ltd., Czech Republic)
Yeast extract, (HiMedia, Czech Republic)
Pepton, (HiMedia, Czech Republic)
Glucose, (Lach-Ner Ltd., Czech Republic)
Mannose, (Lach-Ner Ltd., Czech Republic)
Xylose, (Lach-Ner Ltd., Czech Republic)
Calcium chloride p.a., (Lach-Ner Ltd., Czech Republic)
Citric acid p.a., (Lach-Ner Ltd., Czech Republic)
Na_2-EDTA.$2H_2O$ p.a., (Sigma-Aldrich, Germany)
$(NH_4)_5Fe\ (C_6H_4O_7)_2$ p.a., (Sigma-Aldrich, Germany)
Sodium carbonate p.a., (Lach-Ner Ltd., Czech Republic)
KH_2PO_4 p.a., (Lach-Ner Ltd., Czech Republic)
K_2HPO_4 p.a., (Lach-Ner Ltd., Czech Republic)
$FeCl_3 \cdot 6H_2O$ p.a., (Lach-Ner Ltd., Czech Republic)
Boric acid p.a., (Lach-Ner Ltd., Czech Republic)
$MnCl_2 \cdot 6H_2O$ p.a., (Sigma-Aldrich, Germany)
Sodium molybdate dihydrate p.a., (Sigma-Aldrich, Germany)
$Co(NO3)_2 \cdot 6H_2O$ p.a., (Sigma-Aldrich, Germany)
Zinc chloride p.a., (Lach-Ner Ltd., Czech Republic)
$CoCl_2 \cdot 6H_2O$ p.a., (Lach-Ner Ltd., Czech Republic)
NaCl p.a., (Lach-Ner Ltd., Czech Republic)
Ammonium sulphate p.a. (Lachema, Czech Republic)
Urea p.a., (Lach-Ner Ltd., Czech Republic)
Yeast and Mushroom Beta-glucan Assay Kit (Megazyme, Poland)
Beta-glucan mixed linkage (Megazyme, Poland)

FAME standard mixture (C4 – C24; Sigma Aldrich, USA)
Beta-carotene, HPLC standard, (Sigma-Aldrich, Germany)
Lycopene, HPLC standard, (Sigma-Aldrich, Germany)
Astaxanthis, HPLC standard, (Sigma-Aldrich, Germany)
Thorulene, analytical standard, (CaroteNature, Switzerland)
Torularhodine, analytical standard (CaroteNature, Switzerland)
Ergosterol, HPLC standard, (Sigma-Aldrich, Germany)

4.2 Equipments

Freeze-dryer Labconco FreeZone 4.5 Freeze Dryer (USA)
Termock VWR, (CZ)
Vortex Genie, Scientific Industries, Inc. (USA)
HPLC/PDA: HPLC/MS (Thermo Fischer Scientific, USA)
• LCO 101, Column Oven (ECOM, ČR)
• Detector PDA - PDA Plus Detector, Finnigan SURVEYOR
• Pump - MS Pump Plus, Finnigan SURVEYOR
• Xcalibur
• Column Kinetex Core Shell C18, 150 mm, 5,0 μm, Phenomenex
• Holder - KJ0 - 4282, ECOM (ČR)
• Precolumn - C18, 5,0 μm , Phenomenex
• Filters for HPLC, PRE-CUT, Alltech (GB)
• Evaporator RV 06, IKA (SRN)
Thermo Scientific TRACETM 1300 Gas Chromatograph, (Thermo Fischer Scientific, USA)
• Detector FID (Thermo Fischer Scientific, USA)
• Thermo Scientific AI 1310 Autosampler
• Column Zebron ZB-FAME, 30 m × 0,25 mm × 0,20 μm, Phenomenex
HP 6890 gas chromatograph (Hewlett Packard, Palo Alto, USA)
• SGE BPX70, 60.0 m x 250 μm x 0.25 μm column (SGE Analytical Science, Ringwood, Australia)
Shaker Yellow line RS10, (DE)
Analytical balances Boeco (DE)
LIFE BOX, Esco Technologies, Inc. (US)
High Throughput screening eXTension unit (HTS-XT) coupled to the Vertex 70 FTIR spectrophotometer (Bruker Optik, Germany)
Duetz Microtiter Plate System (Enzyscreen, Netherlands)
MC 1000 - OD multicultivator (Photon System Instruments, Czech Republic)
Precellys evolution homogenizer (Berlin Instruments, Germany)
Software Unscrambler X version 10.5.1 (CAMO Analytics, Norway)
Software Orange data mining toolbox version 3.24 (University of Ljubljana, Slovenia)

4.3 Microorganisms

Yeasts strains of the generi *Saccharomyces, Metschnikowia, Cystofilobasidium, Rhodotorula, Sporidiobolus, Dioshegia* and *Phaffia*, were purchased from the Culture Collection of Yeasts (CCY, Bratislava, Slovakia). *Aureobasidium* and *Candida* yeast were purchased from Czech Collection of Microorganisms, Masaryk University (CCM, Brno). Algal and cyanobacterial strains were obtained from Culture Collection of Autotrophic Organisms (CCALA, Třeboň, Czech Republic). The list of microorgamisms is presented in the Table 4-7. The commertial brewer yeast of *S. cerevisiae* was kindly provided by Ing. Václav Štursa (Table 6).

Table 4: List of used carotenogenic yeasts strains

Number	Microorganisms	Strain collection number
1	*Cystofilobasidium infirmominiatum*	CCY 17-18-4
2	*Cystofilobasidium macerans*	CCY 10-1-2
3	*Phaffia rhodozyma*	CCY 77-1-1
4	*Rhodotorula kratochvilovae*	CCY 20-2-26
5	*Rhodotorula mucilaginosa*	CCY 19-4-6
6	*Rhodotorula mucilaginosa*	CCY 20-9-7
7	*Rhodotorula toruloides*	CCY 62-2-4
8	*Rhodotorula glutinis*	CCY 20-2-47
9	*Rhodotorula glutinis*	CCY 20-2-22
10	*Sporidiobolus metaroseus*	CCY 19-6-20
11	*Sporidiobolus pararoseus*	CCY 19-9-6
12	*Sporidiobolus salmonicolor*	CCY 19-6-4
13	*Sporidiobolus salmonicolor (roseus)*	CCY 19-4-25
14	*Dioshegia hungarica*	CCY 18-1-3

Table 5: List of used non-carotenogenic yeasts strains

Number	Microorganisms	Strain collection number
15	*Metschnikowia pulcherrima*	CCY 29-2-149
16	*Metschnikowia pulcherrima*	CCY 29-2-147
17	*Metschnikowia pulcherrima*	CCY 29-2-129
18	*Aureobasidium pullulans*	CCM 8183
19	*Yarrowia lipolytica*	CCY 29-26-4
20	*Candida glabrata*	CCM 8270
21	*Saccharomyces cerevisiae*	CCY 21-4-102

Number	Microorganisms	Strain collection number
22	*Saccharomyces cerevisiae*	CCY 21-4-81
23	*Saccharomyces cerevisiae*	CCY 48-26
24	*Saccharomyces cerevisiae*	CCY 21-4-64
25	*Saccharomyces cerevisiae*	CCY 48-88

Table 6: List of comertial brewer yeasts

Number	Microorganisms	Fermenting temperature range [^{o}C]
26	Bohemian lager yeast, M84	10-15
27	Cider yeast, M02	12-28
28	Premium gold; Muntons	-
29	Safale S-04	12-25
30	Saflager W-34/70	9-22
31	Saflager S-23	9-22
32	SafAle WB-06	12-25
33	Safale US-05	12-25

Table 7: List of algal and cyanobacterial strains

Number	Microorganisms	CCALA
34	*Botryococcus braunii*	777
35	*Chlorella vulgaris*	924
36	*Chlorella sorokiniana*	260
37	*Desmodesmus acutus*	437
38	*Desmodesmus quadricauda*	463
39	*Scenedesmus dimorfus*	443
40	*Scenedesmus obliqus*	455
41	*Chlamydomonas reinhardtii*	928
42	*Porphyridium cruentum*	415
43	*Porphyridium purpureum*	416
44	*Porphyridium areuginosum*	419
45	*Rhodella violacea*	925
46	*Euglena gracilis*	349

4.4 Media and growth conditions of yeast strains

Cultivation of yeasts was performed, first, on YPD agar medium to recover frozen cultures and then in YPD broth medium to prepare inoculum. For the screening of yeasts for β-glucan, lipids and carotenoids production, inoculum was inoculated into the YPD production medium amd medium with different C/N ratios was used to test the effect of C/N ratio on β-glucan production.

For the YPD agar cultivation, yeast cells from the frozen cryopreserved stock were transferred onto Petri dishes with YPD agar (yeast extract, 10.0 g/L; peptone, 20.0 g/L; glucose 20.0 g/L; agar, 20.0 g/L) (Merck, Darmstadt, Germany) and cultivated for 72 hours at 25 °C. Inoculum was prepared by transferring 1µl of yeasts cells from YPD agar into 50 mL of sterile YPD broth medium (yeast extract, 10.0 g/L; peptone, 20.0 g/L; glucose 20.0 g/L) (Merck, Darmstadt, Germany) in Erlenmeyer flask (250 mL) and cultivated for 24 hours at 25 °C under shaking regime (100 rpm, 50 mm). To remove residual medium after cultivation, the inoculum biomass was washed with sterile water and re-suspended to the original volume. The inoculum added to the YPD broth production medium (composition see above, C/N ratio 2.13:1) and media with other C/N ratios was in the volume ratio of 1:5. The YPD production medium with different C/N ratios was composed of (g/L): N-source yeast extract, 2; KH_2PO_4, 4; $MgSO_4 \cdot 7H_2O$, 0.696 and C-source glucose monohydrate at the C/N ratios 10:1, 40:1, 70:1 and 100:1. For the calculation of the C/N ratio, a carbon content of 40 % in glucose and a nitrogen content of 10.5 % in yeast extract were assumed. The cultivation in all production media was performed in Duetz Microtiter Plate System [18, 22, 23] (Enzyscreen, Netherlands) which consists of 24-well extra deep microtiter plates (MTPs) with low-evaporation Sandwich cowers and Clamp system for mounting MTPs on the top of each other. Cultivation in the control YPD broth production medium was done for 48h and 96h at 25 °C. Cultivations in the YPD broth production media with different C/N ratios were performed for 96h at 25 °C under shaking regime (100 rpm, 50 mm). For investigation of increasing osmolarity on beta-glucan and lipids production, four yeast strains with high beta-glucan production were choose, namely *Saccharomyces cerevisiae* (CCY 21-4-102), *Cystofilobasidium infirmominiatum* (CCY 17-18-4), *Phaffia rhodozyma* (CCY 77-1-1) and *Rhodotorula kratochvilovae* (CCY 20-2-26). Media with C/N ratio 40, 70 and 100 were supplemented with sodium chloride to final concentration 0.2, 0.5, 2, 5, 8 and 11 %. MTPs were used also for the screening of the exopolysaccharide production in red yeast.

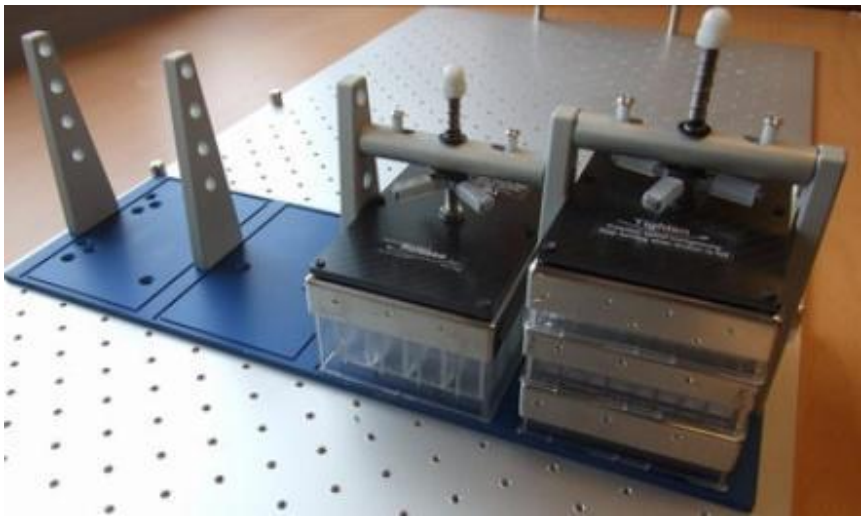


Fig 9: Duetz Microtiter Plate System (Enzyscreen, Netherlands) [106]

These four strains together with *S. pararoseus* CCY 19-9-6 were used for growth in different temperature regime (8, 15, 22 and 30 ^{o}C) at the C/N ratio of 70 and diverse nitrogen sources, where the inoculum was precultivated at the studied temperature. The used nitrogen sources were urea, ammonium sulphate, yeast extract and the combination af yeast extract and urea with the same amount of nitrogen as at C/N ratio experiment (Table 7). Cultivations were done in Erlenmeyer flasks for the time points of 48, 72, 96, 144 and 168h under shaking regime (110 rpm). All cultivation media were sterilized at 121^{o}C for 15 min.

Table 8: Composition of the media used for experiment with different temperature and C/N ratio of 70:1

MED 1		**MED 2**		**MED 3**		**MED 4**	
Compon.	**g/L**	**Compon.**	**g/L**	**Compon.**	**g/L**	**Compon.**	**g/L**
Glucose	40.4	Glucose	40.4	Glucose	40.4	Glucose	40.4
Yeast extract	0.5	Yeast extract	-	Yeast extract	-	Yeast extract	0.5
KH_2PO_4	4.0	KH_2PO_4	4.0	KH_2PO_4	4.0	KH_2PO_4	4.0
$MgSO_4 \cdot 7H_2O$	0.7	$MgSO_4 \cdot 7H_2O$	0.7	$MgSO_4 \cdot 7H_2O$	0.7	$MgSO_4 \cdot 7H_2O$	0.7
$(NH_4)_2SO_4$	0.7	$(NH_4)_2SO_4$	1.0	Urea	0.5	Urea	0.4

The screening of the production of exoglycolipids at *R. kratochvilovae* CCY 20-2-26 started with examination of nitrogen sources in media with glucose as a carbon source. Subsequently, different carbon sources (mannose, glycerol, xylose, galactose and lactose) were explored for production of beta-glucans, exoglycolipids and intracellular lipids. The media composition together with scheme of experiment are vizualized in the Fig. 10 and the Tables 8-9.

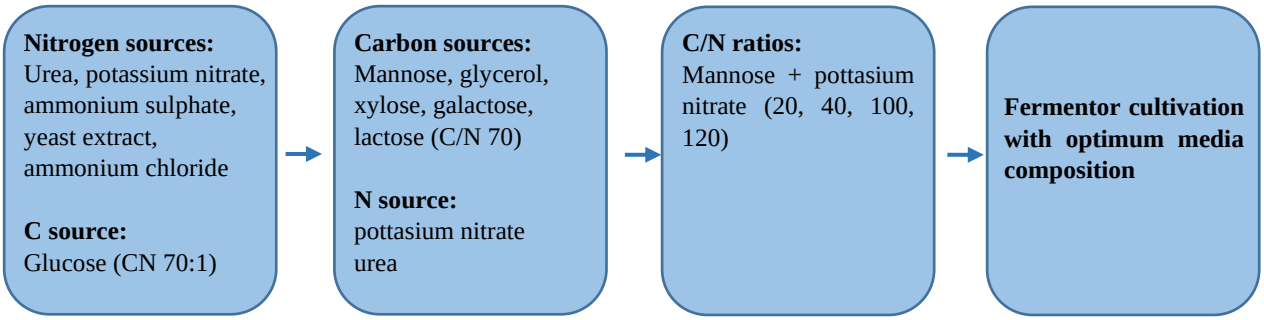


Fig 10: Scheme of the experiment for production of exoglycolipids and beta-glucan from *R. kratochvilovae* CCY 20-2-26

Table 9: Composition of culture media at C/N ratio 70:1 – nitrogen sources

Component	**g/L**
Glucose	40.42
KH_2PO_4	4
$MgSO_4 \cdot 7\ H_2O$	0.7
Nitrogen sources	**g/L**
Yeast extract	2
KNO_3	1.52
NH_4Cl	0.80
$(NH_2)_2SO_4$	0.9906
Urea	0.45

Table 10: Composition of culture media at C/N ratio 70:1 – different carbon sources

Component 1[g/l]	**Component 2 [g/L]**
Urea – 0.45	KNO_3 – 1.52
KH_2PO_4 - 4	KH_2PO_4 - 4
$MgSO_4 \cdot 7\ H_2O$ – 0.7	$MgSO_4 \cdot 7\ H_2O$ – 0.7
Carbon sources	**g/L**
Mannose	40.42
Lactose	34.94
Glycerol	37.57
Xylose	36.75

4.5 Growth of microalgal strains

8.5.1. Screening

The stock cultures of algal and cyanobacterial strains were maintained by inoculation every 20 days into fresh BBM medium (chlorophyta, cyanobacteria), ASW medium (red algae) or Euglena medium (stock culture:fresh medium 1:19) (Table 11) and kept under constant shaking conditions (110 rpm) and light illumination. The 20day old cultures stocks were used for inoculation of two independent Erlenmeyer flasks for each strain by diluting the stock culture to 0.1 OD (680 nm) with fresh medium. The microalgal cultures were grown autophototrophically at 25 ^{o}C and continuously illuminated by white fluorescent light. After cultivation, biomass was centrifuged at 9,500 rpm for 10 min at 15°C and biomass pellet was then washed three times using distilled water. Further, biomass was freeze-dried for 48 h and, subsequently, stored at -80 °C until use. The composition of culture media is introduced in Tables 10 and 11.

Table 11: Composition of culture media

	g/L	
Components	**BBM**	**ASW**
$NaNO_3$	0.75	-
$MgSO_4 \cdot 7H_2O$	0.075	6.6
$CaCl_2 \cdot 2H_2O$	0.025	1.5
$Na_2EDTA \cdot 2H_2O$	$4.5 \cdot 10^{-3}$	-
NaCl	0.01	27
K_2HPO_4	0.075	-
KH_2PO_4	0.175	0.07
$FeCl_3 \cdot 6H_2O$	$5.82 \cdot 10^{-4}$	-
$MnCl_2 \cdot 4H_2O$	$2.46 \cdot 10^{-4}$	-
$Na_2MoO_4 \cdot 2H_2O$	$2.4 \cdot 10^{-5}$	-
$ZnCl_2$	$3 \cdot 10^{-5}$	-
$CoCl_2 \cdot 6H_2O$	$1.2 \cdot 10^{-5}$	-
Kvasničný extrakt	-	-
Acetát sodný	-	-
Beef extrakt	-	-
Trypton	-	-
$MgCl_2 \cdot 6H_2O$	-	5.6
KNO_3	-	1
$NaHCO_3$	-	0.04
1M Tris-HCl; pH 7.6	-	20 ml
Trace Metal	-	1 ml
Chelated Iron	-	1 ml

Table 12: Composition of culture media – micronutrients

Trace metal solution		**Chelated Iron solution**	
$ZnCl_2$	4 mg	$FeCl_3 \cdot 4H_2O$	240 mg
H_3BO_3	60 mg	0,05M EDTA, pH 7,6	100 ml
$ClCl_2 \cdot 6H_2O$	1.5 mg		
$CuCl_2 \cdot 2H_2O$	4 mg		
$MnCl_2 \cdot 4H_2O$	40 mg		
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	37 mg		
Distilled water	100 ml		

8.5.2. Heterotrophic growth

The red algal strains (20days old) were used for inoculation of two independent Erlenmeyer flasks by diluting the stock culture to 0.1 OD (680 nm) with fresh medium ASW supplemented with

glucose (10 and 20 g/L). The microalgal cultures were grown heterotrophically at 25 ºC under constant shaking regime under dark conditions. After cultivation, biomass was centrifuged at 9,500 rpm for 10 min at 15°C and biomass pellet was then washed three times using distilled water. Further, biomass was freeze-dried for 48 h and, subsequently, stored at -80 °C until use.

4.5.1 Cultivation of red algae in multicultivator

The MC 1000 - OD multicultivator (Fig. 8) consists of eight 85cm^3 tubes, which are individually illuminated and aerated. The tubes are placed in a tempered water bath. The instrument measures the absorbance of each tube at 680 nm and 720 nm every 10 min, allowing rapid screening of microscopic algae and cyanobacteria growth. In this doctoral thesis, a multicultural cultivator was used to optimize illumination in red algal strains. The 20day old cultures stocks were used for inoculation of two independent tubes by diluting the stock culture to 0.1 OD (680 nm) with fresh ASW medium. The microalgal cultures were grown autophototrophically at 25 ºC and continuously illuminated by white fluorescent light (50, 100, 150, 200, 250 and 300 µmol photons·m^{-2}·s^{-1}). After cultivation, biomass was centrifuged at 9,500 rpm for 10 min at 15°C and biomass pellet was then washed three times using distilled water. Further, biomass was freeze-dried for 48 h and, subsequently, stored at -80 °C until use.

Fig 11: Multicultivator MC 1 000

4.5.1 Culture conditions of *Euglena gracilis*

The 6day old cultures stocks were used for inoculation of two independent Erlenmeyer flasks by diluting the stock culture to 0.1 OD (680 nm) with fresh medium (Euglena medium or NM medium). The microalgal cultures were grown mixotrophically at 25 ºC and continuous shaking (150 rpm) and illumination or heterotrophically (10 and 20 g/L of glucose; NM medium adapted from Cramer et al. (1952) [107]) without light. After cultivation, biomass was centrifuged at 9,500 rpm for 10 min at 15°C and biomass pellet was then washed three times using distilled water. Further, biomass was freeze-dried for 48 h and, subsequently, stored at -80 °C until use.

Table 13: Composition of culture media for *Euglena gracilis*

	Euglena medium
$NaNO_3$	-
$MgSO_4 \cdot 7H_2O$	-
$CaCl_2 \cdot 2H_2O$	0.01
Yeast extract	2
Sodium acetate	1
Beef extrakt	1
Trypton	2
Chelated Iron	-
Heterotrophic culture: Euglena medium supplemented with 10 or 20 g/L of glucose	

4.6 Biomass determination

After cultivation, biomas sample was centrifuged at 4500 rpm for 5 min at 4°C and biomass pellet was then washed three times using 0,1% NaCl solution. Further, biomass was freeze-dried for 48 h and, subsequently, stored at -20 °C until use.

4.7 Extraction and HPLC analysis of carotenoids, chlorophylls and ergosterol

Into plastic extraction tubes 15±3 mg of freeze-dried biomass was weight and rehydrated by addition of 1 mL distilled water. The water was removed by centrifugation (10,000 rpm/5 min/10 ^{o}C) and to the pellet was added 300 ± 20 mg acid-washed glass beads (250 – 500 μm diameter, Roth, Germany) and 1 mL of methanol. The rupture of the biomass was performed by 10 min of vortexing. The content of the PP tube was transferred into a glass reaction tube by washing it with a 2,000 μL of chloroform and the glass tube was vortexed for 10 min. Then, 1 mL of distilled water was added for the phase separation. After centrifugation (3,000 rpm/5 min/4 ^{o}C), the separated bottom chlorophorm phase with extracted pigments was evaporated under nitrogen at 25 °C followed by the addition of 1 mL of mixture ethylacetate:acetonitrile (20:60). The ethylacetate:acetonitrile mixture containing extracted pigments were filtered through syringe filter (0.45 μm, PTFE membrane, 13 mm) and transferred into glass vials for further HPLC analysis.

The conditions of HPLC separation are presented in the Tables 13 and 14. Contents of individual pigments were calculated according to calibration strandards (beta-carotene, astaxanthin, lycopene, luteine, volaxanthin, neoxanthin, chlorophyll a, chlorophyll b, ergosterol) from regress equation. Chromatogram of the yeast sample (*R. kratochvilovae* CCY 20-2-26) is showed in supplementary material (Fig. S1).

Table 14: Separation conditions

Column	Kinetex, EVO 150 x 4.6 mm, 2.6 μm
Volume of the sample	20 μL
Elution	Gradient 0-13 min: from 100% A to 100% B linearly 13-19 min: 100% B 19-20 min: from 100% B to 100% A linearly 20-25 min: 100% A
PDA	285, 435, 450 and 680 nm
Temperature	25 ^{o}C
Time of analysis	25 min

Table 15: Composition of mobile phases

A		B	
Solvent	Amount	Solvent	Amount
Acetonitrile	840 mL	Methanol	680 mL
Methanol	20 mL	Ethylacetate	320 mL
0.1M Tris-HCl (pH=8)	140 mL	-	-

4.8 Phycobiliproteins isolation

For extraction of phycobiliproteins from cyanobacterial cells several physical and chemical methods have been developed. The most conventional methods are freeze-thaw and ultrasonication into extraction buffer (phosphate, acetate, carbonate or Tris-HCl). Chemical methods include enzymes (lysozyme, cellulase), denaturating agents (Triton X-100) or hydrochloric acid (2 – 10 N) mainly for sheathed cyanobacteria.

In this thesis physical methods (ultrasound and freeze-thaw) were tested for phycobiliproteins isolation with use of neutral pH. Exposure of cells to a low pH leads to pheophytization and creating pheophytin, molecule lacking a central magnesium ion. On the other hand, higher pH induces chlorophyllide formation. Both cases result in large deviations in the determination of chlorophyll (shifted absorption spectra) and other pigments in residual biomass.

One mL of culture (log phase, 14 days) was centrifuged at 10,000 rpm and the pellet was suspended in 1 mL of extraction buffer (or distilled water) contains 0.002 M sodium azide. Phycobiliproteins was extracted by repeated freezing (-80 ^{o}C) and thawing (30 ^{o}C) until the cell biomass become greenish. Cell debris were removed by centrifugation (x rpm/y min) and the crude extracts were filtrated through 0.45 μm nylone filter. Amount of PBP was measured spectrophotometrically by Bennett and Borogad (1973) [108].

The optimization steps are represented in Table 16 and were tested with strains of *Porphyridium cruentum* and *Arthrospira platensis*. The results are presented in supplementary materials (Fig. 2 and 3). The best isolation step is to Freeze-thaw the biomass with 0,1M Tris-HCl buffer, pH=7.

Table 16: Optimization of phycobiliproteins isolation

Method	Extraction solutions	Porphyr.	Arthrosp.
Freeze-thaw (two cycles for one hour in – 80^{o}C)	1,5% CaCl2	P1A	A1A
	0,1M sodium phosphate buffer; pH=7	P1B	A1B
	0,1M Tris-HCl; pH=7	P1C	A1C
Ultrasound (4x15 min)	1,5% CaCl2	P3A	A3A
	0,1M sodium phosphate buffer; pH=7	P3B	A3B
	0,1M Tris-HCl; pH=7	P3C	A3C
Combination (20 kH) (ultrasound 15 min between freeze step)	1,5% CaCl2	P5A	A5A
	0,1M sodium phosphate buffer; pH=7	P5B	A5B
	0,1M Tris-HCl; pH=7	P5C	A5C
Freeze-thaw	Water		

4.9 FAMEs analysis by GC/FID

Method FAMEs analysis from extracted biomas was used for screening of the yeast cultivated in MTPs and salt stress. Lipids from biomass in other experiments were modified to FAMEsby direct transesterification.

8.5.6 FAMEs analysis from extracted biomass

For total lipid extraction a modified Folch-method was used [109, 110]. Into 2 mL polypropylene tubes 15 ± 3 mg of freeze-dried yeast biomass was added together with 250 ± 20 mg acid-washed glass beads (710 – 1180 μm diameter, Sigma-Aldrich, USA) and 600 μL methanol. For disruption of yeast cells, the Precellys evolution homogenizer (Bertin Instruments, Germany) was used with shaking cycles of 5500 rpm (2 x 20 s) three times. The content of the PP tube was transferred into glass reaction tube by washing it with 2400 μL solvent mixture of methanol:chloroform:hydrochloric acid (7.6:1:1 v/v). 1 mg of tridecanoid acid (C13:0) was used as internal standard and added to the reaction mixture. The glass tube was vortexed for 10 s and incubated for 1 hour at 90 °C. After following cooling to room temperature, 1 mL of distilled water and 2 mL hexane:chloroform (4:1 v/v) mixture. The separated upper hexane phase with extracted lipids was evaporated under nitrogen at 30 °C followed by addition of sodium sulphate and dissolving the FAMEs in 1.5 mL hexane containing 0.01% butylated hydroxytoluene (BHT, Sigma-Aldrich, USA). Hexane was transferred into glass vials for further GC analysis. Total lipid content (wt% of total FAMEs of the dry weight) and the fatty acid profile were performed by HP 6890 gas chromatograph (Hewlett Packard, Palo Alto, USA) equipped with an SGE BPX70, 60.0 m x 250 μm x 0.25 μm column (SGE Analytical Science, Ringwood, Australia) and flame ionization detector (FID). Total time of the analysis was 36 min with the initial temperature of 70 °C, holding for 2 min, with increasing of 10 °C/min to 150 °C, then 6 °C/min to 230 °C. 1 μL of the sample was injected in split mode (30:1 split ratio) to inlet tempered to 280 °C. FAME standard mixture (C4 – C24; Sigma Aldrich, Germany) dissolved in hexane was used for identification of the FAMEs. Quantification was based on C13:0 internal standard and relative response factors (RRF) calculated from 5-point calibration curves of the individual FAMEs present in the standard mixture.

8.5.7 FAMEs analysis by direct transesterification

Into 2 mL glass crimp vial 10 ± 2 mg of freeze-dried yeast biomass was weight together with 1.8 mL of transesterification solvent (15% (v/v) H_2SO_4 in methanol (HPLC grade), 0.5 mg/mL C17 as a intermal standard) and the sample was incubated for 2 hours at 85 °C. After following cooling to room temperature, the full volume of vial was transferred into 4mL glass vial with addition of 0.5 mL 0.05M NaOH and 1 mL of hexane (GC grade). The mixture was vortexed for 5 min and after the phase separation, 0.1 mL upper hexane extract and 0.9 mL of pure hexane was transfered into glass vials for further GC analysis. Total lipid content (wt% of total FAMEs of the dry weight) and the fatty acid profile were performed by Thermo Scientific TRACE™ 1300 Gas Chromatograph equipped with an Zebron ZB-FAME column, 30 m x 0,25 mm x 0,20 μm and flame ionization detector (FID). Temperature gradient is presented in the Table 17. 1 μL of the sample was injected in split mode to inlet tempered to 260 °C. FAME standard mixture (C4 – C24; Sigma Aldrich, Germany) dissolved in hexane was used for identification of the FAMEs. Quantification was based on C17:0 internal standard and relative response factors (RRF) calculated from 5-point calibration curves of the individual FAMEs present in the standard mixture. Chromatogram of the yeast sample is showed in supplementary material (Fig. S4).

Table 17: Temperature gradient

Retention time [min]	Gradient [°C/min]	Target temperature [°C]	Hold [min]
0	-	-	-
1	0	80	1
5	15	140	0
21.7	3	190	0
25.5	25	260	1
25.5	STOP	-	-

4.10 Glucan content determination in yeast and mushrooms by enzyme method

The content of total glucans, β- and α-glucans in yeast freeze-dried biomass were determined according to the Yeast and Mushroom β-glucan Assay Procedure K-YBGL (Megazyme Int., Poland). To estimate the total glucans content, yeasts biomass was hydrolysed with ice-cold 12 M sulphuric acid for 2 h and then incubated for 2 h at 100 °C. Further, the neutralization of acidic hydrolysate with 200 mM sodium acetate buffer (pH 5) and 10 M KOH was done, followed by effect of enzymes exo-β-(1→3)-D-glucanase and β-glucosidase in acetate buffer (pH 4.5) for 1 hour at 40 °C. The α-glucan content was determined after enzyme hydrolysis with amylo-glucosidase and invertase. The β-glucan content was calculated from the assay kit procedure as difference between total glucans content and α-glucan content. The absorbance values for total glucans and α-glucans content were obtained spectrophotometrically at 510 nm after adding glucose oxidase/peroxidase reagent to the samples.

4.11 Glucan content determination in cereal grains by enzyme method

Sample was weight (25±5 mg) to a glass centrifuge tube and wetted with 0.2 mL of aqueous ethanol (50% v/v). 4 mL of sodium phosphate buffer (20 mM, pH 6.5) were added and the content was stirred on vortex mixer. The tube was placed into a boiling water bath and incubated for 3 min. Next, the temperature was decreased to 50 °C and the sample was hydrolysed one hour by lichenase (0.2 mL, 10U, 50 °C). 5 mL of sodium acetate buffer were added into tube (200 mM, pH 4.0) and the sample was left to equilibrate to room temperature (5 min) and then centrifuge (1,000 g, 10 min). Aliquots of the sample (0.1 mL) were dispensed into the bottom of three test tubes and β-glucosidase (0.1 mL, 0.2 U) in 50 mM sodium acetate buffer (pH 4.0) was added to two of these tubes (the reaction). To the third (the reaction blank), 50 mM acetate buffer (0.1 mL, pH 4.0) was pippeted. The samples were incubated at 50°C for 10 min. In the end, 3 mL of GOPOD Reagent was added to each tube and incubated at 50 °C for a further 20 min. The absorbance was measured at 510 nm against reagent blank.

Fig 12: β-glucan Assay Kit (Mixed Linkage) from Megazyme [111]

4.12 Isolation of paramylon granules from *Euglena gracilis*

Paramylon was extracted and purified according to Schwartzbach et al. (1975) [112]. 15±3 mg of freeze-dried biomass was weight into glass tube with screw and 2 mL of solution containing 1% (w/v) SDS and 5% (w/v) Na_2EDTA were added. The suspension was vortexed, incubated for 30 min at 37 °C and then centrifuged for 10 min for 1 000 x g. The SDS-Na_2EDTA treatment was repeated. The pellet was washed twice with hot glass-distilled water (70 °C). After the second wash, the suspension was centrifuged, and the granules were dried overnight at 90 °C for dry weight determination. The amount of paramylon was expressed as it's the ratio of paramylon weight per biomass (CDW).

4.13 Determination of total carbohydrates in algal biomass

The total carbohydrate content was determined by method from Zavřel et al. (2018) [113]. Algal biomass was weight into plastic tube with 1 mL of methanol, precooled to 4 °C. The sample was homogenized by pipetting up and down and the pigments were extracted by allowing the samples to sit at 4 °C in the dark for 20 min. Further, the sample was centrifuged (15,000 g/5 min) and the supernatant was discarded. 500 µL of distilled water and 500 µL of 5% phenol (w/w) were added and the sample was kept at laboratory temperature for 15 min. After incubation, 2.5 mL of 96% sulfuric acid was added and the sample was incubated for 5 min (laboratory temperature). The absorbance was measured at 490 nm and the total cellular carbohydrates were recalculated using glucose calibration curve.

4.14 Fourier transform infrared (FTIR) spectroscopy analysis

Biomass of yeasts grown on different C/N ratios was subjected to the biochemical profiling by FTIR spectroscopy. Washed yeast suspension (4 µl) was transferred on 384-well ZnSi microplate (Bruker Optik, Germany) in triplicates. Samples were dried at room temperature for 30 min before analysis. FTIR spectra were recorded in transmission mode using the High Throughput screening eXTension unit (HTS-XT) coupled to the Vertex 70 FTIR spectrophotometer (Bruker Optik,

Germany). The spectra were collected in the spectral range from 4000 to 500 cm^{-1} (spectral resolution of 6 cm^{-1}, and aperture 5.0 mm), with 64 scans as average for each sample.

Prior to data analysis by principle component analysis (PCA), the FTIR spectra were pre-processes. The pre-processing was performed by transforming spectra to the second-derivative using the Savitzky−Golay algorithm with a polynomial of degree 2 and a window size of 11. The second-derivative spectra were pre-processed by Extended Multiplicative Signal correction (EMSC) [24, 25, 26]. Technical replicates (543 spectra in total) were averaged in order to remove technical variability of the measurements, resulting into 181 spectra. PCA was performed for three spectral regions, lipid (3050 – 2800 cm^{-1} combined with 1800 – 1700 cm^{-1}), protein (1700 – 1500 cm^{-1}) and polysaccharide (1200 – 700 cm^{-1}).

The following software packages were used for the data analysis: Unscrambler X version 10.5.1 (CAMO Analytics, Norway), Orange data mining toolbox version 3.24 (University of Ljubljana, Slovenia) [114].

4.15 Isolation of exopolysaccharides

To isolate the exopolysaccharides (EPS) released into the culture broth, supernatants obtained from 10 ml samples, after centrifugation, were treated by two volumes of cold isopropanol followed by an incubation at 4 ^{o}C. Precipitated EPS were removed by centrifugation at 6,000 g for 10 min at 4 ^{o}C, washed again with 5 mL of isopropanol and centrifuged. The samples were dried at 85 ^{o}C overnight and then freeze dried.

4.16 Isolation of exoglycolipids

Exoglycolipids were isolated from culture medium according to procedure from Wang et al. (2019) [89]. 20 mL of culture broth was centrifuged (6,500 rpm/5 min), the supernatant was separated and washed with ethylacetate (5 mL). The exoglycolipids contained biomass was washed with 5 mL of ethylacetate and centrifuged (6500 rpm/5min). The ethylacetate fractions were merged together, evaporated under nitrogen flow and lyophilized. The dry weight of ethylacetate extract was weighed to estimate grams of extracellular glycolipids per liter of culture.

5 RESULTS AND DISCUSSION

5.1 Yeasts prescreening for beta-glucan production

There is very limited information regarding glucan content in red carotenogenic yeasts. In order to perform extensive screening study by involving a relatively large set of red carotenogenic yeasts and testing the effect of cultivation time point, C/N ratio and NaCl supplementation on the glucans production and profile, Duetz-MTPS was utilized, as a system allowing to perform reproducible and high-throughput micro-cultivation-based screenings.

After the cultivation of yeasts in the control YPD broth production medium for 48 h the highest β-glucans content was found in the strain *C. infirmominiatum* CCY 17-18-4 with 13.82 ± 1.15 % per dry weigh of biomass, that was twice higher than in the control strain *S. cerevisiae* CCY 19-6-4 (Fig. 13). In addition to *C. infirmominiatum* CCY 17-18-4, high content of β-glucans was observed also for *P. rhodozyma* CCY 77-1-1, *R. glutinis* CCY 20-2-26, *C. macerans* CCY 10-1-2 and *S. metaroseus* CCY 19-6-20 strains (Fig. 13). The lowest β-glucan content was observed for *R. musilaginosa* CCY 19-4-6, *R. toruloides* CCY 62-2-4, *S. salmonicolor* CCY 19-4-25 and *S. pararoseus* CCY 19-9-6 strains with an average of 4% per dry weight of biomass (Fig. 13). Generally, β-glucans content in ascomycetes yeasts from genera *Saccharomyces* and *Metschnikowia* was slightly over 6 % per dry weight of biomass that was in overall lower than for red carotenogenic basidiomycetes yeasts (Fig. 13).

Most of the studied yeast strains showed the negligible presence of α-glucans, while some of them have relatively high content of it. Interesting, that the highest α-glucan content (2.3 ± 0.32 % per dry weight) was observed in the strain *Sporidiobolus salmonicolor* CCY 19-4-25, which exhibited one of the lowest β-glucans content 3.81 ± 0.62 % per dry weight, meaning that the ratio of α- and β-glucans in this strain was 1:2, while in all other yeast strains β-glucans were in a significantly higher proportion than α-glucans (Fig. 13). The second highest content of α-glucans (2.16 ± 0.35 % per dry weight) was observed for *C. infirmominiatum* CCY 17-18-4 which also had the highest content of β-glucans. High content of α-glucans was measured also for all other strains from *Sporidiobolus* genus (except *S. pararoseus* strain CCY 19-9-6) and strain *P. rhodozyma* CCY 77-1-1 (Fig. 13). The high content of α-glucans for some of the studied red carotenogenic yeasts could be explained by the possible presence of intracellular (poly)saccharides like trehalose (α-1,1-bounded glucose) or glycogen (α-1,4- and α-1,6-glucose polymer) [115, 116].

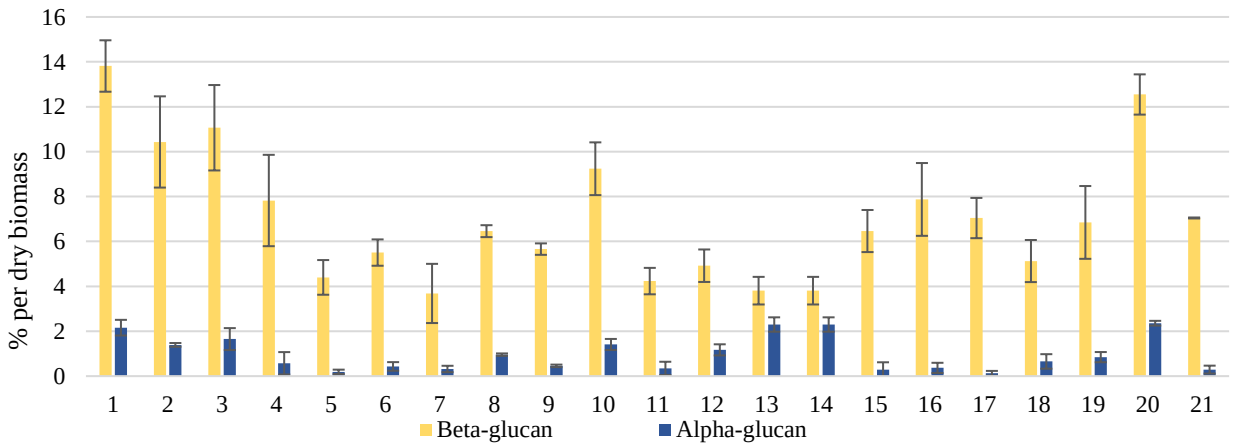


Fig. 13: The content of β- and α-glucans in yeast biomass after 48 h of cultivation in the control YPD broth production medium (the number designation of individual strains is presented in the Table 18 and 19)

Cultivation of yeasts in the control YPD production medium for two different time points – 48h and 96h did not show any significant differences in the content of β- and α-glucans for most of the strains (Fig 14). The most significant difference was obtained in *Phaffia rhodozyma* CCY 77-1-1, in which the level of β-glucans increased from 11.07% after 48 h cultivation to 17.58 % after 96 h of cultivation, while the biomass yield stayed low (Fig 15). Relatively lower β-glucans content after 96 h of cultivation was observed for *M. pulcherrima* CCY 29-2-129, *S. salmonicolor* CCY 19-4-25 and *pararoseus* CCY 19-9-6, *R. glutinis* CCY 20-2-26, R. *mucilaginosa* CCY 20-9-7 and *C. macerans* CCY 10-1-2.

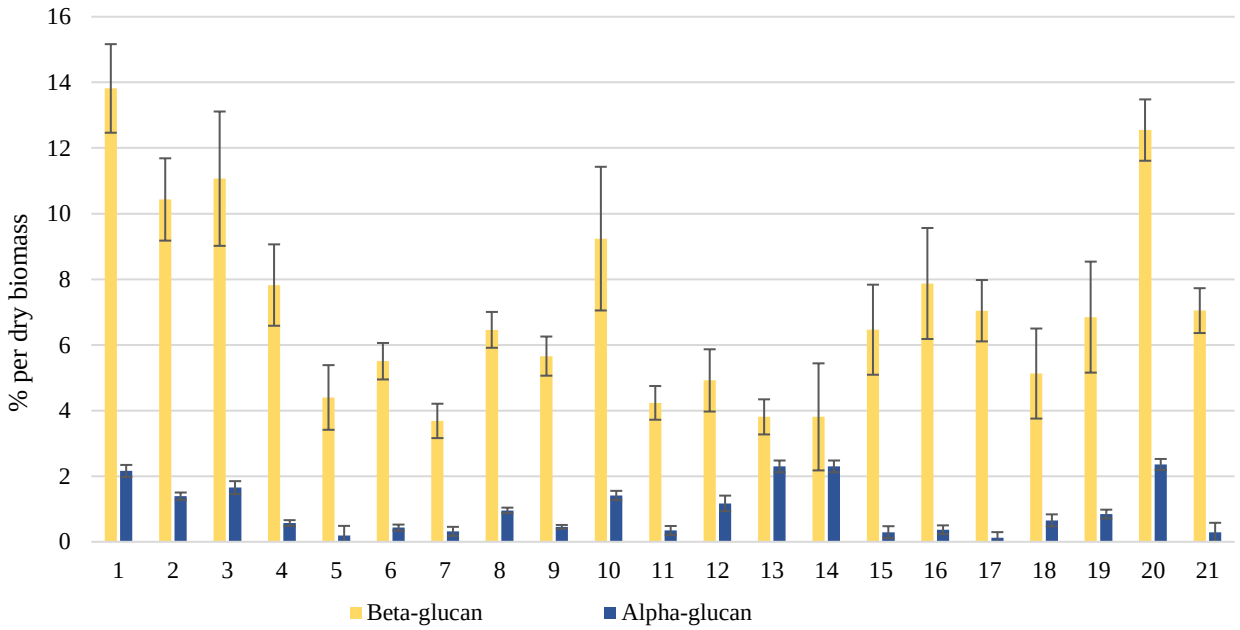


Fig. 14: The content of β- and α-glucans in yeast biomass after 96 h of cultivation in the control YPD broth production medium

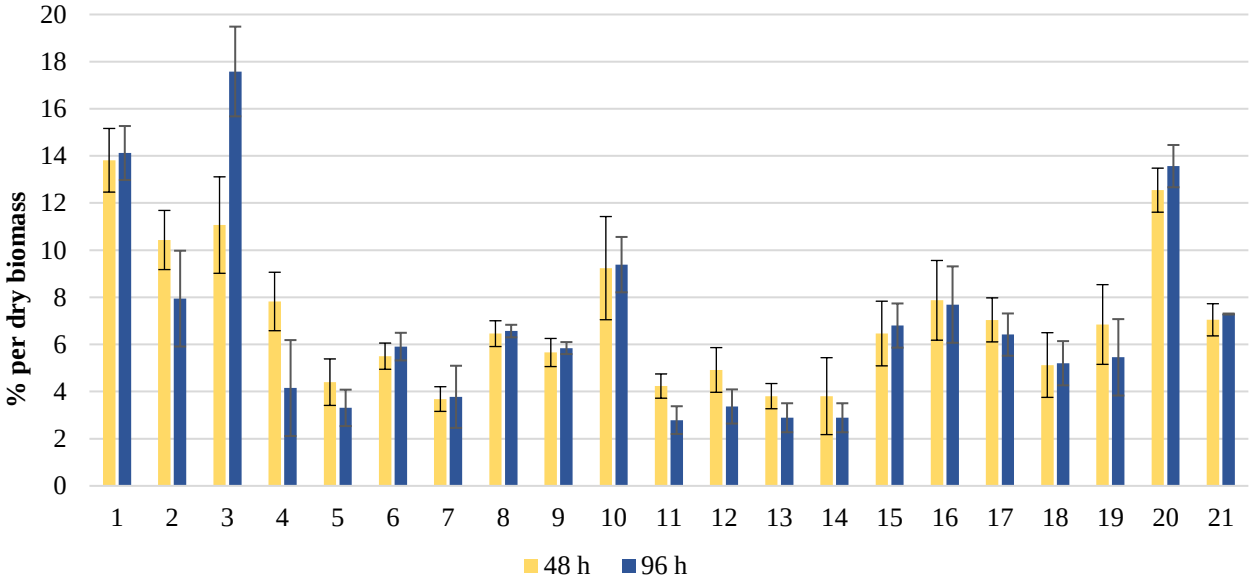


Fig. 15: The content of β-glucans after 48 and 96 hours of cultivation

The biomass yield after the cultivation on the control YPD broth production medium for 96 h was slightly higher than after 48 h for most of the strains (Fig. 16), with the exception for strains *M. pulcherrima* CCY 29-2-149, *M. pulcherrima* CCY 29-2-129, *C. infirmominiatum* CCY 17-18-4 and *C. macerans* CCY 10-1-2. These strains produced higher biomass yield after 48 h of cultivation (Fig. 16). In addition, in some yeast strains we observed only slightly higher β-glucans content after 96 h of cultivation, when compared with growth for 48 h (Fig. 16). The highest biomass yield 11.4 g/L was obtained for *R. glutinis* CCY 20-2-26 after 48h of cultivation (Fig. 16). Two strains of *R. mucilaginosa* CCY 19-4-6 and 20-9-7, *S. salmonicolor* CCY 19-4-25 and *S. pararoseus* (CCY 19-9-6) exhibited the also relatively high biomass yield after 48 h of cultivation slightly exceeding 8.0 g/L. All other strains except *P. rhodozyma* produced biomass yield close to 6 g/L. The lowest biomass yield of 4 and 2g/L for both 48 h and 96 h of cultivations respectively, was registered in *P. rhodozyma* CCY 77-1-1 strain.

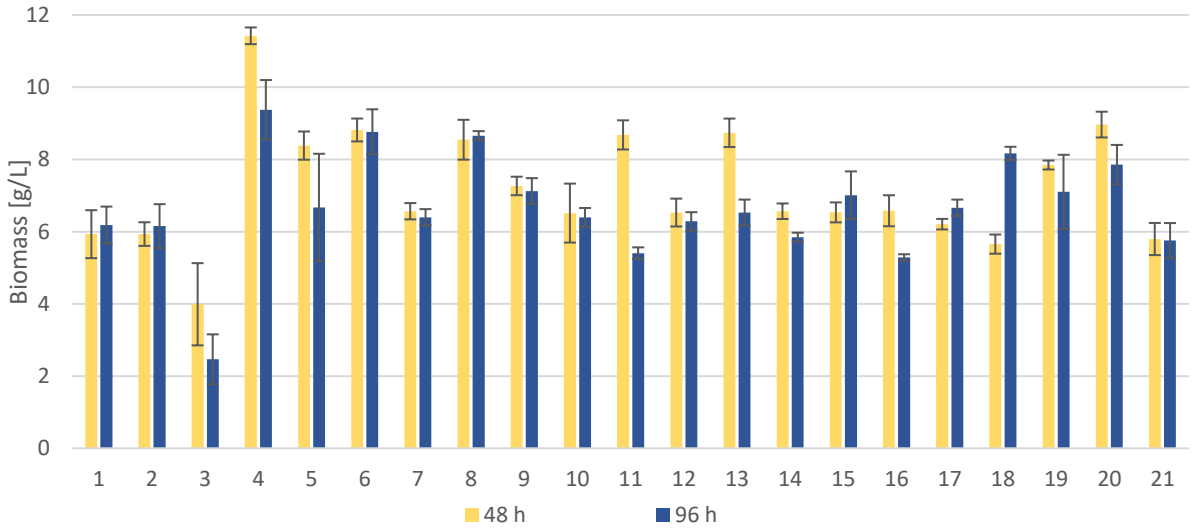


Fig. 16: Biomass yield after cultivation in the control YPD broth production medium for 48 h and 96 h

5.2 Effect of carbon:nitrogen ratio to biosynthesis of diverse metabolites

5.2.1 Growth, total glucan content and co-production of β-glucans and lipids

The screening of yeasts for identifying new potential producers of β-glucans and for the simultaneous glucan and lipid production was performed in a high-throughput set-up by utilizing high-throughput micro-cultivation in Duetz-MTPS. Due to the fact, that glucan production is usually performed in a low C/N ratio media [117], while lipid accumulation in yeasts is triggered by the high C/N ratio and depletion of a nitrogen source [115], media with both low and high C/N ratios were used for the screening. The identification of new promising yeast sources of β-glucans and potential yeast cell factories for the co-production of lipids and β-glucans was based on the estimating the following parameters: biomass yield , total glucan, β- and α-glucans, lipid yield and lipid profile (Tables 18 – 21, Figures 18 – 21).

Cultivation of yeasts in the media with low (10:1), moderate (40:1) and high (70:1 and 100:1) C/N ratios showed a continuous increase in a biomass yield with the increase of C/N ratio (Table 18). Thus, for most of the studied yeast strains the highest biomass yield in a range from 2.67±0.26 to 15.33±1.16 g/L was observed in the medium with the 100 C/N ratio, and strains *R. kratochvilovae* CCY 20-2-26, *R. mucilaginosa* CCY 19-4-6 and CCY 20-9-7, *R. toruloides* CCY

62-2-4 and *S. pararoseus* CCY 19-9-6 showed the highest biomass production in the medium with 70 C/N ratio (Table 18). Basidiomycetes yeast (Table 2). The highest biomass yield 15.3 g/L was obtained for the strain *C. macerans* CCY 10-1-2 at 100 C/N ratio, that was nearly three times higher than for the reference β-glucans producing strain *Saccharomyces cerevisiae* CCY 21-4-102. For all *Rhodotorula* strains and strain *S. salmonicolor* CCY 19-4-25 the significant increase in the biomass production occurred from 10 to 40 C/N ratio, and from 70 to 100 C/N ratio, while little change was observed from 40 to 70 C/N ratio.

Table 18: Biomass yield for yeasts grown in the media with low and high C/N ratios

			Biomass yield (g/L)			
No	**Strain name**	**CCY**	**C/N 10:1**	**C/N 40:1**	**C/N 70:1**	**C/N 100:1**
1	*Cystofil. infirmominiatum*	17-18-4	3.80±0.30	8.37±0.31	13.06±0.43	15.19±0.91
2	*Cystofilobasidium macerans*	10-1-2	3.63±0.24	8.76±0.27	13.34±0.06	**15.33±1.16**
3	*Phaffia rhodozyma*	77-1-1	2.67±0.26	6.78±0.24	10.98±0.50	13.09±1.01
4	*Rhodotorula kratochvilovae*	20-2-26	4.15±0.68	10.23±0.17	12.05±0.29	10.11±1.37
5	*Rhodotorula mucilaginosa*	19-4-6	4.57±0.29	10.36±0.26	11.34±0.23	10.84±0.35
6	*Rhodotorula mucilaginosa*	20-9-7	4.30±0.18	9.80±0.18	9.97±0.49	8.90±1.52
7	*Rhodotorula toruloides*	62-2-4	3.65±0.10	8.70±0.20	11.80±0.61	11.70±0.80
8	*Rhodotorula glutinis*	20-2-22	3.01±0.65	7.34±0.84	11.51±1.02	12.79±0.54
9	*Rhodotorula glutinis*	20-2-47	2.78±0.37	6.93±0.51	10.13±0.16	11.31±0.43
10	*Sporidiobolus metaroseus*	19-6-20	3.67±0.25	8.88±0.05	13.42±0.12	15.11±0.93
11	*Sporidiobolus pararoseus*	19-9-6	3.83±0.44	6.81±1.30	10.88±1.15	10.80±1.88
12	*Sporidiobolus salmonicolor*	19-6-4	2.99±0.15	6.30±0.15	6.55±0.31	8.24±1.09
13	*Sporidiobolus salmonicolor*	19-4-25	3.49±0.15	5.85±0.27	5.88±0.50	5.86±0.36
14	*Dioshegia hungarica*	18-1-3	4.14±0.48	6.61±0.47	7.14±0.21	7.20±0.39

Generally, *Ascomycetes* yeast showed lower biomass production in comparison to carotenogenic yeast (Table 19), except from *Y. lipolytica* CCY 29-26-4, where the biomass content reached 14.95±1.08 g/L. The growth of other yeast strains did not exceeted the yield of 7 g/L. The lowest biomass production can be seen at strain *A. pullulans* CCM 8183, where the 0.81 g/L of biomass was achieved.

Table 19: Biomass yield for yeasts grown in the media with low and high C/N ratios

			Biomass yield (g/L)			
No	**Strain name**	**CCY**	**C/N 10:1**	**C/N 40:1**	**C/N 70:1**	**C/N 100:1**
15	*Saccharomyces cerevisiae*	21-4-102	3.59±0.09	5.25±0.36	5.32±0.74	5.94±0.56
16	*Aureobasidium pullulans*	CCM 8183	1.05±0.04	1.03±0.02	1.12±0.11	0.81±0.16
17	*Candida glabrata*	CCM 8270	2.54±0.24	5.30±0.41	6.38±0.34	6.47±0.67
18	*Yarrowia lipolityca*	29-26-4	4.49±0.54	9.53±0.62	13.57±0.33	**14.95±1.08**
19	*Metschnikowia pulcherrima*	29-2-149	3.57±0.05	5.42±0.06	5.82±0.18	6.78±0.19
20	*Metschnikowia pulcherrima*	29-2-147	3.32±0.06	4.99±0.07	5.66±0.19	5.73±0.10
21	*Metschnikowia pulcherrima*	29-2-129	3.69±0.24	5.49±0.25	6.55±0.34	6.73±0.32

It has been shown previously that variation in a C/N ratio has a significant effect on the accumulation of carbon-based intracellular metabolites (carbohydrates, lipids, pigments, etc.) [115]. Therefore, the increased biomass yield at high C/N ratio could be mainly due to the increased accumulation of glucans (Table 20) and/or lipids (Fig. 18). Thus, we observed the increase in the glucan and lipid content at high C/N ratio for the yeast strains showing the increase in the biomass yield (Table 20, Fig. 18), with the exception of *C. macerans* CCY 10-1-2, *C. infirmominiatum* CCY 17-18-4, *S. metaroseus* CCY 19-6-20 in case of glucan and *S. salmonicolor* CCY 19-6-4 in case of lipids. For the strains *C. macerans* CCY 10-1-2, *P. rhodozyma* CCY 77-1-1 and *R. toruloides* CCY 62-2-4, the highest total glucan content was detected at low C/N ratios (Table 20). This could be due to the fact, that low C/N ratio triggering yeast proliferation and subsequently cell wall formation and carbon is transformed into protein and cell wall components, while lipogenesis is supressed. While the highest β-glucans content 38.84 ± 1.89 % (w/w) was achieved for the strain *C. glabrata* under 100 C/N ratio, and 26.96 ± 2.90% (w/w) for control strain *S. cerevisiae*, that was in accordance with the previously published results reported [1, 31, 32, 33, 34]. Among the carotenogenic yeast the highest total glucan content of 30.15±3.21 (w/w) with the high content of β-glucans 25.34±3.79 (w/w) was recorded for *C. infirmominiatum* CCY 17-18-4 (Table 20). Several other yeast strains showed very similar β-glucans content at different C/N ratios: (i) at 10 C/N ratio, strains *P. rhodozyma* CCY 77-1-1, *S. metaroseus* CCY 19-6-20, *R. kratochvilovae* CCY 20-2-26 and *C. macerans* CCY 10-1-2 had β-glucans content between 21,68% and 24,52% (w/w); (ii) at 40 C/N raio, strain *C. infirmominiatum* CCY 17-18-4 accumulated 25.34 ± 3.79% (w/w) of β-glucans; (iii) at 70 C/N ratio, strain *M. pulcherrima* CCY 29-2-149 accumulated 20.45 ± 0.86% (w/w) of β-glucans and (iv) at 100 C/N ratio, strains *M. pulcherrima* CCY 29-2-129 and *M. pulcherrima* CCY 29-2-147 showed β-glucan content of 22.35 ± 1.68% (w/w) and 21.09±1.68% (w/w), respectively (Table 3). Taking into the account the high biomass yield and β-glucans content for some of the yeast strains, as for example *C. infirmominiatum* CCY 17-18-4, *R. kratochvilovae* CCY 20-2-26 and *P. rhodozyma* CCY 77-1-1, they could be considered as promising new yeast sources of the β-glucans within the carotenogenic yeast.

Most of the studied yeast strains showed the negligible presence of α-glucans, while some of them have a relatively high content of it (Table 20). The highest α-glucan content, over 5 % per dry weight, was observed for the strain *C. macerans* CCY 10-1-2, which exhibited one of the lowest β-glucans content, meaning that the ratio of α- and β-glucans in this strain was 1:2 at 100 C/N ratio,

while in all other yeast strains β-glucans were in a significantly higher proportion than α-glucans (Fig. 20). The second highest content of α-glucans (4.81 ± 1.81 % per dry weight) was observed for *C. infirmominiatum* CCY 17-18-4 which also had the highest content of β-glucans (25.34 ±3.79 % CDW) (Table 20). Relatively high content of α-glucans was measured also for strains from genus *Sporidiobolus* and strain *P. rhodozyma* CCY 77-1-1 (Table 20), where the total glucan content increased at higher C/N ratios. The high content of α-glucans in Basidiomycetes carotenogenic yeasts could be explained by the possible presence of intracellular glucose (poly)saccharides like trehalose (α-1,1-bounded glucose) or glycogen (α-1,4- and α-1,6-glucose polymer) [116]. The lowest α-glucan content was detected for yeasts of *A. pullulans* CCM 8183, *Metschnikowia* genera and *R. mucilaginosa* strains, where it did not exceed 1.5 % of the total cell dry weight (Table 20 and 21).

Table 20: Total glucan, α- and β-glucans content (% CDW) in yeasts grown in the media with low and high C/N ratios

Strain		C/N 10:1	C/N 40:1	C/N 70:1	C/N 100:1
C. infirmominiatum CCY 17-18-4	Total glucan	26.04±0.80	30.15±3.21	24.78±1.64	23.73±2.25
	α-glucans	3.33±0.39	4.81±1.81	4.03±0.94	3.01±0.36
	β-glucans	**22.72±0.41**	**25.34±3.79**	**20.75±1.33**	**20.73±1.39**
C. macerans CCY 10-1-2	Total glucan	26.15±1.38	23.61±2.05	18.53±2.08	16.32±1.14
	α-glucans	2.68±0.37	5.19±3.30	5.08±3.30	5.02±3.25
	β-glucans	**23.43±1.60**	**18.42±2.64**	**13.45±4.42**	**11.31±3.34**
P. rhodozyma CCY 77-1-1	Total glucan	27.13±0.88	24.93±1.61	21.05±1.74	20.35±0.33
	α-glucans	2.61±0.06	2.68±0.75	2.20±0.40	2.03±0.28
	β-glucans	**24.52±0.85**	**22.28±1.05**	**20.04±0.18**	**18.55±0.15**
R. kratochvilovae CCY 20-2-26	Total glucan	23.18±1.19	23.80±1.12	21.84±0.83	18.82±0.29
	α-glucans	1.50±0.34	1.04±0.07	0.98±0.11	1.23±0.19
	β-glucans	**21.68±0.96**	**22.20±1.04**	**21.43±0.34**	**17.59±0.55**
R. mucilaginosa CCY 19-4-6	Total glucan	17.52±0.64	14.60±0.12	15.54±1.04	15.99±1.15
	α-glucans	0.87±0.27	0.49±0.09	0.66±0.10	0.73±0.04
	β-glucans	**16.65±0.67**	**14.11±0.20**	**14.88±1.08**	**15.26±1.15**
R. mucilaginosa CCY 20-9-7	Total glucan	18.18±0.06	19.17±0.67	19.91±1.21	20.31±0.95
	α-glucans	0.96±0.32	0.98±0.21	1.46±0.72	1.18±0.20
	β-glucans	**17.22±0.26**	**18.19±0.65**	**18.45±1.68**	**19.13±1.13**
R. toruloides CCY 62-2-4	Total glucan	19.26±0.84	14.74±0.25	11.75±1.22	11.81±0.58
	α-glucans	1.85±0.30	1.97±0.28	2.07±0.42	1.78±0.31
	β-glucans	**17.41±0.98**	**12.83±0.37**	**9.67±1.63**	**10.03±0.72**
R. glutinis CCY 20-2-22	Total glucan	10.35±0.54	16.02±1.27	6.63±0.28	5.56±0.36
	α-glucans	0.56±0.14	1.03±0.14	1.35±0.21	1.67±0.13
	β-glucans	**9.79±0.36**	**14.99±1.58**	**5.28±0.16**	**3.89±0.22**
R. glutinis CCY 20-2-47	Total glucan	10.23±1.02	9.37±0.34	6.08±0.28	4.13±0.32
	α-glucans	1.02±0.14	1.27±0.11	0.62±0.10	1.35±0.17
	β-glucans	**9.21±0.57**	**8.10±0.26**	**5.46±0.14**	**3.03±0.41**
S. metaroseus CCY 19-6-20	Total glucan	26.75±2.59	22.77±1.32	16.63±1.00	17.68±2.21
	α-glucans	2.60±0.30	4.50±0.36	2.62±0.15	2.92±0.23
	β-glucans	**24.15±2.89**	**18.27±1.32**	**13.99±1.04**	**14.76±2.32**
S. pararoseus CCY 19-9-6	Total glucan	14.30±1.21	16.87±1.91	15.58±0.66	14.73±0.79
	α-glucans	1.26±0.45	2.41±1.60	2.76±1.88	3.51±1.43
	β-glucans	**13.04±0.79**	**14.46±3.13**	**12.81±2.20**	**11.23±1.72**
S. salmonicolor CCY 19-6-4	Total glucan	12.90±1.25	17.12±0.92	17.96±0.50	18.95±2.00
	α-glucans	1.32±0.28	2.28±0.74	2.55±0.57	2.43±0.38
	β-glucans	**11.58±1.03**	**14.83±1.32**	**15.41±0.88**	**16.52±2.28**
S. salmonicolor CCY 19-4-25	Total glucan	15.04±0.67	14.10±0.31	14.95±0.63	17.16±1.73
	α-glucans	1.48±0.14	2.35±0.66	2.67±0.76	2.84±0.57
	β-glucans	**13.56±0.55**	**11.75±0.59**	**12.28±1.29**	**14.32±2.30**
D. hungarica CCY 18-1-3	Total glucan	13.45±1.45	15.34±0.45	11.56±0.68	9.17±0.88
	α-glucans	1.32±0.12	1.32±0.21	2.43±0.23	3.48±0.41
	β-glucans	**12.13±0.75**	**14.02±1.02**	**9.13±0.45**	**5.70±24**

Table 21: Total glucan, α- and β-glucans content (% CDW) in non-yeasts grown in the media with low and high C/N ratios

Strain		C/N 10:1	C/N 40:1	C/N 70:1	C/N 100:1
S.cerevisiae CCY 21-4-102	Total glucan	20.54±0.58	22.91±2.03	26.21±1.14	29.86±3.11
	α-glucans	2.35±0.38	2.35±0.56	3.41±0.80	2.90±0.41
	β-glucans	**18.19±0.36**	**20.57±1.54**	**22.80±0.58**	**26.96±2.90**
A. pullulans CCM 8183	Total glucan	10.45±0.55	10.90±0.55	11.72±1.23	13.54±0.74
	α-glucans	0.12±0.10	0	0.41±0.06	0.64±0.17
	β-glucans	**10.33±1.58**	**10.90±0.55**	**11.31±1.01**	**12.90±0.84**
C. glabrata CCM 8270	Total glucan	25.57±1.38	37.59±1.24	41.48±2.42	44.45±3.14
	α-glucans	3.45±0.16	3.94±0.16	5.35±0.26	5.61±0.37
	β-glucans	**22.12±1.49**	**33.65±1.32**	**36.13±1.57**	**38.84±1.89**
Y. lipolytica CCY 29-26-4	Total glucan	15.65±1.41	18.22±1.42	17.82±1.05	17.81±2.09
	α-glucans	0.84±0.12	1.07±0.13	1.89±0.27	1.89±0.07
	β-glucans	**14.81±0.84**	**17.15±1.41**	**15.93±1.24**	**15.93±1.22**
M. pulcherrima CCY 29-2-149	Total glucan	15.42±0.90	18.26±0.91	21.09±0.94	21.34±1.22
	α-glucans	0.29±0.12	0.57±0.20	0.64±0.18	1.30±0.31
	β-glucans	**15.13±1.02**	**17.69±0.79**	**20.45±0.86**	**20.04±1.12**
M. pulcherrima CCY 29-2-147	Total glucan	18.41±1.55	19.84±1.23	21.70±1.56	21.73±1.21
	α-glucans	0.35±0.04	0.42±0.04	0.77±0.26	0.64±0.11
	β-glucans	**18.06±1.54**	**19.42±1.09**	**20.93±1.80**	**21.09±0.69**
M. pulcherrima CCY 29-2-127	Total glucan	16.84±1.08	21.13±1.25	21.81±1.56	23.25±1.21
	α-glucans	0.47±0.29	0.52±0.19	1.28±0.72	0.90±0.59
	β-glucans	**16.37±1.12**	**20.61±1.09**	**20.54±1.10**	**22.35±1.68**

Some yeast strains, like *Y. lipolytica* CCY 29-26-4 (Fig. 17) exhibit dimorfphizm, i.e. the switch between the mold morphotype (hyphae) and yeast morphotype. This transition results in a change not only in the cell shape, but also in the composition of the cell wall, when the conversion from mold to yeast increase the content of α-(1,3)-glucan and decreased β-(1,3)-glucan content. This occurrence correlates with the level of virulence at pathogenic fungi but can be also interesting task in the production of fungal polysaccharides, like in the case of pathogenic bacteria *Streptococcus* and hyaluronic acid [118, 119].

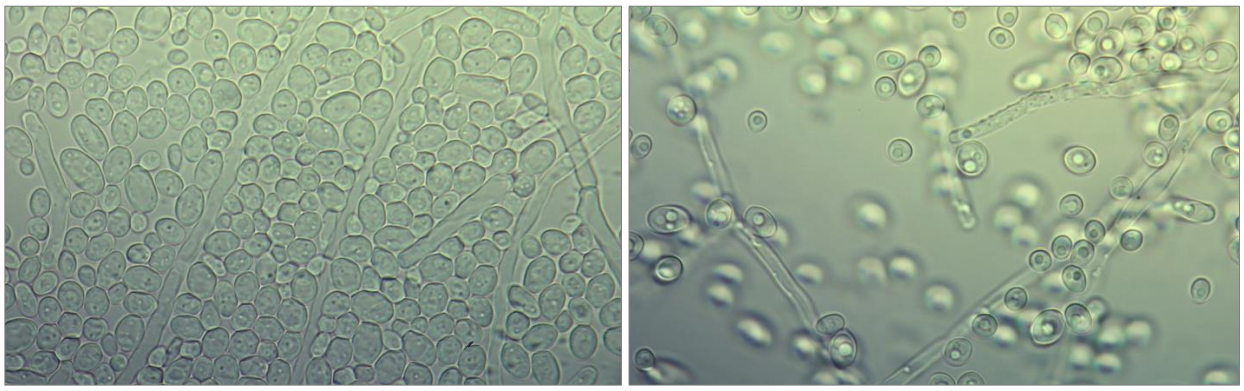


Fig. 17: Dimorphism of *Yarrowia lipolytica* (YPD, CN 100)

The decrease in glucan production for the *Cystofilobasidium* strains, as well as strains *R. toruloides* CCY 62-2-4, *R, kratochvilovae* CCY 20-2-26, and *P. rhodozyma* CCY 77-1-1 under high C/N ratios can be explained by the increased accumulation of lipids in the range from 30 up to 47 % (w/w). It is known that due to the high activity of AMP-dependent isocitrate dehydrogenase some Basidiomyctes carotenogenic yeasts produce lipids up to 70 % lipids/dry weight [115, 120].

In overall, the total lipid content, similarly as a glucan content, was increased in yeast cells when grown in the media with high C/N ratios (Fig. 20), except for the strains *R. kratochvilovae* CCY 20-2-26, *R. mucilaginosa* CCY 19-4-6 and CCY 20-9-7, *S. pararoseus* CCY 19-9-6 and *S.*

salmonicolor CCY 19-4-25, where high amount of glucose (100 C/N) led to the decrease in the lipid content accompanied by the lower biomass production (Table 19). This, could be due to the osmotic pressure stress under the high glucose concentration that negatively influenced the growth of these strains.

Basidiomycetes carotenogenic red yeasts showed oleaginous properties and were able to accumulate lipids from 30 to over 47 % (w/w) while Ascomycetes non-carotenogenic yeast did not accumulate lipids more than 10% (w/w) (Fig. 2). While, it has been reported that non-carotenogenic *Metschnikowia* yeast can accumulate large amount of lipids (up to 40% w/w) [121], in the present study it did not accumulate more than 10 % (w/w) that could be due to the higher temperature and shorter cultivation time used, in comparison to the previsouly reported 15 ^{o}C and 14 days [121]. Generally, Ascomycetes yeasts were not affected by the variation in the amount of glucose in the media, and the biomass and lipid yield were unchanged.

High lipid yield in the range from 33 to 59 % (w/w) was observed for the strains *C. infirmominiatum* CCY 17-18-4, *C. macerans* CCY 10-1-2, *R. toruloides* CCY 62-2-4, *S. metaroseus* CCY 19-6-20, *R. glutinis* CCY 20-2-47 and *S. pararoseus* CCY 19-9-6 when grown under high C/N ratios. The highest lipid content 59.20 ± 1.60 % (w/w) was observed for carotenogenic strain *R. glutinis* CCY 20-2-47 (Fig. 18). The confocal light microscopy of *C. macerans* CCY 10-1-2 cells showed clearly visible large round structures – lipid droplets (Fig. 20). Yeast strains with the highest lipid accumulation (over 45 % w/w), namely *C. macerans* CCY 10-1-2, *R. toruloides* CCY 62-2-4, *R. glutinis* CCY 20-2-47, and *S. metaroseus* CCY 19-6-20 showed low β-glucan content (10 – 14 % w/w) and can be considered as mainly lipid producers. Some yeast strains as for example *C. infirmominiatum* CCY 17-18-4 at 100 C/N and *R. kratochvilovae* CCY 20-2-26 at 70 C/N were able to accumulate relatively high content of both, lipids (38.21 % and 37.92 % of w/w) and β-glucan (20.73 and 21.43 % of w/w) accompanying with the high biomass yield (15.19 and 12.05 g/L) and therefore could be utilised for developing co-production strategies.

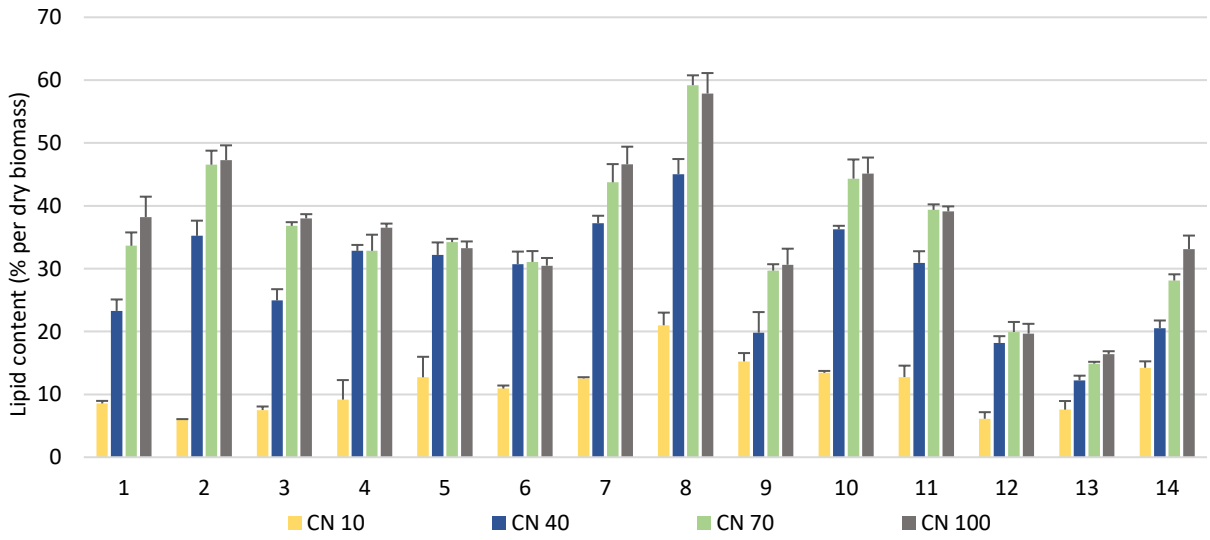


Fig. 18: Lipid content (% w/w) in carotenogenic yeasts grown in the media with low and high C/N ratios. Yeast strain numbers are described in Table 18.

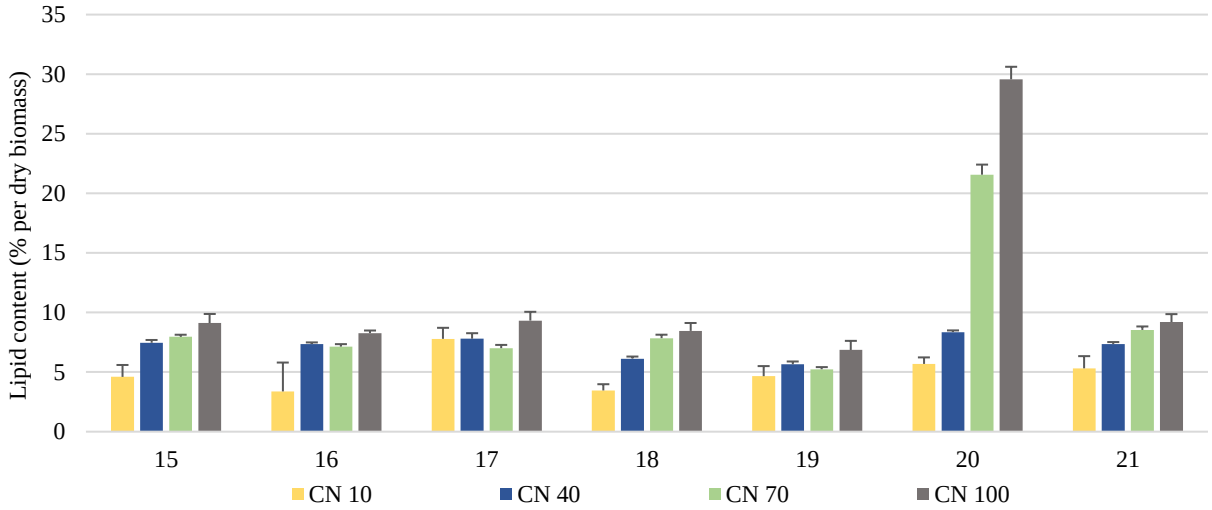


Fig. 19: Lipid content (% w/w) in Ascomycetes yeasts grown in the media with low and high C/N ratios. Yeast strain numbers are described in Table 19.

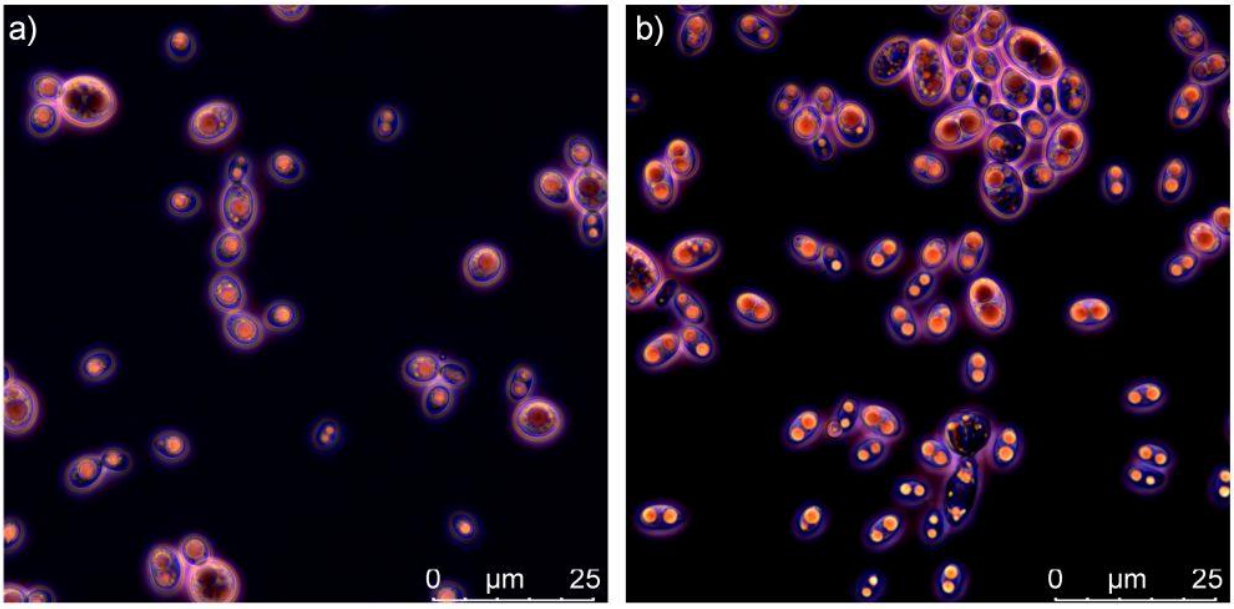


Fig. 20: Microscopy images (false colouring) of *C. infirmominiatum* CCY 17-18-4 (a) and *C. macerans* CCY 10-1-2 (b) grown in 100 C/N ratio medium.

Lipids accumulated in yeasts are represented mainly by triacyl glycerides (TAGs). A detailed fatty acid profile was identified by gas chromatography, where fatty acids present in the amount higher that 2% were taken in a consideration. In all yeast strains the major saturated fatty acids were palmitic (C16:0) and stearic acid (C18:0), monosaturated – palmitoleic (C16:1) and oleic acid (C18:1n9c), and polyunsaturated fatty acids were represented by linoleic (C18:2n6c), γ-linolenic (C18:3n3) and α-linolenic (C18:3n3) fatty acids (Fig. 21). The most abundant fatty acid present in all yeast strains was oleic acid, with a production of over 40 %, except of *S. cerevisiae* CCY 21-4-102, where the major fatty acid was palmitoleic fatty acid. Depending on the C/N ratio and yeast strain, the content of monounsaturated (MUFA), polyunsaturated (PUFA) and saturated (SAT) fatty acids changed significantly (Fig. 21). The content of MUFA, specifically oleic fatty acid, increased with the increase in C/N ratio in all yeast strains (Fig. 21). The highest amount of MUFA

(49 % w/w) was detected for *S. metaroseus* CCY 19-6-20 strain, which showed also one of the highest total lipid and biomass yield (Table 19, Fig. 19) and, thus, it could be considered as a promising candidate for biofuels production, due to the MUFA is the desired oil component for biodiesel production providing low temperature fluidity and oxidative stability [122]. The biggest difference in fatty acid profile can be observed between 10 and 100 C/N ratios, where the highest SAT content in all yeasts was detected at 10 C/N ratio (Fig. 21) while higher C/N ratio favoured oleic acid biosynthesis and suppressed linoleic fatty acid production (Fig. 21). Interesting, that fatty acid profile of strains from genus *Metschnikowia*, belonging to the Ascomycetes phylum, was more similar to the fatty acid profile of carotenogenic Basidiomycetes yeast, differed only for slightly higher production of C16:1 and lower C16:0. Among of the studied Basidiomycetes yeasts, *Rhodotorula* yeasts showed a high production of palmitic fatty acid. Strain *S. cerevisiae* CCY 19-6-4 showed very consistent fatty acid profile not affected by the different C/N ratios, and it had the lowest production of PUFAs and the highest amount of palmitoleic fatty acid (C16:1) at all studied C/N ratios. The highest content of PUFA, with linolenic fatty acid dominating, was detected in *S. salmonicolor* CCY 19-6-4 strain with the yield up to 40 % w/w at 40 C/N ration.

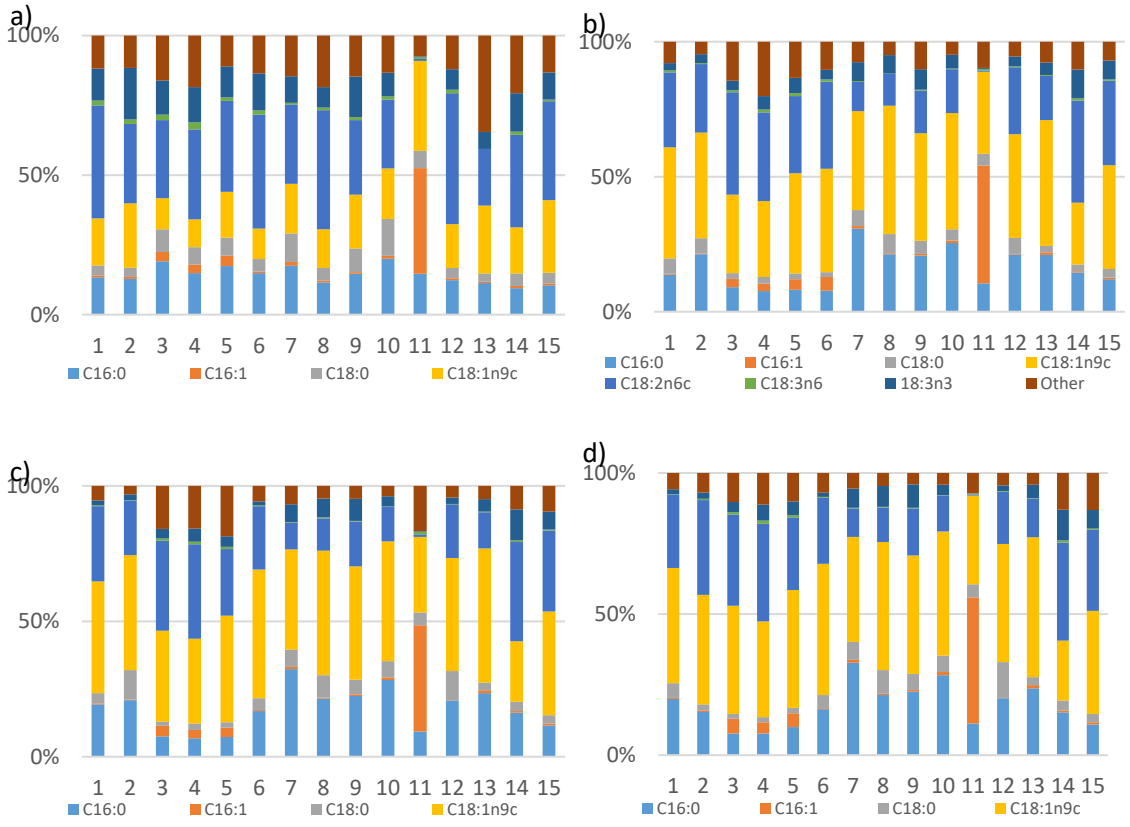


Fig. 21: Fatty acid profile of TAGs accumulated in yeasts grown in the media with (a) 10 C/N, (b) 40 C/N, (c) 70 C/N and (d) 100 C/N ratios (strain numbers are presented in the Table 22)

9.2.1. Total cellular biochemical profiling by FTIR spectroscopy

Fourier transform infrared (FTIR) spectroscopy is a biophysical Next-generation Phenotyping technique, allowing to perform a non-destructive and rapid biochemical profiling of intracellular

and extracellular metabolites of microbial cells by applying High-throughput Screening (HTS) measurement mode. FTIR spectroscopy has been applied in microbiology since 90s, and it has been proven as a highly reproducible and sensitive method for the identification and phenotypic characterization of microorganisms [123], screening for the production of different intracellular metabolites [124, 125], monitoring of substrate consumption and extracellular product release [126], monitoring of metabolite extraction processes [127] etc. In this study, we applied FTIR spectroscopy for obtaining total cellular biochemical profiles of the studied yeasts grown in the media with different C/N ratios.

The obtained FTIR spectra at different spectral regions provide information on all main biochemical building blocks of the yeast cells. Lipids are described mainly by the two spectral regions 3010 – 2800 cm^{-1} and 1800 – 1700 cm^{-1} and some single peaks related to -CH_2 and -CH_3 scissoring in a region 1400 – 1300 cm^{-1} (Table 23, Fig. 22). When analyzing cellular lipid profile based on FTIR spectra, the most important lipid associated peaks usually taken in the consideration, are: (1) peaks 2947 cm^{-1}, 2923 cm^{-1}, 2847 cm^{-1}, 1449 cm^{-1} and 1394 cm^{-1} are related to -CH_3 and -CH_2 stretching and indicating mainly the chain length of carbon skeleton in lipid molecules; (2) peak 1745 cm^{-1} is related to the ester bond stretching and indicating the total lipid content in the cell; (3) peak 1710 cm^{-1} is related to the carboxyl bond vibrations in free fatty acids, and (4) peak 3010 cm^{-1} is related to =C-H stretching in lipids and indicates the unsaturation level of cellular lipids. Proteins are observed in the spectral region 1700 – 1500 cm^{-1} with the main peaks for amide I (1650 cm^{-1}) and amide II (1540 cm^{-1}) bonds and polysaccharides are observed in the region 1200 – 900 cm^{-1} which is mainly related to the sugar ring vibrations (Table 4, Fig. 4).

Table 22. Yeast strains studied with FTIR spectroscopy

No.	Strain	No.	Strain
1	*C. infirmominiatum* CCY 17-18-4	9	*R. mucilaginosa* CCY 20-9-7
2	*C. macerans* CCY 10-1-2	10	*R. toruloides* CCY 62-2-4
3	*M. pulcherrima* CCY 29-2-149	11	*S. cerevisiae* CCY 21-4-102
4	*M. pulcherrima* CCY 29-2-147	12	*S. metaroseus* CCY 19-6-20
5	*M. pulcherrima* CCY 29-2-129	13	*S. pararoseus* CCY 19-9-6
6	*P. rhodozyma* CCY 77-1-1	14	*S. salmonicolor* CCY 19-6-4
7	*R. kratochvilovae* CCY 20-2-26	15	*S. salmonicolor* CCY 19-4-25
8	*R. mucilaginosa* CCY 19-4-6		

Table 23. Peaks assignment for the FTIR spectra of yeast

Peak №*	Wavenumber**	Peak assignment	Reference
1	3010	=C-H stretching in lipids	[128]
2	2947	-C-H (CH_3) stretching in lipids and hydrocarbons	[128]
3	2925	-C-H (CH_2) stretching	[129]
4	2855	CH_2/CH_3 stretching in lipids and hydrocarbons	[128]

Peak №*	Wavenumber**	Peak assignment	Reference
5	1745	C=O ester bond stretching in lipids, esters and polyesters	[129]
6	1680 – 1630	-C=O stretching, α-Helix Amide I in proteins	[130]
7	1530 – 1560	N-H bending and C-N stretching, Amide II in proteins	[130]
8	1465	CH_2/CH_3 stretching in lipids	[128]
9	1377	-C-H (CH_3) bending (sym) in lipids	[131]
10	1265	–P=O stretching of phosphodiesters	[131]
11	1200 - 1100	C-O-C/C-O stretching in polysaccharides	[131]

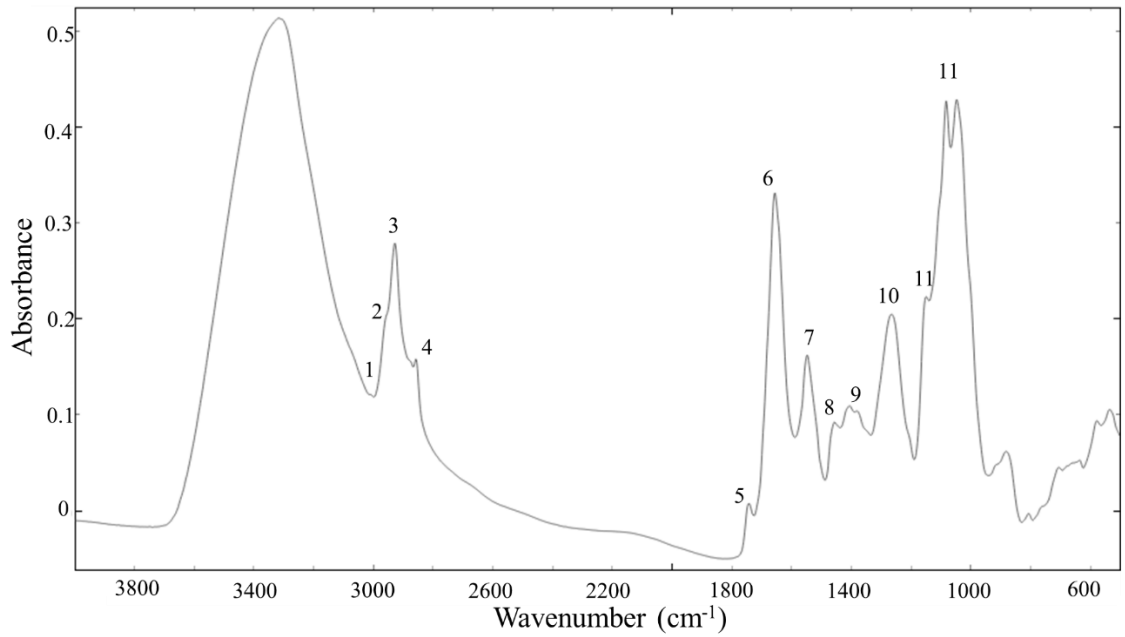


Fig. 22: Pre-processed FTIR spectra *S. cereviseae* CCY 21-4-102 biomass grown in the 70 C/N ratio medium. Peak numbers correspond to the numbers given in Table 23.

For identifying biochemical profile of yeasts grown on different C/N ratios based on the obtained FTIR spectra, a principal component analysis (PCA) was applied. The PCA scatter plot shows that C/N ratio strongly affecting lipid and protein profile in all yeasts, where FTIR spectra of yeasts grown on the medium with 10 C/N ration are grouped distinctly from the spectra of yeasts grown on other C/N ratios (Fig. 23). This indicates, that both total lipid content and fatty acid profile of accumulated TAGs in yeasts cells obtained from 10 C/N medium is significantly different from yeasts grown on other ratios (Fig. 23 A and C). Further, some strain- and species-specific differences in lipid and protein profile were observed from FTIR spectra. Thus, lipid and protein FTIR profile for strains of genus *Metchnikowia* obtained from all types of media, and strains of specie *S. salmonicolor* and *S. cereviseae* grown on 40, 70 and 100 C/N ratios was clearly different

from others (Fig. 23 A and C). These results correlate well with the obtained reference fatty acid profile data, where strains of *Metchnikowia* and *S. salmonicolor* and *S. cererevisiae* differed from others in a content of palmitic, palmitoleic, and polyunsaturated fatty acids (Fig. 21). The polysaccharide profile was not affected by C/N ratio in Ascomycetes yeasts, while significant differences were observed for Basidiomycetes yeasts (Fig. 23B). Polysaccharide profile of strains from genus *Metchnikowia* and specie *S. salmonicolor* differed significantly from other strains (Fig. 23B).

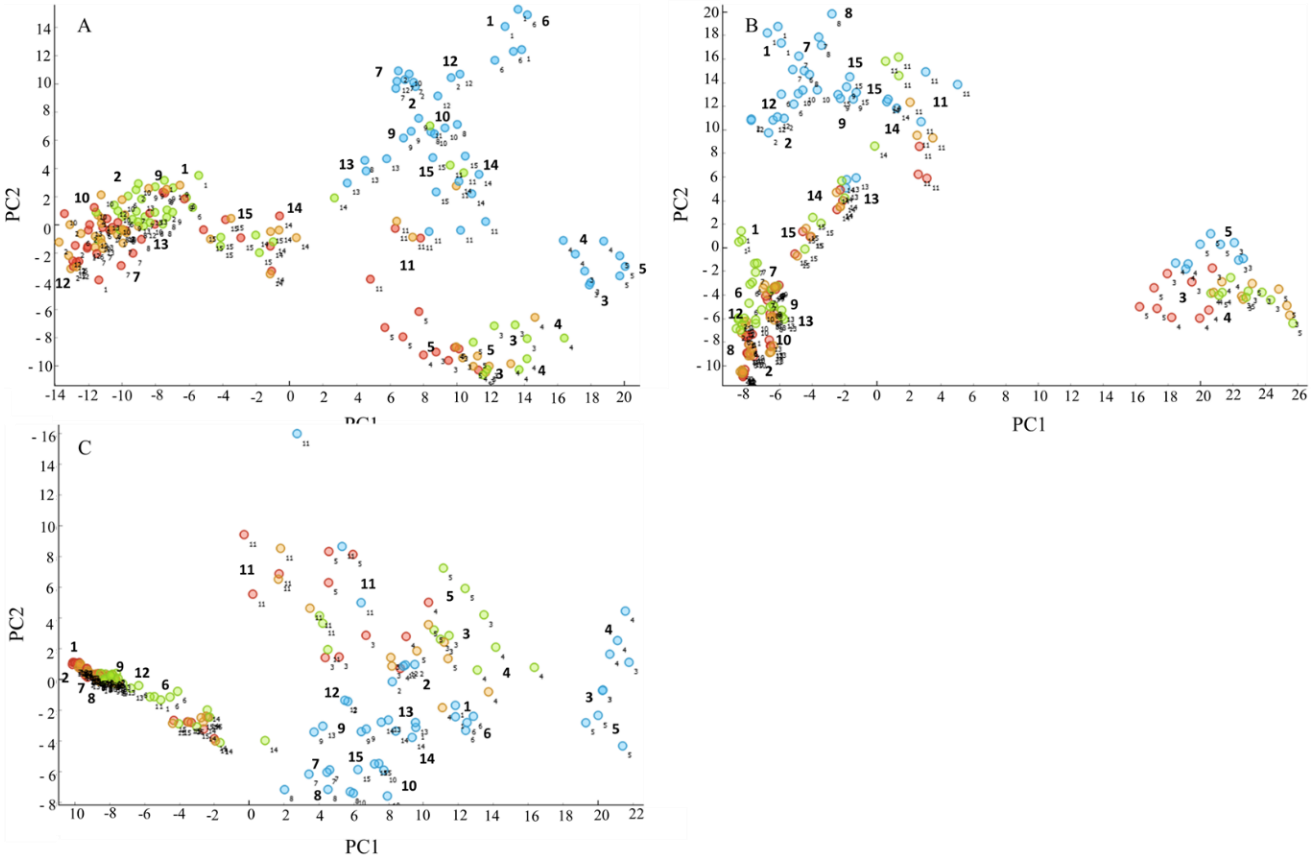


Fig. 23: PCA scatter plot of lipid (A), polysaccharide (B) and protein (C) spectral regions of FTIR spectra of yeasts grown on media with C/N ratios: 10 (blue), 40 (green), 70 (orange) and 100 (red). Strains are labels with numbers according to the Table 22.

9.2.2. Carotenoids and ergosterol production at red yeast

Carotenoids are important by-products of red yeast. They serve as natural colorants, antioxidants used in food industry, pharmaceuticals, aquaculture and poultry industry. Commercial carotenoids are obtained by chemical synthesis and/or extraction from vegetables. However, plants origin's carotenoids are problematic regarding seasonal and geographic variability that cannot be controlled [132].

Carotenoids productivity vary with every publication. The amounts of carotenoids produced by the genus *Rhodotorula* can be classified as high at yields more than 500 μg/g as reported in literature [132]. Total carotenoids content founded in studied 16 carotenogenic strains from genera *Rhodotorula*, *Cystofilobasidium*, *Phaffia*, *Sporidiobolus* and *Dioshegia* uccured in the range from 31.1 μg/g to 3.49 mg/g (Table 24). The main carotenoids occurred in yeast biomass are beta-

carotene, thorulene and small amount of lycopene. Most of the remaining carotenoids (e.g. at *R. kratochvilovae* CCY 20-2-26 more than 50 %) stayed unknown due the lack of the standards.

In general, the increasing C/N ratio decreased in the carotenogenesis, except the strains *P. rhodozyma* CCY 77-1-1, *R. mucilaginosa* CCY 19-4-6 and *S. salmonicolor* CCY 19-6-4. High C/N ratio is benefitial for lipids accumulation in oleaginous microorganisms, which together with increasing carotenoids content is benefitial for commertial production.

Suprisinglly, yeast *R. mucilaginosa* CCY 19-4-6 is able produce lycopene as a main carotenoid. Commertial lycopene production relied on extraction from natural sources (e.g. tomatoes) or chemical synthesis [133]. Microbial production counts on genetic modification or use of specific chemical inhibitors during the fermentation (tertiary amines, aminomethylpyridines or using a heterocyclic nitrogen basis, such as imidazole, pyridine, morpholine, quinolone, methylheptenone, etc.) [133]. Our strain is natural producer, propably with highly inhibited lycopene cyclase, and reach maximum at C/N ratio 100 – 2.66±0.12 g/L.

Table 24: Production of total carotenoids at different C/N ratios

		Total carotenoids (mg/g of biomass)			
Strain name	**CCY**	**C/N 10:1**	**C/N 40:1**	**C/N 70:1**	**C/N 100:1**
Cystofil. infirmominiatum	17-18-4	0.18±0.10	0.60±0.09	0.37±0.08	0.24±0.05
Cystofilobasidium macerans	10-1-2	0.54±0.07	2.52±0.38	2.42±0.36	2.27±0.28
Phaffia rhodozyma	77-1-1	0.76±0.11	2.20±0.21	3.49±0.23	2.67±0.21
Rhodotorula kratochvilovae	20-2-26	0.14±0.02	1.68±0.35	1.13±0.18	1.14±0.20
Rhodotorula mucilaginosa	19-4-6	31.1±4.0 μg/g	2.87±0.45	2.44±0.43	2.98±0.22
Rhodotorula mucilaginosa	20-9-7	35.3±3.3 μg/g	2.31±0.26	1.85±0.29	2.11±0.15
Rhodotorula toruloides	62-2-4	0.21±0.04	2.41±0.43	2.41±0.35	2.13±0.28
Rhodotorula glutinis	20-2-22	Not detected	2.29±0.24	2.22±0.21	1.54±0.16
Rhodotorula glutinis	20-2-47	Not detected	3.33±0.29	2.94±0.24	1.55±0.22
Sporidiobolus metaroseus	19-6-20	Not detected	0.20±0.04	0.27±0.08	0.22±0.07
Sporidiobolus pararoseus	19-9-6	Not detected	0.20±0.04	0.42±0.10	0.41±0.04
Sporidiobolus salmonicolor	19-6-4	0.18±0.10	0.60±0.09	0.37±0.08	0.24±0.05
Sporidiobolus salmonicolor	19-4-25	0.11±0.06	2.52±0.38	2.42±0.36	2.27±0.28
Dioshegia hungarica	18-1-3	0.49±0.03	2.10±0.34	3.26 ±0.29	2.61±0.38

Ergosterol is one of the most crucial components in fungal membranes and it is involved in regulation of membrane fluidity, control of the cellular cycle and distribution and activity of integral proteins. It is a provitamin of vitamin D2 known as ergocalciferol. Exposure to ultraviolet radiation initiates a photochemical reaction in which ergosterol is transformed into ergocalciferol. It is also the target of antifungal drugs [134].

The biggest content of provitamine D was found in *Sporidiobolus pararoseus* CCY 19-9-6 at higher C/N ratio (70:1) (7.42±0.57 mg/g). The lowest values exhibited *R. glutinis* CCY 20-2-47,

C. infirmominiatum CCY 17-18-4 and *D. hungarica* CCY 18-1-3 at low C/N ratios, where the ergosterol content dropped to 1 mg/g of biomass (Table 25).

In general, the production of ergosterol stayed constant through different C/N ratios. The only difference was found only between individually strains.

Table 25: Ergosterol biosynthesis by carotenogenic yeast at different C/N ratios

		Ergosterol (mg/g)			
Strain name	**CCY**	**C/N 10:1**	**C/N 40:1**	**C/N 70:1**	**C/N 100:1**
Cystofil. infirmominiatum	17-18-4	1.15±0.12	2.58±0.84	2.01±0.49	1.76±0.08
Cystofilobasidium macerans	10-1-2	1.87±0.26	3.07±0.65	2.36±0.56	2.25±0.24
Phaffia rhodozyma	77-1-1	1.37±0.19	2.47±0.87	3.12±0.41	2.20±0.10
Rhodotorula kratochvilovae	20-2-26	1.43±0.08	2.34±0.16	1.35±0.20	1.57±0.15
Rhodotorula mucilaginosa	19-4-6	1.72±0.24	3.03±0.64	2.48±0.41	2.70±1.14
Rhodotorula mucilaginosa	20-9-7	1.23±0.16	3.88±0.12	3.26±0.39	3.87±0.63
Rhodotorula toruloides	62-2-4	1.34±0.09	3.32±0.39	2.83±0.37	3.95±0.68
Rhodotorula glutinis	20-2-22	2.03±0.28	3.14±0.39	3.10±0.11	2.05±0.78
Rhodotorula glutinis	20-2-47	1.07±0.09	4.74±0.49	3.14±0.37	3.85±0.36
Sporidiobolus metaroseus	19-6-20	2.57±0.42	4.63±0.56	5.23±0.50	5.76±0.64
Sporidiobolus pararoseus	19-9-6	3.31±0.40	6.84±1.53	7.42±0.57	3.29±0.22
Sporidiobolus salmonicolor	19-6-4	2.52±0.24	2.58±0.84	2.01±0.49	1.76±0.08
Sporidiobolus salmonicolor	19-4-25	2.54±0.36	3.07±0.65	2.36±0.56	2.25±0.24
Dioshegia hungarica	18-1-3	1.19±0.13	2.47±0.87	3.12±0.41	2.20±0.10

9.3. Salt stress

Cell wall remodelling is often a key response event when cells are exposed to the osmotic stress [135, 136]. Due to the fact, that β-glucans are important structural component of the cell wall, applying osmotic stress could potentially have a significant effect on their production. In addition, it is well known that any variations in extracellular osmolarity influence the cell volume, and therefore, the concentration of intracellular macromolecules [137, 138]. Thus, difference in osmolarity could potentially effect lipid accumulation in yeast cells. Recently, several studies reported the influence of extracellular osmolarity on β-glucans' biosynthesis in *Saccharomyces cerevisiae* where a decrease in β-glucan content at higher osmolarity was detected [139]. These studies utilized only a single osmolarity condition (6% of NaCl), while a screening of a wide range of extracellular osmolarity levels is needed if one wants to obtain a deeper understanding of the impact of osmotic stress on glucans production. To the authors knowledge, the impact of osmotic stress on the co-production of the β-glucans and lipids in yeasts was not reported before.

In this study, we used sodium chloride (NaCl) at six different concentrations (0.2; 0.5; 2; 5; 8 and 11%) to investigate the influence of different extracellular osmolarity levels on the production of β-glucans and co-production of lipids and β-glucans [140]. The three yeast strains *C.*

infirmominiatum CCY 17-18-4, *P. rhodozyma* CCY 77-1-1 and *R. kratochvilovae* CCY 20-2-26, showing the highest β-glucans and a high lipid production in this study were selected for the study. The *S. cerevisiae* CCY 19-6-4 strain was used as a control strain for β-glucans production. The supplementation of growth media with C/N ratios of 40 and 70 by NaCl at all studied concentration led to a decrease in the biomass yield for all yeast strains except for *S. cerevisiae* (Table 26). The biomass production for *S. cerevisiae* under low osmolarity conditions (0,2% and 0,5% of NaCl) in C/N ratio growth media with C/N ratios of 40, 70 and 100 was higher or very similar to the standard conditions (Table 5, Table 2), while high osmolarity resulted in the significant decrease of biomass (Table 5). Low extracellular osmolarity (0.2% of NaCl) combined with high C/N ratio resulted in an increase or no change in the biomass yield in comparison to the standard conditions (Table 5, Table 2). The biomass yield was gradually decreasing with the increased amount of NaCl in the media and high concentration of NaCl combined with high C/N ratio resulted in the lowest biomass production that was possibly due to the elevated levels of osmolarity caused by both, high NaCl and glucose content in the medium (Table 5). The strain *P. rhodozyma* CCY 77-1-1 showed the highest sensitivity to the applied osmotic stress, and it growth was highly inhibited in the presence of NaCl 2% and higher (Table 5).

Table 26: The effect of different osmolarity levels on biomass yield in strains *C. infirmominiatum* CCY 17-18-4 (1), *P. rhodozyma* CCY 77-1-1 (6), *R. kratochvilivae* CCY 20-2-26 (7), *S. cerevisiae* CCY 21-4-102 (11)

Strain	C/N	0.2% NaCl	0.5% NaCl	2% NaCl	5% NaCl	8% NaCl	11% NaCl
1	40	7.44±0.04	7.38±0.19	7.08±0.11	6.38±0.07	5.93±0.07	4.81±0.61
	70	11.40±0.10	11.47±0.35	10.77±0.07	10.28±0.07	9.13±0.49	4.54±0.84
	100	15.25±0.39	14.88±0.11	11.40±2.84	11.33±0.05	8.90±0.59	4.73±0.24
6	40	5.62±0.41	5.56±0.08	2.24±0.16	1.05±0.14	0.70±0.16	0.68±0.07
	70	8.86±0.61	8.69±0.27	2.59±0.21	1.06±0.09	0.87±0.16	0.64±0.10
	100	12.35±1.21	11.11±1.48	2.73±0.19	1.00±0.03	0.78±0.23	0.66±0.13
7	40	9.04±0.31	8.57±0.30	8.44±0.07	7.52±0.10	5.71±0.25	3.71±0.09
	70	11.76±0.81	11.26±0.73	10.70±0.04	8.47±0.08	5.80±0.46	3.90±0.39
	100	11.52±0.74	10.83±0.75	10.48±0.04	8.24±0.06	5.69±0.42	3.69±0.47
11	40	5.45±0.11	5.26±0.20	5.03±0.07	3.25±0.04	2.71±0.16	1.78±0.13
	70	5.94±0.14	5.70±0.31	4.97±0.06	3.82±0.05	3.03±0.29	1.69±0.04
	100	6.32±0.40	5.71±0.47	4.95±0.09	4.00±0.03	3.04±0.28	1.57±0.06

The effect of extracellular osmolarity on the total glucan, β- and α-glucan content in yeasts cells was strain-specific and differed depending on the level of osmolarity and C/N ratio used (Table 27). Thus, low (0.2 and 0.5% of NaCl) and in some cases moderate (2 and 5%) levels of osmolarity combined with C/N ratios of 40, 70 and 100 led to an increase in the total glucan and β-glucan content in comparison to the standard conditions (Table 27, Table 18). For example, the addition of 0.2% NaCl caused increase in β-glucan production up to 32.15 ± 0.81 (w/w) in *C. infirmominiatum* CCY 17-18-4 (Table 27), that is about 21 % more than in the standard conditions and significantly higher than for the control strain *S.cerevisiae* CCY 21-4-102 (Table 6). In the case of strain *P. rhodozyma* CCY 77-1-1, β-glucans content was increased at all osmolarity levels when combined with C/N ratios of 70 and 100, while it showed an inhibiting effect on production of β-glucans in other yeast strains (Table 27).

Table 27: Glucans production on media supplemented with sodium chloride (% od DCW)

Strain	C/N	% w/w	0.2% NaCl	0.5% NaCl	2% NaCl	5% NaCl	8% NaCl	11% NaCl
		Total Glucan	35,65±0,86	34,10±1,91	32,87±1,75	22,71±1,67	21,65±0,97	20,80±1,56
	40	Alpha-Glucan	3,50±0,40	3,55±0,39	3,07±0,32	2,96±0,22	3,03±0,48	2,63±0,43
		Beta-Glucan	32,15±0,81	30,55±1,84	29,80±1,43	19,75±1,87	18,62±0,67	18,17±1,55
		Total Glucan	27,15±1,26	25,42±0,38	21,11±0,44	23,33±1,07	19,72±0,56	21,07±0,96
1	70	Alpha-Glucan	3,16±0,21	3,11±0,30	2,79±0,10	3,87±1,16	2,70±0,12	2,79±0,77
		Beta-Glucan	23,99±1,06	22,32±0,63	18,32±0,52	19,46±1,54	17,03±0,66	18,28±1,23
		Total Glucan	21,83±0,27	21,64±1,47	23,16±0,45	19,95±1,58	17,96±0,82	21,52±2,16
	100	Alpha-Glucan	3,04±0,29	3,41±0,36	4,42±1,29	3,04±0,18	3,20±0,03	3,11±0,77
		Beta-Glucan	18,78±0,49	18,24±1,11	18,73±1,71	16,91±1,40	14,76±0,82	18,40±2,02
Strain	**C/N**	**% w/w**	**0.2% NaCl**	**0.5% NaCl**	**2% NaCl**	**5% NaCl**	**8% NaCl**	**11% NaCl**
		Total Glucan	31,69±1,97	32,51±0,51	24,47±1,76	27,75±0,99	29,05±0,46	26,25±0,87
	40	Alpha-Glucan	4,37±2,58	3,25±0,52	3,44±0,17	3,05±0,45	3,25±0,98	2,72±0,56
		Beta-Glucan	27,32±4,55	29,26±1,02	21,03±1,93	24,70±0,60	25,80±1,26	23,53±1,44
		Total Glucan	24,27±2,24	24,30±0,97	26,60±2,10	27,18±0,81	25,65±0,74	24,27±1,37
6	70	Alpha-Glucan	2,42±0,35	2,71±0,32	3,05±0,24	2,69±0,30	3,24±0,66	2,46±0,89
		Beta-Glucan	21,85±2,07	21,59±0,65	23,55±2,31	24,49±0,96	22,41±0,20	21,81±0,69
		Total Glucan	24,97±1,16	27,56±1,29	24,29±1,21	28,11±0,87	26,74±0,83	26,02±1,06
	100	Alpha-Glucan	2,32±0,35	2,73±0,57	3,09±0,13	2,58±0,50	2,87±0,36	2,81±0,88
		Beta-Glucan	22,65±1,19	24,83±1,86	21,20±1,20	25,53±1,28	23,87±0,46	23,21±1,17
		Total Glucan	24,13±2,17	23,53±2,53	17,08±1,09	13,29±0,96	14,67±0,67	12,23±1,90
	40	Alpha-Glucan	1,25±0,28	1,19±0,11	1,13±0,26	0,91±0,14	1,19±0,14	0,84±0,23
		Beta-Glucan	22,88±2,37	22,34±2,53	15,95±0,85	12,38±1,04	13,47±0,61	11,39±1,67
		Total Glucan	18,97±1,45	20,32±0,10	16,41±1,00	11,81±1,09	14,48±1,85	14,84±2,38
7	70	Alpha-Glucan	1,12±0,33	0,83±0,38	0,93±0,31	0,68±0,13	0,90±0,24	1,14±0,19
		Beta-Glucan	17,84±1,20	19,49±0,44	15,48±1,30	11,14±1,05	13,58±1,62	13,69±2,20
		Total Glucan	20,36±0,46	18,18±1,00	13,03±0,53	12,08±0,67	15,68±0,29	9,42±0,38
	100	Alpha-Glucan	1,20±0,24	1,07±0,24	0,68±0,11	0,59±0,07	1,05±0,31	0,81±0,20
		Beta-Glucan	19,16±0,64	17,11±0,78	12,35±0,63	11,49±0,63	14,63±0,31	8,62±0,34
		Total Glucan	30,40 ± 0,72	29,87±1,69	23,95±2,51	23,76±2,93	19,24±1,12	16,31±1,29
	40	Alpha-Glucan	1,49±0,18	1,31±0,35	2,05±0,32	1,21±0,17	0,67±0,21	0,35±0,13
		Beta-Glucan	28,91±0,62	28,56±1,37	21,90±2,81	22,55±2,77	18,57±0,96	15,93±1,45
		Total Glucan	29,16±1,40	27,29±0,36	31,08±2,42	25,83±2,12	18,07±0,45	16,94±2,13
11	70	Alpha-Glucan	1,69±0,39	1,54±0,42	2,19±0,32	1,50±0,10	0,52±0,20	0,58±0,16
		Beta-Glucan	27,47±1,03	25,75±0,57	28,89±2,40	24,33±2,15	17,56±0,58	16,36±2,24
		Total Glucan	33,38±0,93	31,55±2,43	30,06±2,11	24,32±0,92	21,67±0,29	15,59±2,03
	100	Alpha-Glucan	2,06±0,19	1,13±0,47	1,75±0,31	1,03±0,09	1,05±0,32	0,50±0,14
		Beta-Glucan	31,32±0,93	30,42±2,02	28,31±2,27	23,29±0,84	20,62±0,10	15,10±1,92

Extracellular osmolarity at all levels resulted in an decrease of lipid accumulation for all yeast strains in comparison to the standard growth conditions, except for strain *S. cerevisiae*, which did not show any significant changes in the lipid content when NaCl was added to the media (Table 28). Interestingly, yeast cells exposed to relatively moderate amounts of NaCl (2, 5 and in some cases 8%) showed a slight increase in lipid accumulation for most of the studied yeast strains (Table 28), while in the case of *S. cerevisiae* lipid content was increasing with an increased amount of NaCl in the media (Table 28).

Table 28: Lipid content after exposure yeast to salt stress (% od DCW)

Nr.	C/N	0.2% NaCl	0.5% NaCl	2% NaCl	5% NaCl	8% NaCl	11% NaCl
	40	19.66±1.58	18.64±2.13	24.39±0.67	29.07±1.85	26.94±1.28	19.50±1.12
1	**70**	25.58±1.80	25.57±2.15	30.88±0.88	32.97±0.94	36.27±3.05	24.87±0.98
	100	39.85±2.62	36.06±4.36	35.22±4.14	35.26±2.12	31.31±1.74	25.25±2.24
	40	22.08±1.08	21.65±1.70	27.95±2.10	31.40±1.69	16.57±0.65	20.40±0.60
6	**70**	31.84±0.31	35.13±0.68	29.55±1.85	36.64±3.93	22.48±4.36	19.38±2.14
	100	35.49±3.35	31.78±5.69	30.04±0.43	36.32±2.14	20.16±1.22	25.35±1.97
	40	26.13±3.85	26.62±0.97	29.62±0.99	31.33±0.54	29.67±1.11	18.91±1.47
7	**70**	27.66±1.09	32.28±3.04	35.04±2.56	34.18±2.29	31.40±3.72	25.23±2.20
	100	31.82±2.83	36.79±4.17	31.35±1.98	34.96±2.23	30.09±1.60	21.59±1.49
	40	6.62±0.90	8.39±0.71	8.87±1.28	7.03±0.55	7.99±0.41	9.14±0.76
11	**70**	10.24±0.21	10.48±0.65	9.12±0.67	10.99±0.36	7.83±0.88	12.09±1.37
	100	5.68±0.19	8.67±2.49	9.40±0.87	11.73±0.94	10.65±0.45	13.14±1.65

9.4. Influence of the temperature and media composition to β-glucan production

Each of the selected yeast species was cultured in four types of media, differing in composition, resp. nitrogen source (Table 7). Cultivation was carried out in a tempered shaker, ensuring a constant temperature (8, 15, 22, and 30 °C) throughout cultivation. The glucan measurement takes place at higher biomass content, namely at 168 h (8 °C), 144 h (15 °C), 96 h (22 °C), and 144 h (30 °C). The growth curves are visualized in the section of supplementary materials (Fig. S3 – S6).

For all studied media, the combination of ammonium sulphate and urea (MED 4 – Table 8) shows the increase in β-glucan at *S. cerevisiae* CCY 21-4-102, *C. infirmominiatum* CCY 17-18-4 and *P. rhodozyma* CCY 77-1-1 (29.12, 12.32 and 23.48 % of DCW respectively). Concerning *R. kratochvilovae* CCY 20-2-26, the urea as the only nitrogen source exhibit the highest yield (MED 3) of β-glucan. Conversely, the biomass decreases due to low temperature.

Table 29: The effect of the temperature (8 °C) and media composition to β-glucan production (% od DCW)

8 °C **168 h**	*S. cerevisiae* CCY 21-4-102	*C. infirmominiatum* CCY 17-18-4	*P. rhodozyma* CCY 77-1-1	*R. kratochvilovae* CCY 20-2-26
MED 1	26.05±1.58	6.45±0.89	21.74±0.76	20.97±1.08
MED 2	12.59±1.06	5.84±0.67	20.83±0.99	21.01±0.20
MED 3	14.01±1.10	11.72±0.55	15.94±0.45	**22.95±2.14**
MED 4	**29.12±2.37**	**12.32±1.04**	**23.48±2.14**	16.45±1.12

The temperature of 15 °C has a positive effect on *C. infirmominiatum* CCY 17-18-4 (21.94 %, MED 3), *P. rhodozyma* CCY 77-1-1 (29.10 %, MED 3) and *R. kratochvilovae* CCY 20-2-26 (27.19 %, MED 1). The β-glucan content decreased at *S. cerevisiae* CCY 21-4-102. At most of the studied strains (except of *S. cerevisiae* CCY 21-4-102) the increase of the β-glucan production can be seen, but also the decrease in biomass content.

Table 30: The effect of the temperature (15 °C) and media composition to β-glucan production

15 °C	*S. cerevisiae*	*C. infirmominiatum*	*P. rhodozyma*	*R. kratochvilovae*
144 h	CCY 21-4-102	CCY 17-18-4	CCY 77-1-1	CCY 20-2-26
MED 1	16.71±1.25	12.49±0.26	23.68±1.90	**27.19±2.11**
MED 2	13.44±0.73	**21.94±1.49**	28.43±1.05	25.07±3.74
MED 3	14.98±1.06	21.09±2.01	**29.10±2.64**	17.54±1.69
MED 4	**17.03±1.84**	15.86±0.61	28.39±0.55	21.61±1.72

The temperature of 22 °C seemst to be optimal for *C. infirmominiatum* CCY 17-18-4 (26.08±2.03 % of β-glucan on DCW) (Table 31). Oppositelly, the production was inhibited at *S. cerevisiae* CCY 21-4-102, *P. rhodozyma* CCY 77-1-1, *R. kratochvilovae* CCY 20-2-26 when compared with the temperature of 15 and 8 °C (Table 29 and 30).

Table 31: The effect of the temperature (22 °C) and media composition to β-glucan production

22 °C	*S. cerevisiae*	*C. infirmominiatum*	*P. rhodozyma*	*R. kratochvilovae*
96 h	CCY 21-4-102	CCY 17-18-4	CCY 77-1-1	CCY 20-2-26
MED 1	9.54±1.62	10.39±0.20	**24.76±1.05**	13.17±1.02
MED 2	5.09±0.18	**26.08±2.03**	21.15±1.39	9.92±0.45
MED 3	**11.99±0.39**	19.25±1.13	22.72±0.67	**18.06±0.65**
MED 4	10.01±0.72	8.83±0.36	23.46±0.41	16.71±1.14

Table 31 presents the β-glucan content at 30 °C, which was highly inhibited in all culture media and at all studied strains. Thus, the temperature of 30 °C is not benefitial for biotechnological β-glucan production.

Table 32: The effect of the temperature (30 °C) and media composition to β-glucan production

30 °C	*S. cerevisiae*	*C. infirmominiatum*	*P. rhodozyma*	*R. kratochvilovae*
144 h	CCY 21-4-102	CCY 17-18-4	CCY 77-1-1	CCY 20-2-26
MED 1	9.35±0.68	7.59±0.24	**6.08±0.74**	**5.22±0.16**
MED 2	**10.41±1.05**	4.80±0.06	3.98±0.09	3.07±0.04
MED 3	7.12±0.17	**12.45±1.37**	5.15±0.33	2.66±0.13
MED 4	4.89±0.50	3.77±0.43	4.07±0.58	2.38±0.72

In general, increasing the culture temperature decreased the β- glucan content at all studied strains (Table 32). At *C. infirmominiatum* CCY 17-18-4 the highest biomass content occurred at 8 °C, 11.14 g/L, which corresponds with the information from Culture Collection of Yeast, where the optimal conditions for growth is 15 °C. Opossitely, the β-glucan yield was optimal at 22 °C, where it reached 26.08 % w/w (ammonium sulphate as a single nitrogen source), which was even higher than on medium with yeast extract. The highest β-glucan production was measured at *S. cerevisiae* CCY 21-4-102 – 29.12±2.37 % w/w (8 °C, combination of urea and yeast extract), but the biomass was declined (only about 3 g/L). The optimal biomass production was at 22 °C (11 g/L) on medium with urea, but the β-glucan content decrease to 11.99±0.39 % w/w. The highest biomass contents were measured at *R. kratochvilovae* CCY 20-2-26 (12 – 15 g/L) when cultured on medium with urea as the single nitrogen source. For this strain the optimal temperature combined with β-glucan and biomass yield is 22 °C (18.06±0.65 % of DCW**)**.

There are no direct informations about correlation between temperature, medium composition and β-glucan content at carotenogenic yeast. Most of the research is focused on bacteria, where

low temperature can increase the production of extracellular polysaccharides (the same for yeast) or surviving of the yeast during fermentation process [141, 142].

9.5. Exopolysaccharides production

Some microbial strains produce a broath spectrum of extracellular metabolites, such as proteins, DNA, RNA, lipids etc. Particularly, many yeasts synthetize and secrete exopolysaccharides (EPS), biopolymers comprised of monosugar units, and it is related to their secondary metabolism. The biosynthesis and structure depend on many factors, which include the composition of the culture medium and fermentation conditions such as pH, temperature and oxygen concentration [143]. Here, the screening of 11 carotenogenic strains for possibility of EPS biosynthesis were explored. The strains were cultured at MTPs at triplicates on media supplemented with glucose and sucrose, as the most optimal carbon sources according to literature [143].

From all studied yeast only one strain exhibited the biosynthesis of extracellular polysaccharides, namely *Sporidiobolus pararoseus* CCY 19-9-6, with amount of 2.17 g/L (medium with glucose) and 2.28 g/L (medium with sucrose) (Figure 24).

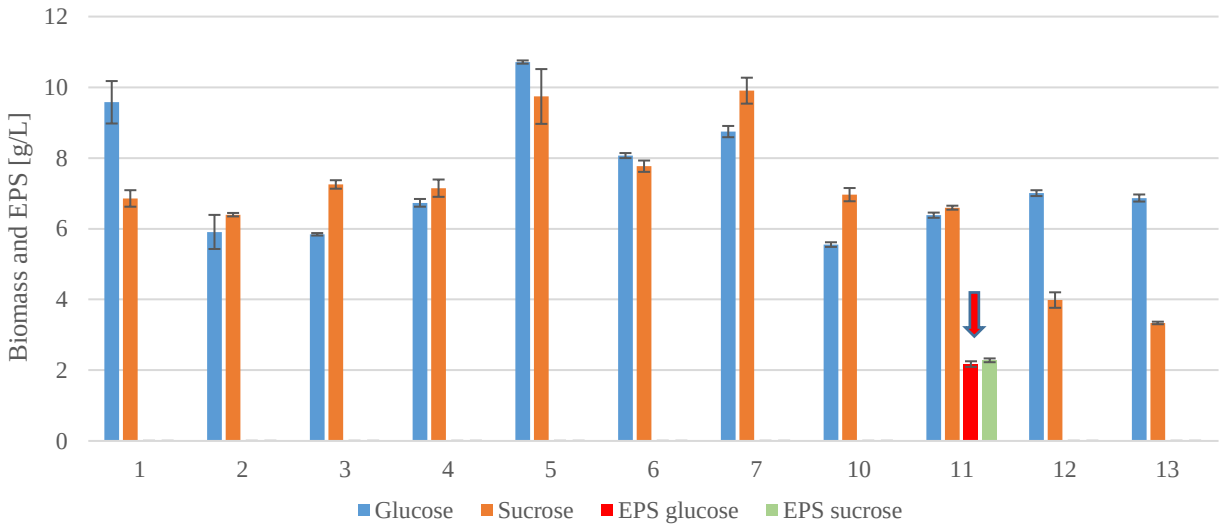


Fig. 24: Screening of exopolysaccharide potential producers (the number designation of individual strains is presented in the Table 18)

After the choice of production strain (*S. pararoseus* CCY 19-9-6), the culture conditions for enhanced production of the exopolysaccharides were explored. From the Table 32, the increase of glucose caused higher yield of EPS, 1.21±0.17 g/L. Ammonium sulphate (AS) or combination AS with yeast extract decreased the bioproduction.

The low temperature (8 °C) with combination of higher C/N ratio exhibited greater accumulation of polysaccharides, on the contrary, the temperature of 30 °C inhibits the secretion of the polymer into culture media.

Another important factor, the pH, has a big influence for EPS biosynthesis, where slightly acidic medium (pH 4) increased in the production to 2.35 g/L. The cause can be the hydrolysis of polysaccharide from cell surface. In highy acidic conditions (pH 3 and 2) the biosynthesis of EPS was inhibited but also the biomass.

Surprisingly, the 2% NaCl has the positive effect on EPS biosynthesis, where the highest yield was achieved, 3.17±0.07 g/L.

Table 33: Exopolysaccharides production in *S. pararoseus* CCY 19-9-6

	EPS [g/L]
Yeast extract (YE), glucose, C/N 40	0.46±0.05
Yeast extract, glucose, C/N 70	0.68±0.08
Yeast extract, glucose, C/N 100	**1.21±0.17**
Ammonium sulphate (AS), glucose, C/N 40	0.20±0.03
Ammonium sulphate, glucose, C/N 70	0.41±0.02
Ammonium sulphate, glucose, C/N 100	0.59±0.07
YE/AS, glucose, C/N 100	0.76±0.12
YE, saccharose, C/N 100	0.86±0.06
YE, glycerol, C/N 100	0.48±0.03
Temperature – 8 ºC	
YE, glucose, C/N 40	1.26±0.07
YE, glucose, C/N 70	0.99±0.05
YE, glucose, C/N 100	**1.92±0.10**
Temperature – 30 ºC	
YE, glucose, C/N 40	0
YE, glucose, C/N 70	0
YE, glucose, C/N 100	0
pH	
pH 2	0.59±0.02
pH 3	1.83±0.13
pH 4	**2.35±0.08**
pH 7	1.62±0.16
pH 8	1.52±0.09
pH 9	1.26±0.11
NaCl	
2% NaCl	**3.17±0.07**
5% NaCl	1.5±0.12
11% NaCl	0.51±0.01

9.6. Exoglycolipids, intracellular lipids and β-glucan biosynthesis at *R. kratochvilovae*

Secretion of extracellular glycolipids has been already reported at *Rhodotorula* strains [89, 90]. Here, for optimization of culture conditions for enhancement of the exoglycolipid biosynthesis the yeast *Rhodotorula kratochvilovae* CCY 20-2-26 was choosen, which was the only strain that exhibited the presence of "oil droplets" on the surface of the culture medium (Fig. 25). The occurrence of extracellular glycolipid was confirmed by FTIR (Fig. S14, supplementary material section).

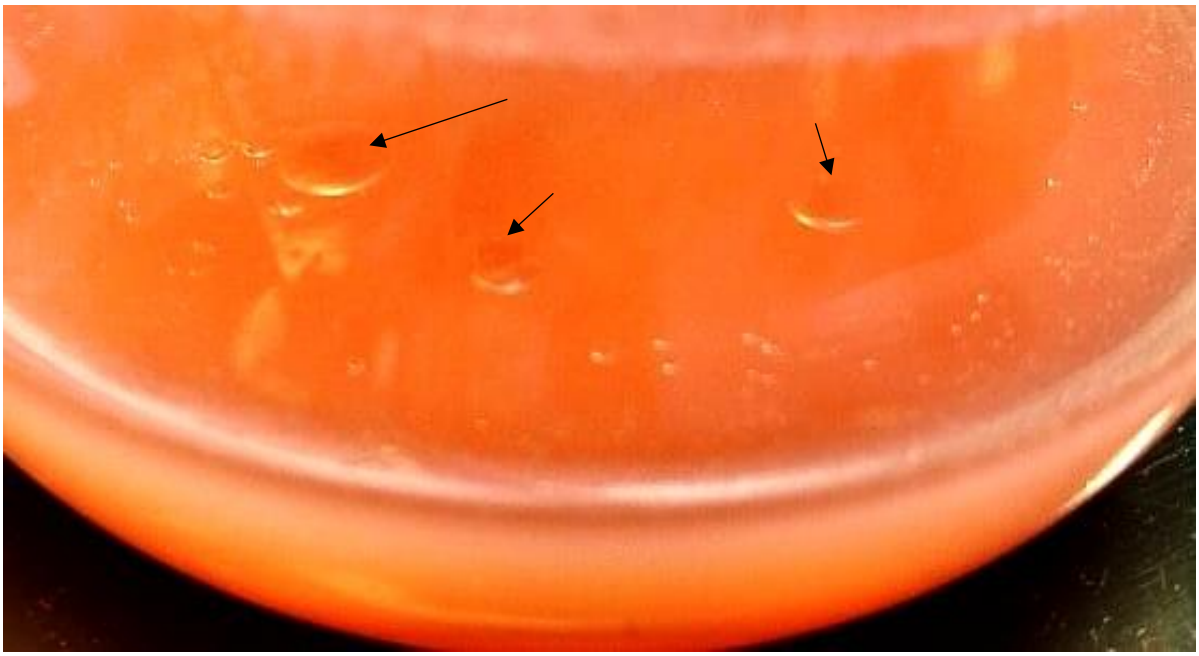


Fig. 25: The presence of "oil droples" (black arrows) on the surface of culture medium of the yeast strain *Rhodotorula kratochvilovae* CCY 20-2-26

9.9.1 Influence of different nitrogen sources on growth and metabolism of *Rhodotorula kratochvilovae* CCY 20-2-26

In the first step, *R. kratochvilovae* CCY 20-2-26 grew in the presence of various nitrogen sources such as potassium nitrate, yeast extract, ammonium sulfate, ammonium chloride, and urea. Here, glucose was used as a carbon source. The conditions and composition of the culture media are presented in chapter 8.4.

Figure 26 shows biomass production with different nitrogen sources. Potassium nitrate, yeast extract, and urea appear to be the most effective sources of nitrogen. Yields for these sources reached a value of about 9.0 g/L of biomass. The highest biomass yield was obtained using potassium nitrate as a nitrogen source in the 144h hour of cultivation, namely 9.5 g/L of biomass. On the contrary, ammonium sulphate and ammonium chloride were evaluated as the worst sources of nitrogen, therefore these sources were no longer used in the following experiments. In general, the highest biomass production was recorded after 96 and 144 hours.

Jiru et al. (2017) state ammonium sulfate in combination with yeast extract and peptone as the best nitrogen source. When cultivated in bioreactors, the amount of biomass reached values of about 17.0 g/L, which is a value higher than ours, but the possible cause is due to the different way of cultivation. As other sources of nitrogen, Jiru et al. (2017) used ammonium chloride again in combination with yeast extract and peptone, but biomass yields were slightly lower there, about 15.0 g/L. As in our experiment, ammonium chloride was a less suitable source of nitrogen [144].

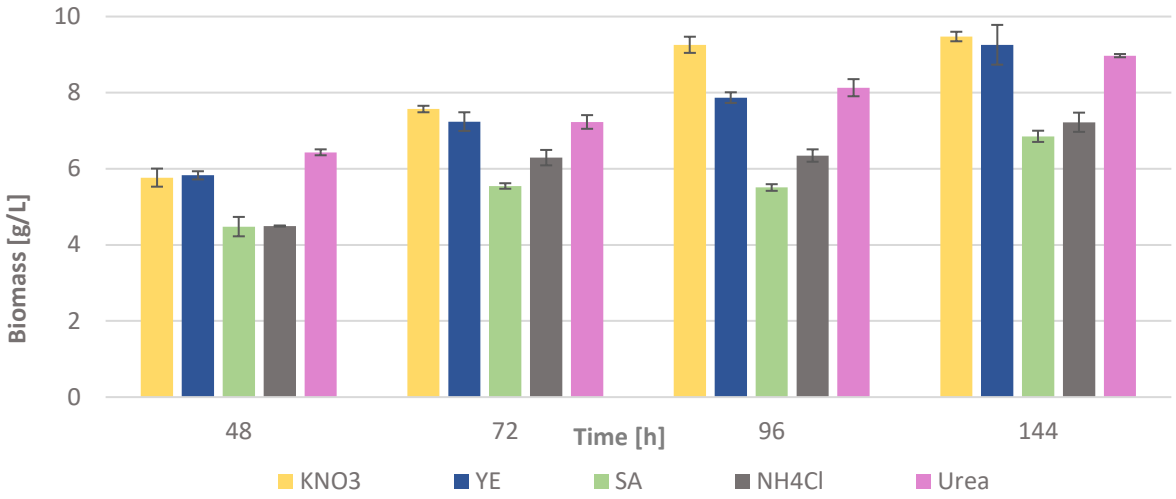


Fig. 26: Biomass production at different nitrogen sources at C/N ratio 70:1

Diverse nitrogen sources affected differently the glucan composition in yeast biomass. Pottasium nitrate, yeast extract and urea decreased the beta-glucan yield through the time. Opositelly, the ammonium sulphate reached maximum in the end of cultivation, 17.60±1.84 % w/w.

Table 34: Glucan content in yeast biomass cultivated on different nitrogen sources

N source	Time	48 h	72 h	96 h	144 h
Pottasium nitrate	Total glucan	24.22±2.14	22.14±1.54	17.26±1.03	16.55±0.38
	Alpha-glucan	2.16±0.21	3.83±0.23	2.02±0.06	2.16±0.04
	Beta-glucan	**22.05±2.21**	**18.31±1.83**	**15.24±1.24**	**14.39±0.77**
Yeast extract	Total glucan	27.11±1.54	23.79±1.36	18.46±1.15	14.30±1.03
	Alpha-glucan	2.12±0.13	2.36±0.20	1.76±0.02	1.69±0.01
	Beta-glucan	**24.99±1.69**	**21.43±1.57**	**16.70±1.33**	**12.62±1.11**
Ammonium sulphate	Total glucan	15.12±0.37	16.59±0.89	17.56±0.67	18.46±1.59
	Alpha-glucan	2.71±0.48	2.62±0.11	1.02±0.04	0.86±0.15
	Beta-glucan	**12.41±1.02**	**13.98±1.04**	**16.54±1.00**	**17.60±1.84**
Urea	Total glucan	23.45±1.00	22.86±1.05	16.65±0.44	17.94±1.07
	Alpha-glucan	2.51±0.33	2.71±0.21	1.98±0.03	1.01±0.09
	Beta-glucan	**20.94±1.25**	**20.15±1.30**	**14.67±0.59**	**16.93±1.13**
Ammonium chloride	Total glucan	17.18±0.19	18.69±0.49	15.49±0.16	12.61±0.41
	Alpha-glucan	2.38±0.04	2.98±0.06	2.15±0.14	1.32±0.12
	Beta-glucan	**14.80±0.81**	**15.71±0.66**	**13.33±0.85**	**11.30±0.75**

Figure 27 shows the biosynthesis of exoglycolipids, where the best sources of nitrogen were also ammonium nitrate, yeast extract, and urea, while the worst sources were again ammonium sulfate and ammonium chloride. Amplitudes were reached at 144 hours using potassium nitrate, where the concentration of exoglycolipids reached up to 1.8 g/L. The lowest yield was observed at 72 and 144 hours of cultivation using ammonium sulfate, only 0.1 g/L.

In potassium nitrate, a slight decrease in the amount of exoglycolipids was observed between 72 and 144 hours, and thus at 96 hours of cultivation, the yield value decreased. In the case of ammonium chloride, the culture also reached the highest value at 96 hours of cultivation, namely 0.3 g/L, and at 144 hours the value was lower. Yields with urea then increased over time from 0.6 g/L at 48 hours of cultivation to 1.6 g/L at 144 hours.

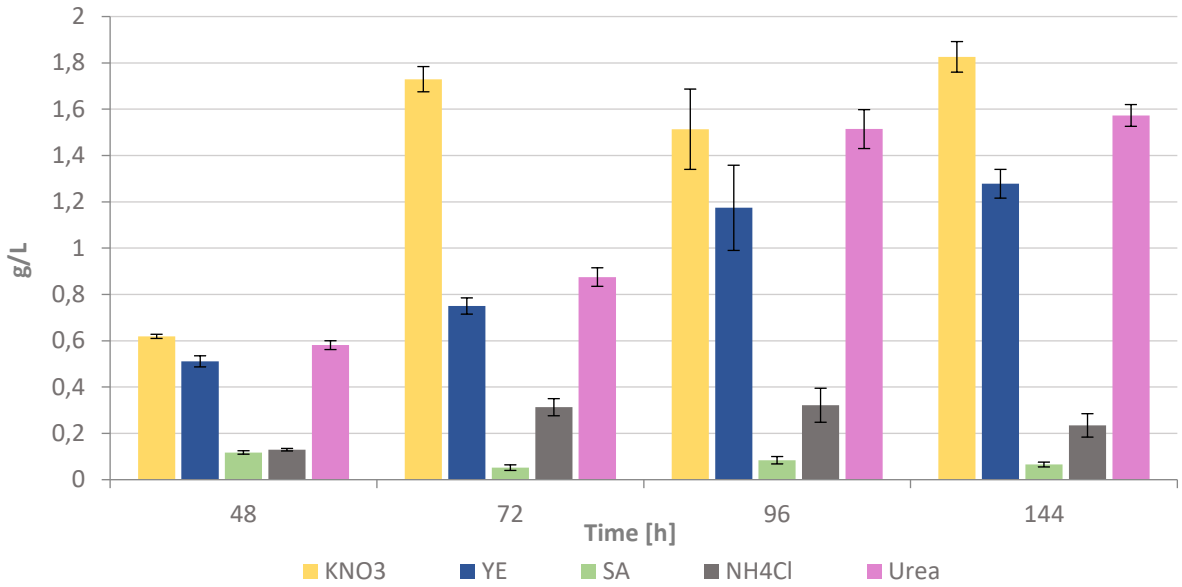


Fig. 27: Extracellular glycolipids yield at media with different nitrogen sources

Figure 28 shows the production of intracellular lipids during cultivation on various nitrogen sources. The results show high yields of intracellular lipid content, almost 60% of the DCW. The highest values were reached in the 96th hour of cultivation, then the number of accumulated lipids rather decreased, so the culture probably got into a stationary phase of growth. The yeast extract, as a source of nitrogen, showed almost the highest yield value, about 58% at 96 hours of cultivation.

The worst source of nitrogen in this experiment was ammonium sulphate with about 37 %. A slightly better source than ammonium sulfate was ammonium chloride, which showed a maximum recovery of up to 40 % of lipids in the cell. However, potassium nitrate was evaluated by the very best source (which can be observed in all previous experiments). Potassium nitrate showed a value of 60 % of the accumulation of intracellular lipids compared to urea, which reached a maximum above 55 % of lipid.

If we compare the production of intracellular lipids with the literature fot this particular strain, ammonium sulfate is the best source of nitrogen in combination with yeast extract, where the accumulation of lipids reached 47% in the dry matter of biomass. However, in our experiment, higher values were achieved, close to 60%. Thus, it is clear, that a more suitable source is potassium nitrate, which was used in our experiment, than ammonium sulfate with the addition of yeast extract, which is reported in the literature [144].

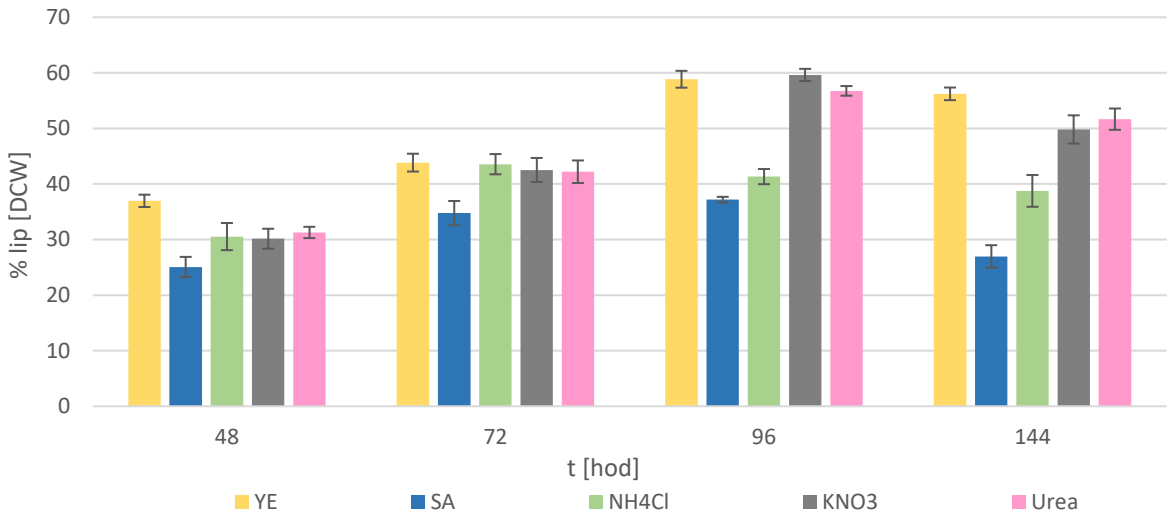


Fig. 28: Intracellular lipid content at media with different nitrogen sources

9.9.2 Influence of different carbon sources on growth and metabolism of *Rhodotorula kratochvilovae* CCY 20-2-26

The next step of the experiment was the selection of suitable carbon sources, which were selected according to drop tests from the experiments of the diploma thesis from previous study [145], when the optimal carbon source were mannose, lactose, glycerol, and xylose. For other monosaccharides, the growth was inhibited and therefore they were not included in this thesis [145].

Potassium nitrate and urea were chosen as the nitrogen source, as they showed the highest yields of biomass, intracellular lipids, and mainly exoglycolipids in the previous experiment. High yields were also observed for the yeast extract, which is, however, a demanding source due to its price compared to the two selected above.

The worst source in the experiment was lactose, which is the least suitable source of carbon for yeast, probably due to the absence of lactase to break down disaccharide into usable monosaccharides (Fig. 29). The biomass yield was maximally only about 1.0 – 1.5 g/L. Lactose was followed, also as a less suitable carbon source, by glycerol, which showed values slightly above 2.0 g/L of biomass, and these values were very similar throughout the whole time horizon of cultivatiom. Xylose appears very similar to glycerol, which showed slightly better results with 3.0 g/L of biomass.

The best carbon source was unrivaled mannose, which at the beginning of the experiment showed values of about 6.0 g/L, up to 10.0 g/L of biomass in the 144th hour of cultivation.

Thus, it can be assessed that urea and mannose appear to be the best sources of carbon and nitrogen for biomass production over time.

In the previous experiment, using urea as a nitrogen source, a maximum yield of about 9.0 g/L was obtained, when the carbon source was glucose. Here it can be observed that even better results (10.0 g/L) were obtained with the same nitrogen and mannose source as the carbon source. Thus, it is clear that mannose is an even better source of carbon than glucose for this experiment, although the difference is not significant.

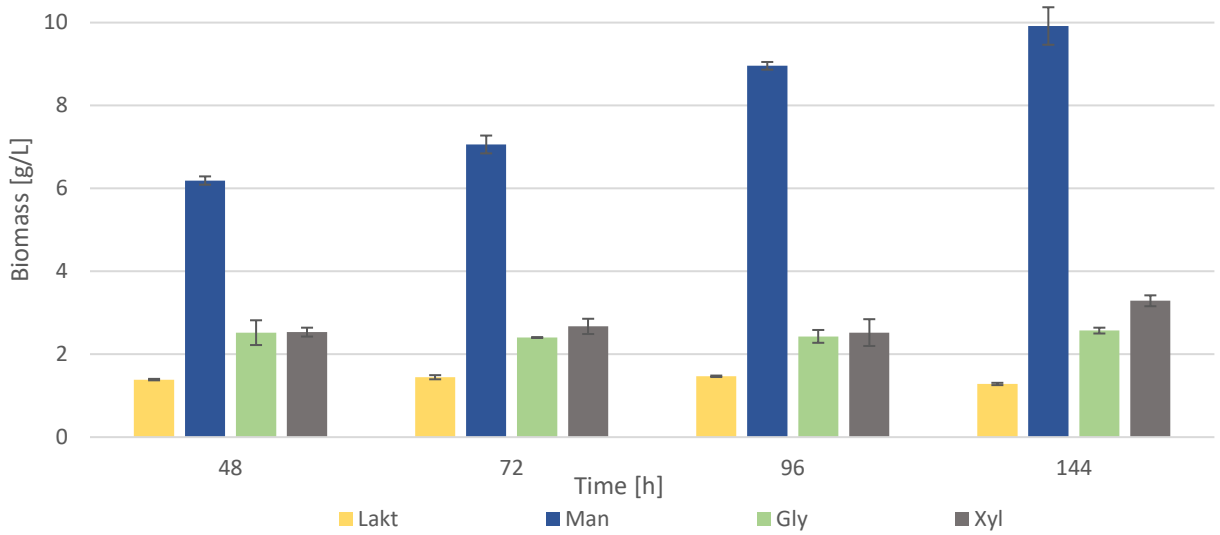


Fig. 29: Biomass production with diverse carbon sources and with urea as nitrogen source

Table 35 shows the production of total, α- and β-glucan in yeast biomass cultured at different saccharides and glycerol with combination of urea. The biomass content for lactose was too low for glucan measurements.

The urea with combination of mannose, glycerol and xylose decreases the β-glucan content when compared to previous experiment with glucose. At mannose as a carbon source, glucan yield dropped from 17.96 to 10.54, probably due to accumulation of other metabolites. Opositelly, glycerol and xylose showed slighty increase at β-glucan production in the end of cultivation.

Table 35: Total, α- and β-glucan content at diverse carbon sources with combination of urea

C source	Time	**48 h**	**72 h**	**96 h**	**144 h**
Lactose	Total glucan Alpha-glucan **Beta-glucan**	-	-	-	-
Mannose	Total glucan	17.96±0.68	11.83±0.43	11.12±1.57	10.54±0.49
	Alpha-glucan	4.21±0.12	1.23±0.02	0.84±0.11	0.67±0.14
	Beta-glucan	**13.75±0.99**	**10.59±0.76**	**10.28±1.83**	**9.87±0.67**
Glycerol	Total glucan	5.64±0.22	5.19±0.33	5.28±0.24	6.31±0.60
	Alpha-glucan	1.82±0.07	1.00±0.04	0.28±0.02	0.95±0.21
	Beta-glucan	**3.83±0.54**	**4.19±0.67**	**5.00±0.37**	**5.37±0.80**
Xylose	Total glucan	4.56±0.16	9.88±1.02	8.87±0.76	8.75±0.55
	Alpha-glucan	1.60±0.01	1.60±0.09	1.28±0.04	1.71±0.13
	Beta-glucan	**2.96±0.39**	**8.28±1.13**	**7.59±0.91**	**7.04±0.76**

The graph below (Fig. 30) shows the production of exoglycolipids using different carbon sources and a single nitrogen source - urea. The situation is repeated by analogy, as with the production of biomass with different carbon sources. Thus, the most suitable carbon source for the production of exoglycolipids is mannose, leaving the remaining sources far behind. Using mannose, values of up to 1.4 g/L of exoglycolipids were achieved.

The next suitable source appears to be glycerol, where the maximum yield was reached after 96 hours of cultivation, slightly above 0.2 g/L of exoglycolipids. Other sources showed very low values compared to mannose, around 0.1 g/L.

The concentration of exoglycolipids was slightly lower in this experiment (formerly 1.8 g/L, now 1.4 g/L) when compared to the previous experiment. Thus, glucose in combination with urea appears to be a slightly more suitable source for the production of exoglycolipids.

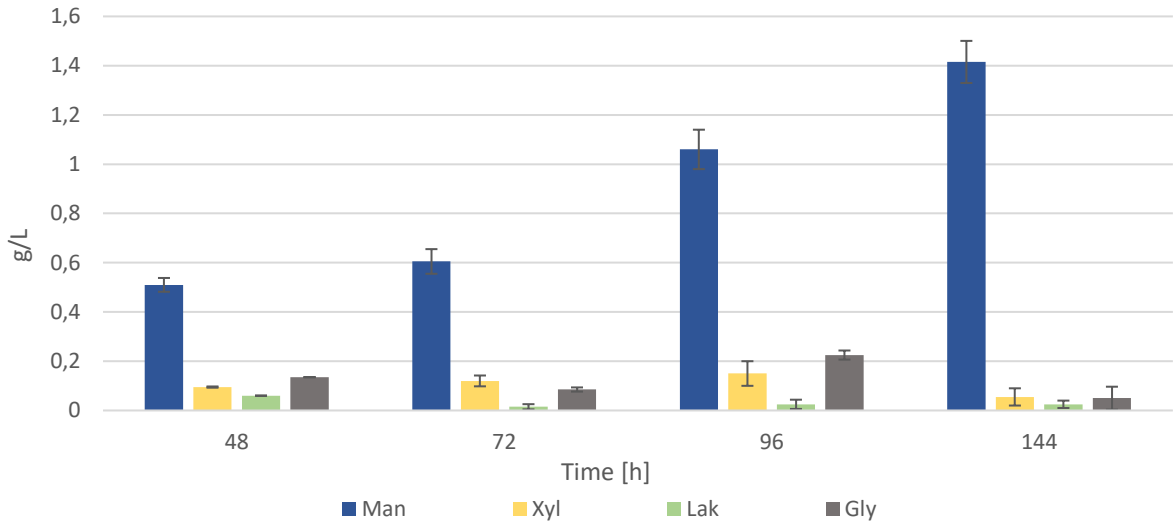


Fig. 30: Extracellular glycolipids yield at media with urea as a single nitrogen source

Figure 31 represents the intracellular lipids content in the dry matter. Here again, various carbon sources (mannose, glycerol, xylose, and lactose) were combined with urea.

Once again, the most suitable carbon source was mannose. However, such high values were not achieved as in the cultivation with potassium nitrate and glucose (see chapter 9.9.1).

Using mannose, the maximum value was reached at 96 hours of cultivation (about 55% of the intracellular lipids in DCW). At 144h, the lipid yield dropped to 51 %, probably because of the exhaustion of carbon source.

For glycerol and xylose, the yields were relatively similar. Glycerol reached a maximum at 72 and 144 hours, approximately 17% of the intracellular lipid content. Xylose reached a maximum of 20 % lipids of DCW at 144h. The least suitable source here was lactose, which overall did not lead to the production of more than 8%.

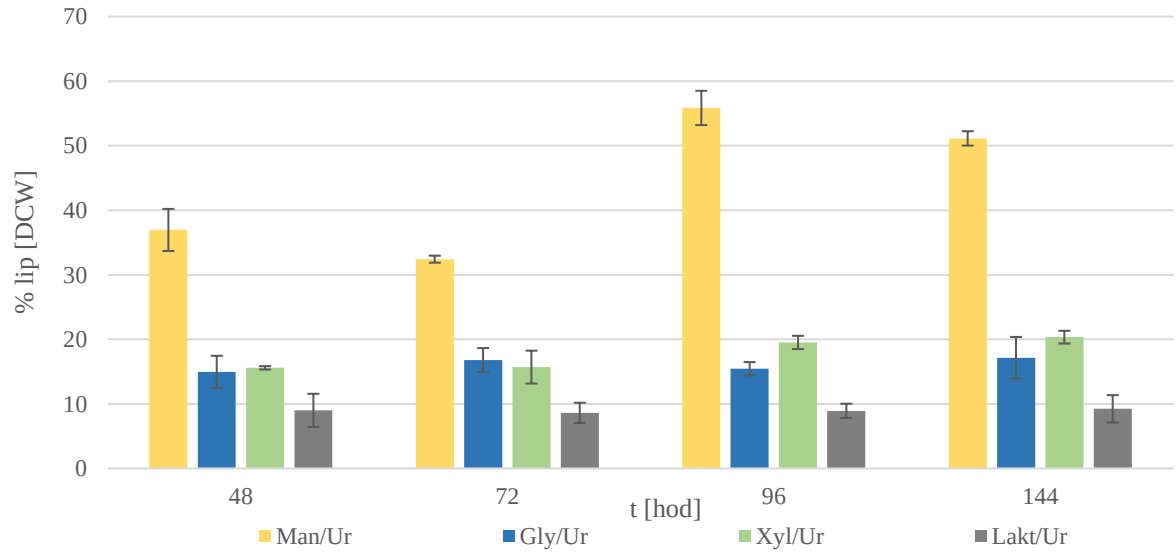


Fig. 31: Intracellular lipid content at media with urea as a single nitrogen source

Here, at the experiment with the potassium nitrate, the best source of carbon is again mannose, specifically at 144 hours, when the yield reached up to about 9.5 g/L of biomass. From the beginning of the cultivation, the yield increased rapidly. This fact indicates that mannose is a suitable source of carbon for yeast in combination with KNO_3 when even with a short exposure of the studied strain to the thus assembled medium resultend in 7.2 g/L biomass after 48h.

In general, potassium nitrate is a less suitable source of nitrogen than urea, because, in the previous experiment, mannose values reached up to about 10.0 g/L, which is the maximum for biomass across of all experiments performed. However, comparable biomass values, i.e. around 9.5 g/L, were obtained in an experiment in which the carbon source was glucose and the nitrogen source, already mentioned, potassium nitrate.

The second most suitable carbon source here was xylose, which reached values of about 6.2 g/L at the end of the cultivation.

Here, too, it is possible to compare the amount of biomass with an experiment using urea and xylose - the values were lower for this combination, only about 3.5 g/L than for the combination of urea and mannose. Thus, it can be concluded that the combination of urea with mannose shows better results than mannose with potassium nitrate, but on the contrary, xylose, as a carbon source, works better in combination with potassium nitrate than with urea.

The worst carbon sources here are glycerol and especially lactose. While glycerol reached at least 3.38 g/L during cultivation, lactose showed much worse results, a maximum of 0.61 g/L.

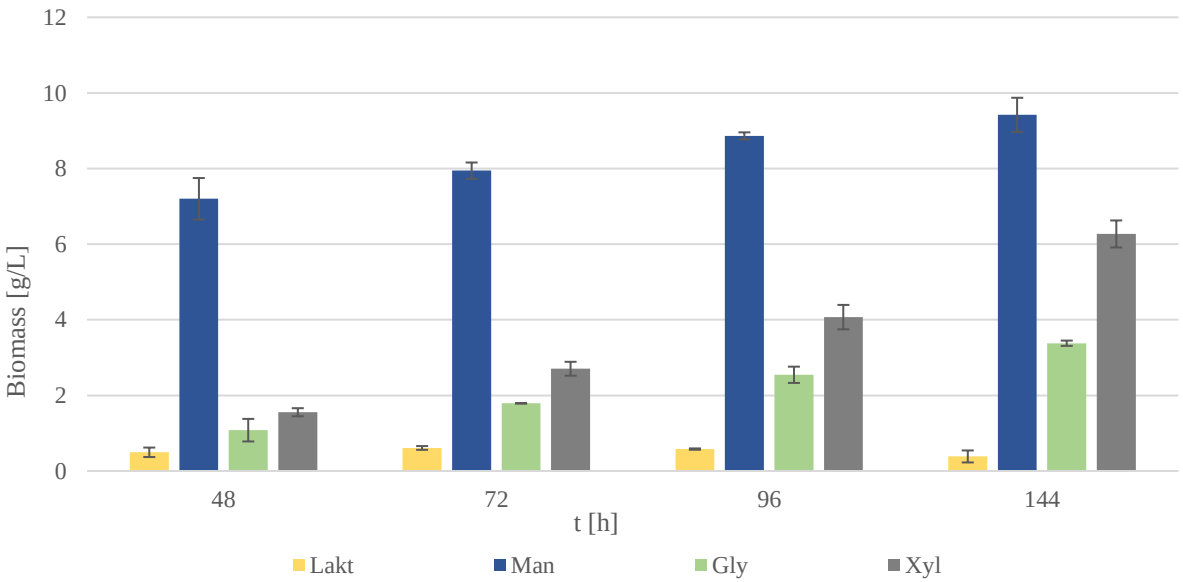


Fig. 32: Biomass production with diverse carbon sources and with potassium nitrate as nitrogen source

The Table 36 shows the production of total, α- and β-glucan in yeast biomass cultured at different carbon sources combined with potassium nitrate as a nitrogen source. Again, the biomass content for lactose was too low for glucan measurements.

When compared with experiment with urea, a rapid increase in β-glucan yield occurred when cultured on mannose. At 144h of cultivation, the high β-glucan content was accompanied with biomass production of 9.42 g/L. On the contrary, the glycerol shows the production only 2.69±0.34 % of DCW.

Table 36: Total, α- and β-glucan content at diverse carbon sources with combination of potassium nitrate

C source	Time	**48 h**	**72 h**	**96 h**	**144 h**
Lactose	Total glucan Alpha-glucan **Beta-glucan**	-	-	-	-
Mannose	Total glucan	18.86±1.08	17.43±1.30	20.70±0.29	21.53±1.46
	Alpha-glucan	1.73±0.13	1.81±0.06	2.57±0.42	2.07±0.13
	Beta-glucan	**17.13±1.24**	**15.62±1.67**	**18.14±0.58**	**19.46±1.85**
Glycerol	Total glucan	8.64±0.36	7.17±0.15	5.39±0.41	7.24±0.20
	Alpha-glucan	2.03±0.08	2.56±0.10	2.31±0.06	4.55±0.08
	Beta-glucan	**6.61±0.49**	**5.36±0.41**	**3.08±0.67**	**2.69±0.34**
Xylose	Total glucan	16.57±0.99	16.43±0.44	15.23±1.00	13.80±1.05
	Alpha-glucan	2.49±0.21	2.85±0.13	3.38±0.32	3.80±0.04
	Beta-glucan	**14.08±1.05**	**13.58±0.81**	**11.85±1.45**	**10.00±1.27**

Figure 33 shows the amount of biosynthesized exoglycolipids when cultured on a medium with different carbon sources and a single nitrogen source – potassium nitrate. Again, the best carbon source is mannose, which achieved the highest yields, up to about 2.0 g/L in 144 hours of cultivation.

Compared with the previous experiment (with urea), potassium nitrate appears to be a more suitable source for exoglycolipid biosynthesis, given that in the previous experiment the maximum values were only about 1.4 g/L exoglycolipids.

For lactose and glycerol, the exoglycolipids production did not exceed the value of 0.2 g/L.

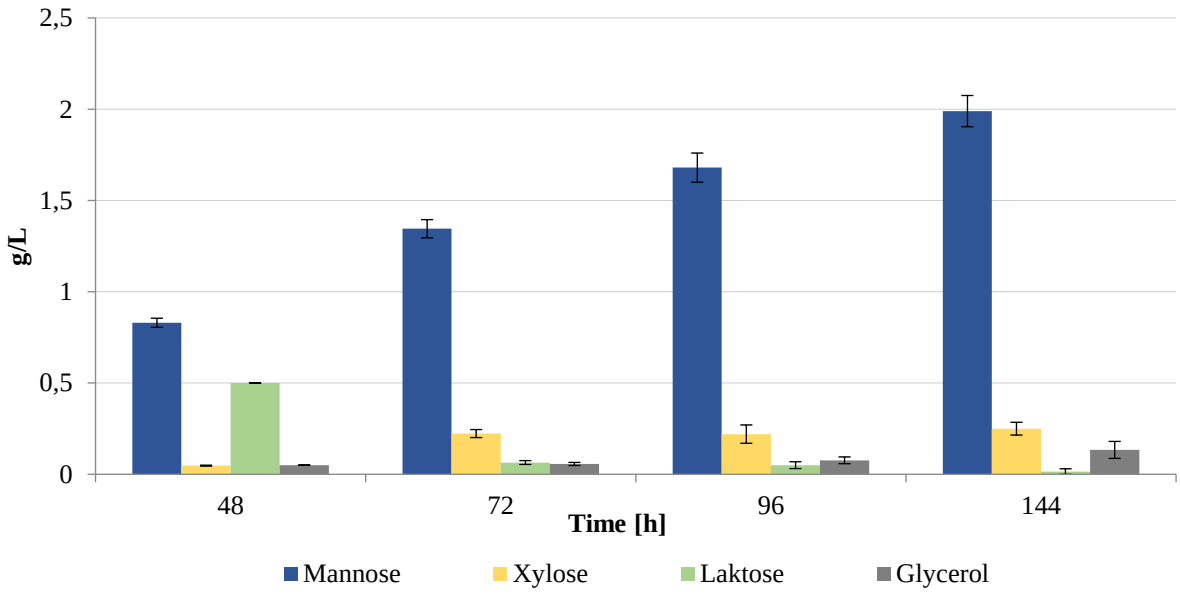


Fig. 33: Extracellular glycolipids yield at media with potassium nitrate as a single nitrogen source

The highest percentage of intracellular lipids in the dry matter, namely 57%, was accumulated in mannose medium with a combination of potassium nitrate. The second reliable carbon source was xylose, which reached about 35% within the 144 hours of cultivation. The least suitable source was again lactose, which was able to produce only 20% of intracellular lipids in DCW.

It can be stated that for the combination of glucose and potassium nitrate the maximum value was reached within 96 hours, namely 60 % of lipids, but the production of exoglycolipids was lower compared to mannose.

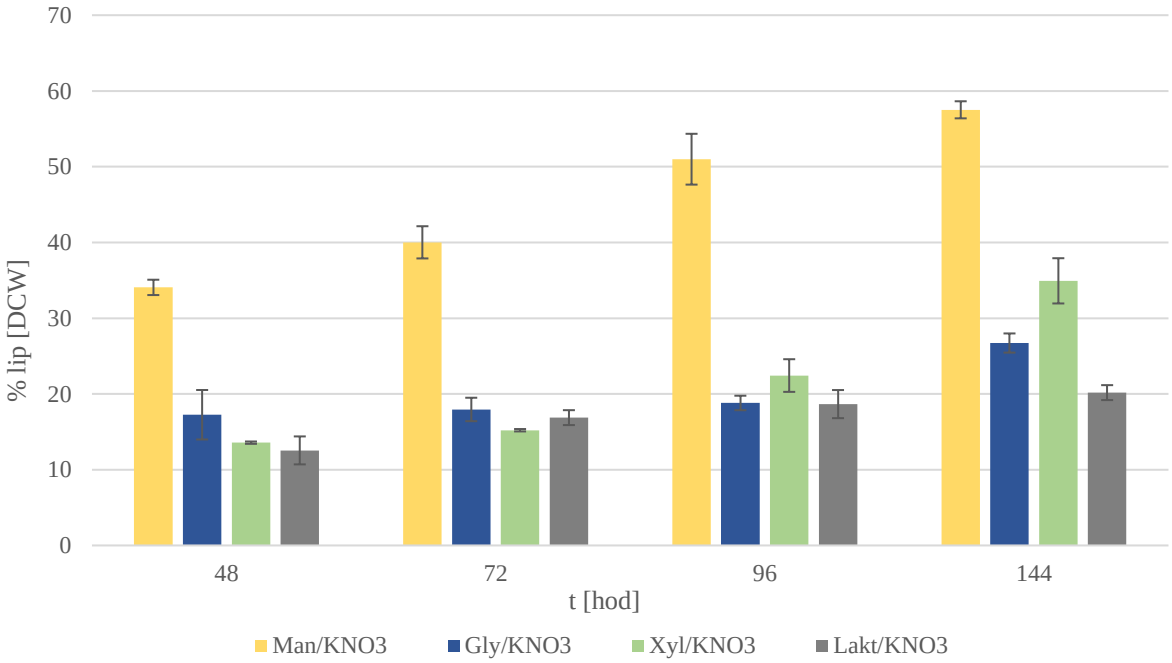


Fig. 34: Intracellular lipid content at media with potassium nitrate as a single nitrogen source

9.9.3 Influence of different carbon:nitrogen ratio on growth and metabolism of *Rhodotorula kratochvilovae* CCY 20-2-26

The C/N ratio is a very important criterion for achieving maximum production of selected metabolites. Regarding its influence on biomass production, it can be said that the higher the C/N ratio, the higher the biomass yields we are able to achieve, mainly due to the accumulation of specific biomolecules [120].

The graph in Figure 35 describes the dependence of biomass production and different concentrations of mannose as a carbon source. Potassium nitrate, previously experimentally tested, was chosen as the nitrogen source with the C/N ratios of 20:1, 40:1, 100:1 and 120:1 (C/N 70:1 was used in previous experiments).

The highest biomass yields were achieved at the highest concentration of mannose, in the 192nd hour of cultivation (around 15.23 g/L). The situation is relatively favorable even with a C/N ratio of 100:1, where the highest yield was achieved in the 168th hour of cultivation, with a value exceeding 14.0 g/L of biomass.

For C/N ratios of 20: 1 and 40: 1, the yields were significantly lower. As for the ratio of 40:1, the highest yield was obtained here at the 144th hour of cultivation (7.16 g/L), then the value rather decreased.

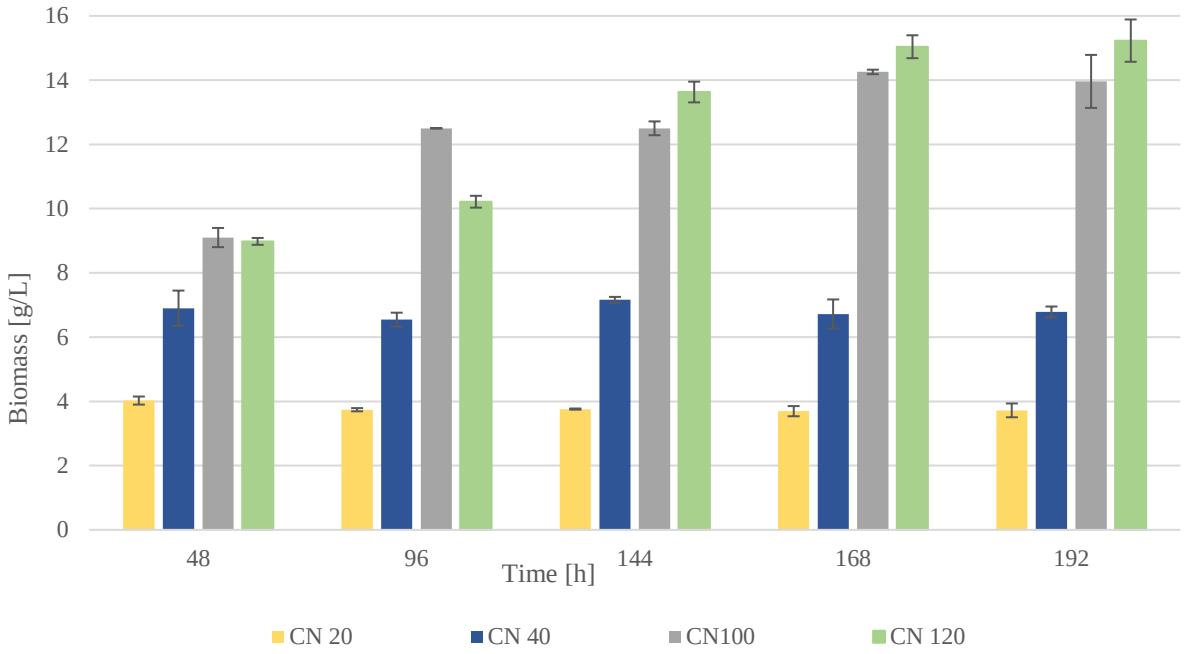


Fig. 35: Biomass production with diverse C/N ratio

The Table 37 shows the production of total, α- and β-glucan in yeast biomass cultured at different C/N ratios, where the C/N ratio 40:1 shows the highest β-glucan yield, 12.83±1.23 % of DCW. Taking together, the C/N ratio of 70:1 (Table 37) shows the best results for β-glucan production, but the C/N ratio 120 exhibited better biomass, intracellular lipids and exoglycolipids yield, thus, is better for biotechnological application.

Table 37: Total, α- and β-glucan content at diverse C/N ratios

C/N ratio	Time	**48 h**	**96 h**	**144 h**	**168 h**
	Total glucan	12.18±0.55	10.28±0.16	8.04±0.11	8.64±0.39
20	Alpha-glucan	1.33±0.02	0.98±0.08	1.49±0.09	1.26±0.12
	Beta-glucan	**10.85±0.68**	**9.30±0.23**	**6.54±0.25**	**7.38±0.63**
	Total glucan	20.82±1.20	16.55±1.36	15.28±1.08	14.51±1.05
40	Alpha-glucan	2.36±0.33	1.07±0.05	1.95±0.03	1.68±0.47
	Beta-glucan	**18.45±1.26**	**15.48±1.41**	**13.33±1.19**	**12.83±1.23**
	Total glucan	22.02±0.84	15.11±1.64	13.18±0.28	11.84±0.91
100	Alpha-glucan	2.26±0.14	1.01±0.91	1.14±0.01	1.59±0.06
	Beta-glucan	**19.75±0.91**	**14.10±1.33**	**12.04±0.42**	**10.25±0.98**
	Total glucan	22.93±0.79	13.29±1.32	16.21±0.83	13.06±1.22
120	Alpha-glucan	2.17±0.17	1.21±0.37	1.45±0.05	1.73±0.51
	Beta-glucan	**20.75±0.85**	**12.08±1.49**	**14.76±0.95**	**11.33±1.34**

As mentioned previously, cultures that are deficient in nitrogen produce intensively metabolites, such as the exoglycolipids. Therefore, the difference between low and high C/N ratios is obvious, up to 12 times the production is higher at a C/N ratio of 120:1 than at a ratio of 20:1.

The highest value for the C/N ratio of 120: 1 was reached at the 192nd hour of cultivation. A very similar situation can be seen with the C/N ratio of 100:1.

The situation was many times worse for the low C/N ratios (20:1 and 40:1), where the very lowest value of the whole experiment was reached at 96 hours, for the lowest selected ratio, namely 0.05 g / l.

Compared to our previous experiment, the exoglycolipids production was higher than with the C/N ratio 70:1. Thus, the C/N ratio played an important role in the production of exoglycolipids, especially in its increase.

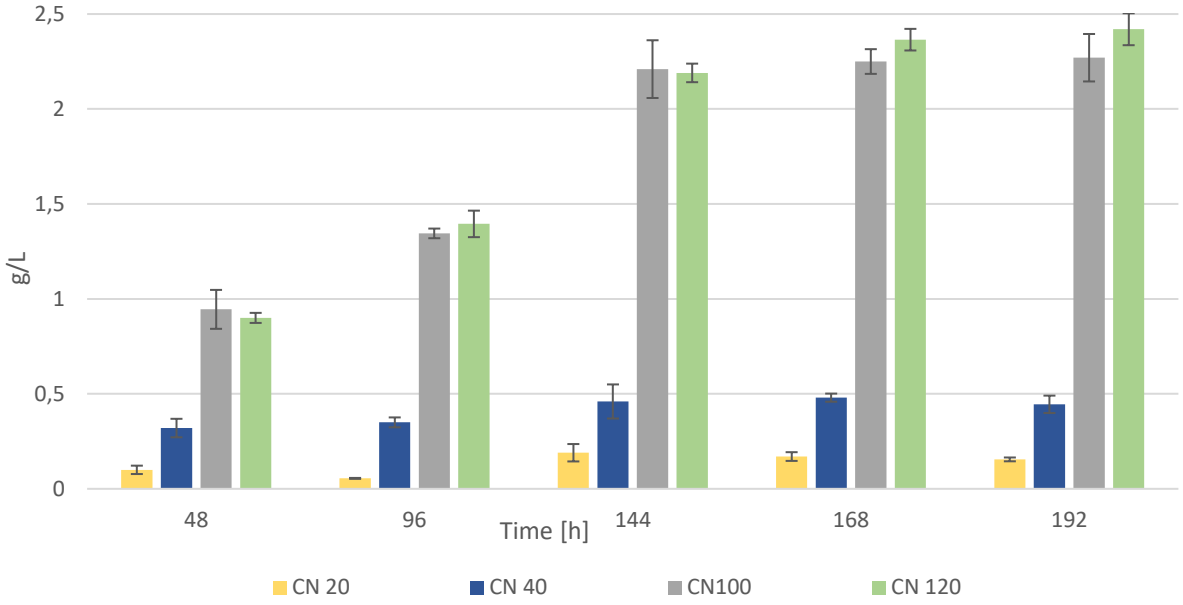


Fig. 36: The exoglycolipid production with different C/N ratios

After previous experiments and the elimination of unsuitable sources of carbon and nitrogen, the C/N ratio was optimized to achieve the highest values of lipid biosynthesis.

For high C/N ratios, values close to 70 % of lipid accumulation was achieved. Specifically, for the 120:1 ratio, it was 68 % at 168 hours. For the 100: 1 ratio, the highest accumulation was observed at 168 hours of culture, 62 %.

For lower C/N ratios, the maximum value was reached at 96 hours, for a ratio of 40: 1, namely 40 %. In general, the lowest value was reached at 144 hours, for the lowest selected ratio, only 14%. In the previous experiment, the maximum (60 %) was reached at 144 hours of cultivation (C/N ratio was 70:1). In the experiment with mannose and urea, the maximum value was equal to 55 %, which is a number slightly lower than in the experiment with potassium nitrate.

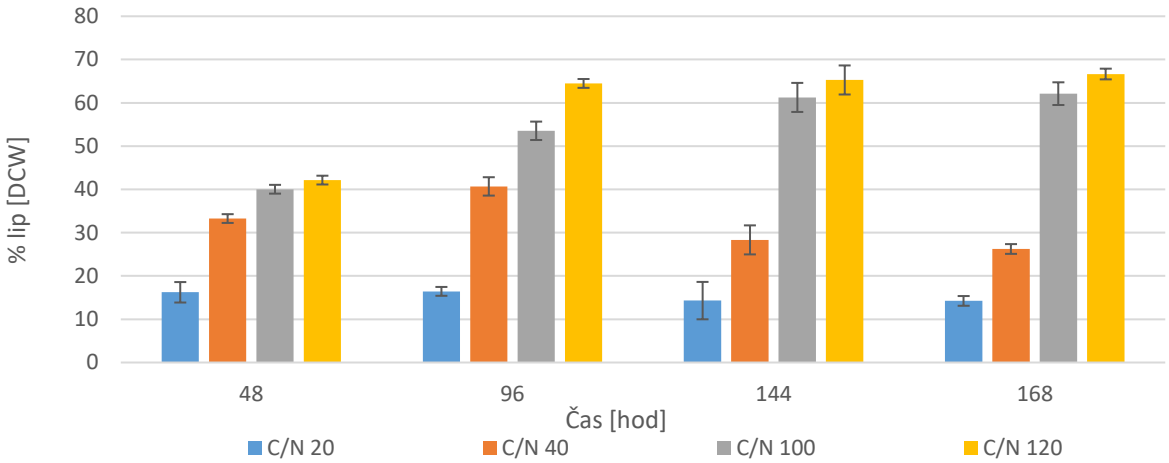


Fig. 37: The intracellular lipid production with different C/N ratios

At section with supplementary materials (Fig. S10-S13) are represented the productions of total carotenoids. The best results were achieved for urea and potassium nitrate with combination of glucose, 2.83 and 2.48 mg/g of biomass respectively. From other carbon sources, glycerol shows the presence of 2.72 mg/g of total carotenoids, accompanied by mannose with 2.21 mg/g at the C/N ratio of 70:1.

So far, the high production cost of biosurfactants is still a main barrier to its broath commercialization, the same as for microbial oils. The promising solution can be the biosynthesis of multiple metabolites and the use of low-cost media or waste substrates [146]. Studied strain *R. kratochvilovae* CCY 20-2-26 represents interesting candidate to study simultaneous production of extracellular glycolipids, intracellular oils, glucans and carotenoids.

9.7. Beta-glucan diversity at *Saccharomyces* collection strains and commertial brewer yeast

Four *Saccharomyces cerevisiae* strains from Culture Collection of Yeasts and eight commertial brewer yeast were used in this study to compare productivity of beta-glucan as a diversity among *Saccharomyces* genera. Collection yeast were choosed to cover different types of the technological use, like wine production (CCY 21-4-81), beer industry (CCY 48-26), baker industry (CCY 21-4-64), and sugar rafinery (CCY 48-88). The commertial brewery yeasts represented the top-fermenting (Safale S-04, Safale S-05, Safbrew WB-06), bottom-fermenting (Bohemian Lager M84, Saflager S-23, Saflager W-34/70), Premium gold yeast, and cider yeast (M02) samples, and were kindly provided, the same as spent biomass after brewing (Safale S-04 and Saflager W-34/70) by Ing. Václav Štursa from Brno University of Technology, Faculty of Chemistry. The culture conditions were the same as for the screening of the carotenogenic yeast in the Chapter 8.4, and biomass was harvested after 96 hours of cultivation.

For biotechnological application the biomass plays the crutial role right after the valuable metabolite production. In this part of the thesis, the biomass yield was in a range from 2.39±0.24 up to 11.78±0.67 g/L. Here, in the nutrient-rich YPD medium, the significant increase occured at the commertial samples of Safale US-05, Cider yeast M02, Bohemian lager yeast M84, SafAle WB-06, collection *S. cerevisiae* CCY 48-88, and, the highest (11.78±0.67 g/L), at the strain *S. cerevisiae* CCY 21-4-64, which is baker strain from Libaň, Czech Republic. After exposure this yeast into medium with different C/N ratios, the biomass dropped up to 50 % suggesting the composition of the medium is not optimal for the growth. The lowest growth was presented at *S.*

cerevisiae CCY 48-26 collection brewer strain where the biomass reached maximum of 3.00±0.15 g/L at the C/N ratio of 100, and, the same as the Saflager W-34/70 and Saflager S-23, show no significant difference between used medium. It is important to note that experiment was performed in Erlenmeyer flasks, thus it is not comparable with high biomass yields from reported batch or fed-batch fermentor cultivations where it can be achieved more than 100 g/L [147].

In general, these is no significant difference between growth of top-fermenting and bottom-fermenting yeast as a two different type of brewing yeast. The biomass production is strain-depended and shows no consistency among diverse biotechnological applications.

Table 38: Biomass yield for biotechnologically important yeast from *Saccharomyces* genera

		Biomass yield (g/L)			
Strain name	**CCY**	**YPD**	**C/N 40:1**	**C/N 70:1**	**C/N 100:1**
Saccharomyces cerevisiae	21-4-81	9.83±0.49	5.56±0.73	6.03±0.62	6.44±1.01
Saccharomyces cerevisiae	48-26	2.50±0.16	2.39±0.24	2.49±0.17	3.00±0.15
Saccharomyces cerevisiae	21-4-64	11.78±0.67	5.56±0.15	5.74±0.30	6.02±0.22
Saccharomyces cerevisiae	48-88	11.09±0.43	6.35±0.37	6.92±0.45	6.63±0.43
Bohemian lager yeast, M84	-	11.10±1.04	7.27±0.26	6.99±0.03	9.21±1.06
Cider yeast, M02	-	10.58±0.53	6.59±0.19	6.06±0.59	8.54±0.66
Premium gold; Muntons	-	6.75±0.06	4.06±0.44	4.12±0.11	3.84±0.09
Safale S-04	-	6.50±0.28	3.71±0.03	3.70±0.33	3.60±0.10
Saflager W-34/70	-	6.26±0.09	5.99±0.22	5.81±0.06	6.42±0.62
Saflager S-23	-	7.24±0.73	6.30±0.81	6.16±1.01	7.55±0.23
SafAle WB-06	-	10.09±0.99	7.60±0.08	7.64±0.55	7.96±0.48
Safale US-05	-	10.34±1.04	6.93±0.33	6.40±0.06	7.69±0.25

From all studied yeast from *Saccharomyces* genera the highest beta-glucan content was detected at *S. cerevisiae* CCY 21-4-102 (26.96±2.90 % w/w) (Table 19). *S. cerevisiae* CCY 48-88 isolated from sugar refinery shows similar results (24.62±1.56 % w/w) at C/N ratio 40. The yield of beta-glucan from other studied *Saccharomyces* strains ranged from 2 – 17 % w/w. The increase in glucose content in the culture media has no significant effect on β-glucan yield, except the Cider yeast M02, where the C/N 100 doubled the glucan content when compared to other ratios. Interesingly, the brewery yeast showed high amount of α-glucans when compared to collection strains. The α-glucan content at Cider yeast represented 83 % of total glucan. The Saflager W-34/70, Saflager S-23, Bohemian lager M84, Safale S-04 and Safale US-05 showed the highest α-glucan yield, over 13 % w/w when cultured at different C/N ratios.

In this thesis, the spent biomass from Safale-S04 and Saflager W-34/70 (Figure 38) after brewery process was analysed with the 4.42 and 8.07 % w/w of β-glucan.

Table 39: Total glucan, α- and β-glucans content (% CDW) in yeasts grown in the YPD media and media with low and high C/N ratios

Strain		YPD	C/N 40:1	C/N 70:1	C/N 100:1
S. cerevisiae CCY 21-4-81	Total glucan	7.43±0.22	14.51±1.23	15.35±0.81	17.27±0.46
	α-glucans	1.57±0.06	3.60±0.14	3.02±0.09	2.86±0.21
	β-glucans	**5.86±0.64**	**10.91±1.36**	**12.33±0.93**	**14.41±0.55**
S. cerevisiae CCY 48-26	Total glucan	9.21±0.12	18.21±0.55	14.87±0.34	13.80±1.02
	α-glucans	0.58±0.09	1.39±0.06	0.67±0.11	0.66±0.17
	β-glucans	**8.63±0.20**	**16.82±0.84**	**14.02±0.38**	**13.14±1.24**
S. cerevisiae CCY 21-4-64	Total glucan	8.14±0.61	18.54±1.01	17.73±1.02	17.10±0.55
	α-glucans	0	6.64±0.22	3.61±0.13	3.46±0.24
	β-glucans	**8.14±0.61**	**11.90±1.35**	**14.12±1.14**	**13.64±0.69**
S. cerevisiae CCY 48-88	Total glucan	9.76±1.64	27.98±1.29	22.75±1.87	25.09±1.47
	α-glucans	1.06±0.94	3.37±0.44	1.08±0.18	1.08±0.03
	β-glucans	**8.70±1.33**	**24.62±1.56**	**21.67±2.03**	**24.00±1.53**
Bohemian lager yeast, M84	Total glucan	11.92±0.88	21.01±1.21	22.15±0.20	22.65±0.61
	α-glucans	1.59±0.39	14.62±0.72	16.03±0.31	17.49±0.37
	β-glucans	**10.34±0.85**	**6.40±1.63**	**6.12±0.48**	**5.17±1.08**
Cider yeast, M02	Total glucan	11.21±0.58	12.44±0.59	14.64±0.03	19.04±0.88
	α-glucans	0.87±0.31	10.35±0.18	9.32±0.02	7.65±0.24
	β-glucans	**10.34±0.72**	**2.09±0.88**	**5.32±0.09**	**11.39±1.03**
Premium gold; Muntons	Total glucan	7.40±0.25	21.70±0.67	19.89±0.50	17.72±0.51
	α-glucans	1.56±0.10	9.94±0.14	9.01±0.57	9.72±0.13
	β-glucans	**5.84±0.46**	**11.76±0.55**	**10.87±0.88**	**8.00±0.82**
Safale S-04	Total glucan	9.05±0.44	20.59±0.45	19.75±0.63	18.96±0.44
	α-glucans	2.33±0.51	13.87±0.21	12.45±0.76	11.22±0.19
	β-glucans	**6.71±0.68**	**6.72±0.75**	**7.03±1.09**	**7.74±0.68**
Saflager W-34/70	Total glucan	5.64±0.26	26.38±0.73	21.67±0.31	19.29±0.25
	α-glucans	0.37±0.01	13.74±0.48	12.06±0.66	11.15±0.43
	β-glucans	**5.27±0.49**	**12.64±1.06**	**9.61±0.59**	**8.14±0.59**
Saflager S-23	Total glucan	7.18±0.70	22.95±0.25	23.58±0.81	24.94±1.33
	α-glucans	0.96±0.05	11.41±0.37	12.95±0.13	**16.35±0.21**
	β-glucans	**6.22±0.76**	**11.54±0.88**	**10.63±0.92**	**8.60±1.24**
SafAle WB-06	Total glucan	12.44±1.03	22.97±0.11	19.48±0.66	19.34±1.06
	α-glucans	0.91±0.02	8.85±0.02	7.39±0.17	8.48±0.22
	β-glucans	**11.54±1.08**	**14.12±0.14**	**12.09±0.69**	**10.86±1.30**
Safale US-05	Total glucan	7.66±0.05	30.26±1.00	20.50±0.39	24.87±0.36
	α-glucans	1.41±0.12	15.14±0.05	15.45±0.22	15.80±0.14
	β-glucans	**6.24±0.16**	**5.12±1.13**	**5.04±0.55**	**9.07±0.42**
Spent biomass from Safale S-04	Total glucan	14.84±0.67			
	α-glucans	10.42±0.41	-	-	-
	β-glucans	**4.42±0.79**			
Spent biomass from Saflager W-34/70	Total glucan	13.69±1.00			
	α-glucans	5.62±0.23	-	-	-
	β-glucans	**8.07±1.04**			

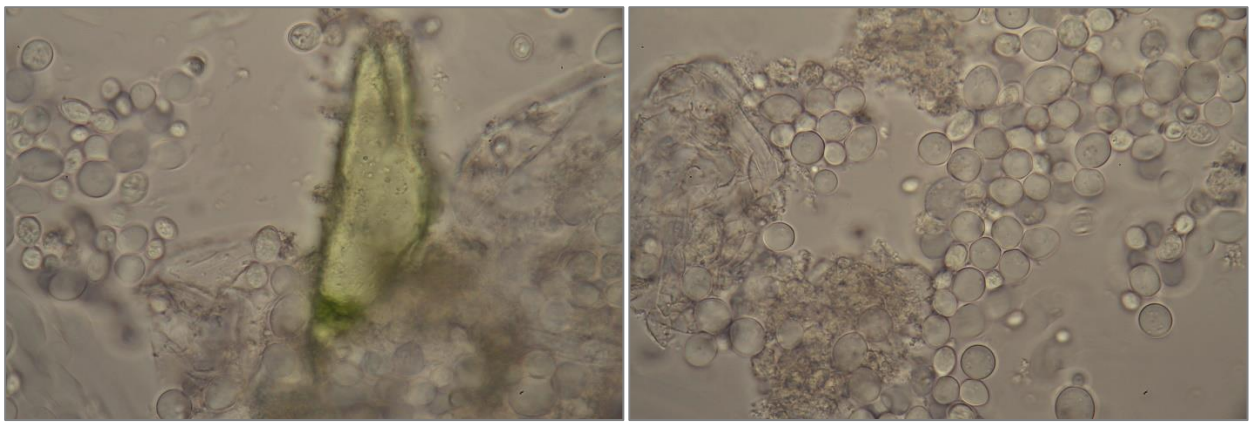


Fig 38: Waste yeast from Saflager W-34/70

The lipid content at diverse *Saccharomyces cerevisiae* strains vary from 6 to 20 % per DCW. The C/N ratio do not afected significantly the lipid production. The only difference occurred at YPD medium, where the lipid biosynthesis was lower.

Table 40: Lipid content at diverse *Saccharomyces cerevisiae* strains

		Lipid yield (% of DCW)			
Strain name	**CCY**	**YPD**	**C/N 40:1**	**C/N 70:1**	**C/N 100:1**
Saccharomyces cerevisiae	21-4-81	7.51±0.54	9.44±0.31	8.84±0.09	10.76±1.02
Saccharomyces cerevisiae	48-26	8.79±0.73	11.89±1.02	12.54±1.14	14.18±1.57
Saccharomyces cerevisiae	21-4-64	6.43±0.24	12.96±0.59	9.70±0.22	11.15±0.19
Saccharomyces cerevisiae	48-88	8.02±1.23	18.46±2.26	19.33±1.58	20.16±2.01
Bohemian lager yeast, M84	-	10.39±0.48	13.70±0.84	15.34±2.06	14.03±0.80
Cider yeast, M02	-	6.18±0.71	11.18±0.33	11.69±0.79	11.84±0.55
Premium gold; Muntons	-	10.59±0.06	15.16±1.17	14.79±0.16	15.78±1.32
Safale S-04	-	10.10±1.00	16.47±0.64	13.77±1.00	14.39±0.69
Saflager W-34/70	-	8.50±0.13	18.56±0.12	18.78±1.43	18.48±1.04
Saflager S-23	-	11.05±0.55	18.70±1.77	17.73±0.22	19.31±1.41
SafAle WB-06	-	7.52±0.42	11.95±0.61	12.03±0.61	15.49±0.42
Safale US-05	-	8.03±0.99	16.19±0.44	14.66±0.18	16.06±2.03

Nowadays, beer is one of the most popular alcoholic beverages and provides 1.5-3 kg/hL wet biomass of residual yeast [148]. The advantage of production of beta-glucan from *Saccharomyces* strains from the "waste" biomass from different fields of industry significant lower the cost of final product.

9.8. Microalgae as a source of β-glucan and other polysaccharides

9.8.1. Screening of microalgal strains

Microalgal carbohydrates are created through number of complex reactions after the capture of light. They serve as structural components in the cell wall, and as intracellular storage components. Cyanobacteria synthetize glycogen (α-1,4 linked glucan) and red algae synthetize floridean starch (hybrid of strarch and glycogen) and green algae synthetize amylopectin-like polysaccharides (starch). The most abundant monosaccharides are glucose, rhamnose, xylose, and mannose [8]. Table 41 represent the biomass yield and total carbohydrates measured at microalgae when growth autophototrophically.

The carbohydrate content at 13 studied algal strains varied from 8.78 to 32.03 % of DCW at green algae, red algae and one protozoa strain. The lowest value was detected at *Euglena gracilis* CCALA 349, but this alga lacks the cell wall and accumulate granules of paramylon, the β-1,3-

glucan. On the contrary, red alga *P. purpureum* CCALA 416 exceeted the sugar content of 30 % of DCW. The value 20 % reached *B. braunii, S. dimorfus* and *R. violacea* CCALA 925.

Table 41: Beta-glucan content in algal and cyanobacterial biomass (green – *Chlorophyta*, red – *Rhodophyta*, blue – *Protozoa*)

Number	Microorganisms	CCALA	Biomass [g/L]	Total carbohydrates [% of DCW]
1	***Botryococcus braunii***	777	0.467±0.012	23.50 ± 2.45
2	***Chlorella vulgaris***	924	0.436±0.007	8.86 ± 1.26
3	***Chlorella sorokiniana***	260	0.377±0.011	18.35 ± 1.43
4	***Desmodesmus acutus***	437	0.829±0.012	18.60 ± 0.37
5	***Desmodesmus quadricauda***	463	0.520±0.023	10.94 ± 0.84
6	***Scenedesmus dimorfus***	443	0.720±0.009	25.65 ± 3.09
7	***Scenedesmus obliqus***	455	0.655±0.013	19.33 ± 0.75
8	***Chlamydomonas reinhardtii***	928	0.683±0.005	11.63 ± 0.34
9	***Porphyridium cruentum***	415	**1.037±0.021**	16.00 ± 1.04
10	***Porphyridium purpureum***	416	0.671±0.014	**32.03 ± 2.65**
11	***Porphyridium areuginosum***	419	0.299±0.022	19.19 ± 1.08
12	***Rhodella violacea***	925	0.607±0.007	26.07 ± 2.49
13	***Euglena gracilis***	349	0.442±0.003	8.78 ± 0.87

Red algae are known for its biosynthesis of sulphated exopolysaccharides which have antiviral, anti-inflammatory, antioxidant and immunomodulatory properties. They consist mainly of xylose (38 %), glucose (24 %), galactose (22 %), and glucuronic acid (10 %). However, minority amount of rhamnose, mannose, and arabinose can also be found [8, 149]. The values measured in presented thesis ranged from 0.2 – 0.5 g/L, with the highest yield reached by *Rhodella violacea* CCALA 925.

Table 42: Production of exopolysaccharides with red algal strains

Strain	CCALA	EPS [g/L]
Porphyridium cruentum	415	0.330±0.018
Porphyridium purpureum	416	0.485±0.024
Porphyridium areuginosum	419	0.208±0.156
Rhodella violacea	925	**0.502±0.046**

Phycobiliproteins consist of three main groups: phycoerythrins, phycocyanins, and allophycocyanins. They have excellent spectroscopic properties and, thus, a wide range of promising application in biomedical research and diagnostics [150, 151]. *P. areuginosum* shows the highest accumulation of phycocyanin (61.93 mg/g), while the lowest was measured in *R. violacea* (6.36 mg/g). As for allophycocyanins, *P. areuginosum* (39.62 mg/g) was the best

producer, and the lowest amount, as for phycocyanin, is observed in *R. violacea* (2.97 mg/g). Finally, phycoerythrin was mostly produced by *P. cruentum* (56.31 mg/g), which, however, shows low levels of phycocyanin and allophycocyanin has not been determined at all. And the worst yield for phycoerythrin was in *P. areuginosum* (9.42 mg/g), which, however, dominated the production of the previous two phycobiliproteins.

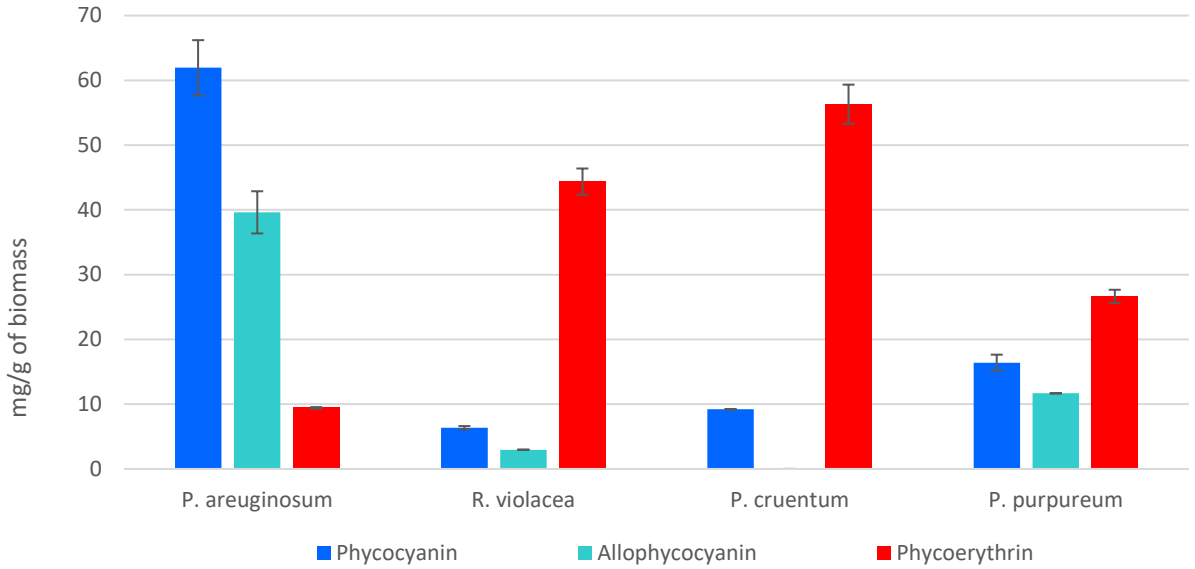


Fig 39: Phycobiliproteins content in red algal biomass

Figure 40 shows the total yield for chlorophyll and carotenoids. The highest value of chlorophylls was found in *Euglena gracilis* CCALA 349, where the content exceeded 28.0 mg/g of this pigment. In contrast, the lowest yield is evident for *Rhodella violacea* CCALA 925, only 2.72 mg/g. Chlorophyll b is completely absent in *Porphyridium* genera and *R. violacea* CCALA 925.

The remaining other chlorophylls were not standardized due to the lack of standards. Most so-called other chlorophylls (which can be pheophytins and pyropheophytins – degradation products of chlorophylls) occur in species number *D. acutus* CCALA 437 (4.04 mg/g), while the least in species number *R. violacea* CCY 925 (0.25 mg/g).

Microalgae are generally capable of more efficient accumulation of secondary metabolites, such as chlorophylls or carotenoids, but with relatively low biomass yields. The measured spectrum of carotenoids produced by microalgae contained mainly lutein, violaxanthin, and neoxanthin and several unknowns. Thus, the quantification may be slightly distorted by the absence of standards.

The highest yield of total carotenoids was also observed for *Euglena gracilis* CCALA 349, 9.86 mg/g. In contrast, the very bottom of carotenoid synthesis inhabits *Desmodesmus quadricauda* CCALA 463 with a value of only 0.85 mg/g. The remaining producers synthesize carotenoid concentrations in biomass in the range of 1.0 - 3.0 mg/g.

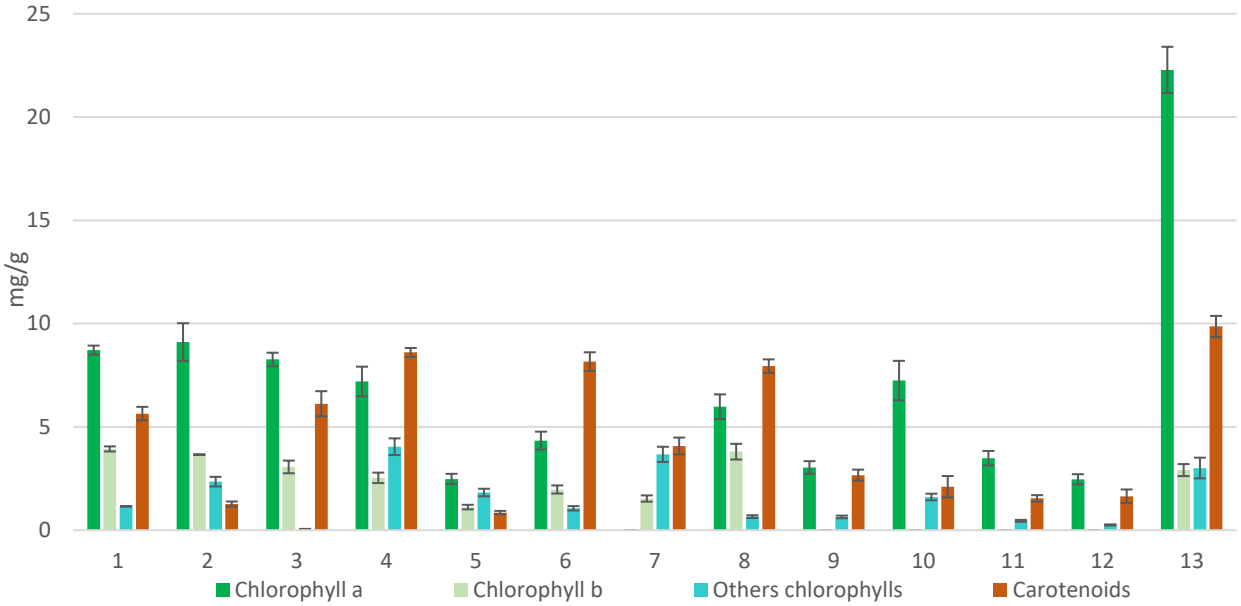


Fig. 40: Chlorophylls and carotenoids content in microalgal biomass (the number designation of individual strains is presented in the Table 41)

The highest biomass production was achieved with *Porphyrium cruentum* (1.04±0.021 g/L), on the contrary, the lowest value can be observed for *Porphyridium areuginosum*, only 0.3±0.02 g/L (Table 43).

The achieved values of biomass yield in autotrophic microorganisms are significantly lower than in yeast (where the values reached up to 15.0 g/L), but it should be taken into account that the cultivation of autotrophic microorganisms takes place without the addition of any nutrients, except inorganic salts and the light source needed for photosynthesis.

Regarding the percentage yields of lipids in biomass dry matter in microalgae, the highest value was reached in *Scenedesmus obliqus* (close to 14%). In contrast, the lowest value can be observed for *Chlamydomonas reinhardtii*, at only about 4%. Here it is clear that the yields in yeast were many times higher when compared to autophototrophic growth (up to about 60%). In general, oil content of microalgae is usually between 5 % and 50 % DCW. Some strains, such *Botryococcus braunii* and *Schizochytrium sp.* can reach up to 77 % [152, 153].

Table 43: Biomass and lipid yield of photoautotrophic microorganisms

No	MO	CCALA	Biomass [g/L]	Lipid yield [%]	SAT : MUFA : PUFA [%]
1	*B. braunii*	777	0.467±0.012	6.47±0.11	19.11 : 22.82 : 58.07
2	*C. vulgaris*	924	0.436±0.007	8.10±0.62	17.66 : 9.10 : 73.24
3	*C. sorokiniana*	260	0.377±0.011	5.60±0.27	19.05 : 17.57 : 63.38
4	*D. acutus*	437	0.829±0.012	11.56±0.84	19.68 : 18.98 : 61.35
5	*D. quadricauda*	463	0.520±0.023	8.96±0.91	20.99 : 10.81 : 68.19
6	*S. dimorfus*	443	0.720±0.009	10.44±0.76	22.02 : 19.00 : 58.98
7	*S. obliqus*	455	0.655±0.013	**13.71±1.05**	36.68 : 12.94 : 50.38
8	*C. reinhardtii*	928	0.683±0.005	4.34±0.93	58.50 : 6.91 : 34.58
9	*P. cruentum*	415	1.037±0.021	6.17±0.29	32.99 : 12.36 : 54.65

No	MO	CCALA	Biomass [g/L]	Lipid yield [%]	SAT : MUFA : PUFA [%]
10	*P. purpureum*	416	0.671±0.014	7.33±0.81	29.66 : 3.76 : 66.58
11	*P. areuginosum*	419	0.299±0.022	7.88±0.36	29.82 : 6.79 : 63.39
12	*R. violacea*	925	0.607±0.007	5.76±0.15	30.16 : 9.43 : 60.42
13	*E. gracilis*	349	0.442±0.003	10.34±0.44	24.94 : 11.30 : 63.76

Red algal strains *Porphyridium cruentum* CCALA 415, *Porphyridium purpureum* CCALA 416, *Porphyridium areuginosum* CCALA 419 and *Rhodella violacea* CCALA 925 were selected due to its potential to produce sulphated extracellular polysaccharides, high amount of intracellular carbohydrates and phycobiliproteins. Next, the *Euglena gracilis* CCALA 149 was cultured to produce paramylon granules, the beta-1,3-glucan polymer.

9.8.2. Optimization of light conditions for *Porphyridium cruentum* CCALA 415

Red alga *Porphyridium cruentum* has sever industrial and pharmaceutical use, especially in the production of intracellular (cell wall and starch) and sulphated extracellular polysaccharides. The culture conditions, such a limited level of nutrients, light intensity and temperature affect the metabolite production [154].

The light intensities of experiment were: 50, 100, 150, 200, 250 and 300 μmol photons·s^{-1}·m^{-2} (chapter 7.4.1). For 50 and 100 μmol photons·s^{-1}·m^{-2} the growth was too inhibited, without any biomass production (Fig. 41). Samples were harvested at the stationary phase, here after 12 days of cultivation.

The highest biomass content (1.17 g/L) can be observed at the 150 μmol photons·s^{-1}·m^{-2} with the highest extracellular polysaccharide yield. The total carbohydrate content seemst to be optimal at higher light conditions, 200 μmol photons·s^{-1}·m^{-2}, but with lower EPS biosynthesis. The increase in total carbohydrate indicate to accumulation of intracellular floridean starch [155].

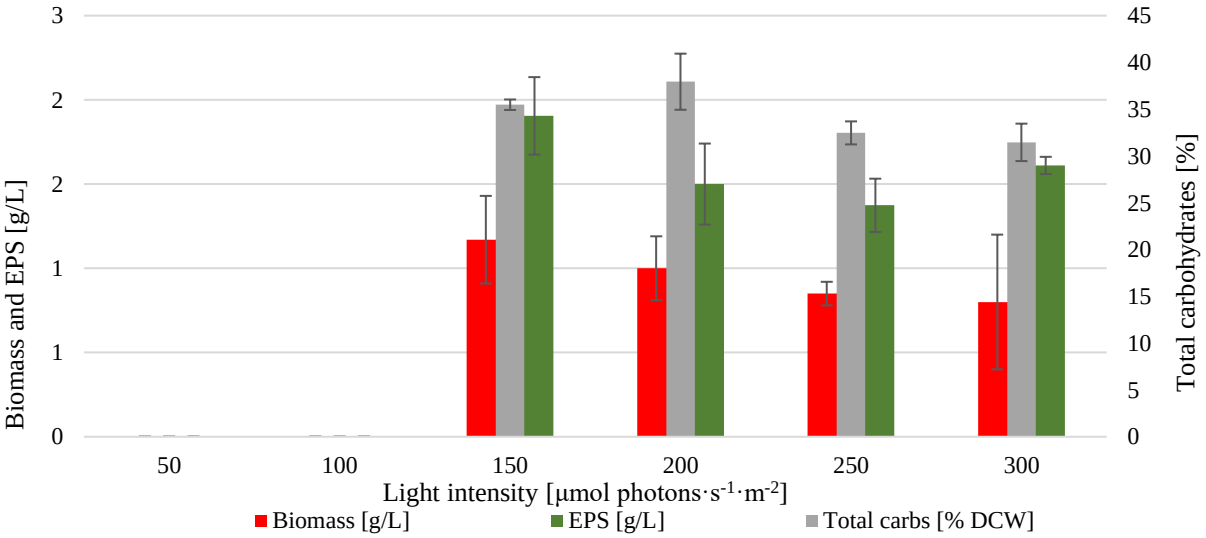


Fig. 41: Influence of light intensity to biomass, exopolysaccharides and total carbohydrates production at *P. cruentum* CCALA 415

9.8.3. Optimization of light conditions for *Porphyridium purpureum* CCALA 416

Red algal species *Porphyridium purpureum* received great attention because of its potential to produce B-phycoerythrin, long-chain polyunsaturated fatty acids, and exopolysaccharides. However, to date, large-scale cultivation and commercial application of *P. purpureum* has not achieved the worldwide implementation [156].

The light conditions were analogous to the experiment with *P. cruentum* CCALA 415 the same as the culture medium. The biomass was harvested after 13 days of cultivation.

The highest achieved biomass content, 5.36 g/L, was measured at 200 µmol photons·s^{-1}·m^{-2}, together with exopolysaccharides (1.95 g/L) and total carbohydrates (47.92 %) (Fig. 42). Opossitelly, the lowest biomass can be found at the illumination by 50 µmol photons·s^{-1}·m^{-2}. Higher light intensity (250 and 300 µmol photons·s^{-1}·m^{-2}) had inhibitory effect on biomass production.

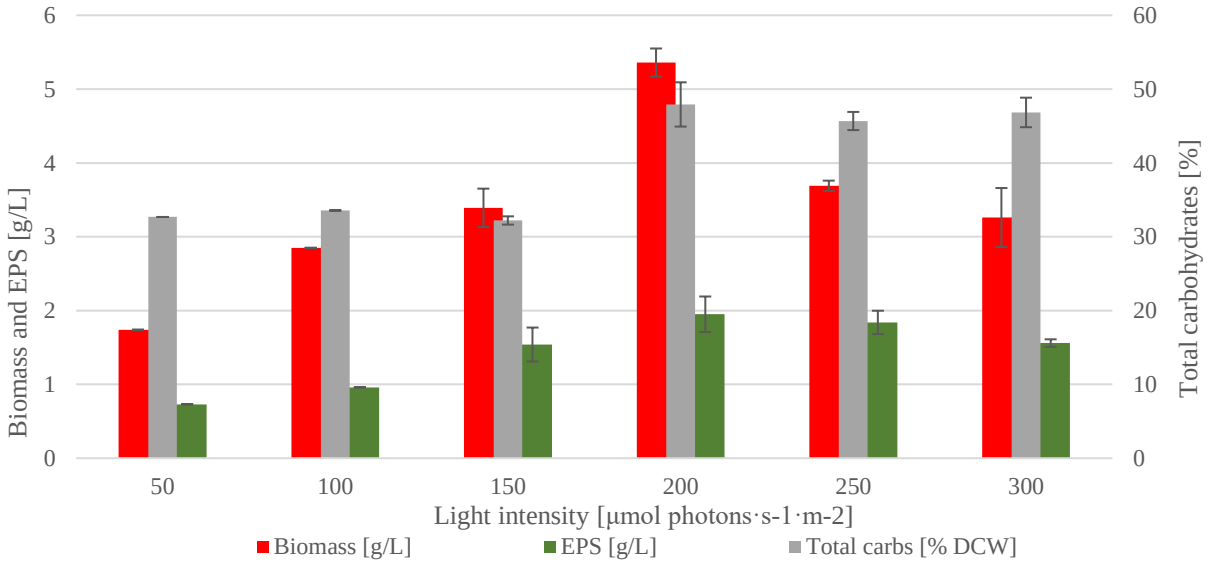


Fig. 42: Influence of light intensity to biomass, exopolysaccharides and total carbohydrates production at *P. purpureum* CCALA 416

9.8.4. Optimization of light conditions for *Porphyridium areuginosum* CCALA 419

The red alga *P. areuginosum* CCALA 419 produces sulphated polysaccharides and contains high amount of phycocyanin, except of phycoerythrin (Fig. 39).

The growth conditions are presented in chapter 7.4.1. The biomass was harvested after 19 days of cultivation. For 50 and 100 µmol photons·s^{-1}·m^{-2} the growth was too inhibited, without any biomass production (Fig. 43), the same, as for the *P. cruentum* CCALA 415. This red alga exhibits the lowest yield of total carbohydrates and EPS at the highest biomass yield (250 µmol photons·s^{-1}·m^{-2}) but interresingly the production of extracellular polysaccharides is increasing with the highering of light intensity.

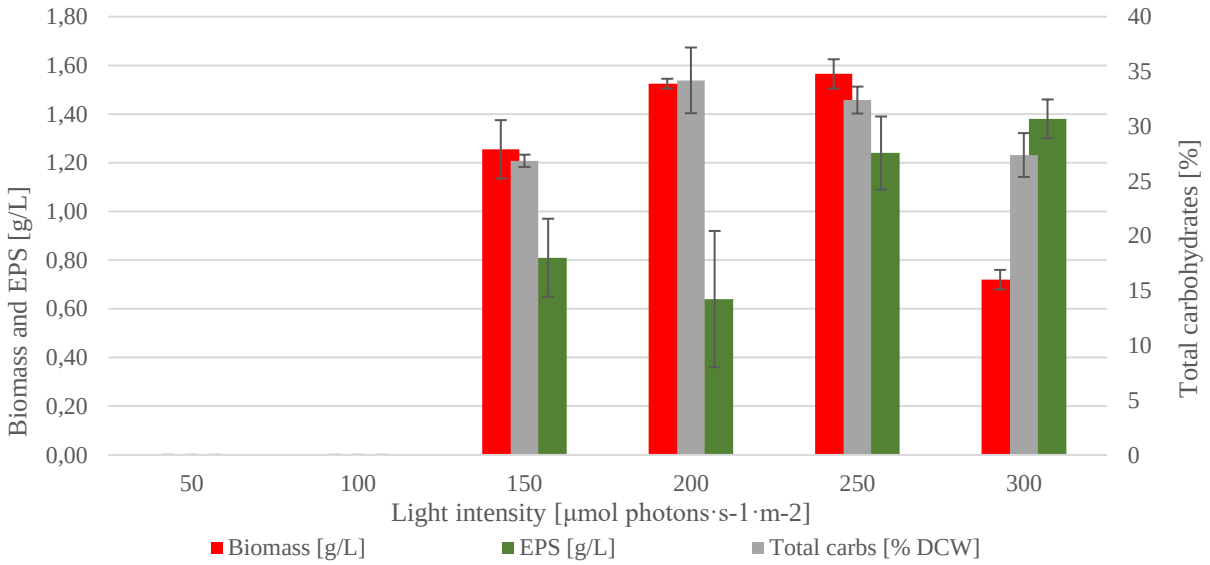


Fig. 43: Influence of light intensity to biomass, exopolysaccharides and total carbohydrates production at *P. areuginosum* CCALA 419

9.8.5. Optimization of light conditions for *Rhodella violacea* CCALA 925

The last studied red alga, *Rhodella violacea*, was cultured at conditions are presented in chapter 7.4.1. The biomass was harvested after 19 days of cultivation.

The highest achieved biomass content, 3.96 g/L, was measured at 200 µmol photons·s^{-1}·m^{-2}, together with total carbohydrates (45.78 %) (Fig. 44). The production of extracellular polysaccharides was constant at all light intensities, about 1.1 g/L.

The culture had a different colours with increasing light intensity, which goes from purple to green shadows (Fig. 45).

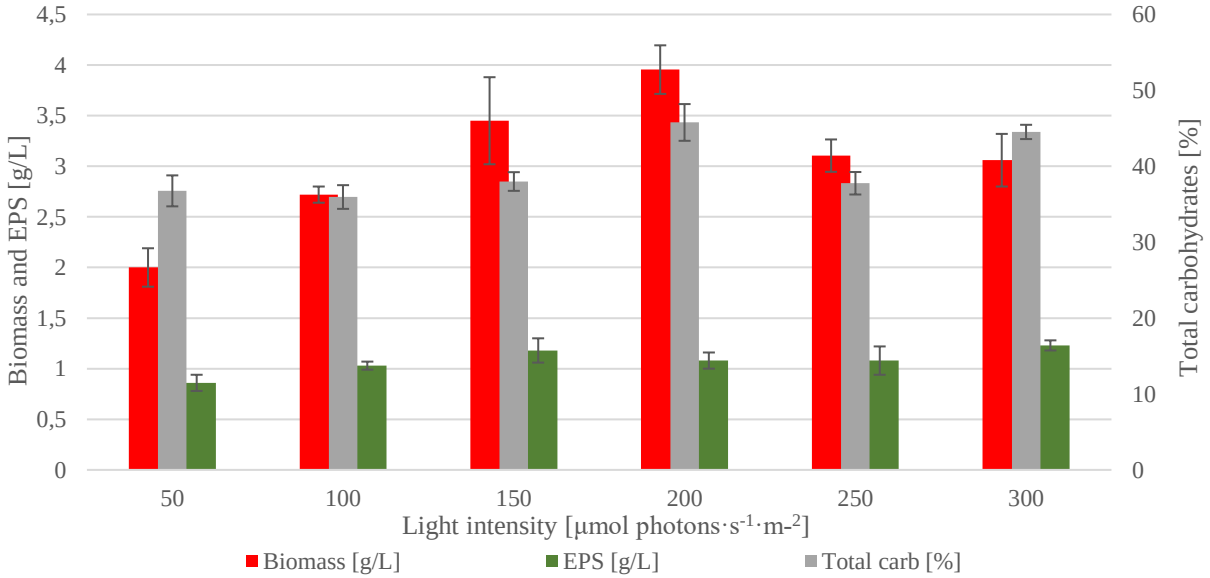


Fig. 44: Influence of light intensity to biomass, exopolysaccharides and total carbohydrates production at *R. violacea* CCALA 925

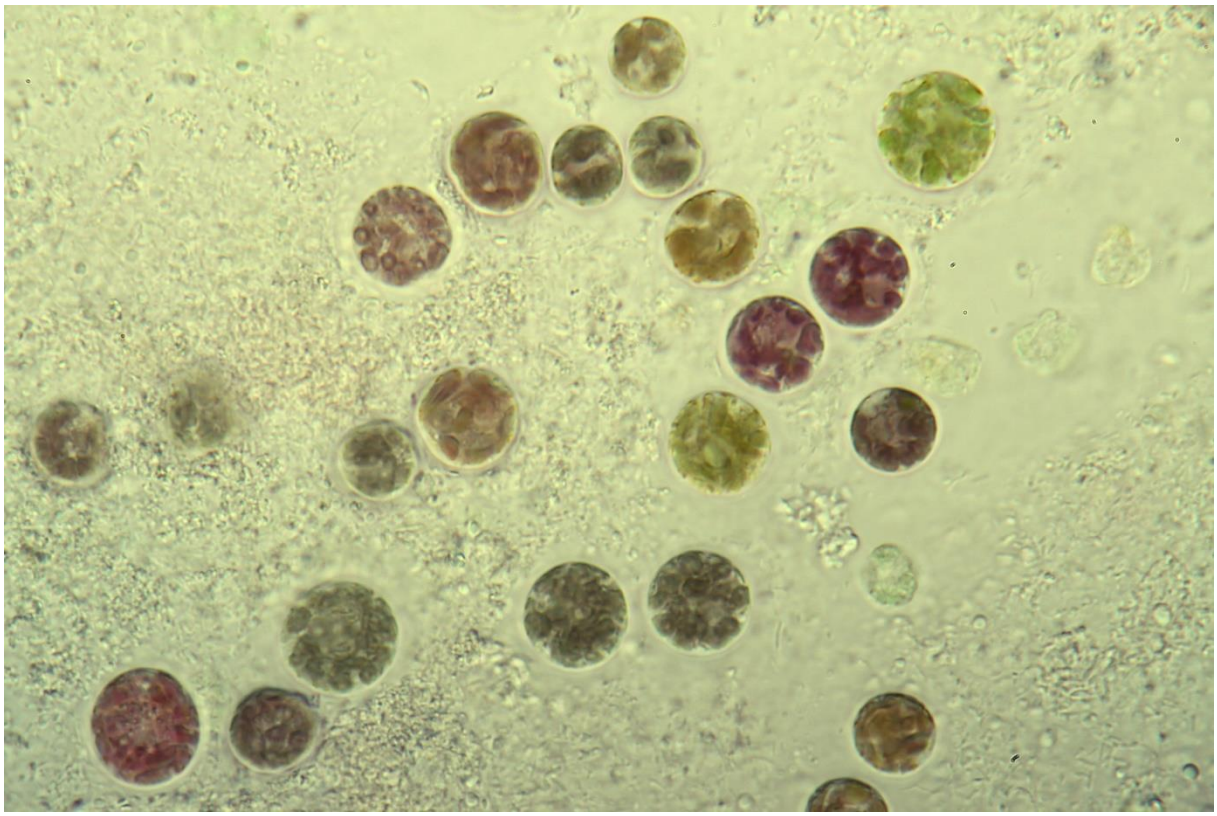


Fig. 45: Diverse coloration of cells of *R. violacea* CCALA 925 caused by different composition of the pigments

Taking together, the *P. purpureum* CCALA 416 represents the highest biomass, total carbohydrate and exopolysaccharide content from all studied red algal species, thus, it is great candidate for the further large-scale cultivation experiments.

9.8.6. Heterotrophic growth of red algae

Heterotrophic metabolism in red algae is not commonly reported but can provide an increase in biomass and metabolite production. Glucose has been shown to be a suitable carbon source for the heterotrophic growth of *Porphyridium cruentum*, not glycerol [157]. In this experiment, ASW medium was supplemented with 10 and 20 g/L of glucose and red algal strains were grow heterotrophically under dark conditions and with constant shaking regime.

Unfortunatelly, after 20 days of cultivation the experiment was stopped due to low level of cell concentration, thus, *Porphyridium cruentum* CCALA 415, *Pophyridium purpureum* CCALA 416, *Rhodella violacea* CCALA 925 and *Pophyridium areuginosum* CCALA 419 are not able to growth heterotrophically.

9.8.7. Metabolic activity of *Euglena gracilis*

Euglana gracilis is protozoic algal strain known for its beta-glucan (called as paramylon) accumulation as the reserve polysaccharide. Here, the mixotrophic and heterotrophic cultivations were performed to evaluate growth and paramylon biosynthesis in Erlenmeyer flasks (culture conditions – chapter 7.4.1).

Figures 45 and 46 represent the growth curves at 680 and 720 nm. The stationary phase begun after 150 h cultivation when *Euglena* grew heterophically with addition of 10 and 20 g/L of glucose.

Growth at constant illumination shorted the exponential phase (after 72h) but the optical density was lower compared with heterotrophic growth. The NM medium caused only slightly increase in growth, reached maximum at 192h.

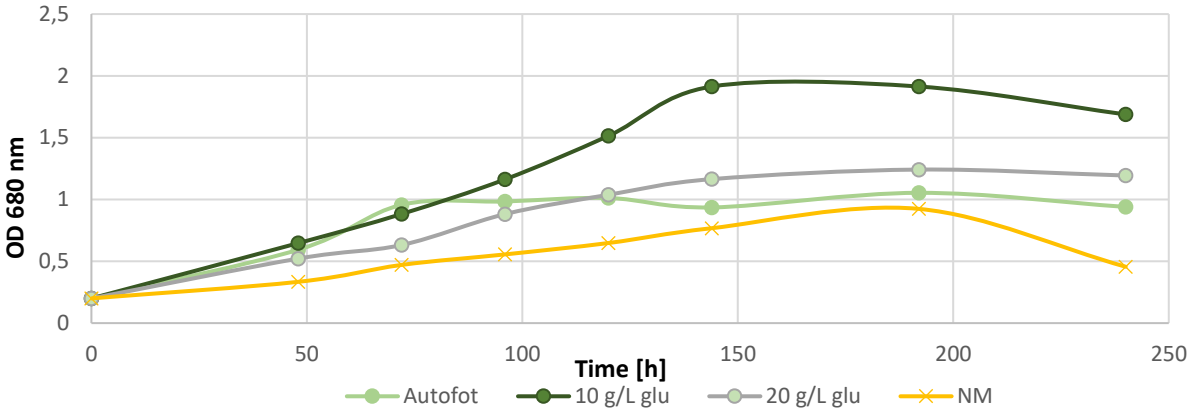


Fig 45: Growth curve of *Euglena gracilis* CCALA 349 at 680 nm

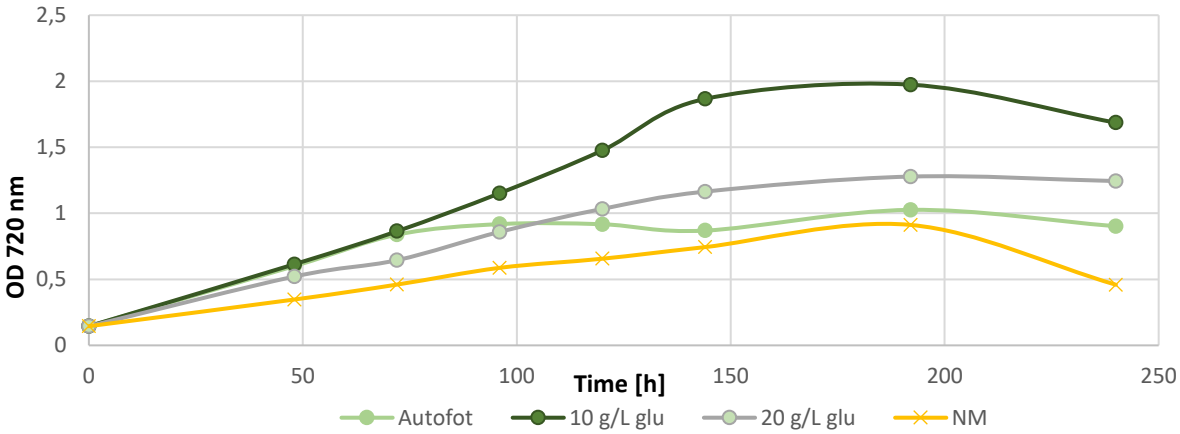


Fig 46: Growth curve of *Euglena gracilis* CCALA 349 at 720 nm

The biomass content reached maxima at 192h hour of cultivation for all culture conditions with the maximum yield of 2.79 g/L on media supplied with 10 g/L of glucose (Fig. 47). During the first 24 h the biomass shows similar production within the hetherotrophic growth, but for the mixotrophic stayed very low from the beginning till the end of cultivation.

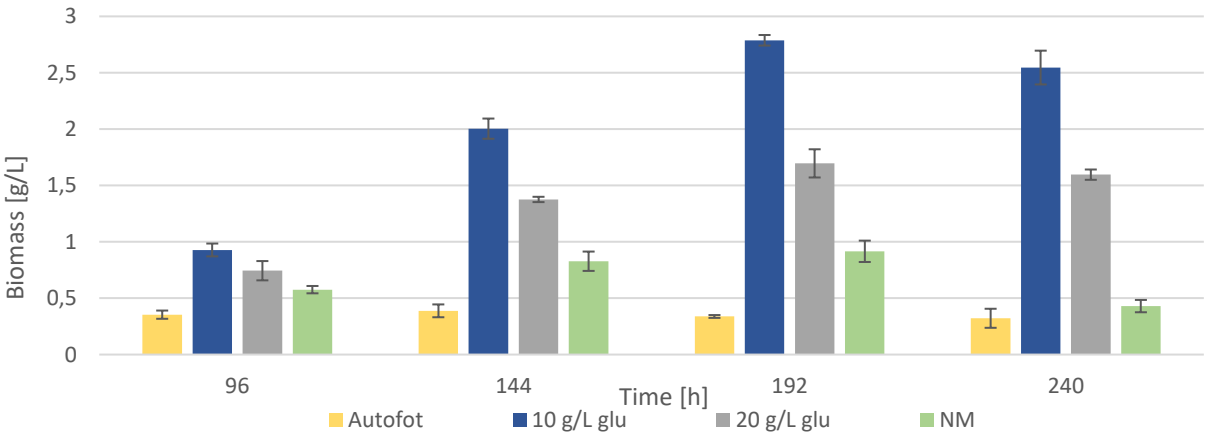


Fig 47: Biomass content of *Euglena gracilis* CCALA 349

The paramylon content isolated from *Euglena gracilis* biomass ranged from 11.9 – 69.29 % of CDW (Fig. 48). The paramylon yield increased rapidly in the medium supplemented with 10 g/L of glucose up to 70 % od DCW, whereas the lowest concentration can be found at growth on light illumination with acetate as a carbon source. The glucose content of 20 g/L lead to decrease the β-glucan content to 61 % of DCW which together with lower biomass yield represent unfavourable conditions for biotechnological purposes. The process of accumulacion within the time is visualized on the Fig. 49 below, where increasing numbers of granules can be observed through the time of cultivation. The composition of other type of culture medium was adapted from Cramer et al. (1952) [107], where the authors stated the highest yields. However, the CCALA 349 strain shows biomass yield below 1 g/L. In general, these types of media are cost-uneffective when compared with yeast, therefore there is need to use waste substrates to alleviate the culture price.

Among the literature, *Euglena gracilis* can accumulate up to 85 % paramylon of DCW. But such a high biosynthesis is caused mostelly by non-photosynthetic mutant strains [28].

Isolation of paramylon granules is simple and quick process due to the absence of the cell wall and presence of pellicle made up of a protein layer instead, when compared to fungal biomass. Mixing the biomass with sodium dodecylsulphate solution cause the rupture of the cells and release of granules which are insoluble in water [158]. On the contrary, yeast β-glucan is a part of the thick cell wall with is covalently bounded to mannan and chitin. Therefore, isolation and subsequent purification of fungal β-glucan represent challenging task for the future.

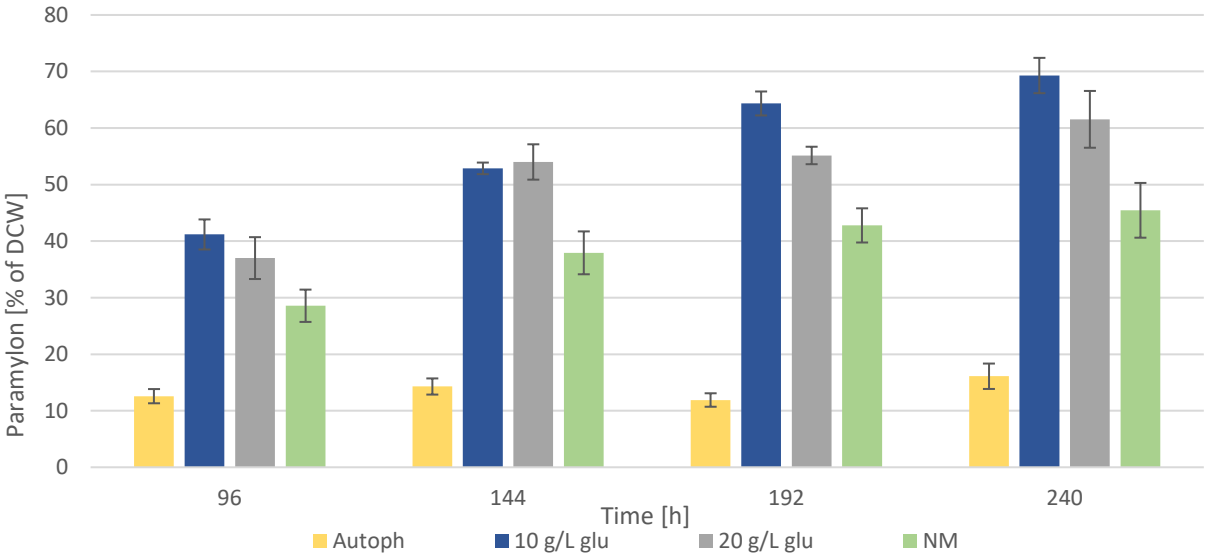


Fig 48: Paramylon accumulation in *Euglena gracilis* CCALA 349

The commercial cultivation of *E. gracilis* began in 2007, after the success of its outdoor mass cultivation and improvement of the harvesting and drying methods. The commercialization of Euglena production is based on the strategy of "5Fs of Biomass," which refers to the development and production of commercial products including food, fiber, feed, fertilizer, and fuel from biomass [159]. Nowadays, Japan-based company, Euglena, is using these microalgae to develop foods and cosmetics as well as conducting research for the production of biofuels. Euglena's sales in 2018 reached $133 million, according to The Investor and the big challenging plan is to produce biofuel for airplanes flying into Tokio for the Summer Olympic Games [160].

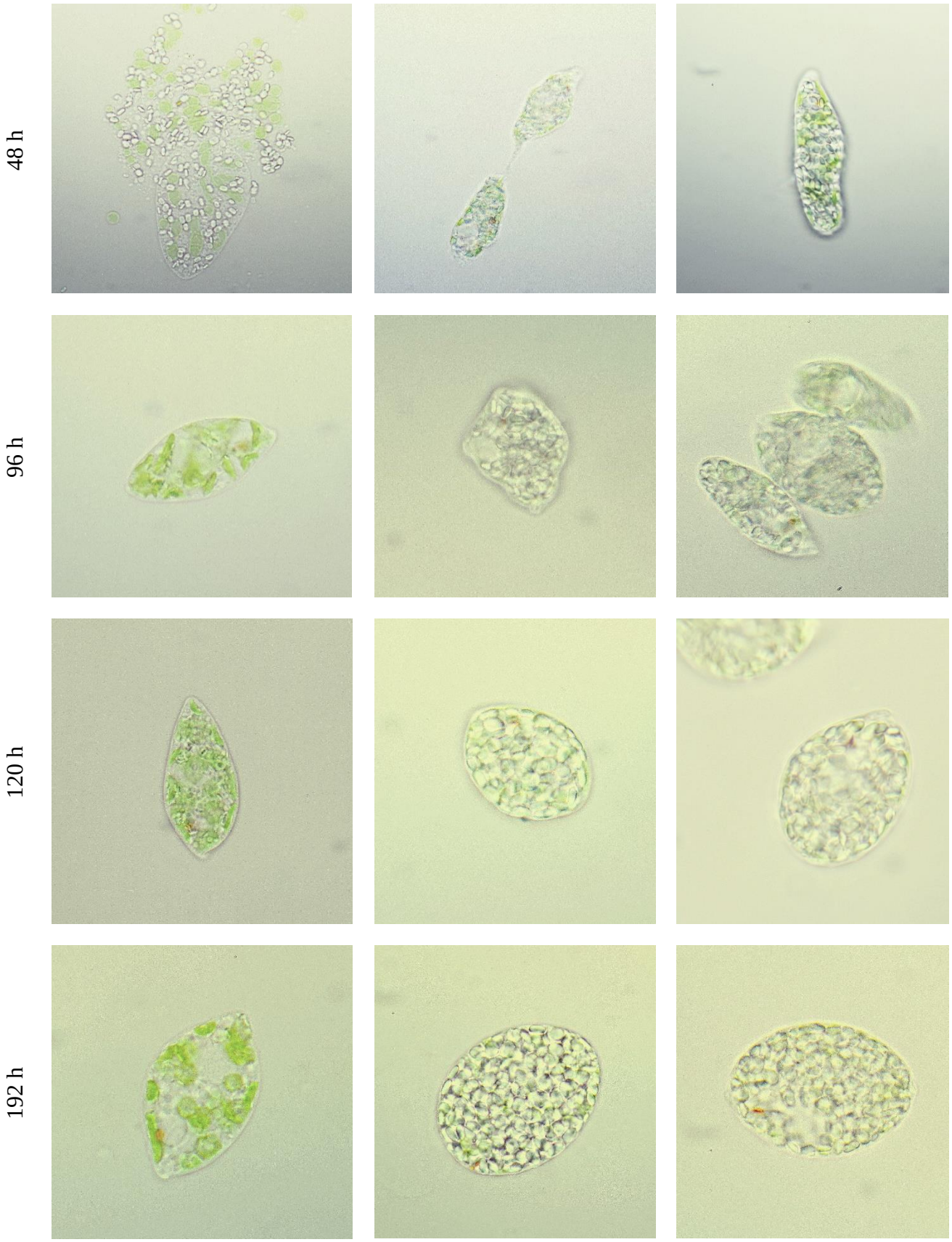


Fig 49: Accumulation of paramylon granules inside the *Euglena* cells

9.8.8. Beta-glucan in edible mushrooms and cereals

Samples of commertial cereals, namely barley (barley grass and barley flour), wheat, rice, oat, and *Secale* (all in the form of flour) and mushrooms (shiitake, Jew's ear, *Pleurotus ostreatus*, *Agaricus campestris*, *Agaricus brunnescens* and *Inonotus obliquus*) were selected to determine the content of glucans. All representatives were purchased in powder form except of *Pleurotus ostreatus*, *Agaricus brunnescens*, and *Agaricus campestris* which were dried at 80 °C for 24 hours and milled into a fine powder before the measurement. Unlike yeast and higher fungi, another kit (Megazyme K-BGLU β-Glucan Assay Kit – Mixed Linkage) had to be used due to the different bonds in the glucan polymer (β-1,3-1,4-glucan). The results are shown in Table 44.

The highest content was measured for *Pleurotus ostreatus* (one of the most important mushroom's sources of β-glucan) where the amount reached the value 27 % DCW. Shiitake (*Lentinus edodes*) contains β-glucan named lentinan, polysaccharide with antitumor and immunopotentiating activity, contain 23 % of DCW. Oppositelly, higher fungi from the genera *Agaricus* and *Inonotus* ranged from 4 to 12 % of DCW.

In rice samples, the quantity determined is too small to show the presence of β-glucans. The highest content was measured for oat (approx. 4.3 % of DCW). It is interesting to observe the difference between barley flour and young barley. This difference may be caused due to different processing in the production of these raw materials. Young barley is harvested at an early stage when it is still green. Therefore, β-glucans may not be produced to the same extent as conventionally harvested barley. The β-glucan content measured in presented samples correlated with the results measured elsewhere [161, 142].

Table 44: Total, α-, and β-glucan content at commertial mushrooms and cereals

	Source	**Total glucan content [% w/w]**	**α-glucan content [% w/w]**	**β-glucan content [% w/w]**
Higher fungi	Shiitake	24.13±1.25	0.79±0.09	23.34±1.77
	Jew's ear	18.86±0.67	0.38±0.04	18.78±0.96
	Pleurotus ostreatus	30.06±0.99	2.62±0.12	**27.44±1.24**
	Agaricus campestris	14.75±0.51	**5.42±0.26**	9.33±0.77
	Agaricus brunnescens	16.64±0.73	4.05±0.43	12.59±1.05
	Inonotus obliquus	5.29±0.48	0.57±0.09	4.72±0.57
Cereals	Barley grass			1.42±0.12
	Barley			3.51±0.21
	Wheat			0.10±0.03
	Secale			1.47±0.22
	Oat			**4.30±0.35**
	Rice			0.04±0.01

6 CONCLUSIONS

The main objective of this work was to optimize the culture conditions for β-glucan production together with intracellular lipids, extracellular polymeric substances and pigments at diverse yeast, and microalgal strains. To enhance the yield of desired metabolites, the broath range of culture conditions were tested, from osmotic stress, temperature to nitrogen and carbon sources.

By applying a high-throughput screening approach, several carotenogenic Basidiomycetes yeast strains were identified as new sources of β-glucans, where the most promising results were obtained for strains *C. infirmominiatum* CCY 17-18-4, *R. kratochvilovae* CCY 20-2-26 and *P. rhodozyma* CCY 77-1-1. Further, several yeast strains showed a high co-production potential, for example strain *C. infirmominiatum* CCY 17-18-4 was able to accumulate 20.73 % (w/w) of β-glucans and 38.21 % (w/w) of lipids, the strain *R. kratochvilovae* CCY 20-2-26 was able to accumulate 38.21 % (w/w) of lipids and 20.73 % of β-glucans, accompanied with the high biomass yield (15.19 g/L). It was that increase in C/N ratio led to an increase in biomass, lipid and β-glucans production for several yeast strains.

Osmotic stress caused by sodium chloride had a negative effect on the biomass and lipid production but positively affected β-glucans production. Osmolarity combined with C/N ratios of 40, 70 and 100 led to an increase in the total glucan and β-glucan content in comparison to the standard conditions. The addition of 0.2% NaCl caused increase in β-glucan production up to 32.15 ± 0.81 (w/w) in *C. infirmominiatum* CCY 17-18-4.

The temperature is the key component affecting the biomass yield in yeasts. The highest β-glucan production was measured at *S. cerevisiae* CCY 21-4-102 – 29.12±2.37 % w/w (8 ^{o}C, combination of urea and yeast extract), but the biomass was declined (only about 3 g/L). The optimal biomass production was at 22 ^{o}C (11 g/L) on medium with urea, but the β-glucan content decrease to 11.99±0.39 % w/w. The highest biomass contents were measured at *R. kratochvilovae* CCY 20-2-26 (12 – 15 g/L) when cultured on medium with urea as the single nitrogen source. The optimal temperature combined with β-glucan and biomass yield is 22 ^{o}C (18.06±0.65 % of DCW**)**.

In other experiment, the production of extracellular polysaccharides was tested. From all studied yeast only one strain exhibited the biosynthesis of EPS, namely *Sporidiobolus pararoseus* CCY 19-9-6, with amount of 2.17 g/L (medium with glucose) and 2.28 g/L (medium with sucrose). Suprisingly, the 2% NaCl has the positive effect on EPS biosynthesis where the highest yield was achieved, 3.17±0.07 g/L.

Secretion of extracellular glycolipids has been already reported at *Rhodotorula* strains. Here, for optimization of culture conditions for enhancement of the exoglycolipid biosynthesis the yeast *Rhodotorula kratochvilovae* CCY 20-2-26 was chosen. The best culture conditions to produce biomass (15.23 g/L), intracellular lipids (68 % of DCW), exoglycolipids (2.42 g/L) and β-glucan (11.33 % od DCW) are mannose as a carbon source and potassium nitrate as a nitrogen source (C/N 120:1).

The red algal strain *P. purpureum* CCALA achieved the highest biomass content, 5.36 g/L, at 200 μmol photons$\cdot s^{-1}\cdot m^{-2}$ and after 13 days of cultivation, together with exopolysaccharides (1.95 g/L) and total carbohydrates (47.92 %) production, thus, it is great candidate for the further large-scale cultivation experiments.

The paramylon content isolated from *Euglena gracilis* biomass ranged from 11.9 – 69.29 % of CDW. The paramylon yield increased rapidly in the medium supplemented with 10 g/L of glucose up to 70 % od DCW, and 2.79 g/L of biomass.

In the end of the experimental part, the samples of commertial cereals (barley grass and barley flour, wheat, rice, oat, and *Secale*) and mushrooms (shiitake, Jew's ear, *Pleurotus ostreatus*,

Agaricus campestris, *Agaricus brunnescens* and *Inonotus obliquus*) were selected to determine the content of glucans. The highest content was measured for *Pleurotus ostreatus* where the amount reached the value 27 % DCW and oat from cereals (approx. 4.3 % of DCW).

7 REFERENCES

[1] Microorganisms: An introduction. *Www.biocote.com/* [online]. United Kingdom, 2014 [cit. 2018-08-02]. Dostupné z: https://www.biocote.com/blog/microorganisms-an-introduction/

[2] SHARMA, Naveen K., Ashwani K. RAI a Lucas J. STAL. *Cyanobacteria: an economic perspective*. Chichester, West Sussex, UK: John Wiley & Sons, 2013. ISBN 978-1-119-94127-9.

[3] GEORGE M. GARRITY a EDITOR-IN-CHIEF. *Bergey's manual of systematic bacteriology*. 2nd ed. New York: Springer, 2001. ISBN 9780387216096.

[4] SONANI, Ravi Raghav. Recent advances in production, purification and applications of phycobiliproteins. *World Journal of Biological Chemistry* [online]. 2016, 7(1), 100- [cit. 2018-08-02]. DOI: 10.4331/wjbc.v7.i1.100. ISSN 1949-8454. Dostupné z: http://www.wjgnet.com/1949-8454/full/v7/i1/100.htm

[5] PERCY M. GAULT AND HARRIS J. MARLER a EDITORS. *Handbook on cyanobacteria biochemistry, biotechnology and applications*. New York: Nova Science Publishers, 2009. ISBN 9781616683009.

[6] TROSCHL, Clemens, Katharina MEIXNER a Bernhard DROSG. Cyanobacterial PHA Production—Review of Recent Advances and a Summary of Three Years' Working Experience Running a Pilot Plant. *Bioengineering* [online]. 2017, 4(4), 26- [cit. 2018-08-02]. DOI: 10.3390/bioengineering4020026. ISSN 2306-5354. Dostupné z: http://www.mdpi.com/2306-5354/4/2/26

[7] SINGH Kumar Singh, MALLICK, Nirupama. Advances in cyanobacterial polyhydroxyalkanoates production, *FEMS Microbiology Letters*, Volume 364, Issue 20, 1 November 2017, fnx189, https://doi.org/10.1093/femsle/fnx189

[8] BOROWITZKA, Michael. *The physiology of microalgae*. New York, NY: Springer Berlin Heidelberg, 2016. ISBN 978-3-319-24943-8.

[9] BARSANTI, L. a Paolo. GUALTIERI. *Algae: anatomy, biochemistry, and biotechnology*. Second edition. Boca Raton, [Florida]: CRC Press/Taylor & Francis Group, [2014]. ISBN 978-1-4398-6732-7.

[10] CYBULSKA, Justyna, Michal HALAJ, Vladimír CEPÁK, Jaroslav LUKAVSKÝ a Peter CAPEK. Nanostructure features of microalgae biopolymer. *Starch - Stärke* [online]. 2016, **68**(7-8), 629-636. DOI: 10.1002/star.201500159. ISSN 00389056. Dostupné z: http://doi.wiley.com/10.1002/star.201500159

[11] SCHULZE, Christian, Martin WETZEL, Jakob REINHARDT, Mandy SCHMIDT, Lisa FELTEN a Sabine MUNDT. Screening of microalgae for primary metabolites including β-glucans and the influence of nitrate starvation and irradiance on β-glucan production. *Journal of Applied Phycology* [online]. 2016, **28**(5), 2719-2725. DOI: 10.1007/s10811-016-0812-9. ISSN 0921-8971. Dostupné z: http://link.springer.com/10.1007/s10811-016-0812-9

[12] MASOJÍDEK, Jiří. *Mikrořasy – solární továrna v jedné buňce*. Praha: Academia, 2016. Věda kolem nás.

[13] RICHMOND, Amos. Handbook of microalgal culture: biotechnology and applied phycology. Ames, Iowa, USA: Blackwell Science, 2004. ISBN 06-320-5953-2.

[14] GLADUE, Raymond a John MAXEY. Microalgal feeds for aquaculture. Journal of Applied Phycology. 1994, 6(2), 131-141. DOI: 10.1007/BF02186067. ISSN 0921-8971. Dostupné také z: http://link.springer.com/10.1007/BF02186067

[15] KOMOR, E. a W. TANNEB. The Hexose-Proton Symport System of Chlorella vulguris: Specificity, Stoichiometry and Energetics of Sugar-Induced Proton Uptake. European Journal of Biochemistry. 1974, 44(1), 219-223.

[16] KOMOR, Ewald, Christian SCHOBERT a Bong-Heuy CHO. Sugar specificity and sugar-proton interaction for the hexose-proton-symport system of Chlorella. European Journal of Biochemistry. 1985, 146(3), 649-656. DOI: 10.1111/j.1432- 1033.1985.tb08700.x. ISSN 0014-2956. Dostupné také z: http://doi.wiley.com/10.1111/j.1432-1033.1985.tb08700.x

[17] GAMBOA-DELGADO, Julián a Julia MÁRQUEZ-REYES. Potential of microbialderived nutrients for aquaculture development. Reviews in Aquaculture. 2018, 10(1), 1- 23. DOI: 10.1111/raq.12157. ISSN 17535123. Dostupné také z: http://doi.wiley.com/10.1111/raq.12157

[18] COLE, Kathleen a Robert SHEATH. *Biology of the Red Algae*. USA: Press Syndicate, 1990. ISBN 0-521-34301-1.

[19] MOREIRA, David, Hervé LE GUYADER a Hervé PHILIPPE. The origin of red algae and the evolution of chloroplasts. *Nature* [online]. 2000, **405**(6782), 69-72 [cit. 2020-07-12]. DOI: 10.1038/35011054. ISSN 0028-0836. Dostupné z: http://www.nature.com/articles/35011054

[20] USOV, Anatolii I. *Polysaccharides of the red algae* [online]. Elsevier, 2011, 2011, s. 115-217 [cit. 2020-07-12]. Advances in Carbohydrate Chemistry and Biochemistry. DOI: 10.1016/B978-0-12-385520-6.00004-2. ISBN 9780123855206. Dostupné z: https://linkinghub.elsevier.com/retrieve/pii/B9780123855206000042

[21] KEIDAN, Marina, Hadas BROSHY, Dorit VAN MOPPES a Shoshana(Malis) ARAD. Assimilation of sulphur into the cell-wall polysaccharide of the red microalga Porphyridium sp. (Rhodophyta). *Phycologia* [online]. 2019, **45**(5), 505-511 [cit. 2020-07-12]. DOI: 10.2216/05-57.1. ISSN 0031-8884. Dostupné z: https://www.tandfonline.com/doi/full/10.2216/05-57.1

[22] YOU, Tao a Stanley M BARNETT. Effect of light quality on production of extracellular polysaccharides and growth rate of Porphyridium cruentum. *Biochemical Engineering Journal* [online]. 2004, **19**(3), 251-258 [cit. 2020-07-12]. DOI: 10.1016/j.bej.2004.02.004. ISSN 1369703X. Dostupné z: https://linkinghub.elsevier.com/retrieve/pii/S1369703X04000282

[23] LI, Shi-Yan, Yossef SHABTAI a Shoshana (MALIS) ARAD. Production and composition of the sulphated cell wall polysaccharide of Porphyridium (Rhodophyta) as affected by CO

2 concentration. *Phycologia* [online]. 2019, **39**(4), 332-336 [cit. 2020-07-12]. DOI: 10.2216/i0031-8884-39-4-332.1. ISSN 0031-8884. Dostupné z: https://www.tandfonline.com/doi/full/10.2216/i0031-8884-39-4-332.1

[24] FRIEDMAN, Orit, Zvy DUBINSKY a Shoshana (Malis) ARAD. Effect of light intensity on growth and polysaccharide production in red and blue-green rhodophyta unicells. *Bioresource Technology* [online]. 1991, **38**(2-3), 105-110 [cit. 2020-07-12]. DOI: 10.1016/0960-8524(91)90139-B. ISSN 09608524. Dostupné z: https://linkinghub.elsevier.com/retrieve/pii/096085249190139B

[25] NASH, Merinda C., Guillermo DIAZ-PULIDO, Adela S. HARVEY, Walter ADEY a Frank MELZNER. Coralline algal calcification: A morphological and process-based understanding. *PLOS ONE* [online]. 2019, **14**(9) [cit. 2020-07-12]. DOI: 10.1371/journal.pone.0221396. ISSN 1932-6203. Dostupné z: https://dx.plos.org/10.1371/journal.pone.0221396

[26] SCHWARTZBACH, Steven D. a Shigeru SHIGEOKA, ed. *Euglena: Biochemistry, Cell and Molecular Biology* [online]. Cham: Springer International Publishing, 2017 [cit. 2020-07-12]. Advances in Experimental Medicine and Biology. DOI: 10.1007/978-3-319-54910-1. ISBN 978-3-319-54908-8.

[27] GISSIBL, Alexander, Angela SUN, Andrew CARE, Helena NEVALAINEN a Anwar SUNNA. Bioproducts From Euglena gracilis: Synthesis and Applications. *Frontiers in Bioengineering and Biotechnology* [online]. 2019, **7** [cit. 2020-07-12]. DOI: 10.3389/fbioe.2019.00108. ISSN 2296-4185. Dostupné z: https://www.frontiersin.org/article/10.3389/fbioe.2019.00108/full

[28] BARSANTI a GUALTIERI. Paramylon, a Potent Immunomodulator from WZSL Mutant of Euglena gracilis. *Molecules* [online]. 2019, **24**(17) [cit. 2020-07-12]. DOI: 10.3390/molecules24173114. ISSN 1420-3049. Dostupné z: https://www.mdpi.com/1420-3049/24/17/3114

[29] TANAKA, Yuji, Takahisa OGAWA, Takanori MARUTA, Yuta YOSHIDA, Kazuharu ARAKAWA a Takahiro ISHIKAWA. Glucan synthase-like 2 is indispensable for paramylon synthesis in Euglena gracilis. *FEBS Letters* [online]. 2017, **591**(10), 1360-1370. DOI: 10.1002/1873-3468.12659. ISSN 0014-5793.

[30] GOLDEMBERG, Sara H a Luis R MARECHAL. Biosynthesis of paramylon in Euglena gracilis. *Biochimica et Biophysica Acta* [online]. 1963, **71**, 743-744. DOI: 10.1016/0006-3002(63)91155-7. ISSN 00063002.

[31] TOMIYAMA, Takuya, Kaeko KURIHARA, Takahisa OGAWA, Takanori MARUTA, Takumi OGAWA, Daisaku OHTA, Yoshihiro SAWA a Takahiro ISHIKAWA. Wax Ester Synthase/Diacylglycerol Acyltransferase Isoenzymes Play a Pivotal Role in Wax Ester Biosynthesis in Euglena gracilis. *Scientific Reports* [online]. 2017, **7**(1). DOI: 10.1038/s41598-017-14077-6. ISSN 2045-2322.

[32] IWASAKI, Keita, Asuka KANEKO, Yuji TANAKA, Takahiro ISHIKAWA, Hemanth NOOTHALAPATI a Tatsuyuki YAMAMOTO. Visualizing wax ester fermentation in single Euglena gracilis cells by Raman microspectroscopy and multivariate curve resolution

analysis. *Biotechnology for Biofuels* [online]. 2019, **12**(1). DOI: 10.1186/s13068-019-1471-2. ISSN 1754-6834.

[33] RUSSO, Rossella, Laura BARSANTI, Valter EVANGELISTA, Anna M. FRASSANITO, Vincenzo LONGO, Laura PUCCI, Giuseppe PENNO a Paolo GUALTIERI. Euglena gracilis paramylon activates human lymphocytes by upregulating pro-inflammatory factors. *Food Science & Nutrition* [online]. 2017, **5**(2), 205-214. DOI: 10.1002/fsn3.383. ISSN 20487177.

[34] PEREZ-GARCIA, Octavio a Yoav BASHAN. Microalgal Heterotrophic and Mixotrophic Culturing for Bio-refining: From Metabolic Routes to Techno-economics. PROKOP, Aleš, Rakesh K. BAJPAI a Mark E. ZAPPI, ed. *Algal Biorefineries* [online]. Cham: Springer International Publishing, 2015, 2015, s. 61-131. DOI: 10.1007/978-3-319-20200-6_3. ISBN 978-3-319-20199-3.

[35] BLANKENSHIP, Robert E. *Molecular mechanisms of photosynthesis*. 2nd ed. Chichester: Wiley Blackwell, 2014. ISBN 978-1-4051-8976-7.

[36] MICHAL, Gerhard a Dietmar SCHOMBURG, ed. *Biochemical pathways: an atlas of biochemistry and molecular biology*. 2nd ed. Hoboken: Wiley, c2012. ISBN 978-0-470-14684-2.

[37] RAINES, C.A. The Calvin cycle revisited. Photosynthesis Research (2003) 75: 1. https://doi.org/10.1023/A:1022421515027

[38] MORALES-SÁNCHEZ, Daniela, Oscar A. MARTINEZ-RODRIGUEZ, John KYNDT a Alfredo MARTINEZ. Heterotrophic growth of microalgae: metabolic aspects. *World Journal of Microbiology and Biotechnology* [online]. 2015, **31**(1), 1-9. DOI: 10.1007/s11274-014-1773-2. ISSN 0959-3993.

[39] PEREZ-GARCIA, Octavio, Froylan M.E. ESCALANTE, Luz E. DE-BASHAN a Yoav BASHAN. Heterotrophic cultures of microalgae: Metabolism and potential products. *Water Research* [online]. 2011, **45**(1), 11-36. DOI: 10.1016/j.watres.2010.08.037. ISSN 00431354.

[40] WALKER, Graeme M. *Yeast physiology and biotechnology*. New York: J. Wiley, c1998. ISBN 0-471-96446-8

[41] *Molecular mechanisms in yeast carbon metabolism*. New York: Springer, 2014. ISBN 978-3-642-55012-6.

[42] SATYANARAYANA, T. a Gotthard. KUNZE. *Yeast biotechnology: diversity and applications*. New York: Springer, [2009]. ISBN 978-1-4020-8292-4.

[43] ŠILHÁNKOVÁ, L. 2008. Mikrobiologie pro potravináře a biotechnology. Praha: ACADEMIA, 2008. s. 60. ISBN 978-80-200-1703.

[44] TANČINOVÁ, D. et al. 2008. Mikrobiológia potravín. 2. Vyd. Nitra: SPU, 2008. s. 15-16. ISBN 978-80-552-0145-0.

[45] ČEJKOVÁ, A. Biotechnologické aplikace mikroorganismů. [online]

[46] Evans, I. H. (Ed.): Yeast Protocols, Methods in Cell and Molecular Biology. Humana Press, Totowa, NJ (1996)

[47] QUEROL, A., FLEET, G. H. 2006. Yeast in food and beverages: The yeast handbook. [online]. Berlin: Springer, 2006. 453 s.

[48] WANG, Q.-M., A.M. YURKOV, M. GÖKER et al. Phylogenetic classification of yeasts and related taxa within Pucciniomycotina. *Studies in Mycology* [online]. 2015, 81, 149-189. DOI: 10.1016/j.simyco.2015.12.002. ISSN 01660616.

[49] ROSA, Carlos a GÁBOR PÉTER (EDS.). *Biodiversity and ecophysiology of yeasts*. Berlin: Springer, 2006. ISBN 9783540309857.

[50] MAROVA, Ivana, Milan CERTIK a Emilia BREIEROV. Production of Enriched Biomass by Carotenogenic Yeasts - Application of Whole-Cell Yeast Biomass to Production of Pigments and Other Lipid Compounds. *Biomass - Detection, Production and Usage* [online]. InTech, 2011. DOI: 10.5772/19235. ISBN 9789533074924.

[51] *Rhodotorula* [online]. South Australia, 2016 [cit. 2018-08-02]. Dostupné z: https://mycology.adelaide.edu.au/descriptions/yeasts/rhodotorula/

[52] HERNÁNDEZ-ALMANZA, Ayerim, Julio CESAR MONTANEZ, Miguel A. AGUILAR-GONZÁLEZ, Cristian MARTÍNEZ-ÁVILA, Raúl RODRÍGUEZ-HERRERA a Cristóbal N. AGUILAR. Rhodotorula glutinis as source of pigments and metabolites for food industry. *Food Bioscience* [online]. 2014, 5, 64-72. DOI: 10.1016/j.fbio.2013.11.007. ISSN 22124292.

[53] KOT, Anna M., Stanisław BŁAŻEJAK, Agnieszka KURCZ, Iwona GIENTKA a Marek KIELISZEK. Rhodotorula glutinis—potential source of lipids, carotenoids, and enzymes for use in industries. *Applied Microbiology and Biotechnology* [online]. 2016, 100(14), 6103-6117 [cit. 2017-02-08]. DOI: 10.1007/s00253-016-7611-8. ISSN 01757598. Dostupné z: http://link.springer.com/10.1007/s00253-016-7611-8

[54] WIRTH, Fernanda a Luciano Z. GOLDANI. Epidemiology of Rhodotorula: An Emerging Pathogen. *Interdisciplinary Perspectives on Infectious Diseases* [online]. 2012, **2012**, 1-7 [cit. 2020-07-12]. DOI: 10.1155/2012/465717. ISSN 1687-708X. Dostupné z: http://www.hindawi.com/journals/ipid/2012/465717/

[55] HERZ, Stephanie, Roland W.S. WEBER, Heidrun ANKE, Adele MUCCI a Paolo DAVOLI. Intermediates in the oxidative pathway from torulene to torularhodin in the red yeasts Cystofilobasidium infirmominiatum and C. capitatum (Heterobasidiomycetes, Fungi). *Phytochemistry*[online]. 2007, 68(20), 2503-2511 [cit. 2018-08-02]. DOI: 10.1016/j.phytochem.2007.05.019. ISSN 00319422. Dostupné z: http://linkinghub.elsevier.com/retrieve/pii/S0031942207003342

[56] SAMPAIO J. P., GADANHO M. BAUER R.: Taxonomic studies on the genus Cystofilobasidium: description of C. ferigula sp. nov. and clarification of the status of C. lari-marini, International Journal of Systematic and Evolutionary Microbiology, 2001, p. 221-229

[57] KOCKOVÁ-KRATOCHVÍLOVÁ A.: Taxonómia kvasiniek a kvasinkovitých mikroorganizmov, Alfa Bratislava, 1990, p. 528, ISBN 3-527-26162-1

[58] LIBKIND, Diego, Ruben SOMMARUGA, Horacio ZAGARESE a Maria VAN BROOCK. Mycosporines in carotenogenic yeasts. *Systematic and Applied Microbiology* [online]. 2005, 28(8), 749-754 [cit. 2018-08-02]. DOI: 10.1016/j.syapm.2005.05.005. ISSN 07232020. Dostupné z: http://linkinghub.elsevier.com/retrieve/pii/S0723202005000925

[59] WACKETT, Lawrence P. Commercial microbial polysaccharides. *Microbial Biotechnology* [online]. 2013, **6**(3), 314-315 [cit. 2020-07-12]. DOI: 10.1111/1751-7915.12053. ISSN 17517915. Dostupné z: http://doi.wiley.com/10.1111/1751-7915.12053

[60] MORRIS, G. a S. HARDING. Polysaccharides, Microbial. *Encyclopedia of Microbiology* [online]. Elsevier, 2009, 2009, s. 482-494 [cit. 2020-07-12]. DOI: 10.1016/B978-012373944-5.00135-8. ISBN 9780123739445. Dostupné z: https://linkinghub.elsevier.com/retrieve/pii/B9780123739445001358

[61] LINTON, J. D., S. G. ASH a L. HUYBRECHTS. Microbial polysaccharides. BYROM, David, ed. *Biomaterials* [online]. London: Palgrave Macmillan UK, 1991, 1991, s. 215-261 [cit. 2020-07-12]. DOI: 10.1007/978-1-349-11167-1_4. ISBN 978-1-349-11169-5. Dostupné z: http://link.springer.com/10.1007/978-1-349-11167-1_4

[62] GENNADY E. ZAIKOV. *Chemistry of Polysaccharides*. Hoboken: CRC Press, 2005. ISBN 9789047415176.

[63] STONE, Bruce. *Chemistry, biochemistry, and biology of 1-3 beta glucans*. Amsterdam: Acad. Press, 2009. ISBN 9780123739711.

[64] KIM, Hyung Sook, Jin Tae HONG, Youngsoo KIM a Sang-Bae HAN. Stimulatory Effect of β-glucans on Immune Cells. *Immune Network*[online]. 2011, 11(4). DOI: 10.4110/in.2011.11.4.191. ISSN 1598-2629.

[65] PENGKUMSRI, Noppawat, Bhagavathi Sundaram SIVAMARUTHI, Sasithorn SIRILUN, Sartjin PEERAJAN, Periyanaina KESIKA, Khontaros CHAIYASUT a Chaiyavat CHAIYASUT. Extraction of β-glucan from Saccharomyces cerevisiae: Comparison of different extraction methods and in vivo assessment of immunomodulatory effect in mice. *Food Science and Technology*. 2017, **37**(1), 124-130. DOI: 10.1590/1678-457x.10716. ISSN 1678-457X.

[66] Rop O, Mlcek J, Jurikova T. Beta-glucans in higher fungi and their health effects. *Nutrition Reviews*. 2009; doi:10.1111/j.1753-4887.2009.00230.

[67] Zielke C, Kosik O, Ainalem ML, Lovegrove A, Stradner A, Nilsson L. Characterization of cereal β-glucan extracts from oat and barley and quantification of proteinaceous matter. *PLoS One*. 2017; doi: 10.1371/journal.pone.0172034.

[68] Javvadi S, Pandey S, Mishra A, Pradhan B, Chatterjee S. Bacterial cyclic β-(1,2)-glucans sequester iron to protect against iron-induced toxicity. *EMBO reports*. 2018; DOI: 10.15252/embr.201744650

[69] BCC Research (2018). https://www.bccresearch.com/partners/verified-market-research/global-beta-glucan-market.html

[70] Vetvicka V, Oliveira C. β(1-3)(1-6)-D-glucans modulate immune status in pigs: potential importance for efficiency of commercial farming. *Ann Transl Med*. 2014; doi: 10.3978/j.issn.2305-5839.2014.01.04.

[71] Bashir KMI, Choi JS. Clinical and Physiological Perspectives of β-Glucans: The Past, Present, and Future. *Int J Mol Sci*. 2017; doi: 10.3390/ijms18091906.

[72] VETVICKA, Vaclav, Luca VANNUCCI a Petr SIMA. B-glucan as a new tool in vaccine development. *Scandinavian Journal of Immunology* [online]. 2019, **91**(2). DOI: 10.1111/sji.12833. ISSN 0300-9475.

[73] De Smet R, Allais L, Cuvelier CA. Recent advances in oral vaccine development: yeast-derived β-Glucan particles. *Hum Vaccin Immunother*. 2014; doi: 10.4161/hv.28166.STIER, Heike, Veronika EBBESKOTTE a Joerg GRUENWALD. Immune-modulatory effects of dietary Yeast Beta-1,3/1,6-D-glucan. *Nutrition Journal* [online]. 2014, 13(1). DOI: 10.1186/1475-2891-13-38. ISSN 1475-2891.

[74] VETVICKA, Vaclav a Miroslav NOVAK, ed. *Biology and Chemistry of Beta Glucan* [online]. BENTHAM SCIENCE PUBLISHERS, 2012 [cit. 2018-08-02]. ISBN 9781608052103.

[75] BROWDER, W., D. WILLIAMS, P. LUCORE, H. PRETUS, E. JONES a R. MCNAMEE. Effect of enhanced macrophage function on early wound healing. *Surgery*. 1988, 104(2), 224-230.

[76] KOH, Timothy a Luisa DIPIETRO. Inflammation and wound healing: the role of the macrophage. *Expert Reviews in Molecular Medicine*. 2011, 13(23), -. DOI: 10.1017/S1462399411001943. ISSN 1462-3994. Dostupné také z: http://www.journals.cambridge.org/abstract_S1462399411001943

[77] SHAHINIAN, Serge a Howard BUSSEY. B-1,6-Glucan synthesis in Saccharomyces cerevisiae. *Molecular Microbiology* [online]. 2000, 35(3), 477-489 [cit. 2018-08-02]. DOI: 10.1046/j.1365-2958.2000.01713.x. ISSN 0950382X. Dostupné z: http://doi.wiley.com/10.1046/j.1365-2958.2000.01713.x

[78] RICHMOND, Amos. *Handbook of microalgal culture: biotechnology and applied phycology*. Ames, Iowa, USA: Blackwell Science, 2004. ISBN 06-320-5953-2.

[79] RUPÉREZ, Pilar a Gema TOLEDANO. Indigestible fraction of edible marine seaweeds. *Journal of the Science of Food and Agriculture*. 2003, 83(12), 1267-1272. DOI: 10.1002/jsfa.1536. ISSN 00225142. Dostupné také z: http://doi.wiley.com/10.1002/jsfa.1536

[80] FRANÇOIS, Jean a Jean Luc PARROU. Reserve carbohydrates metabolism in the yeast Saccharomyces cerevisiae. *FEMS Microbiology Reviews* [online]. 2001, 25(1), 125-145 [cit. 2018-08-02]. DOI: 10.1111/j.1574-6976.2001.tb00574.x. ISSN 1574-6976. Dostupné z: https://academic.oup.com/femsre/article-lookup/doi/10.1111/j.1574-6976.2001.tb00574.x

[81] TORIJA, María-Jesús, Maite NOVO, Anne LEMASSU, Wayne WILSON, Peter J. ROACH, Jean FRANÇOIS a Jean-Luc PARROU. Glycogen synthesis in the absence of glycogenin in the yeast Saccharomyces cerevisiae. *FEBS Letters* [online]. 2005, 579(18), 3999-4004 [cit. 2018-08-02]. DOI: 10.1016/j.febslet.2005.06.007. ISSN 00145793. Dostupné z: http://doi.wiley.com/10.1016/j.febslet.2005.06.007

[82] FRANÇOIS, Jean Marie, Thomas WALTHER a Jean Luc PARROU. Genetics and Regulation of Glycogen and Trehalose Metabolism in Saccharomyces cerevisiae. LIU, Zonglin Lewis, ed. *Microbial Stress Tolerance for Biofuels* [online]. Berlin, Heidelberg: Springer Berlin Heidelberg, 2012, 2012-8-17, s. 29-55 [cit. 2018-08-02]. Microbiology Monographs. DOI: 10.1007/978-3-642-21467-7_2. ISBN 978-3-642-21466-0. Dostupné z: http://link.springer.com/10.1007/978-3-642-21467-7_2

[83] PAALMAN, Johannes W.G., Ren VERWAAL, Sjoukje H. SLOFSTRA, Arie J. VERKLEIJ, Johannes BOONSTRA a C.Theo VERRIPS. Trehalose and glycogen accumulation is related to the duration of the G 1 phase of Saccharomyces cerevisiae. *FEMS Yeast Research* [online]. 2003, 3(3), 261-268 [cit. 2018-08-02]. DOI: 10.1111/j.1567-1364.2003.tb00168.x. ISSN 15671356.

[84] BULÉON, A., P. COLONNA, V. PLANCHOT a S. BALL. Starch granules: structure and biosynthesis. *International Journal of Biological Macromolecules* [online]. 1998, 23(2), 85-112 [cit. 2018-08-02]. DOI: 10.1016/S0141-8130(98)00040-3. ISSN 01418130.

[85] PERCIVAL, Elizabeth. The polysaccharides of green, red and brown seaweeds: Their basic structure, biosynthesis and function. *British Phycological Journal* [online]. 1979, 14(2), 103-117 [cit. 2018-08-02]. DOI: 10.1080/00071617900650121. ISSN 0007-1617.

[86] EDITED BY JOST WINGENDER, THOMAS R. NEU a Hans-Curt FLEMMING. *Microbial Extracellular Polymeric Substances Characterization, Structure and Function*. Berlin, Heidelberg: Springer Berlin Heidelberg, 1999. ISBN 9783642601477.

[87] GIENTKA, Iwona, Stanisław BŁAŻEJAK, Lidia STASIAK-RÓŻAŃSKA a Anna CHLEBOWSKA-ŚMIGIEL. Exopolysaccharides from yeast: insight into optimal conditions for biosynthesis, chemical composition and functional properties – review. *Acta Scientiarum Polonorum Technologia Alimentaria* [online]. 2015, 14(4), 283-292 [cit. 2018-08-02]. DOI: 10.17306/J.AFS.2015.4.29. ISSN 16440730.

[88] KULAKOVSKAYA, Ekaterina a Tatiana KULAKOVSKAYA. *Extracellular Glycolipids of Yeasts* [online]. Amsterdam: Elsevier, 2014 [cit. 2020-07-13]. DOI: 10.1016/C2013-0-12913-3. ISBN 9780124200692.

[89] WANG, Mengqi, Weian MAO, Xiaoxiang WANG, Fengyi LI, Jiming WANG, Zhe CHI, Zhenming CHI a Guanglei LIU. Efficient simultaneous production of extracellular polyol esters of fatty acids and intracellular lipids from inulin by a deep-sea yeast Rhodotorula paludigena P4R5. *Microbial Cell Factories* [online]. 2019, **18**(1) [cit. 2020-07-13]. DOI: 10.1186/s12934-019-1200-3. ISSN 1475-2859.

[90] CAJKA, Tomas, Luis A. GARAY, Irnayuli R. SITEPU, Kyria L. BOUNDY-MILLS a Oliver FIEHN. Multiplatform Mass Spectrometry-Based Approach Identifies Extracellular Glycolipids of the Yeast Rhodotorula babjevae UCDFST 04-877. *Journal of Natural Products* [online]. 2016, **79**(10), 2580-2589 [cit. 2020-07-13]. DOI: 10.1021/acs.jnatprod.6b00497. ISSN 0163-3864.

[91] MÜHLROTH, Alice, Keshuai LI, Gunvor RØKKE, Per WINGE, Yngvar OLSEN, Martin HOHMANN-MARRIOTT, Olav VADSTEIN a Atle BONES. Pathways of Lipid Metabolism in Marine Algae, Co-Expression Network, Bottlenecks and Candidate Genes for Enhanced Production of EPA and DHA in Species of Chromista. *Marine Drugs* [online]. 2013, 11(11), 4662-4697 [cit. 2018-08-02]. DOI: 10.3390/md11114662. ISSN 1660-3397.

[92] BABA, Masato a Yoshihiro SHIRAIW. Biosynthesis of Lipids and Hydrocarbons in Algae. DUBINSKY, Zvy, ed. *Photosynthesis* [online]. InTech, 2013, 2013-06-12 [cit. 2018-08-02]. DOI: 10.5772/56413. ISBN 978-953-51-1161-0. Dostupné z: http://www.intechopen.com/books/photosynthesis/biosynthesis-of-lipids-and-hydrocarbons-in-algae

[93] KLUG, Lisa a Günther DAUM. Yeast lipid metabolism at a glance. *FEMS Yeast Research* [online]. 2014, **14**(3), 369-388 [cit. 2020-07-13]. DOI: 10.1111/1567-1364.12141. ISSN 15671356. Dostupné z: https://academic.oup.com/femsyr/article-lookup/doi/10.1111/1567-1364.12141

[94] FAKAS, Stylianos. Lipid biosynthesis in yeasts: A comparison of the lipid biosynthetic pathway between the model nonoleaginous yeast Saccharomyces cerevisiae and the model oleaginous yeast Yarrowia lipolytica. *Engineering in Life Sciences* [online]. 2017, **17**(3), 292-302 [cit. 2020-07-13]. DOI: 10.1002/elsc.201600040. ISSN 16180240. Dostupné z: http://doi.wiley.com/10.1002/elsc.201600040

[95] ATHENAKI, M., C. GARDELI, P. DIAMANTOPOULOU, S.S. TCHAKOUTEU, D. SARRIS, A. PHILIPPOUSSIS a S. PAPANIKOLAOU. Lipids from yeasts and fungi: physiology, production and analytical considerations. *Journal of Applied Microbiology* [online]. 2018, **124**(2), 336-367 [cit. 2020-07-13]. DOI: 10.1111/jam.13633. ISSN 13645072. Dostupné z: http://doi.wiley.com/10.1111/jam.13633

[96] NIELSEN, Jens. Systems biology of lipid metabolism: From yeast to human. *FEBS Letters* [online]. 2009, **583**(24), 3905-3913 [cit. 2020-07-13]. DOI: 10.1016/j.febslet.2009.10.054. ISSN 00145793. Dostupné z: http://doi.wiley.com/10.1016/j.febslet.2009.10.054

[97] WANG, Yanan, Sufang ZHANG, Zhiwei ZHU, Hongwei SHEN, Xinping LIN, Xiang JIN, Xiang JIAO a Zongbao Kent ZHAO. Systems analysis of phosphate-limitation-induced lipid accumulation by the oleaginous yeast Rhodosporidium toruloides. *Biotechnology for Biofuels* [online]. 2018, **11**(1) [cit. 2020-07-13]. DOI: 10.1186/s13068-018-1134-8. ISSN 1754-6834. Dostupné z: https://biotechnologyforbiofuels.biomedcentral.com/articles/10.1186/s13068-018-1134-8

[98] SANTEK, Mirela Ivancic, Suncica BELUHAN a Bozidar SANTEK. Production of Microbial Lipids from Lignocellulosic Biomass. NAGESWARA-RAO, Madhugiri a Jaya R. SONEJI, ed. *Advances in Biofuels and Bioenergy* [online]. InTech, 2018, 2018-07-04 [cit. 2020-07-13]. DOI: 10.5772/intechopen.74013. ISBN 978-1-78923-286-8. Dostupné z: http://www.intechopen.com/books/advances-in-biofuels-and-bioenergy/production-of-microbial-lipids-from-lignocellulosic-biomass

[99] VETVICKA, Vaclav a Miroslav NOVAK, ed. *Series Title: Biology and Chemistry of Beta Glucan Volume Title: Beta-Glucan, Structure, Chemistry and Specific Application* [online]. BENTHAM SCIENCE PUBLISHERS, 2013 [cit. 2018-08-02]. ISBN 9781608052608.

[100] GUO, Qingbin, Lianzhong AI a Steve CUI. *Methodology for Structural Analysis of Polysaccharides* [online]. Cham: Springer International Publishing, 2018 [cit. 2020-07-14]. SpringerBriefs in Molecular Science. DOI: 10.1007/978-3-319-96370-9. ISBN 978-3-319-96369-3.

[101] KASAAI, Mohammad R. Various Methods for Determination of the Degree of N-Acetylation of Chitin and Chitosan: A Review. *Journal of Agricultural and Food Chemistry* [online]. 2009, **57**(5), 1667-1676 [cit. 2020-07-14]. DOI: 10.1021/jf803001m. ISSN 0021-8561. Dostupné z: https://pubs.acs.org/doi/10.1021/jf803001m

[102] *B-Glucan Assay Kit (Yeast & Mushroom)* [online]. USA: Megazyme [cit. 2018-07-08]. Dostupné z: https://secure.megazyme.com/Beta-Glucan-Assay-Kit-Yeast-Mushroom

[103] Synytsya, A., & Novak, M. (2014). Structural analysis of glucans. *Annals Of Translational Medicine, 2*(2), 7. doi:10.3978/j.issn.2305-5839.2014.02.07

[104] HERBURGER, Klaus a Andreas HOLZINGER. Aniline Blue and Calcofluor White Staining of Callose and Cellulose in the Streptophyte Green Algae Zygnema and Klebsormidium. *BIO-PROTOCOL* [online]. 2016, **6**(20) [cit. 2020-07-14]. DOI: 10.21769/BioProtoc.1969. ISSN 2331-8325. Dostupné z: https://bio-protocol.org/e1969

[105] WOOD, Peter J. a R.G. FULCHER. Specific interaction of aniline blue with (1 → 3)-β-d-glucan. *Carbohydrate Polymers* [online]. 1984, **4**(1), 49-72 [cit. 2020-07-14]. DOI: 10.1016/0144-8617(84)90044-4. ISSN 01448617.

[106] Duetz-System. *Kuhner shaker* [online]. 2020 [cit. 2020-07-1]. Dostupné z: http://old.kuhner.com/en/product/add-ons/replicator/duetz-system.html

[107] CRAMER, Marian a Jack MYERS. Growth and photosynthetic characteristics of euglena gracilis. *Archiv f*r Mikrobiologie* [online]. 1952, **17**(1-4), 384-402 [cit. 2020-07-14]. DOI: 10.1007/BF00410835. ISSN 0302-8933.

[108] BENNETT, Allen a Lawrence BOGORAD. COMPLEMENTARY CHROMATIC ADAPTATION IN A FILAMENTOUS BLUE-GREEN ALGA. *The Journal of Cell Biology* [online]. 1973, **58**(2), 419-435 [cit. 2020-07-14]. DOI: 10.1083/jcb.58.2.419. ISSN 1540-8140.

[109] Kosa G, Kohler A, Tafintseva V, Zimmermann B, Forfang K, Afseth NK, Tzimorotas D, Vuoristo KS, Horn SJ, Mounier J, Shapaval V (2017) Microtiter plate cultivation of oleaginous fungi and monitoring of lipogenesis by high-throughput FTIR spectroscopy. Microbial Cell Factories. Doi: 10.1186/s12934-017-0716-7.

[110] Kosa G, Zimmermann B, Kohler A, Ekeberg D, Afseth NK, Mounier J, Shapaval V (2018) High-throughput screening of Mucoromycota fungi for production of low- and high-value lipids: Structure and potential prebiotic activity. Biotechnology for Biofuels. Doi: 10.1186/s13068-018-1070-7.

[111] K-BGLU | Beta-Glucan Assay Kit (100 Assays). *Deltagen Australia* [online]. Australia, 2020 [cit. 2020-07-16]. Dostupné z: https://deltagen.com.au/product/%CE%B2-glucan-assay-kit-mixed-linkage/

[112] SCHWARTZBACH, Steven D., Jerome A. SCHIFF a Neil H. GOLDSTEIN. Events Surrounding the Early Development of Euglena Chloroplasts. *Plant Physiology* [online]. 1975, **56**(2), 313-317 [cit. 2020-07-16]. DOI: 10.1104/pp.56.2.313. ISSN 0032-0889. Dostupné z: http://www.plantphysiol.org/lookup/doi/10.1104/pp.56.2.313

[113] ZAVŘEL, Tomáš, Petra OČENÁŠOVÁ, Maria SINETOVA a Jan ČERVENÝ. Determination of Storage (Starch/Glycogen) and Total Saccharides Content in Algae and Cyanobacteria by a Phenol-Sulfuric Acid Method. *BIO-PROTOCOL* [online]. 2018, **8**(15) [cit. 2020-07-16]. DOI: 10.21769/BioProtoc.2966. ISSN 2331-8325. Dostupné z: https://bio-protocol.org/e2966

[114] DEMŠAS J, CURK T (2013) Orange: Data Mining Toolbox in Python. Journal of Machine Learning Research. 2013, **14**(8). 2349-2353.

[115] CESCUT, Julien, Luc FILLAUDEAU, Carole MOLINA-JOUVE a Jean-Louis URIBELARREA. Carbon accumulation in Rhodotorula glutinis induced by nitrogen limitation. *Biotechnology for Biofuels* [online]. 2014, **7**(1) [cit. 2020-07-16]. DOI: 10.1186/s13068-014-0164-0. ISSN 1754-6834. Dostupné z: http://biotechnologyforbiofuels.biomedcentral.com/articles/10.1186/s13068-014-0164-0

[116] PLATA, Maria R., Cosima KOCH, Patrick WECHSELBERGER, Christoph HERWIG a Bernhard LENDL. Determination of carbohydrates present in Saccharomyces cerevisiae using mid-infrared spectroscopy and partial least squares regression. *Analytical and Bioanalytical Chemistry* [online]. 2013, **405**(25), 8241-8250 [cit. 2020-07-16]. DOI:

10.1007/s00216-013-7239-9. ISSN 1618-2642. Dostupné z: http://link.springer.com/10.1007/s00216-013-7239-9

[117] VALASQUES, Gildomar L., Flávia O. DE LIMA, Elisangela F. BOFFO, Jener D.G. SANTOS, Bruno C. DA SILVA, Sandra A. DE ASSIS, Wei WANG. Extraction optimization and antinociceptive activity of (1⟶3)-β-d-glucan from Rhodotorula mucilaginosa: an overview. *Carbohydrate Polymers*. 2014, **105**(1), 293-299. DOI: 10.1016/j.carbpol.2014.01.064.

[118] SCHRAGER, H M, J G RHEINWALD a M R WESSELS. Hyaluronic acid capsule and the role of streptococcal entry into keratinocytes in invasive skin infection. *Journal of Clinical Investigation* [online]. 1996, **98**(9), 1954-1958 [cit. 2020-07-16]. DOI: 10.1172/JCI118998. ISSN 0021-9738. Dostupné z: http://www.jci.org/articles/view/118998

[119] OGRODOWSKI, C. S., C. O. HOKKA a M. H. A. SANTANA. Production of Hyaluronic Acid by IStreptococcus/I: The Effects of the Addition of Lysozyme and Aeration on the Formation and the Rheological Properties of the Product. *Applied Biochemistry and Biotechnology* [online]. 2005, **122**(1-3), 0753-0762 [cit. 2020-07-16]. DOI: 10.1385/ABAB:122:1-3:0753. ISSN 0273-2289.

[120] BRAUNWALD, Teresa, Lisa SCHWEMMLEIN, Simone GRAEFF-HÖNNINGER, William Todd FRENCH, Rafael HERNANDEZ, William E. HOLMES a Wilhelm CLAUPEIN. Effect of different C/N ratios on carotenoid and lipid production by Rhodotorula glutinis. *Applied Microbiology and Biotechnology*. InTech, 2013, 97(14), 6581-6588. DOI: 10.1007/s00253-013-5005-8.

[121] SANTAMAURO, Fabio, Fraeya M WHIFFIN, Rod J SCOTT, Christopher J CHUCK. Low-cost lipid production by an oleaginous yeast cultured in non-sterile conditions using model waste resources. *Biotechnology for Biofuels*. InTech, 2014, **7**(1), 1-5. DOI: 10.1186/1754-6834-7-34.

[122] CAO, Yujin, Wei LIU, Xin XU, Haibo ZHANG, Jiming WANG, Mo XIAN. Production of free monounsaturated fatty acids by metabolically engineered Escherichia coli. *Biotechnology for Biofuels*. InTech, 2014, **7**(1), 1-5. DOI: 10.1186/1754-6834-7-59.

[123] SHAPAVAL, V., J. SCHMITT, T. MØRETRØ, H.P. SUSO, I. SKAAR, A.W. ÅSLI, D. LILLEHAUG a A. KOHLER. Characterization of food spoilage fungi by FTIR spectroscopy. *Journal of Applied Microbiology*. InTech, 2013, **114**(3), 788-796. DOI: 10.1111/jam.12092.

[124] SHAPAVAL, Volha, Nils AFSETH, Gjermund VOGT, Achim KOHLER. Fourier transform infrared spectroscopy for the prediction of fatty acid profiles in Mucor fungi grown in media with different carbon sources. *Microbial Cell Factories*. InTech, 2014, **13**(1), 788-796. DOI: 10.1186/1475-2859-13-86.

[125] SHAPAVAL, Volha, Jule BRANDENBURG, Johanna BLOMQVIST, Valeria TAFINTSEVA, Volkmar PASSOTH, Mats SANDGREN, Achim KOHLER. Biochemical profiling, prediction of total lipid content and fatty acid profile in oleaginous yeasts by FTIR spectroscopy. *Biotechnology for Biofuels*. InTech, 2019, **12**(1), 788-796. DOI: 10.1186/s13068-019-1481-0.

[126] KOSA, Gergely, Volha SHAPAVAL, Achim KOHLER, Boris ZIMMERMANN, Volkmar PASSOTH, Mats SANDGREN, Achim KOHLER a A. KOHLER. FTIR spectroscopy as a unified method for simultaneous analysis of intra- and extracellular metabolites in high-throughput screening of microbial bioprocesses. *Microbial Cell Factories*. InTech, 2017, **16**(1), 788-796. DOI: 10.1186/s12934-017-0817-3.

[127] FORFANG, Kristin, Boris ZIMMERMANN, Gergely KOSA, Achim KOHLER, Volha SHAPAVAL, Thomas L. DAWSON, Achim KOHLER a A. KOHLER. FTIR Spectroscopy for Evaluation and Monitoring of Lipid Extraction Efficiency for Oleaginous Fungi. *PLOS ONE*. InTech, 2017, **12**(1), 788-796. DOI: 10.1371/journal.pone.0170611.

[128] GUILLÉN, María D., Nerea CABO, Gergely KOSA, Achim KOHLER, Volha SHAPAVAL, Thomas L. DAWSON, Achim KOHLER a A. KOHLER. Relationships between the Composition of Edible Oils and Lard and the Ratio of the Absorbance of Specific Bands of Their Fourier Transform Infrared Spectra. Role of Some Bands of the Fingerprint Region. *Journal of Agricultural and Food Chemistry*. InTech, 1998, **46**(5), 1788-1793. DOI: 10.1021/jf9705274.

[129] HONG, K., S. SUN, W. TIAN, G. Q. CHEN, W. HUANG, Thomas L. DAWSON, Achim KOHLER a A. KOHLER. A rapid method for detecting bacterial polyhydroxyalkanoates in intact cells by Fourier transform infrared spectroscopy. *Applied Microbiology and Biotechnology*. InTech, 1999, **51**(4), 523-526. DOI: 10.1007/s002530051427.

[130] JACKSON, Michael, Henry H. MANTSCH, W. TIAN, G. Q. CHEN, W. HUANG, Thomas L. DAWSON, Achim KOHLER a A. KOHLER. The Use and Misuse of FTIR Spectroscopy in the Determination of Protein Structure. *Critical Reviews in Biochemistry and Molecular Biology*. InTech, 2008, **30**(2), 95-120. DOI: 10.3109/10409239509085140.

[131] MEYERS, Robert A., ed. *Encyclopedia of Analytical Chemistry*. Chichester, UK: John Wiley & Sons, 2006. ISBN 0471976709.

[132] MATA-GÓMEZ, Luis, Julio MONTAÑEZ, Alejandro MÉNDEZ-ZAVALA a Cristóbal AGUILAR. Biotechnological production of carotenoids by yeasts: an overview. *Microbial Cell Factories* [online]. 2014, **13**(1) [cit. 2020-07-16]. DOI: 10.1186/1475-2859-13-12. ISSN 1475-2859. Dostupné z: http://microbialcellfactories.biomedcentral.com/articles/10.1186/1475-2859-13-12

[133] LÓPEZ-NIETO, M. J., J. COSTA, E. PEIRO, E. MÉNDEZ, M. RODRÍGUEZ-SÁIZ, J. L. DE LA FUENTE, W. CABRI a J. L. BARREDO. Biotechnological lycopene production by mated fermentation of Blakeslea trispora. *Applied Microbiology and Biotechnology* [online]. 2004, **66**(2), 153-159 [cit. 2020-07-16]. DOI: 10.1007/s00253-

004-1669-4. ISSN 0175-7598. Dostupné z: http://link.springer.com/10.1007/s00253-004-1669-4

[134] BLAGA, Alexandra Cristina, Corina CIOBANU, Dan CAŞCAVAL a Anca-Irina GALACTION. Enhancement of ergosterol production by Saccharomyces cerevisiae in batch and fed-batch fermentation processes using n -dodecane as oxygen-vector. *Biochemical Engineering Journal* [online]. 2018, **131**, 70-76 [cit. 2020-07-16]. DOI: 10.1016/j.bej.2017.12.010. ISSN 1369703X. Dostupné z: https://linkinghub.elsevier.com/retrieve/pii/S1369703X17303443

[135] TURK, Martina, Zrinka ABRAMOVIĆ, Ana PLEMENITAŠ and Nina GUNDE-CIMERMAN. Salt stress and plasma-membrane fluidity in selected extremophilic yeasts and yeast-like fungi. *FEMS Yeast Research*. InTech, 2007, **7**(4), 550-557. DOI: 10.1111/j.1567-1364.2007.00209.x.

[136] GARCÍA, Raúl, Enrique BRAVO, Sonia DIEZ-MUÑIZ, Cesar NOMBELA, Jose M. RODRÍGUEZ-PEÑA and Javier ARROYO. A novel connection between the Cell Wall Integrity and the PKA pathways regulates cell wall stress response in yeast. *Scientific Reports*. InTech, 2017, **7**(1), 550-557. DOI: 10.1038/s41598-017-06001-9.

[137] SINGH, Gunjan, Arshad JAWED, Debarati PAUL, Kalyan K. BANDYOPADHYAY, Abha KUMARI and Shafiul HAQUE. Concomitant Production of Lipids and Carotenoids in Rhodosporidium toruloides under Osmotic Stress Using Response Surface Methodology. *Frontiers in Microbiology*. InTech, 2016, **7**(1), 550-557. DOI: 10.3389/fmicb.2016.01686.

[138] KOT, Anna M., Stanisław BŁAŻEJAK, Marek KIELISZEK, Iwona GIENTKA, Joanna BRYŚ, Lidia RECZEK and Katarzyna POBIEGA. Effect of exogenous stress factors on the biosynthesis of carotenoids and lipids by Rhodotorula yeast strains in media containing agro-industrial waste. *World Journal of Microbiology and Biotechnology*. InTech, 2019, **35**(10), 550-557. DOI: 10.1007/s11274-019-2732-8.

[139] VARELAS, Vassileios, Evangelia SOTIROPOULOU, Xara KARAMBINI, Maria LIOUNI and Elias NERANTZIS. Impact of Glucose Concentration and NaCl Osmotic Stress on Yeast Cell Wall β-d-Glucan Formation during Anaerobic Fermentation Process. *Fermentation*. InTech, 2017, **3**(3), 550-557. DOI: 10.3390/fermentation3030044.

[140] SINGH, Gunjan, Arshad JAWED, Debarati PAUL, Kalyan K. BANDYOPADHYAY, Abha KUMARI and Shafiul HAQUE. Concomitant Production of Lipids and Carotenoids in Rhodosporidium toruloides under Osmotic Stress Using Response Surface Methodology. *Frontiers in Microbiology*. InTech, 2016, **7**(3), 550-557. DOI: 10.3389/fmicb.2016.01686.

[141] STACK, Helena M., Niamh KEARNEY, Catherine STANTON, Gerald F. FITZGERALD a R. Paul ROSS. Association of Beta-Glucan Endogenous Production with Increased Stress Tolerance of Intestinal Lactobacilli. *Applied and Environmental Microbiology* [online].

2010, **76**(2), 500-507 [cit. 2020-07-16]. DOI: 10.1128/AEM.01524-09. ISSN 0099-2240. Dostupné z: https://AEM.asm.org/content/76/2/500

[142] ZHU, Fengmei, Bin DU a Baojun XU. A critical review on production and industrial applications of beta-glucans. *Food Hydrocolloids* [online]. 2016, **52**, 275-288 [cit. 2020-07-16]. DOI: 10.1016/j.foodhyd.2015.07.003. ISSN 0268005X. Dostupné z: https://linkinghub.elsevier.com/retrieve/pii/S0268005X15300126

[143] GIENTKA, Iwona, Stanisław BŁAŻEJAK, Lidia STASIAK-RÓŻAŃSKA a Anna CHLEBOWSKA-ŚMIGIEL. Exopolysaccharides from yeast: insight into optimal conditions for biosynthesis, chemical composition and functional properties – review. *Acta Scientiarum Polonorum Technologia Alimentaria* [online]. 2015, **14**(4), 283-292 [cit. 2020-07-16]. DOI: 10.17306/J.AFS.2015.4.29. ISSN 16440730. Dostupné z: http://www.food.actapol.net/volume14/issue4/abstract-1.html

[144] JIRU, Tamene Milkessa, Marizeth GROENEWALD, Carolina POHL, Laurinda STEYN, Nicholas KIGGUNDU a Dawit ABATE. Optimization of cultivation conditions for biotechnological production of lipid by Rhodotorula kratochvilovae (syn, Rhodosporidium kratochvilovae) SY89 for biodiesel preparation. *3 Biotech* [online]. 2017, **7**(2) [cit. 2020-05-18]. DOI: 10.1007/s13205-017-0769-7. ISSN 2190-572X. Dostupné z: http://link.springer.com/10.1007/s13205-017-0769-7

[145] ROUBALOVÁ, M. Studium metabolismu karotenogenních kvasinek na molekulární úrovni. Brno: Vysoké učení technické v Brně, Fakulta chemická, 2017. XY s. Vedoucí diplomové práce prof. RNDr. Ivana Márová, CSc.

[146] KOT, Anna M., Stanisław BŁAŻEJAK, Marek KIELISZEK, Iwona GIENTKA a Joanna BRYŚ. Simultaneous Production of Lipids and Carotenoids by the Red Yeast Rhodotorula from Waste Glycerol Fraction and Potato Wastewater. *Applied Biochemistry and Biotechnology* [online]. 2019, **189**(2), 589-607 [cit. 2020-07-16]. DOI: 10.1007/s12010-019-03023-z. ISSN 0273-2289. Dostupné z: http://link.springer.com/10.1007/s12010-019-03023-z

[147] Kim, Y.H.; Kang, S.W.; Lee, J.H.; Chang, H.I.; Yun, C.W.; Paik, H.D.; Kang, C.W.; Kim, S.W. High cell density fermentation of Saccharomyces cerevisiae JUL3 in fed-batch culture for the production of β-Glucan. *J Ind Eng Chem*. **2007**. *13*(1), 153-158.

[148] FILLAUDEAU, Luc, Pascal BLANPAIN-AVET a Georges DAUFIN. Water, wastewater and waste management in brewing industries. *Journal of Cleaner Production* [online]. 2006, **14**(5), 463-471 [cit. 2020-07-16]. DOI: 10.1016/j.jclepro.2005.01.002. ISSN 09596526. Dostupné z: https://linkinghub.elsevier.com/retrieve/pii/S0959652605000041

[149] BALTI, Rafik, Romain LE BALC'H, Nicolas BRODU, Marthe GILBERT, Benjamin LE GOUIC, Sophie LE GALL, Corinne SINQUIN a Anthony MASSÉ. Concentration and purification of Porphyridium cruentum exopolysaccharides by membrane filtration at various cross-flow velocities. *Process Biochemistry* [online]. 2018, **74**, 175-184 [cit. 2020-

07-16]. DOI: 10.1016/j.procbio.2018.06.021. ISSN 13595113. Dostupné z: https://linkinghub.elsevier.com/retrieve/pii/S1359511318304847

[150] GLAZER, Alexander N. Phycobiliproteins — a family of valuable, widely used fluorophores. *Journal of Applied Phycology* [online]. 1994, **6**(2), 105-112 [cit. 2020-07-16]. DOI: 10.1007/BF02186064. ISSN 0921-8971. Dostupné z: http://link.springer.com/10.1007/BF02186064

[151] PUZORJOV, Anton, Alistair J MCCORMICK a Rodrigo GUTIÉRREZ. Phycobiliproteins from extreme environments and their potential applications. *Journal of Experimental Botany* [online]. 2020, **71**(13), 3827-3842 [cit. 2020-07-16]. DOI: 10.1093/jxb/eraa139. ISSN 0022-0957. Dostupné z: https://academic.oup.com/jxb/article/71/13/3827/5809840

[152] NAUTIYAL, P., K.A. SUBRAMANIAN a M.G. DASTIDAR. Recent Advancements in the Production of Biodiesel from Algae: A Review. *Reference Module in Earth Systems and Environmental Sciences* [online]. Elsevier, 2014, 2014 [cit. 2020-07-16]. DOI: 10.1016/B978-0-12-409548-9.09380-5. ISBN 9780124095489.

[153] MEIRELES, M.A.A. Supercritical CO 2 extraction of bioactive components from algae. *Functional Ingredients from Algae for Foods and Nutraceuticals* [online]. Elsevier, 2013, 2013, s. 561-584. DOI: 10.1533/9780857098689.3.561. ISBN 9780857095121.

[154] WANG, Juan, Bilian CHEN, Xiaozhen RAO, Jian HUANG a Min LI. Optimization of culturing conditions of Porphyridium cruentum using uniform design. *World Journal of Microbiology and Biotechnology* [online]. 2007, **23**(10), 1345-1350. DOI: 10.1007/s11274-007-9369-8. ISSN 0959-3993.

[155] JONES, Raymond F., Henry L. SPEER a Wendell KURY. Studies on the Growth of the Red Alga Porphyridium cruentum. *Physiologia Plantarum* [online]. 1963, **16**(3), 636-643 [cit. 2020-07-16]. DOI: 10.1111/j.1399-3054.1963.tb08342.x. ISSN 0031-9317.

[156] LI, Tao, Jin XU, Houbo WU, Peiliang JIANG, Zishuo CHEN a Wenzhou XIANG. Growth and Biochemical Composition of Porphyridium purpureum SCS-02 under Different Nitrogen Concentrations. *Marine Drugs* [online]. 2019, **17**(2) [cit. 2020-07-16]. DOI: 10.3390/md17020124. ISSN 1660-3397.

[157] LI, Shaohua, Liang JI, Qianwen SHI, Haizhen WU a Jianhua FAN. Advances in the production of bioactive substances from marine unicellular microalgae Porphyridium spp. *Bioresource Technology* [online]. 2019, **292**. DOI: 10.1016/j.biortech.2019.122048. ISSN 09608524.

[158] WANG, Yanming, Tuulikki SEPPÄNEN-LAAKSO, Heiko RISCHER, Marilyn G. WIEBE a Adrianna IANORA. Euglena gracilis growth and cell composition under different temperature, light and trophic conditions. *PLOS ONE* [online]. 2018, **13**(4). DOI: 10.1371/journal.pone.0195329. ISSN 1932-6203.

[159] SUZUKI, Kengo. Large-Scale Cultivation of Euglena. SCHWARTZBACH, Steven D. a Shigeru SHIGEOKA, ed. *Euglena: Biochemistry, Cell and Molecular Biology* [online].

Cham: Springer International Publishing, 2017, 2017-04-21, s. 285-293. Advances in Experimental Medicine and Biology. DOI: 10.1007/978-3-319-54910-1_14. ISBN 978-3-319-54908-8.

[160] Euglena sets sights as an Olympic hopeful for algae-based aviation jet fuel. *Biofuels Digest* [online]. 2019 [cit. 2020-07-16]. Dostupné z: https://www.biofuelsdigest.com/bdigest/2019/08/03/euglena-sets-sights-as-an-olympic-hopeful-for-algae-based-aviation-jet-fuel/

[161] SARI, Miriam, Alexander PRANGE, Jan I. LELLEY a Reinhard HAMBITZER. Screening of beta-glucan contents in commercially cultivated and wild growing mushrooms. *Food Chemistry* [online]. 2017, **216**, 45-5. DOI: 10.1016/j.foodchem.2016.08.010. ISSN 03088146.

8 LIST OF ABBREVIATIONS

ATP	adenosine monophosphate
BRMs	biological response modifiers
C/N ratio	carbon:nitrogen ratio
DCW	dry cell weight
ECM	extracellular matrix
EMP	Embden–Meyerhof–Parnas
EPS	exopolysaccharides
PAR	photosynthetically active radiation
GRAS	Generally Recognized as Safe
TAG	triacylglycerol
UDP	uridine diphosphate
GTP	guanosine triphosphate
PAMPs	Pathogen Associated Molecular Patterns
ROS	reactive oxygen species
FTIR	Fourier-transform infrared spectroscopy
NMR	nuclear magnetic resonance
HPLC	high-performance liquid chromatography
GC	gas chromatography
FAME	fatty acid methyl esther
PCA	principal component analysis
MTP	Microtiter Plate System

9 SUPPLEMENTARY MATERIALS

Fig. S1: Chromatogram of carotenoids separation – *R. kratochvilovae* CCY 20-2-26

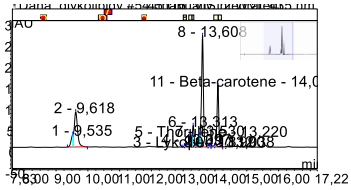


Fig. S2: Growth curves of *S. cerevisiae* CCY 21-4-102 at different temperature and media composition

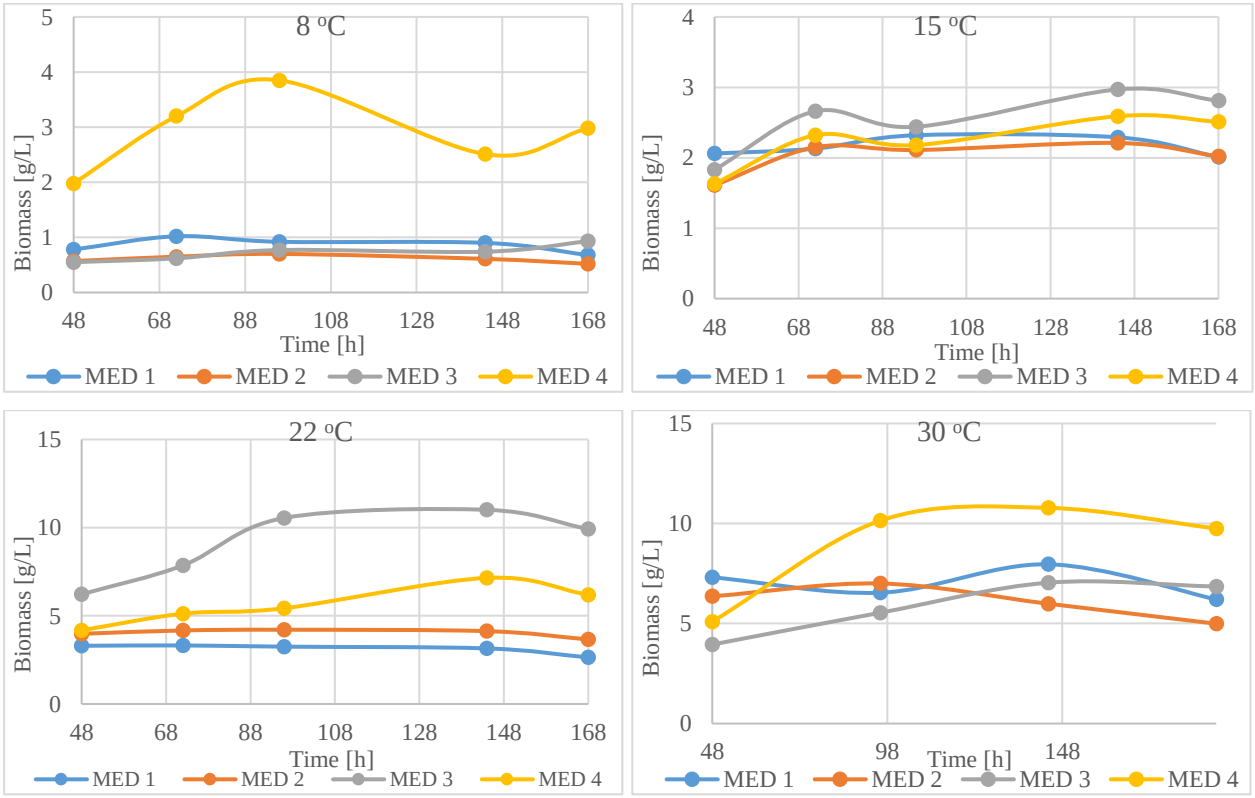


Fig. S3: Growth curves of *C. infirmominiatum* CCY 17-18-4 at different temperature and media composition

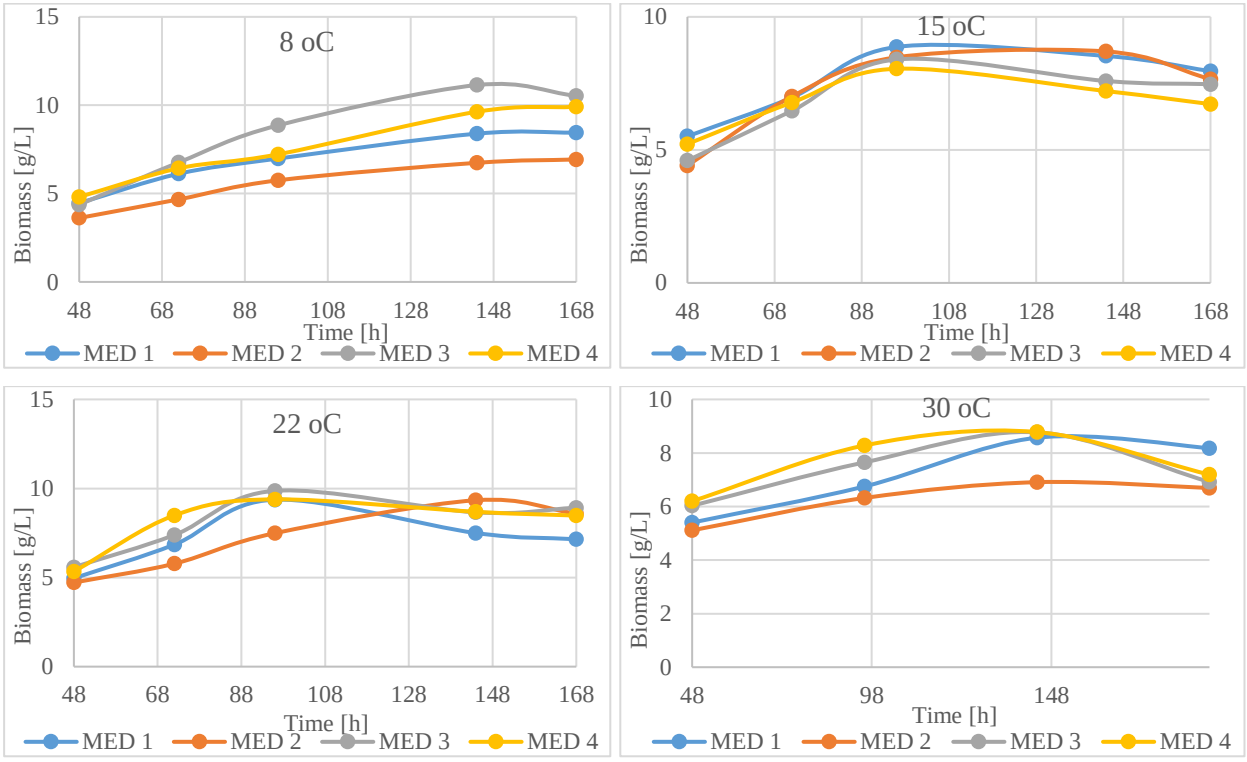


Fig. S4: Growth curves of *P. rhodozyma* CCY 77-1-1 at different temperature and media composition

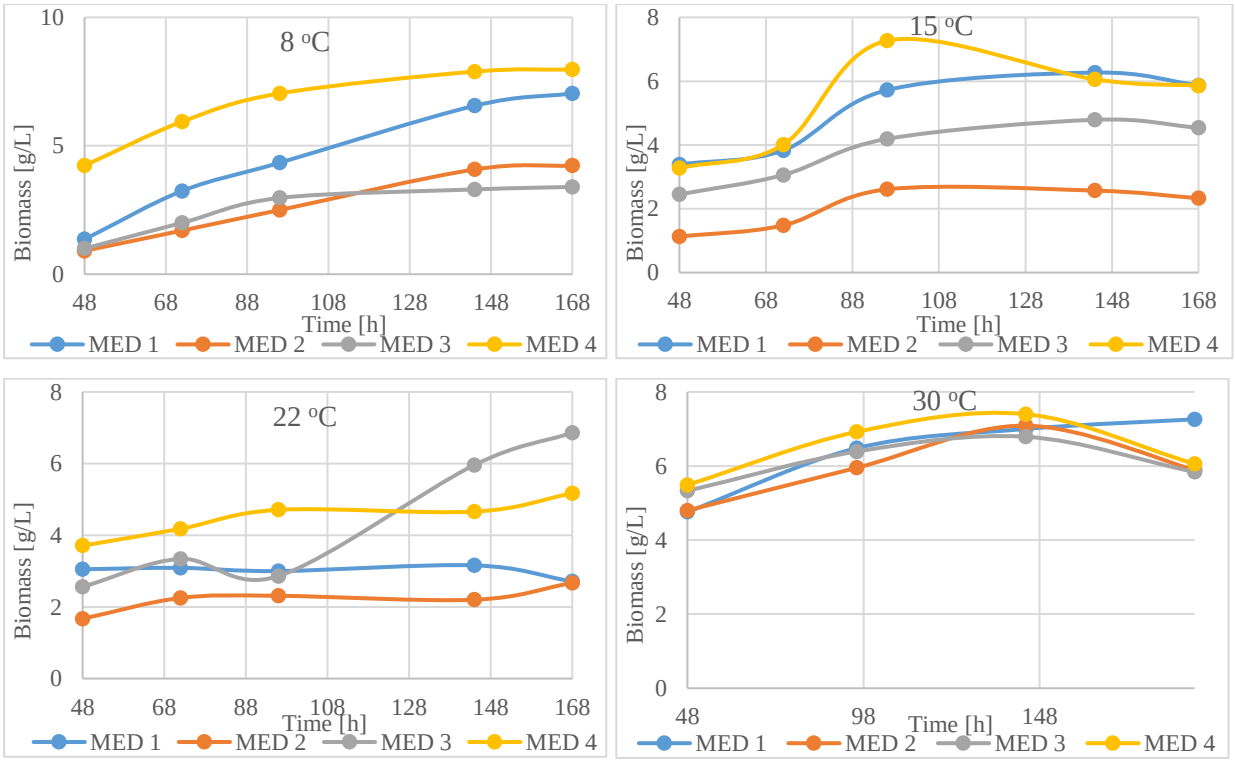


Fig. S5: Growth curves of *R. kratochvilovae* CCY 20-2-26 at different temperature and media composition

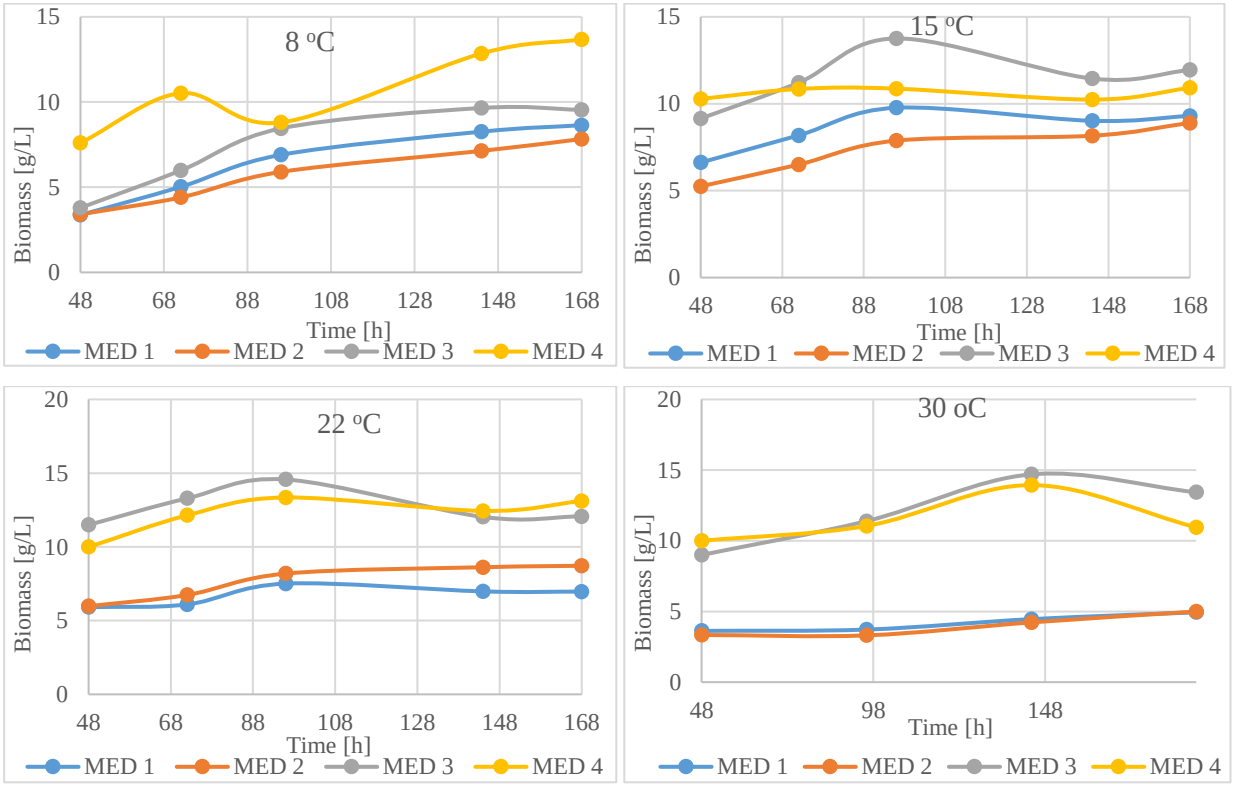


Fig. S6: Optimization of phycobilin extraction from *P. cruentum* biomass

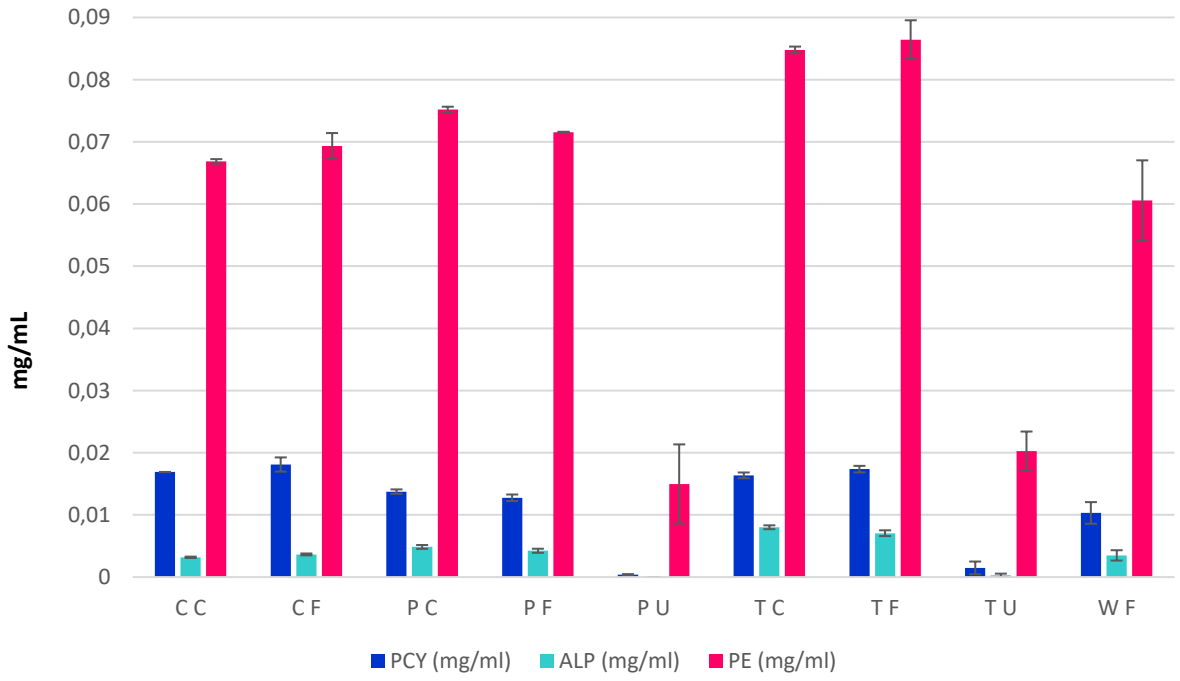


Fig. S7: Optimization of phycobilin extraction from *A. maxima* biomass

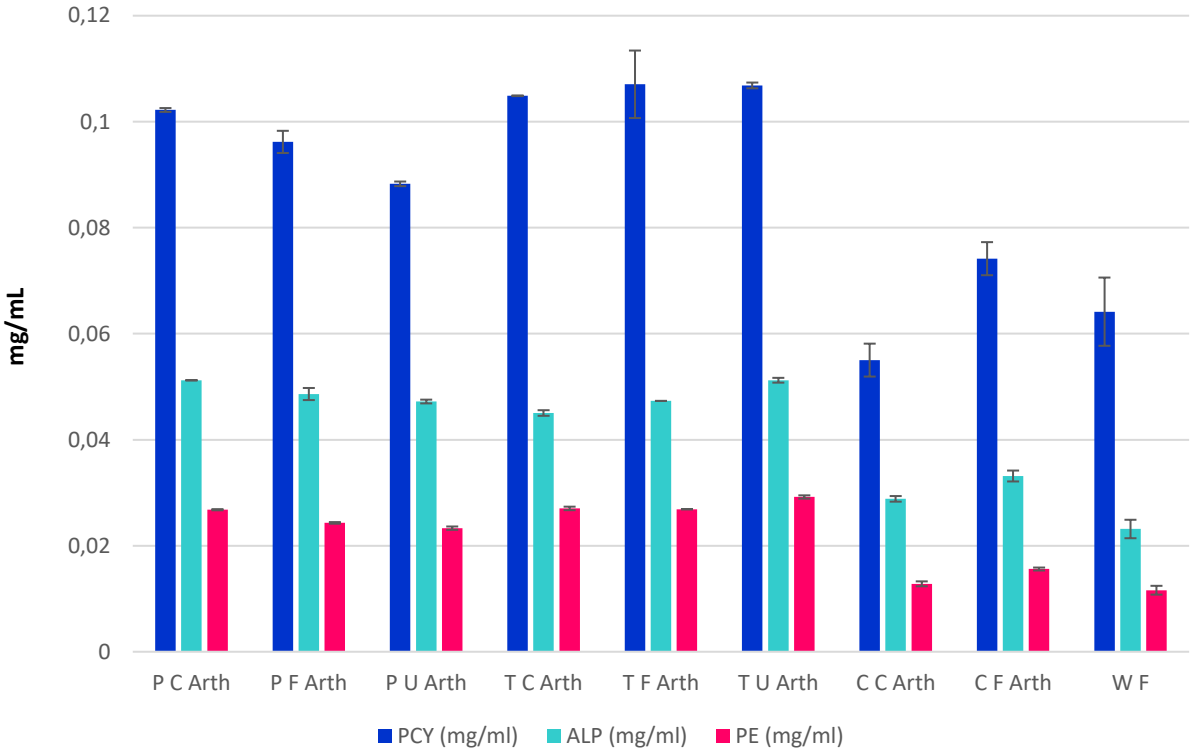


Fig. S8: Separation of FAMEs by gas chromatography – *R. kratochvilovae* CCY 20-2-26

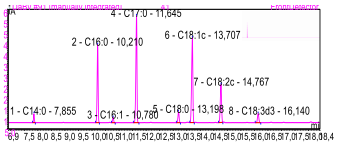


Fig. S9: Total carotenoids content at *R. kratochvilovae* CCY 20-2-26 (yeast extract – YE, ammonium sulphate – SA, ammonium chloride – NH_4Cl, potassium nitrate – KNO3 a urea – Urea)

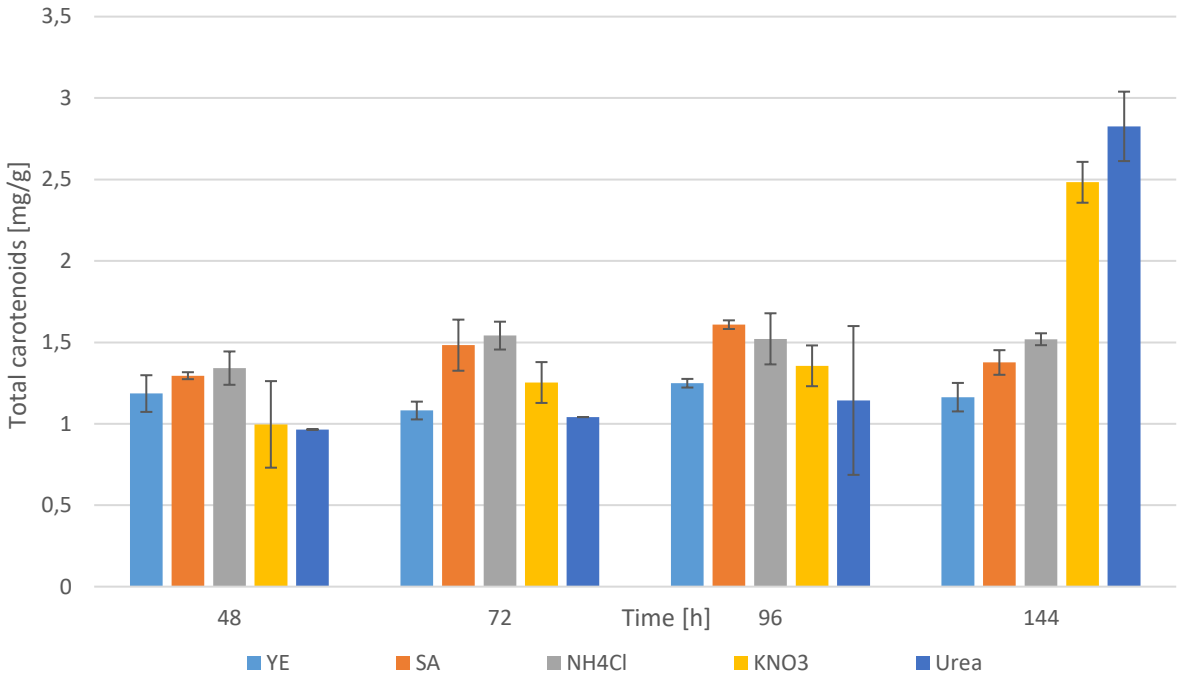


Fig. S10: Total carotenoids content at *R. kratochvilovae* CCY 20-2-26 at diverse carbon sources and urea

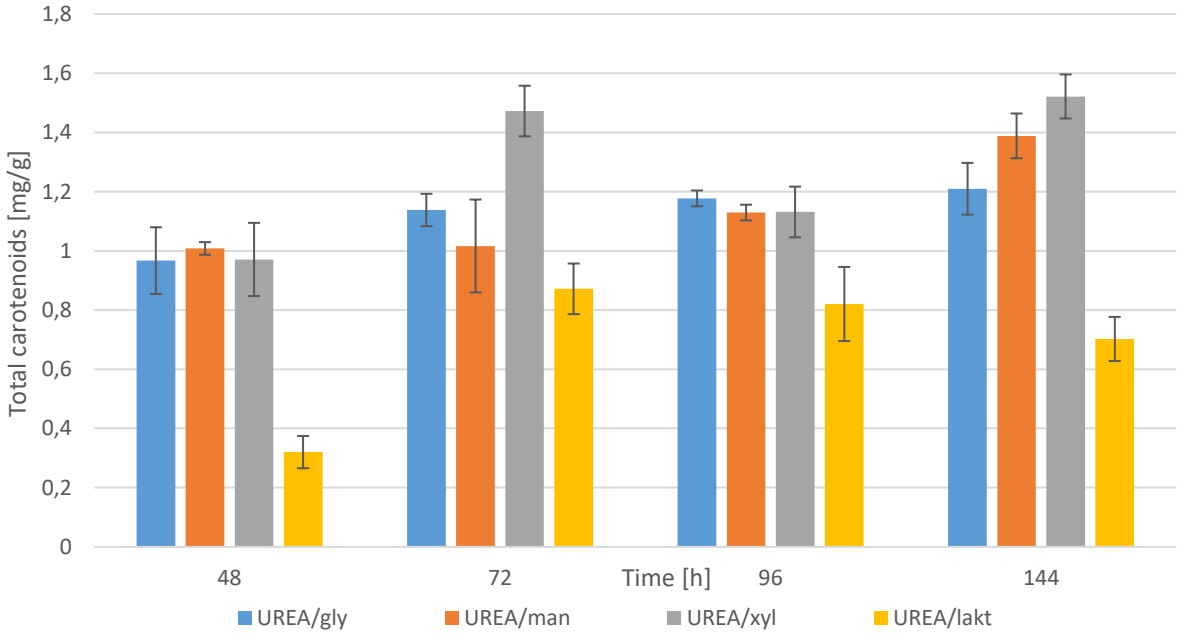


Fig. S11: Total carotenoids content at *R. kratochvilovae* CCY 20-2-26 at diverse carbon sources and potassium nitrate

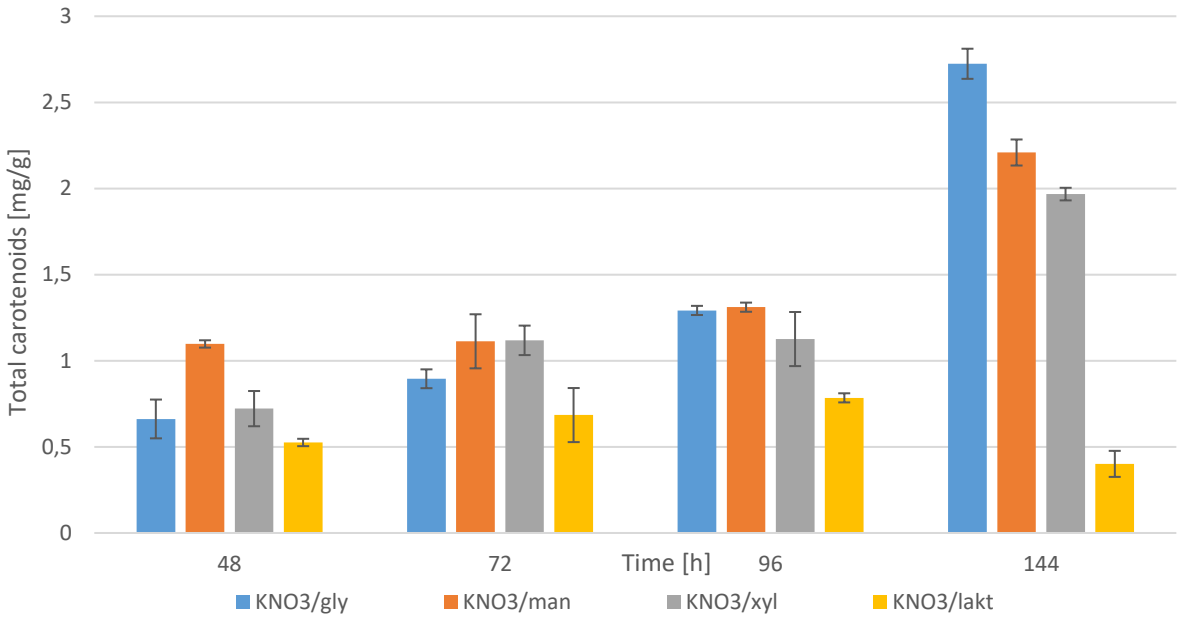


Fig. S12: Total carotenoids content at *R. kratochvilovae* CCY 20-2-26 at diverse C/N ratios

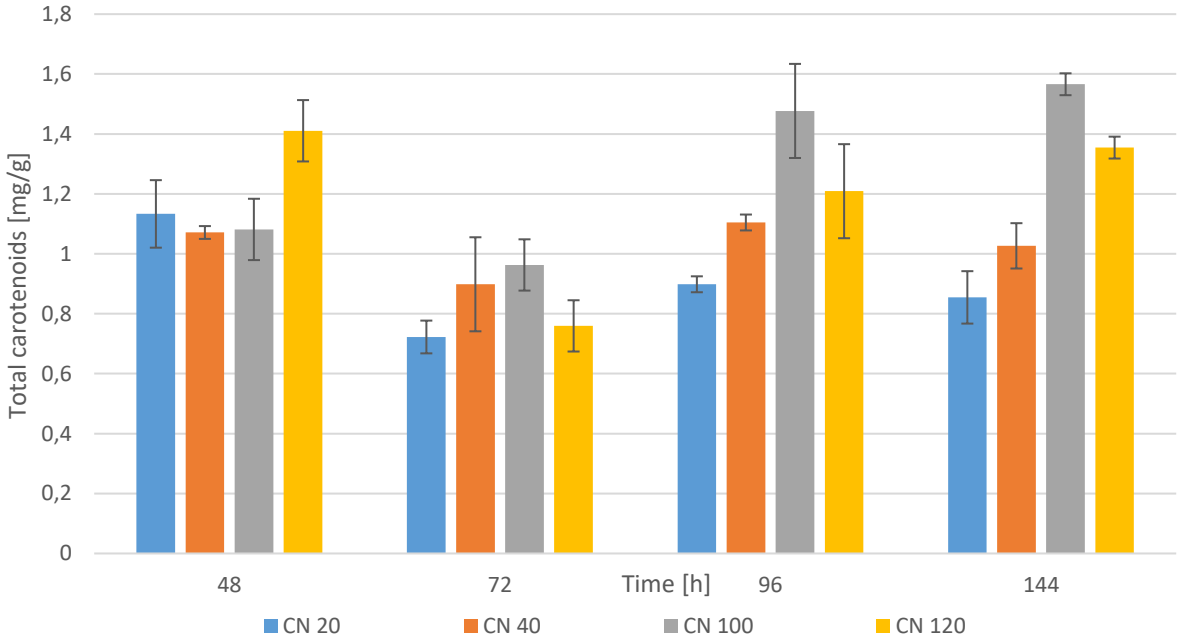
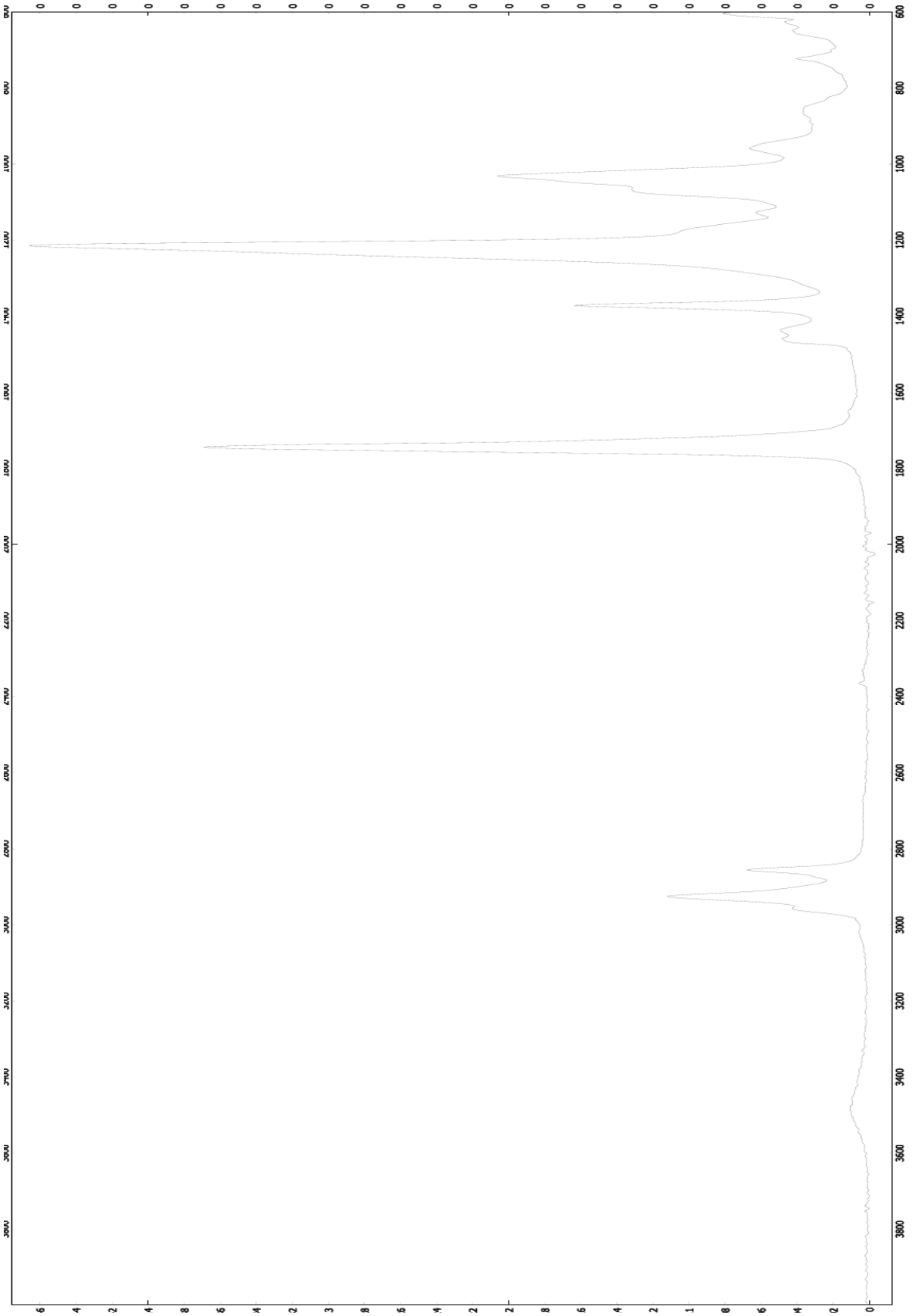


Fig. S13: FTIR spectrum of exoglycolipid from *R. kratochvilovae* CCY 20-2-26



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2007 – 2011	Gymnázium s polským jazykem vyučovacím v Českém Těšíně
2011 – 2014	Vysoké učení technické v Brně, Fakulta chemická Bakalářské studium zakončené státní zkouškou, titul Bc. Téma práce: Příprava a stabilita organických mikro- a nanočástic typu core-shell.
2014 – 2016	Vysoké učení technické v Brně, Fakulta chemická Magisterské studium zakončené státní zkouškou, titul Ing. Téma práce: Optimalizace kultivace řasových kultur ve fotobioreaktorech.
2016 – nyní	Vysoké učení technické v Brně, Fakulta chemická Doktorské studium

Stáže

Místo:	Contipro, Dolní Dobrouč, Česká republika
Doba trvání:	Srpen, 2013
Řešená problematika:	Optimalizace syntézy upconverzních nanočástic typu core-shell
Místo:	Photon System Instruments, Drásov, Česká republika
Doba trvání:	Akademický rok 2015/2016
Řešená problematika:	Experimentální část diplomové práce, optimalizace kultivace kmenů řasy *Haematococcus pluvialis* ve fotobioreaktorech
Místo:	University of Life Sciences, Ås, Norsko
Doba pobytu:	1.10.2017 – 16.12.2017
Řešená problematika:	Možnosti produkce beta-glukanů kvasinkami a jejich analýza pomocí FTIR-HTS spektroskopie
Místo:	University of Huelva, Huelva, Španělsko
Doba pobytu:	30.05.2018 – 30.07.2018
Řešená problematika:	Možnosti analýzy chlorofylu, karotenoidů a fykobiliproteinů extremofilní sinici *Croocchosidiopsis sp.*
Místo:	University of Life Sciences, Ås, Norsko
Doba pobytu:	12.08.2018 – 4.11.2018
Řešená problematika:	Vliv stresových podmínek na produkci beta-glukanů

Místo:	University of Life Sciences, Ås, Norsko
Doba pobytu:	1.6.2019 – 1.8.2019
Řešená problematika:	Vliv stresových podmínek na produkci beta-glukanů; možnost produkce extracelulárních polymerních substancí kvasinkami

PŘEHLED PUBLIKAČNÍ ČINNOSTI

Publikace v časopisech:

BYRTUSOVÁ, D., SHAPAVAL V., HOLUB J., ŠIMANSKÝ S., RAPTA M., SZOTKOWSKI M., KOHLER A. a MÁROVÁ I.. Revealing the Potential of Lipid and β-Glucans Coproduction in Basidiomycetes Yeast. *Microorganisms*. 2020, **8**(7). DOI: 10.3390/microorganisms8071034. ISSN 2076-2607.

VANEK M., MRAVEC F., SZOTKOWSKI M., **BYRTUSOVA D.**, HARONIKOVA A., CERTIK M., SHAPAVAL V., MAROVA I: Fluorescence Lifetime Imaging of Red Yeast *Cystofilobasidium capitatum* During Growth. *The EuroBiotech Journal,* 2018, roč. 2, č. 2, s. 114-120. ISSN: 2564-615X.

MAROVA I., SZOTKOWSKI M.; VANEK M.; RAPTA M.; **BYRTUSOVA D.**; MIKHEICHYK N.; HARONIKOVA A. Utilization of animal fat waste as carbon source by carotenogenic yeasts – a screening study. *The EuroBiotech Journal,* 2017, roč. 1, č. 4, s. 310-318. ISSN: 2564-615X.

SZOTKOWSKI M.; **BYRTUSOVA D.**; HARONIKOVA A.; VYSOKA M.; RAPTA M.; SHAPAVAL V.; MAROVA I. Study of Metabolic Adaptation of Red Yeasts to Waste Animal Fat Substrate. *Microorganisms* 2019, *7*, 578.

Konferenční příspěvky s plným uvedením textu:

MÁROVÁ, I.; SZOTKOWSKI, M.; RAPTA, M.; BYRTUSOVÁ, D.; VANĚK, M.; HÁRONIKOVÁ, A.; MIKHEICHYK, N. *Simultaneous production of lipids and pigments by red yeasts grown on animal fat using the concept of biorefinery.* Madrid, Spain: Formatex Research Centre, 2017. s. 199-202.

MÁROVÁ, I.; RAPTA, M.; SZOTKOWSKI, M.; BYRTUSOVÁ, D. *Lipofungi - report.* NMBU As, Norsko: NMBU As, Norsko, 2017. s. 1-17.

Abstrakty:

Márová I., Szotkowski M., Vanek M., Byrtusova D., Rapta M., Haronikova A., Certik M., Shapaval V: Pigmented yeasts as biotechnological factories for bioproducts and biofuels. 45th Annual Conference on Yeast. May 15-18, 2018, Smolenice. Book of Abstracts, p. 33-33. ISSN: 1336-4839.

Byrtusová D., Szotkowski M., Holub J., Bradáčová K., Vysoká M., Rapta M., Márová I.: TRANSFORMATION OF WASTE FAT INTO HIGH VALUABLE METABOLITES BY RED YEASTS. 45th Annual Conference on Yeast. May 15-18, 2018, Smolenice. Selected to the session Poster highlights. Book of Abstracts, p. 42-42. ISSN: 1336-4839.

Rapta M., Shapaval V., Zimmermann B., Tafintseva V., Kohler A., Byrtusová D., Márová I.: VIBRATIONAL SPECTROSCOPY AS HIGH-THROUGHPUT TOOL FOR LIPID

ACCUMULATION IN RED YEASTS. 45th Annual Conference on Yeast. May 15-18, 2018, Smolenice. Book of Abstracts, p. 58-58. ISSN: 1336-4839.

RAPTA, M.; SHAPAVAL, V.; ZIMMERMANN, B.; TAFINTSEVA, V.; SZOTKOWSKI, M.; BYRTUSOVÁ, D.; MIKHEICHYK, N.; MÁROVÁ, I. *THE EFFECT OF NITROGEN SOURCE ON LIPID AND CAROTENOID ACCUMULATION IN RED YEAST*. 44th ANNUAL CONFERENCE ON YEAST. 44th Annual Conference on Yeasts, Book of abstracts. Bratislava: Chemický ústav SAV Bratislava, 2017. s. 62-62. ISSN: 1336-4839.

KOSTOVOVÁ, I.; ROUBALOVÁ, M.; POKRÝVKOVÁ, Z.; BYRTUSOVÁ, D.; SZOTKOWSKI, M.; HLAVÁČEK, V.; MÁROVÁ, I. *Improvement of lipid compound prooduction by red yeasts though random mutagenesis.* 44th Annual Conference on Yeast. Bratislava: Slovak Academy of Sciences, Bratislava, 2017. s. 58-58.

RAPTA, M.; SHAPAVAL, V.; KOHLER, A.; TAFINTSEVA, V.; SZOTKOWSKI, M.; BYRTUSOVÁ, D.; MIKHEICHYK, N.; MÁROVÁ, I. *OPTIMIZATION OF SIMULTANUOUS CAROTENOID AND LIPID PRODUCTION IN RED YEAST.* BOOK OF ABSTRACTS. Mikulov: Czech Society of Industrial Chemistry, 2017.

HÁRONIKOVÁ, A.; BYRTUSOVÁ, D.; SZOTKOWSKI, M.; RAPTA, M.; MIKHEICHYK, N.; MÁROVÁ, I. *POTENTIAL USE OF CAROTENOGENIC YEASTS AND MICROALGAE FOR PRODUCTION OF HIGH VALUE PRODUCTS.* BOOK OF ABSTRACTS 8th International Symposium on RECENT ADVANCES IN FOOD ANALYSIS. 1. Prague: University of Chemistry and Technology, Prague, 2017. s. 339-339. ISBN: 978-80-7080-999-0.