RIGA TECHNICAL UNIVERSITY

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SINGLE-CELL PROTEIN AND SINGLE-CELL OIL PRODUCTION FROM AGRO-INDUSTRIAL BY-PRODUCTS

Summary of the Doctoral Thesis

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I hereby declare that the Doctoral Thesis submitted for the review to Riga Technical University for the promotion to the scientific degree of Doctor of Science (Ph. D.) is my own. I confirm that this Doctoral Thesis had not been submitted to any other university for the promotion to a scientific degree.

Kriss Spalvins ……………………………. (signature)

Date: ………………………

The Doctoral Thesis has been written in English. It consists of an Introduction; 4 chapters; Conclusion; 12 figures; 13 tables; one appendix; the total number of pages is 222. The Bibliography contains 420 titles.

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INTRODUCTION

Fish, crustaceans, and aquatic plants are a good source of proteins, unsaturated fatty acids, minerals and vitamins and inclusion of these products in the daily diet is advisable as a health benefit. Wild capture fisheries have always been the main source of these products. However, this sector could not increase global landing volumes since the early 90s, while the world's population has risen by more than two billion over the same period. In order to compensate and meet the ever-increasing demand for seafood, the production volumes were rapidly boosted by the aquaculture industry, and as the world's fastest growing food industry, by 2014 its production volume for the first time in terms of output surpassed the wild capture, and currently, most of the consumed fish and crustaceans come from aquaculture farms, not from the world's seas and oceans. Aquaculture not only supplies the world with predictable and regular production volumes, but also promotes reduction of wild capture intensity and the associated negative impacts on the exploited biotopes. Despite the fact that aquaculture helps to meet the demand for seafood, it is still partly dependent on wild capture, since a large proportion of aquaculture fish is fed with raw materials (fish meal and fish oil) that come directly from wild capture. Thus, a situation has developed, where wild fish are actively caught in order to enable farming of fish in captivity in sufficient quantities. Due to the rapid development of aquaculture, today about 70 % of globally produced fish meal and fish oil are used in aquaculture as a feed, but the production of fish meal and fish oil cannot be increased due to the stagnation of wild capture, which in general threatens the further development of the aquaculture industry.

In order to ensure that the aquaculture industry continues to develop, it is necessary to replace the existing fish meal and fish oil with new protein and oil rich sources, which could meet the demand for qualitative fish feeds in the future. Fish meal and fish oil can be replaced with the following alternatives: (1) by-products of wild capture fish processing (heads, scales, fins, skin, viscera); (2) various protein-rich and oleaginous plants (canola, sunflower, soy, etc.); (3) genetically modified (GM) plants; (4) by using plant derived sugars as ingredients for cultivation of SCP and SCO producing microorganisms; (5) by using biodegradable by-products, alien plant species or other agro-industrial residues (hereafter called "by-products") as ingredients for cultivation of SCP and SCO producing microorganisms. All of these alternatives have their pros and cons, but SCP and SCO produced from by-products are considered to have the highest prospect of becoming the most competitive, while also being the least impactful on the environment. Currently SCP and SCO derived from by-products are not widely used in fish feeds, as despite the large number of studies carried out so far, no sufficiently competitive solutions have been introduced in the market. Therefore, additional research is needed on the by-products suitable for the production of SCP and SCO, on the procurement of by-products, and on various combinations of by-products and microorganisms.

Topicality

The aquaculture industry is the world's largest supplier of seafood, which continues to grow rapidly and increase in production volumes. However, the fish feed currently used in aquaculture is produced from ingredients that are not available in sufficient quantities, compete directly over agricultural areas, threaten species diversity, contribute to climate change, cause health problems for farmed fish, and reduce the nutritional value of farmed fish products. Consequently, solutions are needed to replace the feed used so far with more sustainable solutions. The problem is topical, because the study analyses and further develops better technological solutions, which are single-cell proteins and single-cell oils produced from biodegradable by-product.

Although SCP and SCO technology is theoretically superior to currently applied solutions, it is still not widely used in fish feed production due to a lack of research on which by-products are most suitable for SCP and SCO production, lack of research on the availability of various by-products and optimal combinations of by-products and SCP and SCO producing microorganisms. As this dissertation analyses various by-products and reports on utilization of new by-products and microorganisms not previously used in SCP and SCO production, the results of this work further develop SCP and SCO production technologies, which directly justify the relevance of the work in the overall context of the problem.

The aim and tasks of the dissertation

The aim of the work is to analyse the most suitable by-products for the cultivation of SCP and SCO-producing microorganisms, to search for new combinations of by-products and SCP or SCO-producing microorganisms, as well as to draw conclusions about the most promising by-products and provide recommendations.

In order to achieve the goal, the following tasks were set:

1. Carry out a literature analysis of all suitable by-products:
   a) describe the properties, availability, reported SCP and SCO yields of each by-product and categorize all by-products;
   b) based on the analysis performed, identify the potentially most suitable by-products from each category.
2. Develop a by-product supply optimization model:
   a) develop an easy-to-use model that does not require the user to have prior knowledge in working with dedicated optimization software or experience working with similar models;
   b) carry out a case study for one by-product using the developed model.
3. Create a laboratory stand for practical experiments:
   a) attract financing for the purchase of inventory;
   b) organize inventory purchases;
   c) outfit the laboratory and validate the functionality of the purchased inventory to achieve the relevant research goals.
4. Perform practical experiments in the laboratory to obtain SCP from a previously unexplored by-product and microorganism strain combination:
   a) based on the analysis performed in Task 1, select by-products and perform practical experiments with SCP-producing microorganisms and determine the growth rate, biomass amount, protein concentration in biomass and accumulation of harmful compounds.

Scientific novelties of the dissertation

During the development of the dissertation, several scientific novelties have been created:

1. For the first time in the scientific literature, all agro-industrial by-products and residues suitable for the cultivation of microorganisms are collected, categorized, and described in one place. The necessity of such a summary is evidenced by the citation of the author's published review articles on this topic:
    a) publication "Single cell protein production from waste biomass: review of various agricultural by-products. Agronomy Research 2018; 16(S2):1493-1508" by Spalvins, K., Ivanovs, K., Blumberga, D. has received 15 citations (95rd percentile, self-citations excluded), and field-weighted citation impact (FWCI) – 3.77.
    b) publication "Single cell protein production from waste biomass: comparison of various industrial by-products. Energy Procedia 2018; 147:409-418" by Spalvins, K., Zihare, L., Blumberga, D. has received 5 citations (96th percentile, self-citations excluded), and FWCI – 4.86.
    c) publication "Single cell oil production from waste biomass: Review of applicable agricultural by-products. Agronomy Research 2019; 17(3):833-849" by Spalvins, K., Blumberga, D. has received 3 citations (82th percentile, self-citations excluded), and FWCI – 1.67.
2. A patent for an innovative single-cell oil extraction technology has been submitted to and accepted by the Patent Office of the Republic of Latvia.
3. Highest reported *Yarrowia lipolytica* biomass concentrations were achieved when SCP production was done with waste cooking oil used as the main carbon source. The batch experiments showed very high biomass concentrations of yeast *Yarrowia lipolytica* in the medium (57.37 g/L).
4. The resulting *Yarrowia lipolytica* biomass accumulated low concentrations of toxic malondialdehyde (MDA) (2.32 mg MDA/kg) when compared to concentrations initially detected in the WCO itself (30.87 mg MDA/kg). Attempts of decreasing MDA via microbial fermentations have not been previously reported.

The proposed hypothesis

1. Single-cell proteins and single-cell oils derived from agro-industrial by-products are more suitable for fish feed production than currently used raw materials.
2. The highest yields of single-cell proteins and single-cell oils can be obtained when monosaccharide and disaccharide rich sources are used as the main carbon source.
3. *Yarrowia lipolytica* biomass cultivated from waste cooking oil as the main carbon source will accumulate low enough lipid peroxidation products to be considered safe (<2.00 mg MDA/kg).
4. *Yarrowia lipolytica* is an effective microorganism (at least 6 g/L pure protein or 30 g/L biomass with the protein concentration of at least 20 %) in the utilization of waste cooking oil to produce SCP.

Practical value

The reviews prepared within the dissertation and the performed experiments with the acquisition of SCP and SCO using by-products as substrates are directly related to the researched problem – the lack of adequate fish feed raw materials in the aquaculture industry. Both the characterization of the by-products and the experiments performed on SCP and SCO-producing microorganisms using by-products are further developing the SCP and SCO production technologies to ensure that in the future the use of SCP and SCO in fish feeds becomes mainstream.

During the development of the dissertation, the Biosystems Laboratory of Institute of Energy Systems and Environment (IESE) of Riga Technical University was outfitted with new equipment, which allowed to perform experiments in microbiology, molecular biology and chemistry and will also enable students and IESE researchers to do so in the future as well.

The analysis of by-products was concluded with the development of a resource supply optimization model and a case study for one by-product. The validated model was designed to be as simple as possible for users with no prior knowledge of dedicated software or no experience with optimization models. Thus, this model has a direct practical application in the procurement of various resources and in the search for the most suitable by-products.

Scientific approbation of the dissertation

The work is based on the following scientific publications:

1. Spalvins, K., Blumberga, D. Production of fish feed and fish oil from waste biomass using microorganisms: overview of methods analyzing resource availability. Environmental and Climate Technologies 2018:22:149–154.
2. Spalvins, K., Ivanovs, K., Blumberga, D. Single cell protein production from waste biomass: review of various agricultural by-products. Agronomy Research 2018:16(S2):1493–1508.
3. Spalvins, K., Zihare, L., Blumberga, D. Single cell protein production from waste biomass: comparison of various industrial by-products. Energy Procedia 2018:147:409–418.
4. Spalvins, K., Blumberga, D. Single cell oil production from waste biomass: review of applicable agricultural by-products. Agronomy Research 2019:17(3):833–849.
5. Spalvins, K., Vamza, I., Blumberga, D. Single cell oil production from waste biomass: review of applicable industrial by-products. Environmental and Climate Technologies 2019:23(2):325–337
6. Spalvins, K., Blumberga, D. A simple tool for resource availability optimization: A case study of dairy whey supply for single cell protein and oil production in Latvia. Agronomy Research 2020 (accepted).
7. Spalvins, K., Geiba, Z., Blumberga, D. Waste cooking oil as substrate for single cell protein production by yeast *Yarrowia lipolytica*. Environmental and Climate Technologies 2020 (accepted).

Other scientific publication related to the topic, but not included in the dissertation:

8. Zihare, L., Spalvins, K., Blumberga, D. Multi criteria analysis for products derived from agro industrial by-products. Energy Procedia 2018:147:452–457.
9. Racko, E., Blumberga, D., Spalvins, K., Marciulaitiene, E. Ranking of by-products for single cell oil production. Case of Latvia. 2020 (submitted).

Other scientific publications:

10. Spalvins, K., Blumberga, D. Analysis of *Arabidopsis* defensin-like genes and ovule development. Agronomy Research 2017:15(5), 2144–2160.
11. Priedniece, V., Spalviņš, K., Ivanovs, K., Pubule, J., Blumberga, D. Bioproducts from Potatoes. A Review. Environmental and Climate Technologies 2017:21:18–27.
12. Romagnoli, F., Balina, K., Spalvins, K. Eutrophication Reduction, Using Latvian Lake Macroalgae For Biogas Production. From: 4th world Lettigallian congress "Latgales Simtgades kongress", Latvia, Rezekne, 5–6 of May, 2017. Rezekne: Rezekne Academy of Technologies 2017:67–68. ISSN 2500-9591.
13. Ivanovs, K., Spalvins, K., Blumberga, D. Approach for modelling anaerobic digestion processes of fish waste. Energy Procedia 2018; 147:390–396.
14. Zihare, L., Muizniece, I., Spalvins, K., Blumberga, D. Analytical framework for commercialization of the innovation: case of thermal packaging material. Energy Procedia 2018:147:374–381.
15. Blumberga, A., Fraimanis, R., Muizniece, I., Spalvins, K., Blumberga, D. Trilemma of historic buildings: smart district heating systems, bioeconomy and energy efficiency. Energy 2019: 186:115741.
16. Zihare, L., Gusca, J., Spalvins, K., Blumberga, D. Priorities Determination of Using Bioresources. Case Study of *Heracleum sosnowskyi*. Environmental and Climate Technologies 2019:23(1):242–256.

Participation in scientific conferences:

1. Spalvins, K., Blumberga, D. Analysis of *Arabidopsis* defensin-like genes and ovule development: The Conference of Environmental and Climate Technologies, CONECT 2016, October 12–14, 2016, Riga, Latvia.
2. Spalvins, K., Blumberga, D. Production of fish feed and fish oil from waste biomass using microorganisms: overview of methods analyzing resource availability: The Conference of Environmental and Climate Technologies, CONECT 2017, May 10–12, 2017, Riga, Latvia.
3. Spalvins, K., Ivanovs, K., Blumberga, D. Single cell protein production from waste biomass: review of various agricultural by-products: Conference of Biosystems Engineering, BSE 2018, May 10, 2018, Tartu, Estonia.
4. Spalvins, K., Zihare, L., Blumberga, D. Single cell protein production from waste biomass: comparison of various industrial by-products: The Conference of Environmental and Climate Technologies, CONECT 2018, May 16–18, 2018, Riga, Latvia.

Zihare, L., Spalvins, K., Blumberga, D. Multi criteria analysis for products derived from agro industrial by-products: The Conference of Environmental and Climate Technologies, CONECT 2018, May 16-18, 2018, Riga, Latvia.

5. Spalvins, K., Blumberga, D. Single cell oil production from waste biomass: review of applicable agricultural by-products: Conference of Biosystems Engineering, BSE 2019, May 9, 2019, Tartu, Estonia.
6. Spalvins, K., Vamza, I., Blumberga, D. Single cell oil production from waste biomass: review of applicable industrial by-products: The Conference of Environmental and Climate Technologies, CONECT 2019, May 15–17, 2019, Riga, Latvia.
7. Spalvins, K., Blumberga, D. A simple tool for resource availability optimization: A case study of dairy whey supply for single cell protein and oil production in Latvia. Conference of Biosystems Engineering, BSE 2020, May 6, 2020, Tartu, Estonia.
8. Spalvins, K., Geiba, Z., Blumberga, D. Waste cooking oil as substrate for single cell protein production by yeast *Yarrowia lipolytica*: The Conference of Environmental and Climate Technologies, CONECT 2020, May 13–15, 2020, Riga, Latvia.

Scientific projects:

1. Project for the commercialization of research results "Supercritical Omega-3 Oil from Production By-Products" co-funded by ERDF and supervised by LIAA. Project implementation period: 29 January 2018 – 28 April 2021.

Patents:

1. Kriss Spalvins, Dagnija Blumberga. Method for producing single cell oil from biodegradable by-products, P-18-63, 22.04.2020.

Supervised and co-supervised bachelor and master's thesis:

1. Elīna Račko. Single cell oil yields in *Yarrowia lipolytica* and *Umbelopsis isabellina* microbiological cultures when using biodegradable by-products. Bachelor thesis. RTU, 2018 (In Latvian).
2. Diāna Veršiņina. Effect of various biodegradable by-products on single cell protein yields in microbiological cultures. Bachelor thesis. RTU, 2019 (In Latvian).
3. Laura Karīna Pizāne. Single cell oil production from production by-products. Bachelor thesis. RTU, 2019 (In Latvian).
4. Zane Kušnere. Single cell protein production from biodegradable waste products in *Cyberlindnera jadinii*, *Rhizopus oryzae*, *Paecilomyces marquandii* and *Paecilomyces carneus* microbiological cultures. Master's thesis. RTU, 2019.
5. Ilze Vamža. Omega-3 fatty-acids production from biodegradable by-products, by using single cell organisms. Master's thesis. RTU, 2019 (In Latvian).
6. Elīna Račko. Multicriteria analysis of Labyrinthulomycetes class microorganism strains and applicable agro-industrial by-products. Master's thesis. RTU, 2020.
7. Reičela Pētersone. Single cell protein production from fruit pulp and dairy residues by using *Candida utilis*. Bachelor thesis. RTU, 2020 (In Latvian).

1. SUMMARY OF SCP AND SCO ADVANTAGES OVER OTHER ALTERNATIVES

As mentioned previously, it is possible to produce proteins and oils, applicable as feeds for farmed fish, from the following sources:

1. From wild capture fisheries (low value fish for fish meal and oleaginous fish for fish oil and fish meal).
2. From by-products of wild capture fish processing (heads, scales, fins, skin, viscera).
3. From various protein-rich and oleaginous plants (soy, canola, sunflower etc.).
4. From genetically modified (GM) plants, selected for either increased protein or oil concentrations or improved amino acid or fatty acid profiles.
5. By using plant derived sugars as feed for cultivation of SCP and SCO producing microorganisms.

However, when compared to SCP and SCO production from by-products, these alternatives have multiple flaws that limit use of these sources.

1. Proteins and oils acquired from resources of wild capture fisheries cannot satisfy the growing aquaculture demand for fish meal and fish oil. Production volumes of wild capture fisheries have been stagnant for the last 20 years. Produced volumes are also affected by fishing quotas and inconsistent landings of low value fish and oleaginous fish.
2. Use of by-products of wild capture fish processing is hampered just like the use of low value and oleaginous fish, because both of these sources are depending on limited landings of wild capture fisheries. Additionally, use of by-products for the production of fish meal and fish oil is limited by quality of the used waste products and small amounts of oil that can be acquired from these by-products [1].
3. Using plant derived protein meals in diets of captive fish is considered adequate in regard to their feed conversion ratios. However, plant derived feeds are unsuitable for intestinal tract of predatory fish (salmonids, etc.), which is one of the main causes of poor health of aquaculture fish, fish are more likely to die and large amounts of antibiotics are needed to treat various diseases [2]. By replacing agriculture derived feeds with SCP, the health of the fish is considerably increased because these proteins are more easily digested and in its composition SCP is much more similar to feed these species of fish can acquire in wild (plankton: microalgae, bacteria, fungi, etc.) [3]. Fish produced with SCP also have a higher nutritional value and are therefore healthier for human consumption.
4. Using oils derived from plants in diets of captive fish is also considered adequate to feed conversion ratios. However, doing so reduces the concentration of long-chain omega-3 fatty acids, such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) in the tissue of the fish. By using fish that was fed with plant derived oils in diet human body does not receive enough DHA and EPA anymore. These fatty acids are vital for human body and regular uptake of these fatty acids improve cell membranes, function of the brain, nerve impulses, oxygen transfer to blood plasma, hemoglobin synthesis, processes of cell division and brain development of the newborns; overall these fatty

acids are considered very important because human body is not capable of synthesizing them on its own [4]–[6]. Another negative effect of switching feed source is the change of omega-3 to omega-6 ratio, where increased concentration of omega-6 fatty acids is paralleled with reduction of omega-3 [4]. Such change in ratio can be alarming when considering that high omega-6 to omega-3 ratio in diet coincide with multiple cardiovascular and neurodegenerative diseases, inflammation and cancer [7], [8]. Therefore, use of oils derived from plants as feed for farmed fish can have negative impact on human health if fish fed with such diets is consumed for prolonged periods of time, therefore it is necessary to find alternative sources of fish feed, which could be used in aquaculture without creating risks to human health.

5. It is possible to considerably increase protein concentrations and adjust amino acid profiles of the plant meals by genetically modifying (GM) the plants. It is also possible to derive omega-3 fatty acids from various sources, of which soybeans, wheat, linseed, and canola have shown positive effects on growth of farmed fish when used as feed in aquacultures. Nevertheless, non-genetically modified (non-GM) plants do not have the ability to synthesize such fatty acids as DHA and EPA, therefore genetically modified plants are used for that purpose. Although there are GM plants that are capable of producing omega-3 fatty acids, their DHA and EPA concentrations are lower than in oil produced by microorganisms [4]. GM cultures have great potential in food production sector in general, but society still considers GM crops as unsafe and in European Union and other parts of the world growing, selling and importing of the GM crops is completely or partially restricted [9]–[15].
6. Microorganisms can be used in order to satisfy the high demand for proteins and omega-3 fatty acids. Produced SCO does not lower the levels of the high value fatty acids in the fish tissue and does not disrupt the omega-3 to omega-6 ratio, as it happens in the case of using plant derived oils in fish diet. Produced SCP amino acid profiles can be adjusted via selection of various SCP producing microorganisms, thus increasing feed conversion ratios and decreasing the generated amount of detritus, which in turn reduces the load on filtration systems. However, cultivation is very expensive if the main materials used as feed for microorganisms are grown in arable lands (sugars derived from plants). Available area of arable lands is limited [16], [17], therefore growing of plants rich in sugar for microbial fermentation competes directly with arable land areas which could be used for growing plants used as food for human or animal consumption. Cultivation by using sugars derived from plants can be cost-effective only if produced proteins or oils are sold with very high added value (pharmacy, baby formula, etc.). Using these proteins and oils as feed for aquacultures is not financially viable, therefore it is necessary to cultivate microorganisms by using cheaper materials (by-products of other industries).

1.1. Advantages of using microorganisms for production of either SCP or SCO

1. The growth of microorganisms is considerably faster than growth of plants, animals or fish. Microscopic algal populations double within 2–6 hours, yeast and microscopic fungi populations in 1–3 hours, and bacterial populations double within 0.5–2 hours. Therefore, microbial reactors require protein production cycles of 12–72 h for bacteria and 5–10 days for yeast, fungi and algae, while it is possible to harvest agricultural produce only once a season (1–2 times a year).
2. Thanks to the rapid growth of microorganisms, suitable strains of microorganisms can be chosen and artificially selected in few weeks or months, while breeding takes years with plants and animals.
3. Microorganisms can use a variety of carbon sources as nutrients. As a result, proteins and oils can be obtained by utilizing variety of biodegradable residues and by-products, thus significantly reducing the production costs.
4. Autotrophic microorganisms (microscopic algae, photosynthetic bacteria) are capable of growing using CO_2 as a carbon source. Thanks to the Wood-Ljungdahl biochemical cycle or reverse Krebs cycle, microorganisms are 3 to 10 times more efficient CO_2-absorbers than plants [18], which generally ensures faster biomass growth and reduced negative environmental impacts.
5. The cultivation of microorganisms for protein and oil production is independent of seasonal weather conditions and climatic changes. The process of cultivation in the reactors is protected from extreme weather conditions, which usually destroy plant crops grown for feed production. Unlike plants, microorganisms that do not require light for growth, can be cultivated around the clock.
6. Cultivation of microorganisms consumes considerably less water than cultivation of crops in agricultural areas. Due to the evaporation, transpiration and leakage of water, average water consumption per 1 kg of cereal is an average of 1800 litres [19]. When cultivating microorganisms in bioreactors, none of these factors have any effect on water consumption.
7. The cultivation of microorganisms does not require fertile land, so it does not compete with agriculture. Due to low water consumption, SCP and SCO production can also be done in dry climate regions where the availability of fertile land is limited.

1.2. SCP advantages

In general, replacing of existing feed sources (agriculture, wild capture) with single-cell proteins provides a number of advantages:

1. A wide range of microorganisms can be used for SCP production. So far, hundreds of microorganisms from 48 genera have been identified as suitable for SCP production, capable of accumulating at least 20 % protein relative to its biomass dry weight [20], [21]. Due to the wide selection, it is possible to select microorganisms that have the appropriate amino acid profiles for the specific purpose (the animal to be fed with SCP) and are

able to efficiently use certain by-products [20], [21].

2. Microorganisms have similar protein content to fish and several times higher than that of plants (for microorganisms 20–80 %, for plants 6–40 %, for fish 30–63 %) [22]–[24]. Consequently, the cultivation of microorganisms for protein production is much more efficient than from traditional feed sources.
3. The composition of SCP is of significantly higher quality than the protein derived from plants. Plant-based feeds contain low levels of 4 essential amino acids – lysine, methionine, threonine and tryptophan, while all nine essential amino acids in SCP are in line with FAO recommendations [22], [25].

1.3. SCO advantages

Single cell oils are similar in composition to those that can be obtained from plants and animals, but SCO has several advantages over traditional oil sources:

1. A wide range of microorganisms can be used for SCO production. So far, hundreds of microorganisms from more than 60 genera suitable have been identified as suitable for SCO production, capable of accumulating at least 20 % oil relative to its biomass dry weight [4], [26]–[28].
2. Microorganisms have several times higher oil content in dry matter than plants and animals (20–80 % for microorganisms, 5–35 % for plants, 2–30 % for fish) [4], [29].
3. The composition of SCOs is of higher quality than that of oils derived from plants or fish. For example, the concentration of high-quality omega-3 fatty acids (EPA and DHA) from the total oil content of SCO can reach up to 40 %, while for plants and fish it is 4.9 % and 3 %, respectively [4].

2. AVAILABILITY ANALYSIS OF BY-PRODUCTS APPLICABLE FOR SCP AND SCO PRODUCTION

2.1. Summary of agricultural by-products applicable for SCP production

In this first review paper, most of the agricultural wastes that can be used in the production of SCP have been categorized and discussed more closely. Each agricultural waste group has its own advantages and disadvantages if used as substrate for SCP production.

Monosaccharides and disaccharides rich sources require minimal pre-treatment, which give these wastes distinct technological and economical advantage over other waste types, since simpler bioreactor designs can be used, and no sophisticated pre-treatment processes are required.

Fermentation of polysaccharides, protein or lipid rich sources improve the overall digestibility of these by-products, which makes them more applicable as animal feeds. More extensive pre-treatment of these wastes can result in higher SCP yields, but cost effectiveness of applied pre-treatments needs to be considered in order to justify the expenses.

Structural polysaccharides rich wastes are available in huge quantities all over the world; therefore, using these wastes have limited competition with other industries which use waste as resource for production of other value-added products. In comparison, competition over monosaccharides, disaccharides and starch rich sources is greater, since those wastes are not so abundant and are more easily used. If other waste types have limited local availability and efficient and economically reasonable pre-treatment process can be used for hydrolysis, structural polysaccharides rich wastes can be used extensively for production of SCP.

In general, the key considerations for choosing the most suitable waste product for SCP production are: (1) local availability of the particular waste product; (2) pre-treatment costs of the waste product before using it in fermentation; (3) the costs of transportation of the waste product; (4) SCP concentrations in the final microbial biomass after fermentation.

2.2. Summary of industrial by-products applicable for SCP production

In this second review paper, most of the industrial wastes that can be used in the production of SCP have been categorized and discussed more closely. Each industrial waste group has its own advantages and disadvantages if used as substrate for SCP production. Use of polymer-rich sources is problematic mostly due to extensive pre-treatments these wastes require before efficient SCP fermentation can take place. Carbon compounds, especially waste gases and glycerol, have the highest potential in becoming widely used carbon sources for various types of microbial fermentations, including SCP production, but further advancements in these technologies are required for these sources to become more widely accepted. Basic infrastructure for using various wastewaters for SCP production already exists, however, reasonable concerns over heavy metal and other admixtures in biomass and inefficient waste and biomass separation solutions are holding back the use of wastes applicable for photosynthetic microorganisms.

The key considerations for choosing the most suitable waste product for SCP production remains the same as concluded in previous review [20] (2.1.1. Agricultural by-products applicable for SCP production) with few additions. Key considerations are: 1) local availability of the particular waste product; 2) pre-treatment costs of the waste product before using it in fermentation; 3) the costs of transportation of the waste product; 4) maximum obtainable cell densities in substrate; 5) SCP concentrations in the final biomass after fermentation; 6) estimation whether cultivation conditions can be efficiently maintained (energy and heat consumption); 7) efficiency of biomass and waste separation, and SCP extraction (protein extraction from biomass and removal of impurities) methods.

2.3. Summary of agricultural by-products applicable for SCO production

In this review, most of the agricultural wastes that can be used in SCO production have been categorized and discussed more closely. Since agricultural waste groups were categorized in the same way as it was done previously for SCP production [20], the same advantages and disadvantages can be referred to these wastes as well with few additions.

Monosaccharides and disaccharides rich sources require minimal pre-treatment, which gives these wastes technological and economic advantages over other waste types. However, these wastes are already widely used in other fermentation processes and as feedstock in animal feeds. Therefore, each waste material must be evaluated in regard to its economic feasibility and compared with already existing or potentially emerging competing sectors.

Use of starch, protein or lipid rich sources and their hydrolysates in SCO production result in comparatively lower SCO yields than if monosaccharaides and disaccharides or fibre-rich materials are used in fermentations. Regardless of this, waste products such as food waste, potato and corn starch processing wastewaters and waste cooking oils are generated in huge amounts each year in all parts of the world. In order to reduce the negative environmental impact and improve SCO production efficiency, additional research is needed to develop more efficient methods of waste hydrolysis and medium detoxification.

Structural polysaccharides rich wastes are available in huge quantities all over the world; therefore, using these wastes have limited competition with other industries, which use waste as resource for production of other value-added products. These wastes require extensive pre-treatments and during hydrolysation microbial growth inhibiting compounds may be released, which, in turn, require additional detoxification of the substrate, before these wastes can be used in microbial fermentations.

The key considerations for choosing the most suitable waste product for SCO production are similar with the ones concluded in previous reviews [20], [21] with few changes for details specific to SCO production. Key considerations are: target market for the final oil (biodiesel production; animal feeds); which microorganism strain produces necessary fatty acid profile for the target market; local availability of the particular waste product; pre-treatment costs of the waste product before using it in fermentation; the costs of transportation of the waste product; maximum obtainable cell densities in the substrate; SCO concentrations in the final biomass after fermentation; estimation whether cultivation conditions can be efficiently maintained (energy and heat consumption); efficiency of biomass and waste separation, and SCO extraction (oil extraction from biomass and removal of impurities) methods.

2.4. Summary of industrial by-products applicable for SCO production

In the scope of this review only a few industrial by-products were considered for SCO production. As shown in this review, every waste or by-product could be used for higher value-added product production, as well as for reduction of industry's burden on environment as in the case with wastewaters and butanol wastewaters.

2.5. Summary of the optimization model and case study

By interviewing two companies for this study, it became apparent that companies lack capacity to provide the researchers with requested data, since data acquisition takes a long time and usually employees are unwilling to spend additional hours for data gathering. Also, the provided information about current use of cheese and cottage cheese whey at dairy plants should be taken with some scepticism, as company representatives are unlikely to disclose if some of the whey is discharged into local wastewater system or in natural water bodies without prior treatment. Both companies stated that they process or treat 100 % of the generated whey, although estimates [30] state that at least 25 % of the generated whey in EU is not disposed of properly or is not reprocessed in new products. Of course, this might also not be the case in Latvia and possibly all of the generated whey is treated properly or reprocessed, but with currently conflicting information it is difficult to know for sure. Complete data for generated aged and fresh cheese volumes was provided by CSB, thus it was possible to perform analysis with accurate data for the generated whey amount in each of the 22 dairy companies.

The current model assumes that all generated whey is used in SCP/SCO production, which is not accurate, because in reality some of the dairy plants use whey for production of other value-added products. Such products as whey powder, whey protein powder, lactose powder, whey drinks, etc., are also products with higher added value than SCP or SCO. Therefore, further data acquisition is required in the future to calculate the available whey volumes in each dairy plant. This could be done by further interviews about the production volumes of alternative products, which use whey as main ingredient, or by acquiring data via other sources – CSB, media, etc. Since most of the generated whey in Latvia is aged cheese whey (95.88 %), the SCP and SCO production should focus on using only this type of whey. By using the reported SCP and SCO yields when using whey as substrate [31]–[33], in Latvia, it would be possible to produce up to 800 tonnes of pure SCP [32] or up to 2250 tonnes of pure SCO [33]. SCO also has wider possible applications (feeds, biofuel, building block chemicals, etc.) [27], [34] and higher market price [22], therefore it can be concluded that SCO is the preferable end-product if whey is used as a substrate.

After validation, the developed model confirms that it is possible to calculate the optimal SCP/SCO plant location. Use of model itself is simple, quick, and does not require any prior knowledge in using dedicated optimization or dynamic modelling software. This model can be used to optimize the sourcing of any raw material, in any industry where the situation requires the raw material to be gathered from multiple sources and transported to single processing plant. Therefore, the proposed model can be used for modelling the sourcing of by-products not only applicable for SCP or SCO production, but also for other purposes, such as sourcing of starch rich by-products for ethanol fermentations, sourcing of vegetable oil rich by-products and wastes for fuel production (biodiesel), sourcing of lignocellulose rich by-products, and waste materials for lignocellulose hydrolysis followed by ethanol production, etc.

In the future, the model needs to be refined by the following improvements:

- by taking into account the number and capacity of transporting trucks and optimization of the route from raw material supplier to another, if it is not possible to load the truck fully in a single source;
- by introducing a dynamic model of the SCP or SCO production process to find the optimal production capacity (bioreactor volumes), while also taking into account the amount of feedstock supplied daily and the very short shelf life of the feedstock (for whey it is 24 hours);
- improving the dynamic model of the transport and production process by introducing a cost estimation that could also demonstrate whey viability for use in SCP and SCO production, taking into account the market price of the final product;
- ensuring that the developed model is easily adjustable for different types of feedstocks applicable for SCP and SCO production.

3. DEVELOPMENT OF THE LABORATORY STAND

3.1. Attracting the funding

In 2017, part of the performance funding allocated to the institute was used for the improvement of the RTU IESE Biosystems Laboratory (Financing of scientific activity development in higher education institutions: Financing of higher education institutions' performance, Ministry of Education and Science).

In November 2017, a project application for a project competition was submitted "Support for the commercialization of research results", which is co-funded by European Regional Development Fund (ERDF) and managed by Investment and Development Agency of Latvia (LIAA) [377]. On January 2018, the project proposal titled "Supercritical Omega-3 oil from production by-products" was approved for the first stage of the project. During the first stage technical and economic feasibility study and commercialization strategy was developed. The project entered the second stage of the project competition in July and after successful pitching of the technology it was approved in November 2018 for the second stage of the project where the main funding of 274 500 EUR was provided for the development of the proposed technology. The project is still ongoing (the end of the project is April 28, 2021), and thanks to the received funding it was possible to outfit the IESE Biosystems Laboratory with most of the equipment required for experiments in microbiology, molecular biology, chemistry, etc.

3.2. Outfitting the IESE Biosystems Laboratory

Initially, the IESE Biosystems Laboratory lacked the necessary equipment to provide a sterile environment for working with microorganisms and to prepare sterile microbiological media for culturing microorganisms under controlled conditions, as well as specialized equipment and reagents specialized in microbiology, molecular biology, and chemistry. Thanks to the attracted funding, several procurements were organized and the IESE Biosystems Laboratory was equipped with the following inventory:

- chemically resistant work surface;
- laminar flow hood;
- fume box;
- incubator shaker with refrigeration;
- large volume (400 L) incubator with refrigeration;
- medium volume (256 L) incubator;
- autoclave;
- refrigerated centrifuge;
- UV-Vis spectrophotometer;
- solvent based extraction system;
- rotary evaporator;
- electrophoresis apparatus;

- ball mill;
- press filter system;
- magnetic stirrers;
- −40 °C freezer;
- pipette sets;
- grinder;
- fridges;
- vortexes;
- low value inventory;
- materials;
- chemicals;
- analytical tests.

The following equipment was upgraded or modified:

- 5 L bioreactor:
  - equipped with DO probe;
  - equipped with pH meter.
- Gas chromatograph:
  - new columns for detection of fatty acid methyl esters;
  - new septums, ferrules and injector liners.

The obtained and improved inventory and tested methods allow the laboratory to perform the following:

- manipulation of microorganisms under sterile conditions;
- factorial cultivation experiments of microorganisms in various volumes and temperature regimes;
- prepare microbiological media;
- perform polymer hydrolysis;
- extract oils, biomass, volatile compounds, etc.;
- liquid filtration and separation;
- biomass concentration analysis;
- protein concentration analysis;
- oil concentration analysis;
- amino acid analysis;
- fatty acid analysis;
- mechanical and chemical treatment of biomass;
- storage of sensitive samples.

6. LITERATURE

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