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# **From biomass to value: a decision support analysis for market-driven microalgal biorefineries**

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Master of Science Thesis in Technology

Author(s):  
Sara Makkonen

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Turku

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**Author(s):** Sara Makkonen

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**Supervisor(s):** PhD Elina Peltomaa and Prof. Yagut Allahverdiyeva-Rinne

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**In this thesis a decision support analysis is done for Aircohol Oy to aid strategic decision-making. Aircohol Oy cultivates microalgae for sustainable circular economy applications. The aim of the decision support analysis is to evaluate the most profitable biorefinery strategies in the current market situation.**

**Microalgae are versatile raw material that contains valuable compounds for various industries. The ratio of the compounds within a microalgae cell can be manipulated by adjusting cultivation parameters. This is an important feature that makes microalgae production flexible as the cell composition dictates the product portfolio. In this thesis, the compositions of five microalgae cultivations are compared to identify the one with the highest profit potential.**

**While the intrinsic value of the microalgal compounds is dictated by the biochemical properties, their market value is ultimately dictated by demand, which varies between the products. The market values are investigated with a market analysis and interviewing potential customers. Moreover, these products not only differ in value but also in refining cost. Biorefining is a concept with which all valuable microalgal products are isolated to maximise overall value. However, the choice of a biorefinery strategy must be carefully justified by a decision support analysis. The most profit is created when the selected biorefinery strategy is aligned with the desired product fractions, as dictated by both the biomass composition and market demand. In this thesis different biorefinery strategies are evaluated through techno-economic assessment (TEA). Only the process related costs are within the scope of this assessment, and other costs such as plant establishment, maintenance and labour costs are excluded, although they are commonly included. Based on this evaluation, ten biorefinery strategies are designed. The techno-economic parameters identified in the decision support analysis are compiled in a flexible decision support system.**

**The most profitable biorefinery strategies in current market situation were the production of broken and dried microalgae cells and isolating protein, mono- and polysaccharides from the insoluble fraction with two-step filtration. This decision support analysis must be updated as prevailing market situation and regulations change. Also, the secondary data used in this TEA should be updated and replaced with primary data as it becomes available.**

**Decision support analysis is crucial to become and remain competitive in changing market situation where market values and costs fluctuate and new regulations emerge. Finally, the development of microalgae strains and biorefinery technologies enable new applications and generate novel process parameters.**

**Key words:** Microalgae, biorefinery, circular economy, sustainable business, strategic decision-making

# Table of contents

<b>Abbreviations</b>	<b>5</b>
<b>1 Introduction</b>	<b>6</b>
<b>1.1 Global challenges demand application of new sustainable technologies</b>	<b>6</b>
<b>1.2 Microalgae biorefinery as value creator</b>	<b>7</b>
1.2.1 Microalgae as bioeconomy promoters	7
1.2.2 Generating and recovering microalgae biomass	8
<b>1.3. From biomass to value: the biorefinery concept</b>	<b>8</b>
1.3.2 Cell disruption releases microalgal components	12
1.3.3 Extracting the released components	13
1.3.4 Preparing products for sale	13
<b>1.4 Commercial microalgae production</b>	<b>14</b>
1.4.1 Use of microalgal compounds in various industries	14
1.4.2 Scaling up microalgae biorefinery downstream operations	16
1.4.3 Techno-economic bottlenecks in commercialising microalgae products	16
1.4.4 Contribution of commercial microalgae solutions to the Sustainable Development Goals	17
1.4.5 Aircohol – Cheers, planet!	18
<b>1.5 Economic feasibility of a biorefinery</b>	<b>20</b>
1.5.1 Evaluating the minimum selling price with techno-economic assessment	20
1.5.2 Defining the market value of microalgae components	21
1.5.3 Maximising biorefinery value with a decision support analysis	22
<b>1.6 Aims of the thesis</b>	<b>23</b>
<b>2 Materials and methods</b>	<b>24</b>
<b>2.1 Laboratory tests on microalgal biomass</b>	<b>24</b>
2.1.1 Cell composition data	25
2.1.2 Cultivation density measurements	27
2.1.3 Total pigment protocol development and measurements	27
2.1.4 Total protein protocol development and measurements	28
2.1.5 Total sugar protocol development and measurements	29
2.1.6 Compiling the total compositions of the studied microalgae strains	30
<b>2.2 Analysis of the microalgae market</b>	<b>31</b>
2.2.1 Market knowledge generation approach	31
2.2.2 Defining product scope and value proposition	31
2.2.3 Desk research – secondary market data collection	31
2.2.4 Market selection analysis approach	31
2.2.5 Customer discovery and willingness to pay	32
<b>2.3 Conducting techno-economic assessment</b>	<b>32</b>
2.3.1 Inventory analysis	33
2.3.2 Impact analysis	33
<b>2.4 Decision support system</b>	<b>34</b>
<b>2.5 Use of artificial intelligence</b>	<b>34</b>
<b>3 Results and discussion</b>	<b>34</b>
<b>3.1 Protocol development and measurements of microalgae compositions</b>	<b>34</b>
3.1.1 Total pigment protocol development and content	35
3.1.2 Total protein protocol development and content	36
3.1.3 Total sugar protocol development and content	39
3.1.4 Overall composition	41
<b>3.2 Microalgae markets</b>	<b>42</b>
3.2.1 Existing prominent customer industries	42
3.2.2 Prominent industries for cultivated biomass according to its composition	46
3.2.3 Willingness to pay for microalgae products in the proposed markets	48
3.2.4 Added value created with microalgal products	49
<b>3.3 Biorefining target products</b>	<b>50</b>

<b>3.4 Techno economic analysis of biorefinery methods</b>	<b>51</b>
3.4.1 Goal definition	51
3.4.2 Scope definition	52
3.4.3 Inventory analysis	53
3.4.4 Impact analysis	54
<b>3.5 Decision support system</b>	<b>59</b>
<b>3.6 Other parameters to take into consideration in the microalgae industry</b>	<b>64</b>
3.6.1 Other production costs	64
3.6.2 Regulatory and legal aspects	64
3.6.3 Future opportunities and challenges of microalgae industry in Finland	65
<b>3.7 Evaluation of the study</b>	<b>66</b>
<b>4 Conclusion</b>	<b>67</b>
<b>References</b>	<b>69</b>
<b>Appendices</b>	<b>84</b>

## **Abbreviations**

B2B Business-to-business

CAGR Compound annual growth rate

CAPEX Capital expenditure

DSA Decision support analysis

DSS Decision support system

EU European Union

GHG Greenhouse gas

GMO Genetically modified organism

GRAS Generally recognised as safe

HPH High pressure homogenisation

HPLC High performance liquid chromatography

MSP Minimum selling price

OPEX Operating expense

PBR Photobioreactor

R&D Research and development

ROI Return on investment

SDG Sustainable development goals

TEA Techno-economic assessment

TRL Technology readiness level

UN United Nations

US Ultrasonication

WTP Willingness to pay

# 1 Introduction

## 1.1 Global challenges demand application of new sustainable technologies

As the global population continues to grow, the demand for daily commodities and nutrition accelerates. The current production system is not capable of responding to this demand without causing crises like the climate change due to increasing greenhouse gas (GHG) emissions and food crises due to irrational agricultural habits. The increasing production and consumption rates challenge energy security as more electric power is required to run our daily lives while health crises like global pandemics and antibiotic resistance take advantage of urbanisation (World Health Organisation, 2024). Simultaneously, concern regarding sustainability and biodiversity are increasing, demanding innovative production methods for compounds in various industries (Safi et al., 2017). Many industrial compounds either originate from fossil resources or require other fossil-derived inputs for their production (J. S. Singh et al., 2016), which places a heavy burden on the environment.

These negative environmental and climate impacts are then further allocated to the finished products (Michael Z. Hauschild et al., 2018). Moreover, the price and availability of fossil resources fluctuate with geopolitical factors (Kliopova et al., 2016), rendering some societies into more vulnerable position than others. Replacing these fossil-derived compounds with naturally sourced alternatives and existing side streams can reduce both the climate and environmental impacts while simultaneously lowering international dependencies and improving resource and food security (Mendes et al., 2024; Ruiz et al., 2016).

These issues have been noticed in the international regulatory organisations such as the European Union (EU) and the United Nations (UN). The EU adopted a bioeconomy strategy (European Commission. Joint Research Centre., 2024) and an algae strategy (European Commission, 2022a) which aim to tackle these challenges by efficiently utilising biological resources. The bioeconomy has been recognised as a feasible approach to enhance sustainability in various industries while also contributing more broadly to the Sustainable Development Goals (SDGs) established by UN (Solarte-Toro & Cardona Alzate, 2021).

## 1.2 Microalgae biorefinery as value creator

### 1.2.1 Microalgae as bioeconomy promoters

Microalgae are a versatile group of photoautotrophic organisms that synthesise organic carbon compounds from atmospheric carbon dioxide and light. They have an essential role in the evolution of life on planet Earth, as atmospheric oxygen is the byproduct of their energy metabolism (Brocks et al., 1999). At present, microalgae produce up to 60 % of the Earth's oxygen (Show et al., 2017). They also function as a carbon sink since they use the CO<sub>2</sub> of a system to synthesise organic compounds (Dutta et al., 2025). This property can be harnessed to reduce the GHG emissions of industrial systems and thereby mitigate the climate impact, while simultaneously generating valuable biomass.

Microalgae grow extremely rapidly compared to terrestrial plants, making them a highly renewable process feedstock (Safi et al., 2017). Cultivating microalgae does not require land area (Rumin et al., 2021), as they can be grown in closed systems wherever needed. For some applications, the cultivation can be carried out in effluents or sea water, which decreases the cultivation's water footprint of the process, while contributing to wastewater purification (Dutta et al., 2025). These microalgae, however, have limited applications and cannot be used for high-sanitary products for dietary or medical purposes unless the effluent has been proven to be free of contaminants (Mendes et al., 2024).

Several microalgal processes and products have already been commercialised, particularly in food, fuels and cosmetics sectors (Ruiz et al., 2016). The most widely commercialised microalgae are prokaryotic *Limnospira* or *Arthrospira*, (commonly referred to as *Spirulina*), but many other species are also used (Gallego et al., 2025). Often both prokaryotic cyanobacteria and eukaryotic microalgae are considered as one group, microalgae, due to their fundamental similarities (McFadden, 2001). *Spirulina* and *Chlorella* have been cultivated commercially from the 1960's in Japan (Bhattacharya & Goswami, 2020). The vast diversity of this group of organisms provides tools for various applications and optimising these processes. The advantage of eukaryotic organisms to their prokaryotic relatives is their ability to more complex metabolism such as post translational modifications, which leads to more versatile metabolic products (Russell et al., 2022).

### 1.2.2 Generating and recovering microalgae biomass

The cultivation process has developed significantly from open ponds used in the 1960's that were exposed to the environment into highly controlled closed systems, photobioreactors (PBRs) (Ruiz et al., 2016). Cultivating in closed PBRs offers more possibilities in design, construction and operation, enabling new applications, despite their higher capital costs. PBRs are also the most reliable cultivation method because the cultivation conditions can be controlled to a higher degree. This provides the possibility for optimisation and added value creation. (Greenwell et al., 2009; Harun et al., 2010; Pulz, 2001.) Closed cultivation also prevents various cellular stress factors such as contamination, nutrient and CO<sub>2</sub> availability, self-shading, oxygen accumulation and pH fluctuations caused by cellular respiration during dark periods (Barkia et al., 2019; Ruiz et al., 2016). The PBR design affects the productivity and cost structure of microalgae cultivation, underlining the importance of selecting the design according to the production setup. Variables that affect growth rate and can be regulated by PBR are gas-liquid transfer and light penetration rates, avoidance of contamination and harvesting efficiency (Dos Santos et al., 2022). Some PBR design available are flat plane, air-lift, bubble column, horizontal tubular and stirred tank, from which hybrid versions are also available (Tan et al., 2020).

Nowadays microalgae are cultivated in all parts of the world. Within the EU alone, 153 companies were engaged in cultivating or harvesting microalgae in 13 EU member states in 2022 (Vazquez Calderon & Sanchez Lopez, 2022). The development of PBR technologies in particular has supported the global spread of microalgal cultivation. Some of the largest microalgae companies in the EU are Portuguese Necton, Icelandic Algalif and Dutch Algaspring. Necton has a broad portfolio with aquafeed, cosmetics and food products produced with natural sunlight in closed systems. Algalif also uses closed cultivation systems but relies on synthetic light to produce astaxanthin products for health benefits. Algaspring, however, uses a controlled open pond system and natural daylight to cultivate *Nannochloropsis gaditana* for aquafeed and dietary supplements.

### 1.3. From biomass to value: the biorefinery concept

Recently, the focus in the microalgae sector has altered from a single-product approach to valorising the entire biomass, through several extraction methods (Ruiz et al., 2016). This is



the biorefinery approach that aims to extract simultaneously multiple high-value products while minimising the generated side streams (IEA Biotechnology, 2019; Vázquez-Romero et al., 2022). A closely related approach is the cascade approach and these two are sometimes considered the same (Slegers et al., 2020). According to the European Commission, cascading refers to the efficient use of biomass to extend its availability within a system (European Commission. Directorate General for Internal Market, Industry, Entrepreneurship and SMEs. et al., 2016). This approach increases the utilisation potential of the used biomass from 7-28 % to over 97 %, simultaneously increasing revenue potential (Slegers et al., 2020). Ruiz et al. (2016) reported the largest microalgal processing expenses, 70-80 %, were created in extracting and purifying soluble proteins. Hence, cascading residual microalgae biomass into medium-to-high value products enables the maximum utilisation of biomass and greater value creation (Karan et al., 2023).

The aim of cascading and biorefinery approaches is to fully valorise the biomass while generating minimal waste streams. They both follow the circular bioeconomy concept, while biorefining emphasises processing the raw material to various products whereas cascading lengthens the biomass value chain by reusing the residuals and once-used biomass. (Eppink et al., 2019; Malik et al., 2022.) Although these multi-product chains require higher investments and operational costs, these are generally more than compensated by revenue generated from a diverse product portfolio (Slegers et al., 2020). Since single-product biorefineries are not considered economically viable, multiproduct biorefinery approaches should be adopted (Bhattacharya & Goswami, 2020; *Multi-Product Integrated bioRefinery of Algae*). Nevertheless, single-product value chains often serve as the basis for the development of multi-product value chains.

In practice, the extraction processes are often carried out sequentially (Dutta et al., 2025; Izanlou et al., 2023). For efficient valorisation of the biomass, detailed knowledge of the composition is required, since the physical and chemical properties of the extracted compounds affect the entire biorefinery structure. (Dutta et al., 2025.) Izanlou et al. (2023) demonstrated that the sequential extraction of algal compounds can reduce resource use significantly, saving 79 % in initial biomass, 67 % in time, 34 % in chemicals and 58 % in energy, compared with single-product extractions.

Also, the different extracts have varying market values. To maximise profitability, the products with the highest market values should be prioritised over those of lower value. The relation of product quantity in biomass to its market value is roughly inversely proportional and is often depicted with a biorefinery value triangle (Figure 1).

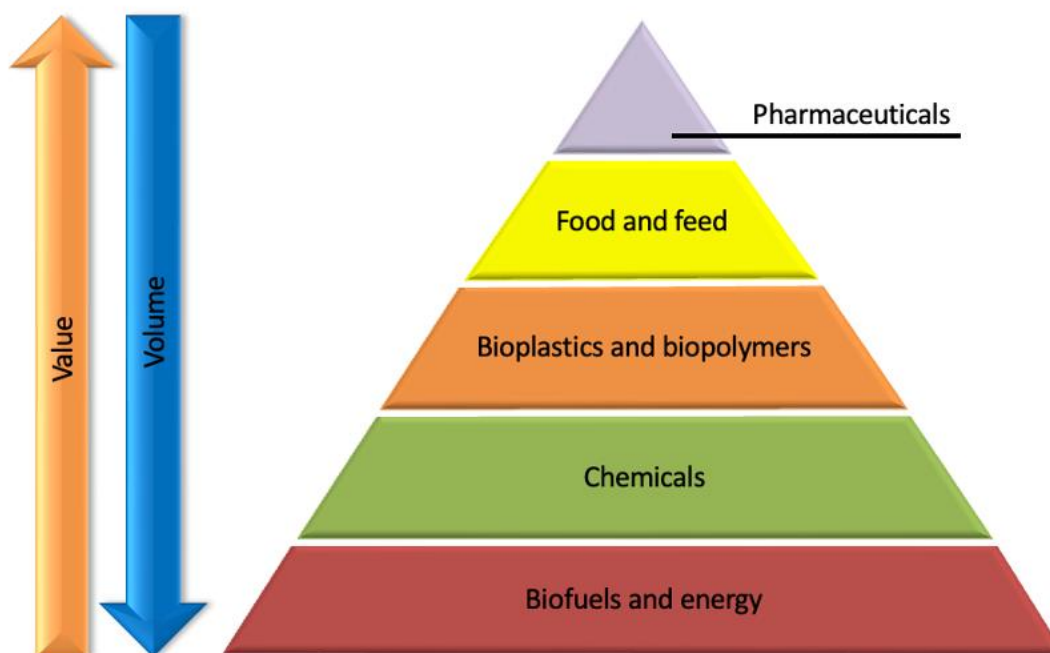


Figure 1. Biorefinery value triangle. The highest value sector of the biomass is the smallest and the largest is of the lowest value. Based on Allahverdiyeva-Rinne (2024).

### 1.3.1 Maximising the value of the microalgae biomass via biorefinery

The microalgae biorefinery products are protein, lipids, pigments, carbohydrates, vitamins and minerals, to name a few. Each product can be separated individually, but often at the expense of the other products (Slegers et al., 2020). Moreover, each additional refinery step increases the overall costs of the final product while decreasing the total product yield (Quiroz-Arita et al., 2022). Due to this, the refinery process must be carefully planned and feasibility-tested, to avoid unnecessary costs and waste.

Detailed knowledge of the initial biomass composition is essential for optimising the refining processes to maximise both product yields and value creation. The product portfolio strongly

influences the chosen biorefining strategy (Ruiz et al., 2016) and as a result the portfolio must be carefully selected according to the cell composition. A broader product portfolio allows flexibility in the markets and improves economic feasibility. Optimising the value created from microalgae biomass may also require entering more than one market segment, leading to diverse market combinations (Ruiz et al., 2016).

A versatile product portfolio provides flexibility on the markets, as target products can be changed according to the market demand. However, the production rates of individual compounds are often relatively low if none of the components dominates the cell composition clearly. In a situation in which the cell has somewhat equal concentrations of multiple components it is more difficult to prioritise the refining of any main product.

Fortunately, the composition of the microalgal biomass can be manipulated by changing cultivating conditions. For example, nitrogen deficit directs the composition towards higher lipid concentration while reducing protein concentration (Ördög et al., 2012) and higher CO<sub>2</sub> concentrations support biomass generation and lipid synthesis (Swarnalatha et al., 2015). Also, the selection of the microalgae strain, due to the diversity of these organisms, allows manipulating the biomass compositions (Russell et al., 2022). These strains can also be developed towards optimal compositions with modern biotechnology (A. Singh et al., 2022). By altering the cultivation conditions and selecting optimal strains, microalgal production can be rapidly adapted to the changing market demands.

For commercial feasibility and maximum profit creation new markets should be penetrated (Ruiz et al., 2016). Microalgal products hold different values depending on its application sector, which allows for optimising profits by exploiting price differences across markets (Gallego et al., 2025).

Proteins are a feasible target product among other microalgae extraction products, as they have relatively a high TRL (Quiroz-Arita et al., 2022). They can be used for animal feed, human nutraceuticals, dietary supplement, and industrial bulk material (Mendes et al., 2024; Quiroz-Arita et al., 2022). However, the sensory properties of the final meal remain a challenge limiting its acceptance by both human and pet consumers, (Mendes et al., 2024) and must be solved for market penetration. Proteins from *Chlorella* also provide higher value bioactive functions. Such peptides, when extracted from natural sources via biosynthetic

routes, have reduced side effects and are overall healthier compared to synthetic alternatives. Producing these peptides could be more inexpensive and sustainable when extracted from a natural source rather than produced synthetically. (Barkia et al., 2019.)

Microalgal pigments have various health benefits and have established markets in nutraceuticals, functional dietary supplements and cosmetics (Minhas et al., 2016). They could also be used as sustainable colourants (Ngamwonglumlert et al., 2017) but the challenges with their stability and colouring efficiency must be addressed to achieve economically viable selling prices (Karan et al., 2023). Tackling these hurdles may be the key for more comprehensive market penetration also in other industries. Currently, 80-90 %, of produced carotenoids for dietary supplements for animal feed and human food are synthetic (Saini & Keum, 2019), indicating there would be room for more sustainable production and natural non-synthetic compounds.

The microalgae strains used in this study are known to contain low lipid concentrations and contain insignificant amounts of polyunsaturated fatty acids: thus, extracting lipids was not considered viable. However, some microalgal strains accumulate higher lipids content, which is known to have a direct positive impact on human health in the form of nutraceutical supplements and indirectly by improving the quality of animal products (Mendes et al., 2024). Microalgal lipids have also been used as a source of sustainable biofuel. However, the TRL for economically viable fuel production remains too low (Quiroz-Arita et al., 2022).

Microalgae contain carbohydrates of different lengths and structures. Long chain polysaccharides can be used in cosmetics for various skin benefits (Ariede et al., 2017). In food applications, the positive effects of these soluble fibres can be enhanced by having them in appropriate ratio with dietary fibres. Moreover, these microalgal carbohydrates have anti-inflammatory, antimicrobial and antiviral properties which could be used, in addition to health and skin vitality support, for preserving food products naturally. This in turn would reduce the reliance on synthetic chemicals in food. Originating from a natural, food grade source, they are safe for consumers and the environment. (Mendes et al., 2024.)

### 1.3.2 Cell disruption releases microalgal components

The refining chain begins with cell disruption. Some microalgae, especially those belonging to the division *Chlorophytae*, have a rigid cell wall, which restricts the release of cellular

components (Van De Walle et al., 2025). This makes breaking the cell wall extremely important, because without it no intracellular products can be extracted. The efficiency of the extraction subsequent downstream steps are strongly influenced by the effectiveness of this step (Corrêa et al., 2020; Quiroz-Arita et al., 2022). Given its importance, cascading the disrupted biomass into multiple medium-to-high value products maximises the overall utilisation of biomass and value creation (Karan et al., 2023).

The cell wall may be disrupted with physio-mechanical, chemical or biological methods. Physio-mechanical methods consume the most energy and are hence expensive to use. Yet, they do not alter the structures or biological activities of the target compounds nor limit the selection of following refining steps. (Günerken et al., 2015.) Chemical methods may be less expensive at the operations stage, but they often alter the structure of target components (Corrêa et al., 2020). The chemicals used may restrict the use of some following refining steps, making the refining chain less flexible (Di Caprio et al., 2023). In addition, many chemicals are organic solvents that impose environmental burdens due to solvent production and disposal, often making them economically unfeasible (Corrêa et al., 2020). Biological methods, such as enzymatic treatments, are typically efficient and produce high quality extracts but remain expensive when compared to other methods (Y. Zheng et al., 2016). Discovering an efficient cell disruption method is important to reach low operating expenses (OPEX) while reaching high product recovery and high-quality extraction products (Günerken et al., 2015).

### 1.3.3 Extracting the released components

After cell disruption the released intracellular components can be extracted from the cellular solution. The sequence in which the target compounds are extracted in a biorefinery significantly influences the product yields (Sadukha et al., 2023). Thus, extracting the products of the highest value must be prioritised in the extraction chain, in accordance with the biorefinery value triangle (Figure 1).

### 1.3.4 Preparing products for sale

Most often the extracted products must be purified for sales and concentrated for efficient distribution. The degree of purity depends on application requirements. Each additional

purification steps increase the production cost and hence the product should not be purified beyond necessarily specifications to minimise operation costs, although each purification step increases product specificity. (Quiroz-Arita et al., 2022.) Most purification methods require product concentration for which membrane filtration is a sustainable and economically feasible method at an industrial scale (Bleakley & Hayes, 2017). The membranes are available in various pore sizes (micro, ultra, nano and reverse osmosis) enabling separation across a range of purity levels. They are particularly suitable for concentrating and purification of protein and peptides. (Quiroz-Arita et al., 2022.)

Different chromatographic methods can be used to purify almost all compounds found from microalgae (Barkia et al., 2019): Ion exchange chromatography can be used to purify any molecules that ionize such as peptides and nucleic acids (Corrêa et al., 2020); Affinity chromatography is the most selective, but it requires designing of the chromatography matrix; Reverse phase high performance liquid chromatography (RP-HPLC) is considered the most efficient chromatographic method for purifying peptides. (Barkia et al., 2019.) Two- and three-phase separation offer optimisation of operating conditions to purify pigments, proteins and amino acids. Additionally, electro-assisted processes can increase the protein yield by almost 20 %. (Corrêa et al., 2020.)

Product concentration is commonly achieved by evaporating the solvent. Concentrated products are more valuable to the customer and easier to distribute than ones in solution. There are multiple ways of drying the product with or without thermal treatment such as spray drying and freeze drying. The drying methods vary in energy and time consumption. Each technique differs in time and energy requirements, making it necessary to balance processing speed with cost efficiency when selecting the most suitable approach. (Vázquez-Romero et al., 2022.)

## **1.4 Commercial microalgae production**

### **1.4.1 Use of microalgal compounds in various industries**

Microalgal compounds can be utilised in various industries from pharmaceuticals to industrial compounds and semiconductors (Karan et al., 2023; Safi et al., 2017) as well as whole-cell products. The most common applications of these compounds are as dietary supplements, feed and cosmetics, but their value as biostimulants and vegan protein has been recognised

(Rumin et al., 2021). In addition, microalgae can also be used for CO<sub>2</sub> sequestration i.e. valorising the emissions of industrial processes into high-value biomass (Vázquez-Romero et al., 2022). Simultaneously the process reduces the climate impact of the selected industry and adds value to the stakeholders.

The wide range of microalgal applications make it important to define the target use of the cultivated biomass and its derivatives. This is due to the differing purity requirements and production standards across industries (Quiroz-Arita et al., 2022). For example, dietary products must be food grade, whereas pharmaceutical applications demand an extremely high purity (Barkia et al., 2019).

Equally important is the selection of microalgal species, since only a limited are approved for commercial applications in the European Atlantic Area (Rumin et al., 2021). Some species like *Chlorella* are generally recognised as safe (GRAS) by the United States Food and Drug Administration and allowing them to be used for a wide range of purposes including food. In Europe, however, commercialisation is complicated by the Novel Food Regulation (*Regulation (EU) 2015/2283*), which restricts the use of certain microalgal strains and purified compounds, in addition to other food-related legislations and limitations (Klein et al., 2023; Slegers et al., 2020). The Novel Food Regulation ensures that all food products in the EU are safe for consumers, leading to extensive investigations for products entering the market after May 15<sup>th</sup> 1997 (*Regulation (EU) 2015/2283*). The EU legislation also places limitations on the commercial use of genetically modified organisms (GMOs), making it essential to select a native strain to avoid regulatory barriers. The legislation concerning microalgae as both a food product and in other industries, however, is not unequivocal and is according to Rumin et al. (2021) “the most critical obstacle limiting the industrial development of microalgae”. For example, if the produced microalgae product is considered a novel food, its approval costs several hundreds of thousands of euros and takes on average two years (Rumin et al., 2021).

Cultivating food approved strains opens the doors for many other applications, since the strain has been recognised safe for humans. For example, in feed, the safety of the whole food chain is evaluated (*Regulation (EU) 2017/625*), at the top of which humans are. Microalgae are most often used in feed for their favourable fatty acid composition, especially the high concentration of polyunsaturated fatty acids (PUFAs). The fatty acids are supplied to animals

either as whole-cell meal or extracted fatty acids are added to other meal form (Kratzer & Murkovic, 2021).

However, the extraction products of non-food approved strains might hold value in cosmetics and pharmaceuticals. These bioactive compounds can originate from various strains and the use of genetic engineering fosters this development. (Nethravathy et al., 2019.)

For fuel productions, microalgae strains with high lipid content are selected. Microalgal carbohydrates may also be fermented into hydroxylic fuels and hydrogen gas. (Show et al., 2017).

#### 1.4.2 Scaling up microalgae biorefinery downstream operations

Scaling up a production is very capital intensive and typically requires external investments and the support of stakeholders. Planning the refining chain carefully in advance, according to the composition of the biomass, facilitates the upscaling process. When the refining chain is well designed and justified, stakeholders may support the scaling up decision with more ease.

Upscaling is often the stumbling block of the microalgae industry, which is why it must be approached systematically with a strong justification (Dutta et al., 2025; Ruiz et al., 2016). New technologies that promote this industry and improve economic feasibility have emerged continuously throughout the past years (Shitanaka et al., 2024). These technological advances continuously increase the TRL of microalgal biorefineries, thereby enhancing the probability of commercial success.

#### 1.4.3 Techno-economic bottlenecks in commercialising microalgae products

Despite the prospects of the microalgal industry, relatively few products have been fully commercialised. This is due to high production costs, technological limitations and variation in biomass composition, all of which restrict the expansion of the sector (Ruiz et al., 2016). In addition, significant gaps in scientific knowledge and expertise in large-scale microalgae production (Araújo et al., 2021; Rumin et al., 2021) alongside regulatory challenges (Rumin et al., 2021) all limit the progression of the industry.

One of the biggest obstacles for commercialisation of microalgal products remains the high production costs. The costs are derived from both the cultivation phase as well as the



biorefining of microalgal biomass. Furthermore, the technology readiness level (TRL) for different microalgal products varies, which has to be taken into account when selecting the target compounds. (Quiroz-Arita et al., 2022.)

Also, establishing the production costs of different products is difficult (Vázquez-Romero et al., 2022), which slows down strategic decision making. Yet, both the biomass production and downstream processing contribute independently in the value chain (Vazquez & Sanchez, 2022) and value created in each must be assessed separately.

#### 1.4.4 Contribution of commercial microalgae solutions to the Sustainable Development Goals

Microalgae have been recognised as one of the most sustainable process feedstocks (Ruiz et al., 2016). According to Solarte-Toro & Cardona Alzate (2021), biorefineries contribute either directly or indirectly to 8 out of the 17 SDGs set by the UN (Figure 2). The direct impacts are based on the rapid growth of microalgae and their high energy content that can be made use of as food or fuel. The indirect impacts are related to increased and better employment opportunities. In addition, microalgae support the SDGs 11, 13, 14, and 15, sustainable cities and communities, climate action, life below water and life on land, respectively (Figure 2). This is due to their autotrophic metabolism, which utilises atmospheric carbon dioxide as a carbon source. With their natural metabolism, microalgae sequester a strong GHG and reduce pollution in cities and nature. (Olabi et al., 2023.)

Although algae-based production is not yet efficient enough for representing the most environmentally sustainable alternative, the socio-economic impacts are significant. The social problems related to conventional oil and protein sources caused by human actions caused by social unsustainability, including overfishing and deforestation caused by social unsustainability, could be solved by at least partly by replacing such resources with algae derived alternatives. (*Multi-Product Integrated bioRefinery of Algae*). This would reduce the exploitation of those resources and provide more just employment opportunities.

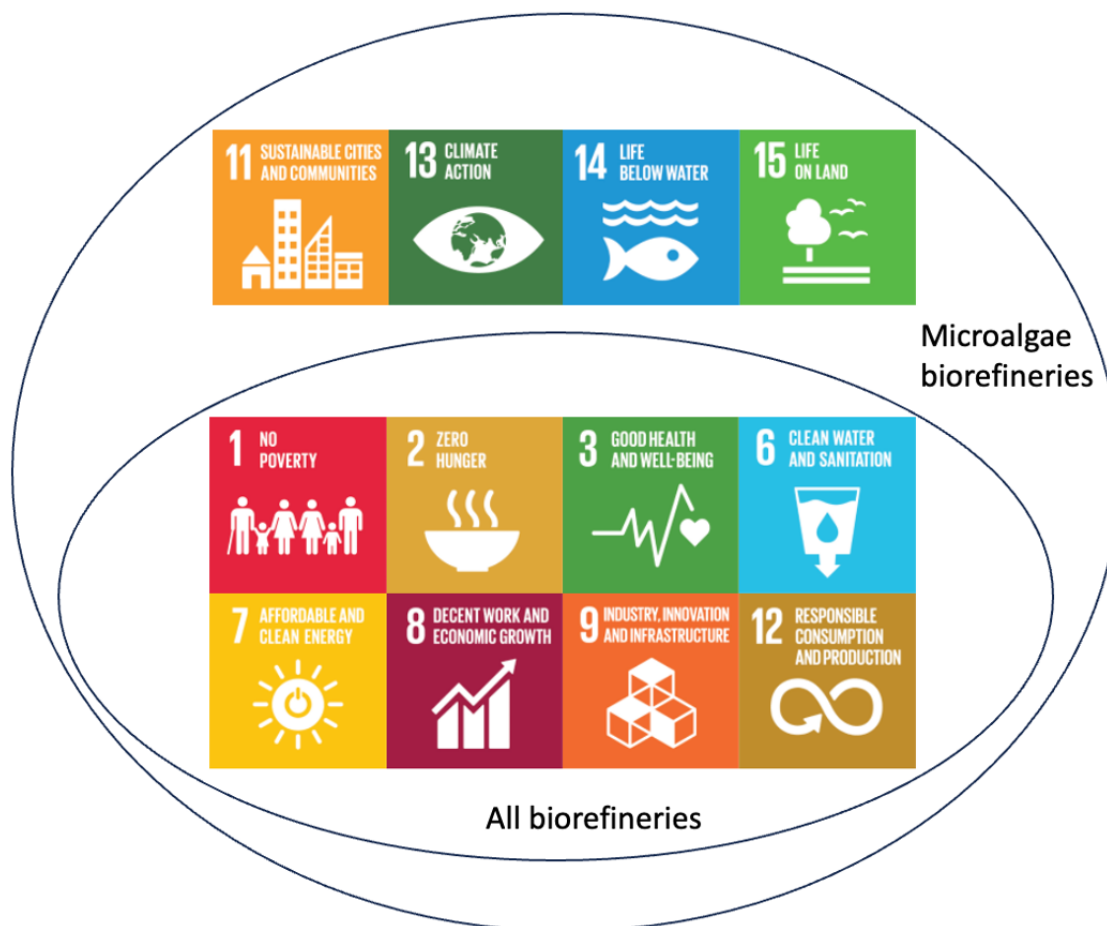


Figure 2. The sustainable development goals that biorefineries and microalgae biorefineries help accomplish. (Olabi et al., 2023; Solarte-Toro & Cardona Alzate, 2021.)

The environmental sustainability of microalgae production, however, is not uncomplicated. Bioreactors consume a considerable amount of electricity and building the necessary infrastructure for commercial microalgae biorefineries has a significant environmental impact. Most data about the environmental impacts originate from laboratory scale experiments and the scopes, the methods and the presentation of the results of the studies vary making it difficult to form a uniform judgement. (Gurreri et al., 2023.) Microalgal products are often marketed with overoptimistic sustainability claims, yet they provide true opportunities for sustainable business, if the business model is well planned and executed. Microalgae can be harnessed into dematerialising business models, in which value is created out of waste streams and circulated within a system. These foster the opportunity for nature regeneration, even if electricity consumption remains high. (Pakseresht et al., 2025.)

#### 1.4.5 Aircohol – Cheers, planet!

Aircohol Oy is a biotechnology company, focused on providing green solutions, with its primary application in the alcohol industry. Alcohol is produced by the anaerobic fermentative metabolism of heterotrophic yeasts, most often *Saccharomyces cerevisiae*. Their metabolism produces a significant amount of CO<sub>2</sub>, which is not considered in carbon emission calculations, because it originates from a biogenic source (Cordova et al., 2025). Hence, these emissions are not part of the global carbon market and sequestering it does not bring direct value for the customer company. Instead, reducing the climate impact of beverages adds value for the final consumer. Also, by mitigating emissions from own processes, the customer company demonstrates voluntary environmental responsibility.

The production of Aircohol's biomass is based on the principles of the circular economy. The CO<sub>2</sub> generated in their customers' fermentation process is valorised in Aircohol's system, where it is converted into fermentable sugars. This process simultaneously reduces both the carbon impact and the environmental footprint of the customer company, as some of the fermentable sugars used in breweries are produced in closed systems with zero environmental impacts.

Since the produced biomass only partially consists of fermentable sugars, some side streams are in turn created. To minimise final waste, also the residual biomass should be valorised as commercial products. As the goal of any company is to make profit, the biomass composition enabling the creation of the greatest value should be identified.

The company is currently at the verge of scaling up its production and to make strategic decisions regarding target markets. Using strains from the genera *Chlorella* and *Scenedesmus*, the company holds a broad product portfolio. They both are eukaryotic microalgae from the division of *Chlorophytae*. Both are considered GRAS and are not novel food organisms in the EU. Only one strain of *Chlorella* was used in this study, but two strains of *Scenedesmus* were examined. Due to the controversial history of the microalgae taxonomy, the other *Scenedesmus* strain has been named *Coelastrella*. Despite this differing naming, the strain *Coelastrella* is also GRAS and not a novel food. Both *Chlorella vulgaris* and *Scenedesmus obliquus* have been previously used for producing various compounds for different industries (Gallego et al., 2025) and are approved as valid raw materials.

Aircohol aims to operate on a business-to-business (B2B) model, in which microalgal products are to be refined into high value industrial raw materials for other companies. B2B approach allows the company to provide high value microalgae compounds without having to manufacture consumer products. Thus, the produced compounds may be used in multiple industries without the company having to invest in machineries or familiarise itself with varying industry-specific legislations in depth.

## **1.5 Economic feasibility of a biorefinery**

These target compounds set the product portfolio and hence the scope for the decision support analysis (DSA) and its sub-analyses, the market analysis and TEA.

The target biorefinery compounds of this study are protein, pigments and fermentable sugars, which are quantified from each cultivation. This allows the comparison between different cultivations. The data acquired is quantitative but does not tell the ultimate quantities of each product in each cultivation. Yet, it is sufficient comparative purposes and for supporting strategic decision making and techno-economic comparison.

### **1.5.1 Evaluating the minimum selling price with techno-economic assessment**

A TEA is used to evaluate the production efficiency of a process or a product. The efficiency is defined according to the product yields and production costs within a defined timeframe. The costs consist of the capital expenditures (CAPEX) and OPEXs of the production line. TEA is a useful tool to define the minimum selling price (MSP), the lowest price with which the product can be sold to break even and to compare the financial differences between production technologies.

Systematic up-scaling improves the probability of long-term success. While formulating the plan, both the limitations set by the biomass composition and the economic feasibility of technologies required for refining must be considered. Importantly, it is not practical to refine even the highest value product if its market is saturated or its access to suitable customers is limited. Therefore, conducting a market analysis plays a critical role in determining the actual product value of the microalgae products on the markets, identifying whether paying customers exist and evaluating the competitiveness of the production system.

The result of the market analysis, however, is the price that customers are willing to pay (WTP) for the product. If the MSP exceeds the WTP, the production is not profitable (Eq. 1). To avoid unnecessary capital costs and technological investments, this investigation must be conducted beforehand.

$$WTP - MSP > 0 \quad (1)$$

The most reliable results of TEA are obtained when primary data is used in the analysis. Primary data refers to direct measured values from a technology in operation. However, this is not possible when the technologies or production lines are not yet implemented, comparisons must rely on secondary data. This data may come from existing databases, suppliers or downstream actors. While less accurate, secondary data can provide meaningful insights for indicative decision making. (Michael Z. Hauschild et al., 2018) In this study, secondary data is used to compare the economic efficiencies between biorefinery methods and technologies to support future strategic decision making. Uncertainties are always included when using secondary data because they are estimated values. Hence, a sensitivity analysis should be conducted to evaluate the magnitude of the uncertainty and take it into consideration in decision making.

### 1.5.2 Defining the market value of microalgae components

To produce maximum profit from the biomass, the production costs of target products should be as low as possible and the selling price as high as possible. This maximises the profit margin. To define the actual selling price of a product, its potential on the markets should be investigated in theory and in practice. First it is investigated, which products could be derived from the cultivated microalgae biomass. Since microalgae are novel raw material in most, established markets are limited, and therefore the industries should be prioritised according to their market potential (European Commission, 2022b). Market analysis is used to determine the market sizes of microalgae and different products derived from them. Market analysis studies market sizes and future development of those markets. It is an essential tool when a company is planning on launching a new product. Selecting the correct market is crucial for successful product launch and market penetration (Moore, 2007). At this stage of the company's development, the product portfolio can be optimised relatively easily according to

the results of the market analysis. In contrast, established companies may also use market analysis, but adapting their existing production line is typically more costly. Thus, early stages of development are the most optimal for market analysis, because no capital has been invested yet.

All techno-economic results are used for DSA. This analysis gathers relevant information and defines the parameters that affect the economic feasibility of a business. Analysis results help to design and optimise microalgae refining strategies according to market demand and techno-economic feasibility (Rumin et al., 2021).

### 1.5.3 Maximising biorefinery value with a decision support analysis

By combining the results from the market analysis and TEA, the most profitable target compound can be identified in relation to the biomass composition. Since there are different biomass composition options available in the cultivation phase, the most profitable cultivation method can be selected according to TEA and market analysis results.

All techno-economic data is compiled into a decision support system (DSS) which can be used to compare different cultivations, technologies and market values. The information flow of the DSS is depicted in Figure 3. In this DSS primary and secondary data is mixed. All data may be updated and secondary data replaced with primary data, improving accuracy over time. The DSA framework supports both strategic and tactical decision making when either the market demand or technologies change to ensure maximal profit.

DSA is valuable for companies of all sizes, especially when determining new production strategies (Kinnunen et al., 2011). The purpose of decision support is to provide decision-makers with structured information on the parameters that influence the strategic outcomes (Bohanec, 2003). Related terms such as *Business Analysis* and *Business Intelligence* are also used in the same context with decision support (Arnott & Pervan, 2014). Based on DSA a highly automated DSS may be compiled with a spreadsheet software, commercial decision support software or object-oriented programming. The DSA in this study aims to answer two research questions “Which products should be produced in the current market situation to gain maximum profit?” and “How should this product be refined from the microalgae

biomass?”. The outcome of the DSA is intended to be used as a support for future production decision-making, and it may serve as the foundation for developing a more advanced DSS.

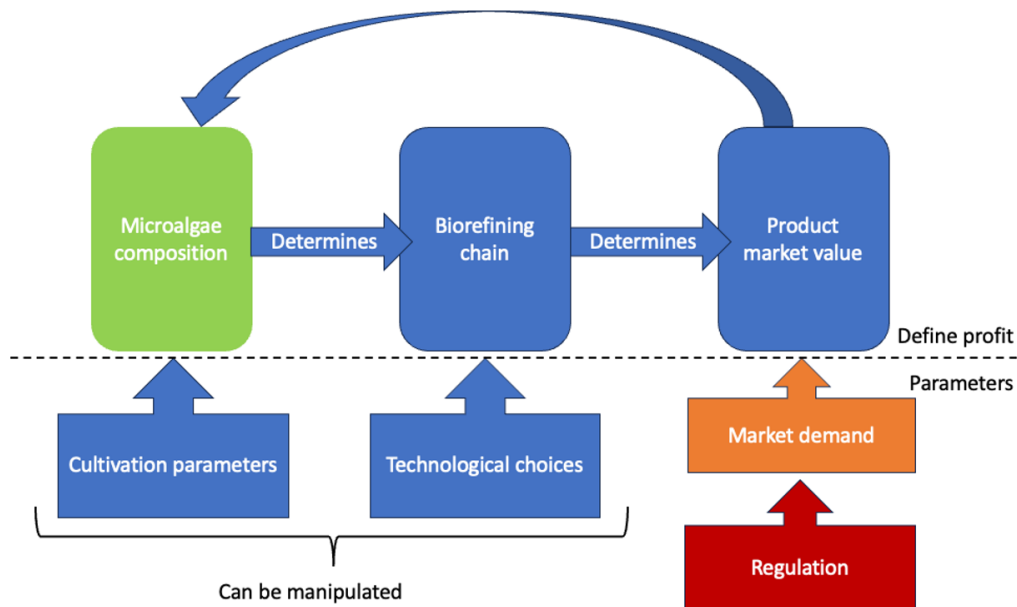


Figure 3. Information flow chart that describes how the data in the decision support system is related to each other. Market demand dictates the cultivation parameters and technological choices that should be made to maximise value created by a biorefinery.

## 1.6 Aims of the thesis

The aim of the thesis is to create a DSA based on Aircohol’s process that can also be used as part of their strategic decision making. This tool will enable the company to identify the most economically profitable large-scale refining chains under varying microalgae biomass compositions and dynamic market conditions, which has been the struggle in the microalgae industry. To reach this goal the following steps must be taken:

- 1) Quantify and compare the compositions of five microalgae cultivations.
- 2) Investigate which products could be refined from the produced biomass.
- 3) Assess what are the market prices of these products.
- 4) Evaluate, which methods are the most economic to produce the products of interest.

## 2 Materials and methods

### 2.1 Laboratory tests on microalgal biomass

In this study, both microbiological laboratory work and a literature review-based TEA were conducted. In the laboratory, the pigment, protein and fermentable sugar contents of five different microalgal samples were measured. The three different microalgae strains of the subject were *Chlorella vulgaris*, *Scenedesmus vacuolatus* and *Coelastrella vacuolata*. The cultivation conditions of these cultures are listed in Table 1.

The final cultivation is performed in a commercial 1250 L vertical photobioreactor (PBR) (Industrial Plankton) with continuous illumination at temperatures between 23 and 28 °C and pH 6.50-7.00. Harvested samples were stored at -18 °C freezer in approximately 2 L blocks and subsamples for analysis were thawed in water bath at  $\leq 35$  °C. The phosphate and nitrate contents were measured from the growth medium spectrophotometrically: phosphate at 690 nm using Spectroquant Phosphate (Merck) and nitrate at 220 nm.

The impact of nutrient source and harvesting method was further investigated for *Chlorella*. Two harvesting methods were compared: Vibro-I 7.5-20 m<sup>2</sup> Series 1 membrane filter (SANI membranes) and flocculation using chitosan. The flocculation was tested on two *Chlorella* cultivations, one which nutrient medium was commercial JWP (Varicon aqua) and another with a JWP-YARA nutrient mixture.

Table 1. Microalgae growth conditions and harvesting methods.

The microalgae strains that were investigated in this study as well as their cultivation conditions and harvesting methods. The nitrate and phosphate concentrations were measured from the growth medium just before harvesting. The Phosphate and nitrate contents were measured from the growth medium with Spectroquant Phosphate kit (Merck) and spectrophotometrically at 220 nm, respectively.



Strain	<i>Chlorella vulgaris</i>	<i>Chlorella vulgaris</i>	<i>Chlorella vulgaris</i>	<i>Scenedesmus vacuolatus</i>	<i>Coelastrella</i>
Nutrient	JWP + YARA	JWP	JWP	HP	HP
c(N-NO <sub>3</sub> ) mg/l	159.50	110.87	110.87	89.04	42.16
c(P-PO <sub>4</sub> ) mg/l	46.00	19.00	19.00	13.34	8.72
Cultivation period days	10	9	9	17	13
Dry weight g/l	18.0	35.74	31.4	60.4	33.9
Harvesting method	Flocculation	Vibro-I membrane filter (SANI membranes)	Flocculation	Vibro-I membrane filter (SANI membranes)	Vibro-I membrane filter (SANI membranes)

### 2.1.1 Cell composition data

Detailed compositional data of *Chlorella* harvested with membrane filter were provided by the company. The analyses were made by two third-party laboratories. It was assumed that the compositions of individual fractions, for example the amino acid composition, remained constant across while the total quantities, like total protein, varied. The datasets included carotenoid, carbohydrate, amino acid and lipid compositions (Figure 5A-D) as well as the overall composition (Figure 4).

The biomass contained only a small portion of lipids (16.67 %DW) and valuable fatty acids (7.58 %DW). Especially PUFAs that composed less than 5 % of the biomass dry weight. However, the cultivation contained a lot of protein. The protein digestibility corrected amino acid score (PDCAAS) of the whole biomass was 0.26. The PDCAAS depicts the amino acid availability in metabolism. Despite this, the biomass contained all nine essential amino acids (Figure 5 C). The biomass also contained four different carotenoids, neoxanthin, zeaxanthin,

$\beta$ -carotene and lutein, from which lutein was the dominant, presenting 65.2 % of the total carotenoids (Figure 5 A).

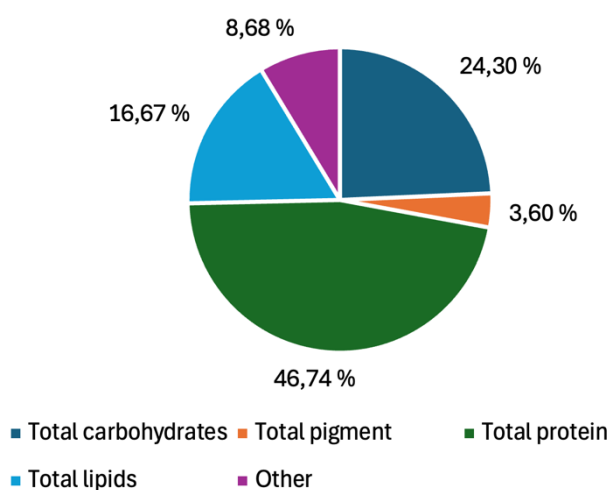
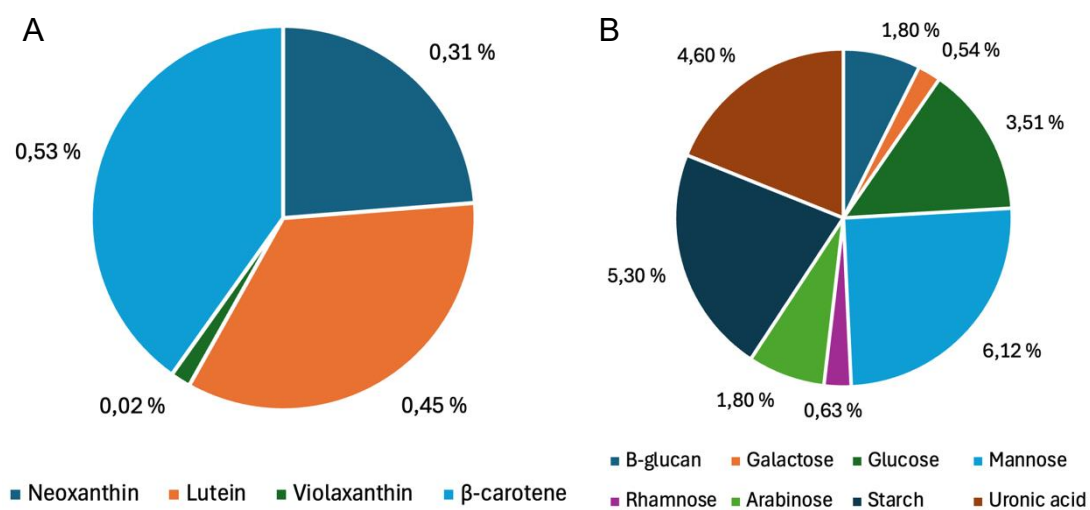


Figure 4. Overall composition of Chlorella biomass cultivated in Aircohol Oy. Values are presented as percentages of fraction weight per cell dry weight. The compositional analyses have been conducted by two third-party laboratories from whose results these charts have been compiled.



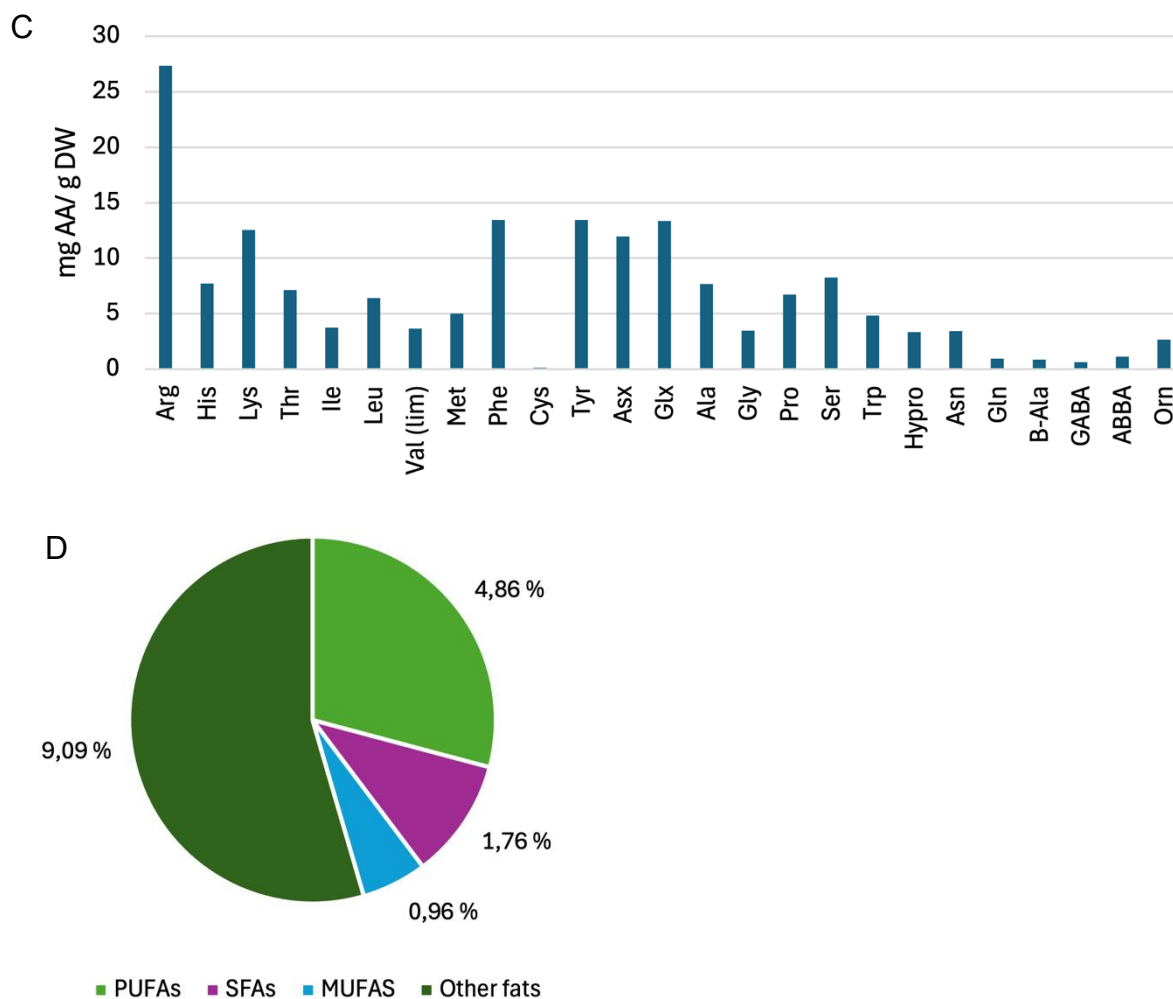


Figure 5 A-D. The A) carotenoid, B) carbohydrate, C) amino-acid and D) lipid compositions of *Chlorella* biomass cultivated in Aircohol Oy. Values are presented as percentages of fraction weight per cell dry weight. The compositional analyses have been conducted by two third-party laboratories from whose results these charts have been compiled.

### 2.1.2 Cultivation density measurements

Both wet and dry weights of each cultivation were determined by loading five replicates of 2 mL samples of each cultivation to weighing boats and dried in 70 °C oven (Termaks) overnight. Sample weights were measured before and after drying with FAS64 analytical precision balance (fisherbrand analytical series).

### 2.1.3 Total pigment protocol development and measurements

Sample volumes between 0.5- and 5-mL were screened to determine a suitable range for quantitative pigment analysis. The samples were centrifuged depending on the sample size either with Rotina 380 (Hettich Zentrifugen) for 10 minutes at 5000 rpm or with Centrifuge

5424 (Eppendorf) for 10 minutes at 14500 g. Pellets were resuspended into 94 % ethanol to a final volume of 10 mL and incubated in room temperature (~25 °C) for 2 hours. Turbidity was reduced by centrifuging the samples again with the Centrifuge 5424 (Eppendorf) for 5 minutes at 14500 g.

The pigments were measured at the wave lengths of 470.0, 649.0, 665.0 and 670.0 nm with Easy-UV spectrophotometer (Mettler Toledo) using quartz cuvettes. Pigment concentrations were calculated according to Lichtenthaler (1987) study (as cited in Kulkarni & Nikolov, 2018), except for using 665 nm instead of 664 nm. Sample dry weights were calculated using previously measured sample water contents.

Protocol was optimised for dense cell cultures by gradually reducing the sample size from 5 mL to 0.5 mL using samples from *Chlorella vulgaris* harvested with the membrane filtration, until the absorbance values fell within the linear reading range of the spectrophotometer.

For *Chlorella vulgaris* cultivated in JWP and collected with flocculation, pipetting, which was done for the other samples, was not possible due to the formation of large floccules. Instead, the samples had to be scooped with a spoon for measurement. The sample weights were measured with FAS64 analytical precision balance (fisherbrand analytical series) to normalise the values to sample weight. Six technical replicates were measured per cultivation.

#### 2.1.4 Total protein protocol development and measurements

Samples were prepared for protein extraction by centrifuging 10 mL samples from each cultivation using Rotina 380 (Hettich Zentrifugen) centrifuge for 10 minutes at 5000 rpm and first washing the pellet with 10 mL of MQ-H<sub>2</sub>O and then resuspending the recentrifuged sample to 35 mL of MQ-H<sub>2</sub>O.

To investigate which method available gave the highest protein yield in this scenario, five cell disruption methods were tested. The cells were disrupted with two osmotic shock treatments for 15 mL samples of 1/10 dilution of previously prepared sample, using 1 M NaOH or 1 M HCl to reach pH 8.5 and 5.0, respectively. Samples were incubated for 3 hours in 50 °C water bath after which they were neutralised back to pH 6.6. The autoclaving experiment was conducted using 200 mL of similar 1/10 dilution and autoclaved for 70 minutes at 121 °C.

For bead milling 1 mL samples of prepared dilutions were centrifuged for 10 minutes at 14500 g with Centrifuge 5424 (Eppendorf). Then 0.6 mm diameter glass beads (Sigma Aldrich) were added approximately in 1:1 (v/v) to the pellet size with 1 mL of ice cold MQ-H<sub>2</sub>O. Bead milling was done by vortexing 3 times for 60 seconds and storing them on cold racks for 2 minutes in between vortex cycles.

Also, the pellets from pigment extraction were used after 1 hour incubation. From the ethanol treatment samples, the effect of effect of pigments on the Bradford method absorbance at 595 nm was measured from pigment extraction samples. The ethanol was washed once by centrifuging the 10 mL pigment samples for 10 minutes at 5000 rpm with Rotina 380 (Hettich Zentrifugen) and resuspending the pellet into 2.5 mL of MQ-H<sub>2</sub>O. The pigmented sample was taken here but to get a non-pigmented sample, the pigments were spun down once more and resuspended into 1 mL of MQ-H<sub>2</sub>O.

Cells were pelleted down from all samples by centrifuging 10 minutes at 14500 g with Centrifuge 5424 (Eppendorf) and the supernatant was used for the protein quantification. All samples were stored in a refrigerator whenever not under handling. The protein content of the samples was determined spectrophotometrically with Thermo Scientific™ Pierce™ Bradford Protein Assay Kit according to its instructions using Easy-UV spectrophotometer (Mettler Toledo) spectrophotometer at 595 nm wavelength. The calibration curve was prepared in duplicate with three replicates each by adjusting the kit protocol to a more suitable working range of 10-200 µg/mL of soluble protein by calculating suitable dilutions of the Bovine Albumin Serum concentrate.

Finally, protein measurements from the subjected samples were done by autoclaving 200 mL of 1:10 dilution of the twice-washed cultures at 121 °C for 1 hour. The dilutions were the same in these as in protocol optimisation, but the original sample size was 15 mL instead of 10 mL. From each cultivation sample, six technical replicates were measured.

### 2.1.5 Total sugar protocol development and measurements

The concentrations of glucose, galactose, fructose and sucrose in the cultivation samples were determined with a HPLC Dionex UltiMate 3000 (Thermo Fisher Scientific). The sugars were released either by two-step acid hydrolysis or enzymatic hydrolysis.

The samples two-step acid hydrolysis started first with 72 % (w/w) sulphuric acid with 1.5 hour incubation in 30 °C water bath followed with treatment with 4 % (w/w) sulphuric acid and autoclaving for 1 hour at 121 °C (Sluiter et al., 2010; Templeton et al., 2012). The samples were neutralised with NaOH to pH 5.5-8.0. The salt that was formed in the neutralisation of the strong acid was removed by using watered ion-removing solid phase extraction packed bed cartridges (Agilent Technologies).

Enzymatic hydrolysis of fermentable sugars was based on previous experiments made in the company by using 4 % (w/w DW) Rapidase Fiber carbohydrase pectinase (DSM) and Saczyme Yield mixture of glucoamylase, acid amylase and cellulase (Novozymes) for samples with pH set to 4.1-4.2 using 1 M HCl. Samples with enzymes were incubated for 2 hours in 47 °C water bath.

All samples were further centrifuged until the supernatant was clear and filtered through 0.45 and 0.22 nm pore size filters into HPLC vials. From each sample, two technical replicates were prepared and each was measured twice.

The chromatography was done using a SP0810 (Shodex) styrene divinylbenzene copolymer column at 80 °C with a guard precolumn. MQ-H<sub>2</sub>O was used as the mobile phase at a flow rate of 0.600 mL/min, and injection volume of 10 µL. Detection was carried out using a refractive index detector (RID) RI-101 (Shodex).

Two standard curves with 1:10, 2:5 and 4:5 dilutions were made using D(-)-Fructose, D(+)-Galactose, D(+)-Sucrose (Thermo Scientific) and D-(+)-Glucose (Sigma Life Science). An additional standard was made and diluted to 2/5 to verify the standard curve.

### 2.1.6 Compiling the total compositions of the studied microalgae strains

The results of total protein, pigment and sugar analyses were used to compare the studied microalgae strains to one another. The results of each analysis were normalised to the highest

results, receiving percentual values. These relative values were used to evaluate the final cellular composition, using the third-party analyses done on *Chlorella* cultivated with a membrane filter (Figures 4 and 5 A-D).

## **2.2 Analysis of the microalgae market**

To gain knowledge about the profit margin that could be created with the cultivated microalgal biomass, a market analysis was conducted. The framework of Kuada (2008) was used as a guideline for selecting the strategy for this market analysis.

### **2.2.1 Market knowledge generation approach**

Market knowledge was generated using a universalistic approach. Both, analytical and etic approaches were applied, generating market knowledge while seeking to discover general behaviour of the market.

### **2.2.2 Defining product scope and value proposition**

The scope of different microalgal products was defined according to the biomass composition of the cultures used. Their attributes like form, purity requirements and potential applications were searched from literature using Web of Science and World Intellectual Property Right databases.

### **2.2.3 Desk research – secondary market data collection**

Secondary data were collected on the current market sizes in which algae-derived products could be sold via extensive desk research. In addition, the historical and projected demand trends in each market were investigated. Information concerning product pricing, upcoming sustainability drivers and regulatory trends were also compiled.

### **2.2.4 Market selection analysis approach**

A systematic approach was taken when selecting the target markets and data was collected and analysed in a formal manner. This market analysis focused on national and Western

markets, as the expansive approach to market selection is generally accepted in the literature as the most effective approach to penetrate new markets. The target markets are prioritised according to their size and how the composition of the cultivated microalgae suit the demands of a market. Also, the compound annual growth rate (CAGR) and regulatory drivers of each market are assessed. Data were obtained from market analytics and literature. All results were normalised to 2024 value by using CAGR. From the five components of market opportunity analysis that are portrayed in Kuada (2008), only the three first are completed here. Also, demand behaviour is analysed using Kuada (2008) as a framework.

### 2.2.5 Customer discovery and willingness to pay

From the results of market segmentation, potential B2B customers were searched for. The potential companies found were prioritised according to their previous use of algae, company size and innovation capacity, relevance of industry, research and development (R&D) orientation and geographic and regulatory advantage (Appendix Table 1).

The potential customers established were contacted via email, phone calls and LinkedIn - communication channel for short interviews in the order of priority. Alternatively, the interviewees could answer questions in writing. Each invitation was tailored to the recipient and included a brief description of algae-based products and how they could fit to their product portfolio. Particular emphasis was given to health and sustainability benefits.

During interviews, the respondents were asked questions about microalgal applications and market potential in their respective industry. Questions and discussions were specified to applications that the composition of the investigated biomass could suit. Missing data were supplemented through desk research, including literature searches, Alibaba.com, and other appropriate sourcing platforms. From the responses, the WTP for different microalgal products in each market was estimated.

## 2.3 Conducting techno-economic assessment

This TEA followed steps similar to life-cycle analysis (LCA), which is an applicable method for conducting a TEA, according to Hauschild et al. (2018). The steps are i) goal definition, ii) scope definition, iii) inventory analysis and iv) impact analysis. A key difference from the ISO



14040 standard for conducting LCAs (*Environmental Management. Life Cycle Assessment. Principles and Framework (ISO 14040:2006)*, 2020, p. 14040) is that in TEA, results are interpreted continuously throughout each step, rather than only in the final phase.

### 2.3.1 Inventory analysis

The inventory analysis for this techno-economic assessment was conducted through a comprehensive literature review. This review was conducted using Web of Science and World Intellectual Property Organisation databases. The review focused on methods that were already in commercial use or intended for large-scale application. The primary aim was to identify processes for refining the products defined in the market analysis. The most used keywords were microalga\*, extraction, isolation, biorefinery, cascade, sequential, economic, valorisation, production and “downstream processing”.

The technological suitability for the used biomass was also assessed. For example, processes made for producing microalgal oil were left out due to their inexpediency for cultivated strains. Data for this analysis were also obtained from literature similarly to the search of biorefinery methods. The equipment prices were estimated for a processing capacity of 500 L of harvested biomass per day. Specific information for commercial prices was gathered from the internet and by requesting quotations from equipment and utility suppliers.

During the inventory analysis all possible microalgae cell disruption and product extraction methods were considered. For each method, the used technologies and other inputs such as chemicals and electricity consumption were found out. Process flow charts were made for each option. Processes that clearly were not applicable in a commercial scale or did not support the biomass composition were excluded.

### 2.3.2 Impact analysis

Based on the results of each inventory analysis, an impact analysis was made for each refinement method. In a TEA impact analysis, the impact category is defined as monetary costs. The inventory analysis results were then further defined into OPEX and CAPEX categories. The impact analysis provided the estimated total costs of each process, which allowed the comparison of financial feasibilities across methods. Those processes that were

tested in laboratory scale, were assumed to perform similarly in larger scale (Deenu et al., 2013).

## 2.4 Decision support system

The decision support analysis tool DSS was compiled in Microsoft Excel® spreadsheet application. The tool consists of multiple input sheets with input values, determining the output values of the system. The inputs contain OPEX and CAPEX parameters for each value chain as well as global input values such as electricity price. The results are automatically obtained via built-in calculation paths, enabling assessing different multi-parameter scenarios. (Vázquez-Romero et al., 2022.) The value chains were designed based on the TEA results. Both whole-cell and multi-product value chains were designed to allow comparison of diverse product portfolios. The comparison between the value chains was done by calculating the estimated profit (Equation 1). The profits for each fraction and value chain were evaluated per kilogram dry weight (Equation 2).

$$Profit \left( \frac{\text{€}}{\text{kg DW}} \right) = (WTP - MSP) * fraction\ yield\ (\%) \quad (2)$$

Investment calculations return on investment (ROI) (Equation 3) was added to the DSA.

$$ROI = \frac{(Income - Investment)}{Investment} \times 100 \quad (3)$$

## 2.5 Use of artificial intelligence

Artificial intelligence model ChatGPT (OpenAI) was used for more efficient information retrieval similarly to any search engine.

## 3 Results and discussion

### 3.1 Protocol development and measurements of microalgae compositions

The first objective was to discover the cellular composition of the five microalgae cultivations. Total soluble protein, total pigments and total fermentable sugars were quantified to allow comparing the cultivations, that were the subject of this study, with each other. First,

the quantification protocols were to be developed, since the company did not yet have established protocols. These laboratory scale experiments were performed to enable comparison between the cultivations rather than to generate primary data for modelling larger scale operations.

The developed protocols were applied to characterise the cellular compositions of the five cultivations. While the results do not necessarily reflect the absolute concentrations of total pigments, proteins and sugars, they are sufficiently precise for comparison purposes. All results were normalised to sample dry weights.

### 3.1.1 Total pigment protocol development and content

Pigment protocol was modified from Lichtenthaler (1987, as cited in (Kulkarni & Nikolov, 2018) to ensure that the absorbance values of the samples fit the linear range of the used spectrophotometer. The sample size of 0.5 mL for the absorbances to be sufficiently low to remain within the linear range of spectrophotometer. In addition, sample turbidity had to be minimised. This meant that cultivations with smaller cell sizes had to be spun down for the biomass to settle down. High turbidity disturbs spectrophotometric reading and distorts the resulting values. For more accurate pigment quantification chromatographic methods could be used, yet it is not necessary for the scope of this study.

The pigment quantities of five microalgae cultivations were measured to identify the most promising cultivation for industrial pigment production. Pigment quantities of examined samples were measured according to the Lichtenthaler (1987 study, as cited in Kulkarni & Nikolov, 2018), dissolving the pellets from a 0.5 mL cultivation sample into 10 mL of 94 % ethanol.

The highest total pigment content was found in *Chlorella*, cultivated with JWP and Yara nutrients (Figure 7). The high pigment content was from due to the high amounts of chlorophylls in the cells, whereas the carotenoid content was not that high compared to the other cultures (Figure 7). Most likely, the increased nutrient availability supported the pigment production (Magyar et al., 2024).

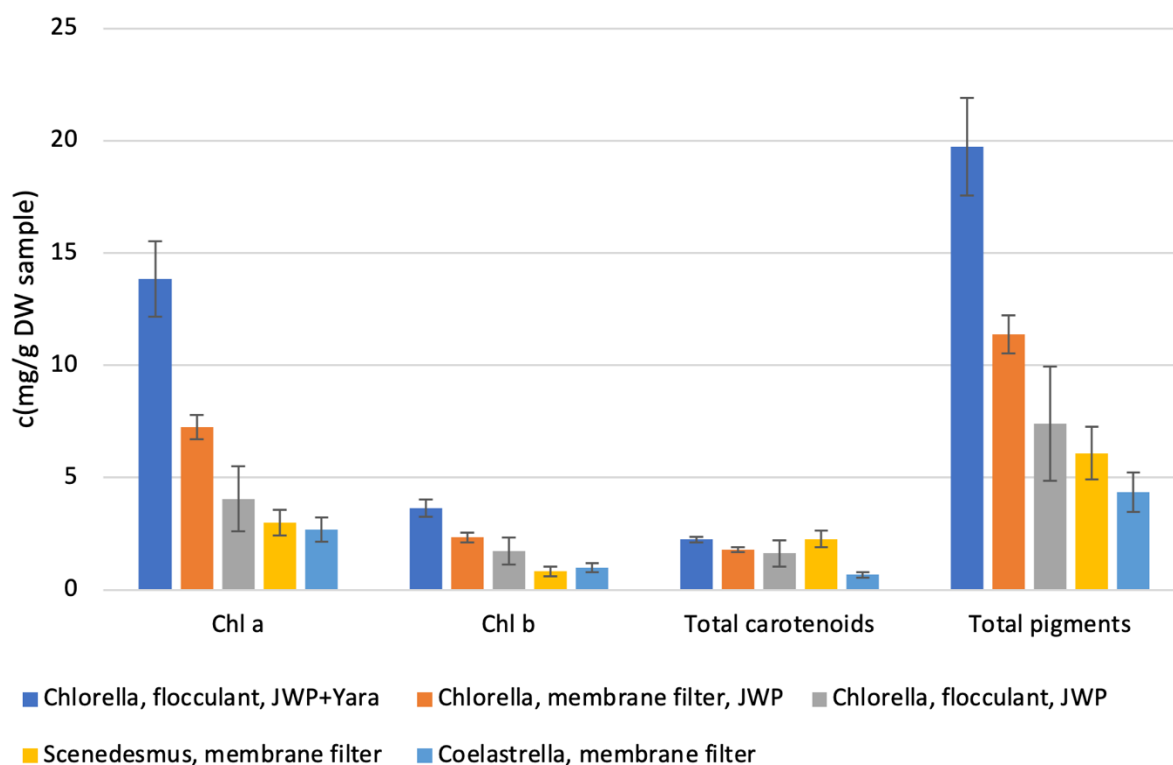


Figure 6. Concentrations of chlorophyll a, b, total carotenoids and total pigments of five microalgae cultures varying in species and strain selection, quality of cultivation, nutrients and harvesting method (Table 1). All cultivations were done in 1000 L photobioreactor, harvested and frozen. Samples were thawed prior to the analysis. For pigment quantification 0.5 mL samples were diluted into 10 mL of 94 % ethanol and incubated for 2 hours. Total pigments were calculated as the sum of chlorophyll a, b and total carotenoids. Error bars depict the standard deviation of the replicates.

### 3.1.2 Total protein protocol development and content

For protein quantification, the intracellular proteins must first be released. Several cell disruption protocols with varying efficiencies were tested to determine which would lead to the highest protein yield. As the protein were quantified with the Bradford method, only the soluble proteins are quantified. It can be assumed that the amino acid compositions are the same in each cultivation, but only the total protein quantity varies. As Bradford method is also spectrophotometric, the impact of the most abundant microalgal pigment chlorophyll was investigated.

Ethanol was used as a one cell disruption method. Its challenge was that simultaneously it released the pigments from the cell to the solution, affecting the spectrophotometric reading. When investigating the influence of chlorophylls to the Bradford method, they increased the

absorbance by 60.97 % compared to samples that had them removed. This verified that green and blue toned pigments should be carefully removed from the samples for protein quantification with the Bradford method. However, after pigment removal, the protein quantity of samples disrupted with ethanol was significantly lower than of other methods (Figure 6). This could be caused by protein denaturation and precipitation caused by the ethanol, leading to a lower amount of soluble protein in water solution. Ethanol, however, should not affect the method, because it was washed off from the samples. Autoclaving the samples lead to the highest soluble protein yield and thus the method was used for cell disruption in comparative measurements (Figure 6).

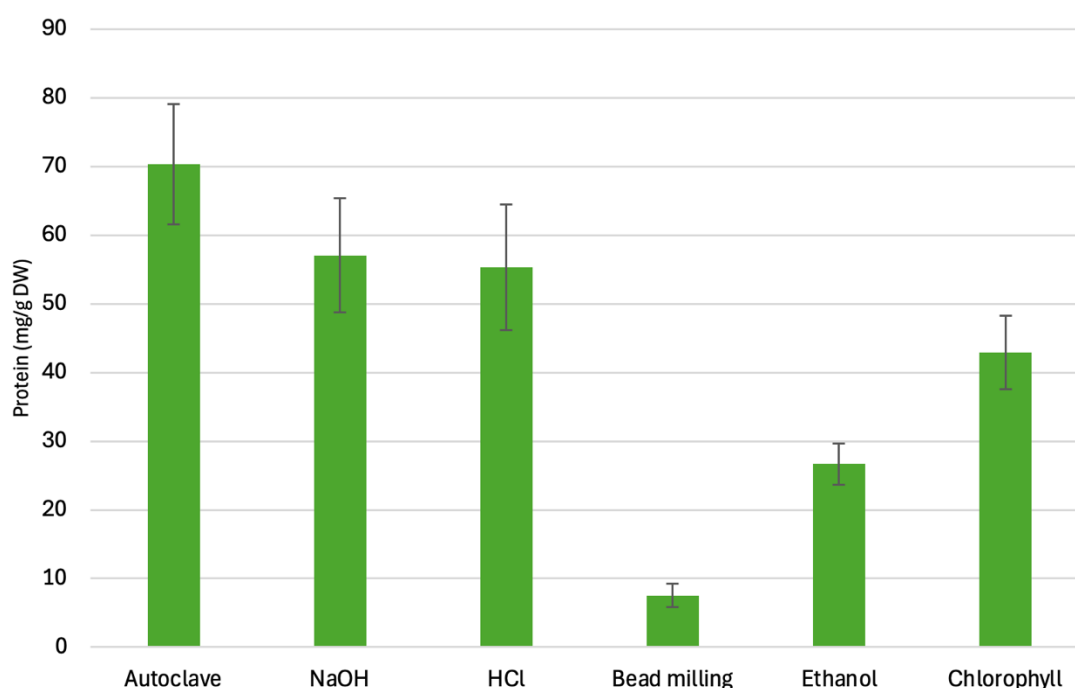


Figure 7. Yields of soluble protein using different cell disruption methods. The tests were conducted using *Chlorella*. Autoclaving was done for 70 minutes in 121 °C, using 1:10 dilution of harvested biomass. 1M NaOH and HCl were used for alkali and acid hydrolysis, to reach pH 8.5 and 5.0, respectively, and incubated for 3 hours in 50 °C, using the same sample dilution as previously. For bead milling, 1 mL on harvested biomass was centrifuged and 1:1 ratio of 0.6 mm glass beads to the pellet volume were added with 1 mL of ice-cold water. The bead milled samples were kept cold and vortexed 3 x 60 seconds with 2-minute breaks between vortex cycles. The 94 %-ethanol treated samples are from pigment extraction and washed once with MQ-H<sub>2</sub>O and diluted into 2.5 ml of MQ-H<sub>2</sub>O. For samples with pigments removed, the 94 %-ethanol treated samples were spun down another time and diluted into 1 mL of MQ-H<sub>2</sub>O. Error bars depict the standard deviation of the replicates.

The examined samples were also subjected to total soluble protein quantification. This was done to discover, in which of the cultivations the most protein is synthesised. The quantitative protein analysis was done by diluting the harvested biomass into MQ-H<sub>2</sub>O in 1:9 ratio and disrupting the cells by autoclaving them for one hour at 121 °C. The Bradford method was

used to quantify protein from the supernatant of centrifuged samples according to Thermo Scientific™ Pierce™ Bradford Protein Assay Kit instructions.

Protein content was highest in *Chlorella* that was cultivated with both JWP and Yara nutrients (Figure 8). Although, this cultivation contained more nitrogen than ones cultivated solely with JWP (Table 1), nitrogen availability was not limiting in either case. This suggests that the form of nitrogen in Yara nutrients may be more favourable for microalgae protein synthesis. Other factors such as nitrogen uptake efficiency or slight differences in growth phase may also have influenced the protein levels.

The results indicate only the amounts of soluble protein and the actual total protein quantities are significantly higher. According to a third-party analysis, the solubility index of the whole biomass is only 21.34 %. In addition, proteins are molecules with limited solubility (Bai et al., 2021), reducing their water solubility index. The actual protein content may thus be over five-fold higher than what these results indicate. Also, because the samples have gone through a thermal treatment, all protein should be in their denatured form. The hydrophobic peptides exposed by this treatment may further decrease the measured values of total protein (Dent et al., 2024). However, it can be generalised that the protein profile in each cultivation is the same, and only the total quantity varies, meaning that the soluble protein quantity is sufficient for comparative analysis.

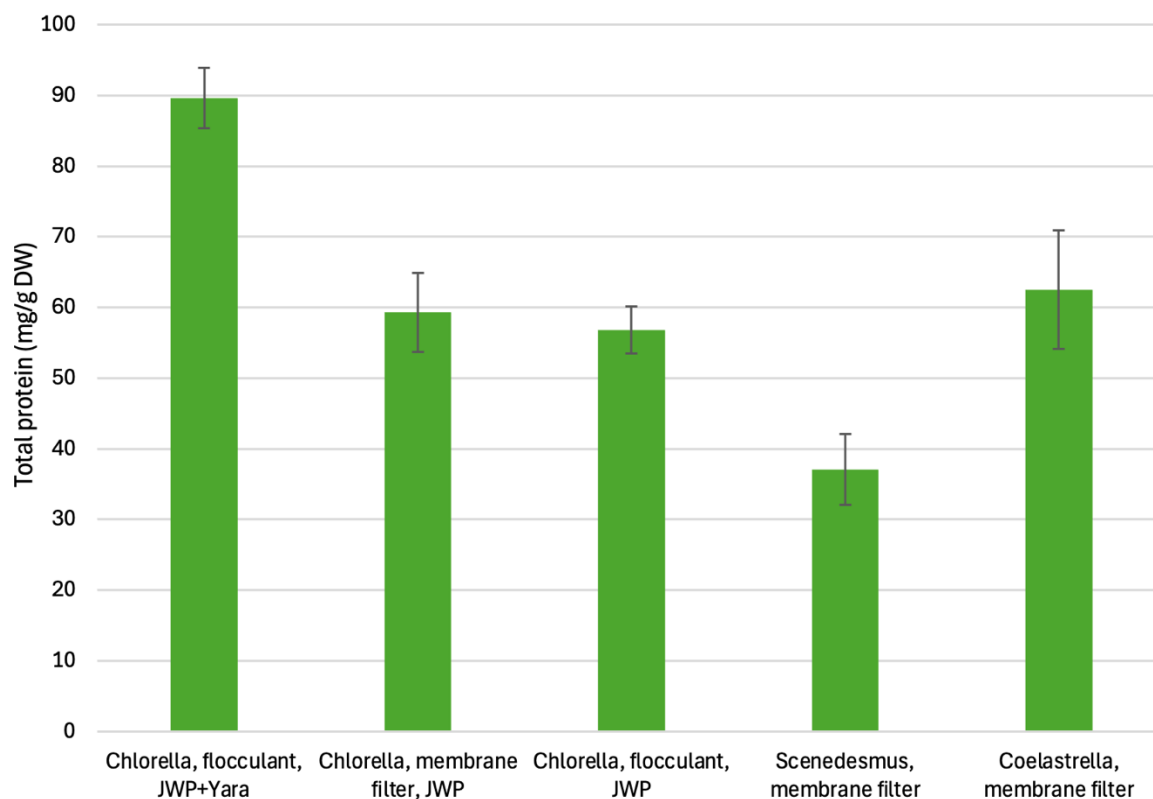


Figure 8. The total protein content of five microalgae cultures varying in species and strain selection, quality of cultivation nutrients and harvesting method (Table 1). All cultivations were done in 1000 L photobioreactor, harvested and frozen. Samples were thawed for analysis. Cells were disrupted by autoclaving for 1 hour at 121 °C 1:10 dilution of harvested microalgae biomass. The protein quantification was performed spectrophotometrically with Bradford method according to Thermo Scientific™ Pierce™ Bradford Protein Assay Kit. Error bars depict the standard deviation of the replicates.

### 3.1.3 Total sugar protocol development and content

Although fermentable sugars are the target compounds of the company, there is not yet established protocol for releasing intracellular polysaccharides and convert them to monosaccharides. Two cell disruption methods were tested to release the sugars into the water solution for quantitative sugar analysis with HPLC: more conventionally used two-step acid hydrolysis (Templeton et al., 2012) and enzymatic hydrolysis. The two-step acid hydrolysis protocol was unsuitable as the samples became too diluted and high salt concentrations formed during acid neutralisation, interfering with the HPLC reading. Ion-exchange cartridges were tested to remove the salt, but it failed to reproducibly remove salts and sugar peaks masked by salt signals in the chromatograms.

By contrast, enzymatic hydrolysis produced clear chromatographic peaks were detected. This method therefore proved more suitable than acid hydrolysis for this study. However, the

company has not yet been able to maximum yields with these enzymes' combinations. In addition, enzymes are more expensive, potentially limiting feasibility at larger scale. For future operations, it would be important to discover the most efficient method for maximising the monosaccharide yield.

Finally, the amounts of fermentable sugars were quantified from the five cultivations for sugar production optimisation. Intracellular polysaccharides were converted into monosaccharides with enzymatic hydrolysis using both fibre and starch degrading enzymes at pH 4.1-4.2 and incubating them for 2 hours in a 47 °C water bath. The enzymes used facilitate degradation of starches and fibres into simpler sugars, meaning these samples should not contain significant amounts of polysaccharides after hydrolysis.

*Chlorella* harvested by membrane filtration contained the highest concentrations of glucose, fructose and total sugars (Figure 9), when normalised with cultivation dry weight. It could be hypothesised that the flocculant binds some of the sugars into itself, reducing the sugar concentration in the solution. As the samples vary only in the harvesting method, the effect of growth phase may be excluded. Because the cultivation harvested with the flocculant had a lower dry weight than one harvested with membrane filter, the difference in dissolved sugar amounts is even larger per cell. The highest amounts of galactose were found from the *Scenedesmus* strains rather than *Chlorella*, which was in accordance with Martins et al. (2025) study, unlike the higher glucose concentrations in *Chlorella* than *Scenedesmus*. The *Chlorella* strains used by the company, however, are selected for their maximal sugar concentration, causing the dissimilarity between results.



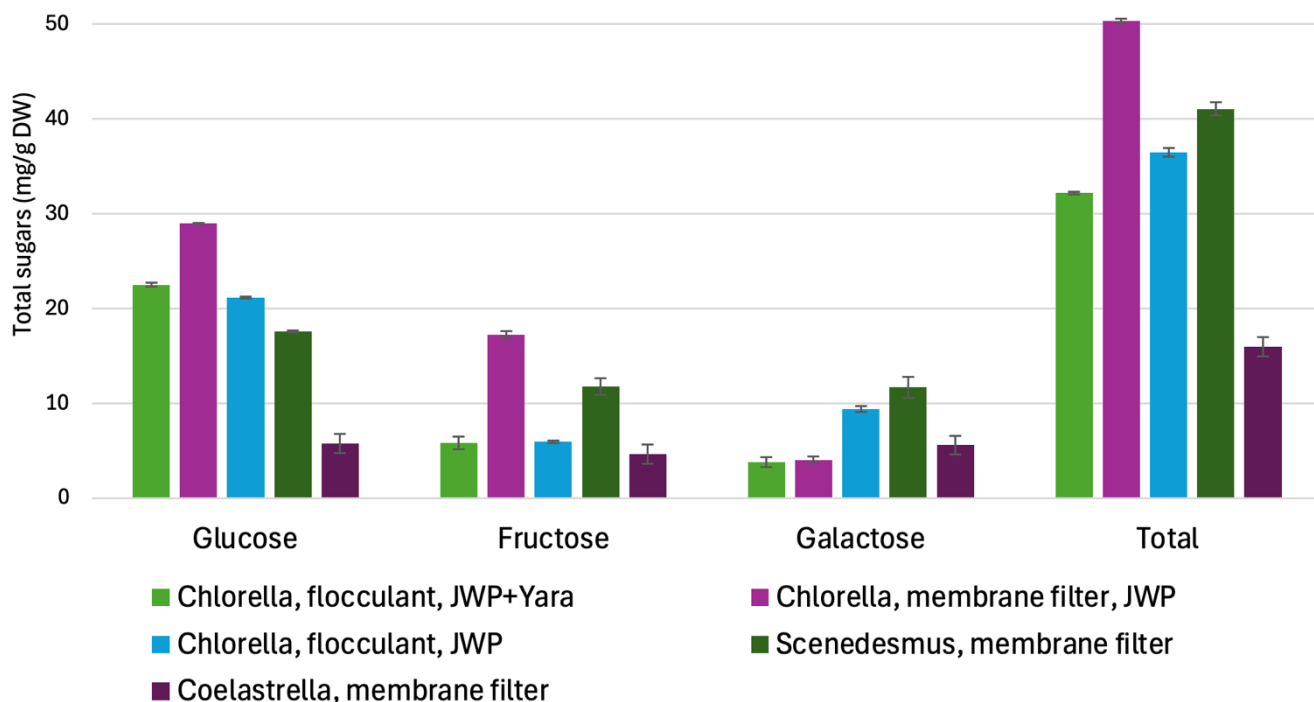


Figure 9. Concentrations of glucose, fructose, galactose and total sugars in five microalgae cultivations with varying microalgae species, strains, cultivation nutrients and harvesting method. All cultivations were performed in 1000 L photobioreactor, harvested and frozen. Samples were thawed for analysis. The samples were lysed with enzymatic hydrolysis using 4 % (w/w) fibre and starch degrading enzymes that also release the resulting monosaccharides to the solution. The samples were incubated for 2 hours at 47 °C in pH 4.1-4.2. Error bars depict the standard deviation of the replicates.

### 3.1.4 Overall composition

The overall composition of the five microalgae cultivations was examined to know determine cultivations should be used for maximal single-product production and total revenue creation with a multi-product biorefinery strategy.

The cultivations vary significantly in their composition (Figure 10 A-D). As hypothesised, nutrient supply, harvesting method, and the choice of species and strain all impact the composition. For maximal protein production, *Chlorella* cultivated with two types of nutrients and harvested with flocculant appeared most suitable. Likewise, for pigment production the *Chlorella* cultivated with two nutrient sources was optimal, while for sugar production *Chlorella* harvested with a membrane filter was the best option. It should be noted that these quantitative protein, pigment and sugar analyses do not reflect absolute concentrations of these compounds and do not account for all components in the biomass. As a result, substantial fractions of the biomass that were undetected in the conducted analyses.

Nevertheless, the cultivation compositions can be compared and results used for further profit analyses.

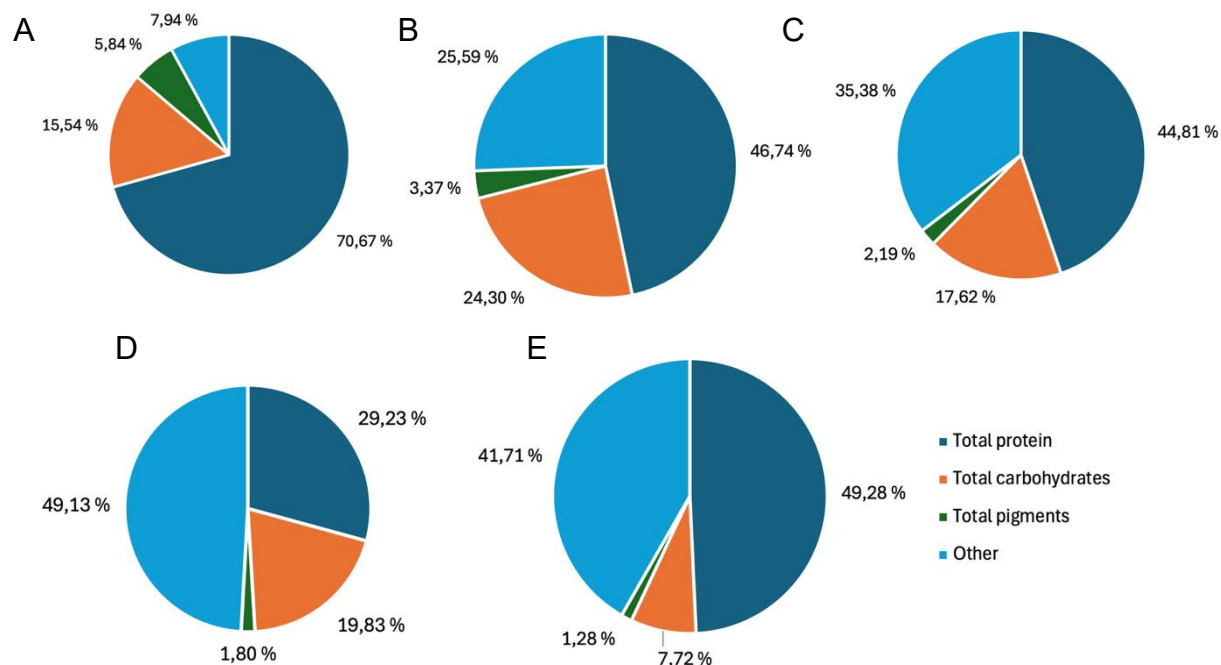


Figure 10. The overall compositions of A) *Chlorella vulgaris* cultivated with JWP and Yara nutrients harvested with flocculation, B) *Chlorella vulgaris* cultivated with JWP and harvested with membrane filter, C) *Chlorella vulgaris* cultivated with JWP and harvested with flocculation, D) *Scenedesmus vacuolatus* and E) *Scenedesmus* strain Coelastrella. All microalgae were cultivated in 1000 L photobioreactor. The pigment and protein concentrations were analysed spectrophotometrically and sugars with HPLC. The protocols used were optimized in this study.

## 3.2 Microalgae markets

### 3.2.1 Existing prominent customer industries

The second objective was to investigate the commercial products in which microalgae could be used. To generate revenue from the cultivated microalgae, the potential industries were identified through a literature review. This was done using a universalistic approach, i.e. investigating markets that the prospective products could fit into rather than targeting the products to a particular market (Figure 4), to suit the microalgae cell composition and the size of the market. The industries, in which compounds derived from microalgae are currently used, were looked for from the literature. Analytical and etic approach was used to study the behaviour of the markets.

Products derived from microalgae can be used in multiple industries. Different industries use different components of the microalgae cells, yet many use multiple microalgal compounds (Table 2). These compounds can be used for various purposes. For example, polysaccharides can be used as active ingredients in cosmetics, for inflammation reduction in feed and for erosion reduction in soil conditioning (Chamizo et al., 2018; Chew et al., 2017; Mendes et al., 2024).

Table 2. Industries in which microalgal compounds are in use.

Different industries in which microalgal compounds are currently used, which compounds are being used in the corresponding industry and for what purposes the compounds are used for.

<b>Industry</b>	<b>Compounds</b>	<b>Purpose of use</b>	<b>Reference</b>
<b>Food</b>	<ul style="list-style-type: none"> <li>- Whole biomass</li> <li>- Protein</li> <li>- Oils</li> <li>- Vitamins and minerals</li> <li>- Pigments</li> <li>- Dietary fibres</li> </ul>	<ul style="list-style-type: none"> <li>- Product fortification</li> <li>- Improvement of nutritional value</li> <li>- Texture manipulation</li> <li>- Dyeing</li> </ul>	(Barkia et al., 2019; Nunes et al., 2024; Quiroz-Arita et al., 2022)
<b>Cosmetics and personal care</b>	<ul style="list-style-type: none"> <li>- Whole biomass</li> <li>- Protein</li> <li>- Oils</li> <li>- Vitamins and minerals</li> <li>- Pigments</li> <li>- Soluble carbohydrates</li> </ul>	<ul style="list-style-type: none"> <li>- UV-block</li> <li>- Anti-age</li> <li>- Inflammation reduction</li> <li>- Dyeing</li> <li>- Texture manipulation</li> </ul>	(Ariede et al., 2017; Nunes et al., 2024)
<b>Animal and aqua feed</b>	<ul style="list-style-type: none"> <li>- Whole biomass</li> <li>- Protein</li> <li>- Oils</li> <li>- Vitamins and minerals</li> <li>- Polysaccharides</li> <li>- Pigments</li> </ul>	<ul style="list-style-type: none"> <li>- Product fortification</li> <li>- Improvement of nutritional value</li> <li>- Inflammation reduction</li> <li>- Immunity improvement</li> </ul>	(Quiroz-Arita et al., 2022)
<b>Soil conditioning and fertilizing</b>	<ul style="list-style-type: none"> <li>- Whole biomass</li> <li>- Exopoly-saccharides</li> </ul>	<ul style="list-style-type: none"> <li>- Biodiversity improvement</li> <li>- Erosion reduction</li> <li>- Soil aeration and structure improvement</li> </ul>	(Chamizo et al., 2018)
<b>Nutraceuticals and dietary supplements</b>	<ul style="list-style-type: none"> <li>- Whole biomass</li> <li>- Protein</li> <li>- Lipids</li> <li>- Vitamins and minerals</li> </ul>	<ul style="list-style-type: none"> <li>- Inflammation reduction</li> <li>- Protein</li> <li>- Essential amino acids</li> <li>- Health improvement</li> <li>- Immunity improvement</li> </ul>	(Barkia et al., 2019; Dutta et al., 2025)
<b>Pharmaceuticals</b>	<ul style="list-style-type: none"> <li>- Pigments</li> <li>- Protein</li> </ul>	<ul style="list-style-type: none"> <li>- Antioxidants</li> <li>- Anti-inflammatory agents</li> </ul>	(Barkia et al., 2019; Nunes et al., 2024)
<b>Bioplastics and biopolymers</b>	<ul style="list-style-type: none"> <li>- Protein</li> </ul>	<ul style="list-style-type: none"> <li>- Polymers</li> </ul>	(Quiroz-Arita et al., 2022)
<b>Electronics</b>	<ul style="list-style-type: none"> <li>- Pigments</li> </ul>	<ul style="list-style-type: none"> <li>- Photovoltaics</li> <li>- Semiconductors</li> </ul>	(Gsänger et al., 2016; Orón-Navar et al., 2021)
<b>Water purification</b>	<ul style="list-style-type: none"> <li>- Whole biomass</li> </ul>	<ul style="list-style-type: none"> <li>- Pollutant accumulation</li> </ul>	(Quiroz-Arita et al., 2022)
<b>Fuels</b>	<ul style="list-style-type: none"> <li>- Oils</li> </ul>	<ul style="list-style-type: none"> <li>- High energy compounds</li> </ul>	(Quiroz-Arita et al., 2022)

### 3.2.2 Prominent industries for cultivated biomass according to its composition

To maximise the probability of successful market entry, suitable markets should be selected (Moore, 2007), which was the third objective of the study. The most prominent markets were selected by using a systematic approach (Kuada, 2008). Market size data were compiled from the many market analysis providers on the internet ([The Business Research Company](#), [IMARC group](#), [Precedence Research](#), [Grand View Research](#), [Straits Research](#), [Fortune Business Insight](#) and [Globe News Wire](#)) accessed in June 2025. From the markets compiled in Table 2, food products, nutraceuticals and dietary supplements, cosmetics and personal care and animal and aqua feed were selected as target markets. These four industries are the greatest in which microalgae products can be utilised both in Europe and globally (Figure 11). In Europe, the largest portion of cultivated or harvested microalgae biomass is used for human food with a relative size of 32 %. The next largest sectors to which microalgae are used for in Europe are nutraceuticals and dietary supplements, cosmetics and personal care and animal feed with 22, 17 and 13 % relative sizes, respectively. These four sectors make up 84 % of all microalgae product target markets. (Gallego et al., 2025.) Also Adarchenko et al. (2024) and Araújo et al. (2021) support the selection of these markets.

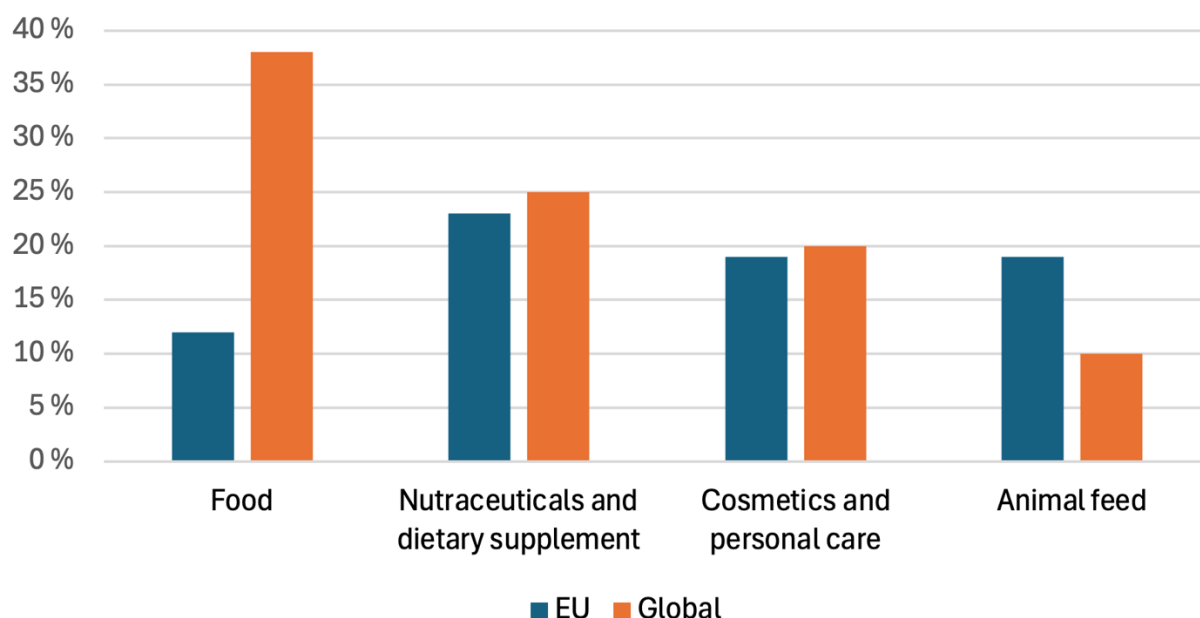


Figure 11. Portions of microalgae product markets per market segment. Data compiled from Gallego et al. (2025) and *Microalgae-Based Products Market Outlook 2025–2033*.

According to Vázquez-Romero et al. (2022), market saturation is not an issue in any of the current markets where microalgae could be adopted. Figure 12 depicts that each selected target market is predicted growth annually for the coming 4 to 10 years.

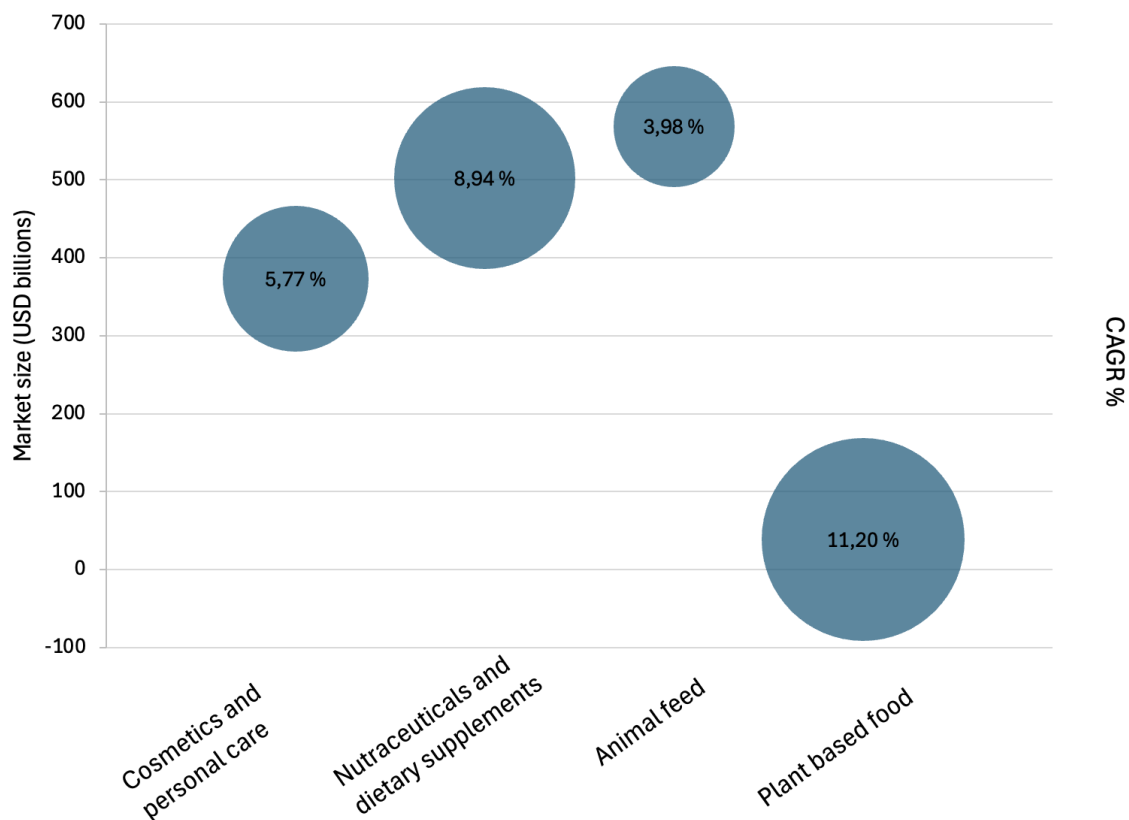


Figure 12. Sizes of markets and their compound annual growth rates. Shows how much money is in the game and how much growth potential is foreseen. Data is compiled from multiple market analysis companies from the years 2022-2025. Market size values were normalized to 2024 using compound annual growth rate. Values were found from [The Business Research Company](#), [IMARC group](#), [Precedence Research](#), [Grand View Research](#), [Straits Research](#), [Fortune Business Insight](#) and [Globe News Wire](#).

When the sizes of the selected markets were studied, but from the perspective of microalgae used, the market sizes were significantly smaller (Figure 13), but they showed only slightly smaller CAGRs, indicating that there is potential in bringing microalgae products on the markets. As CAGR constitutes an indicative projection, it should not be considered a sufficient basis for marketing strategy on its own, although it can be utilised on a broader level.

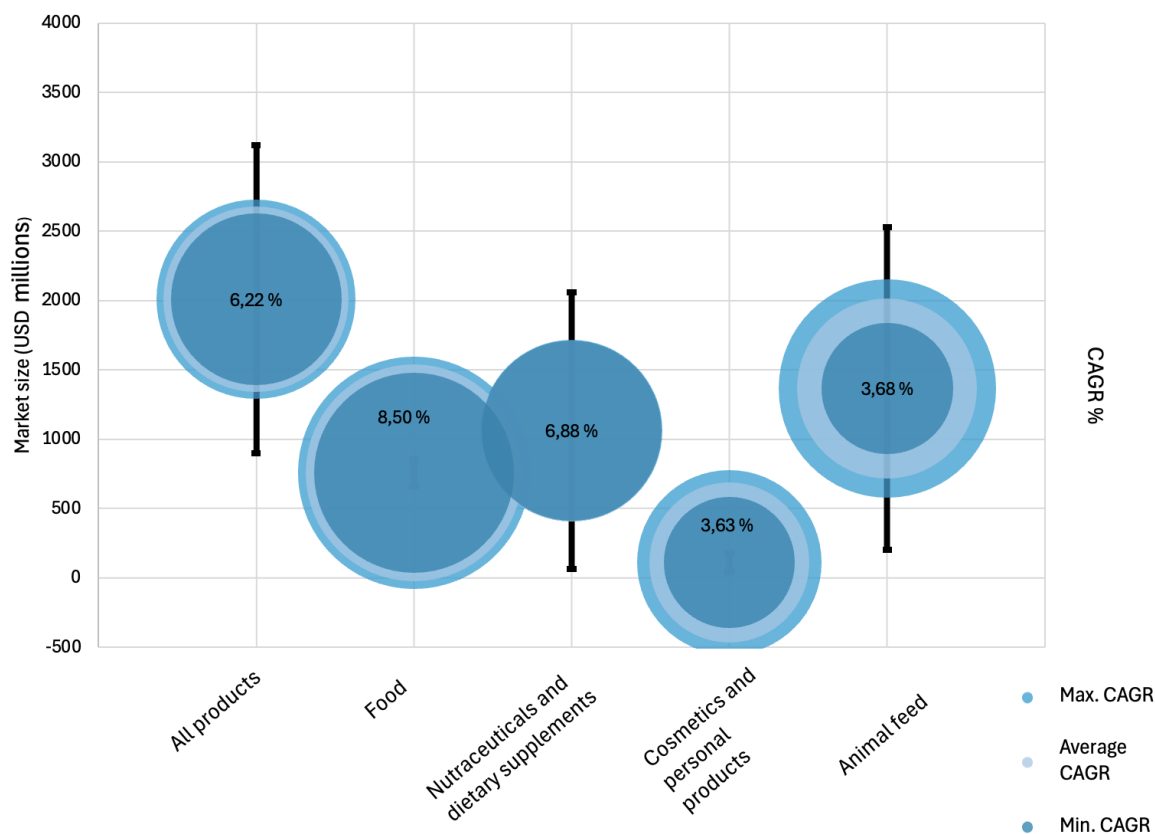


Figure 13. Market sizes microalgae in the most prominent customer industries and the CAGR of each. Market size values are normalized to year 2024 by calculating using the given CAGR. The bubble sizes indicate the compound annual growth rates. Error bars depict the standard deviation of the values found in desk research.

### 3.3.3 Willingness to pay for microalgae products in the proposed markets

The WTP for the produced microalgal compounds was assessed among customers by contacting prioritised B2B customers (prioritisation based on to their previous use of algae, company size and innovation capacity, relevance of industry, R&D orientation and geographic/regulatory advantages (Appendices Table 1). WTP values were also retrieved from the literature. Results were received from six companies and are compiled in Table 3. The results indicate that high protein content is valued, but as a bulk material, it is relatively low value. Companies that are specialised in microalgae products are willing to pay more for microalgae product than others. Active ingredients for cosmetics such as vitamins are of the highest value from investigated sources.



Table 3. WTP for microalgal products.

Product price results from potential customers. Prices are either existing or latent demand.

Industry	Product	Price	Reference
<b>Food</b>	- Protein	10-15 €/kg DW	Confidential
	- Whole biomass	2-10 €/kg DW	
	- High in iron		
<b>Nutraceuticals and dietary supplements</b>	- Whole biomass	20-30 €/kg	Confidential
	- Broken biomass		
<b>Cosmetics and personal care</b>	- Active compounds	20-200 €/kg 2- thousands €/kg	Confidential
	- Texture modifiers		
	- $\beta$ -carotene		
<b>Animal feed</b>	- High-protein low-oil whole biomass	0.37 €/kg Peptides higher	Confidential <i>(Average Prices for Soybeans Worldwide from 2014 to 2026, n.d.)</i>
	- Peptides		

### 3.2.4 Added value created with microalgal products

While establishing the WTP for microalgae products, the added value created by microalgae was investigated. For Finnish companies, local production added value to the product, and it was seen as a significant marketing advantage. For bulk commodities like food and feed, sustainability is yet not an added value, at least of a sort that would increase the customers WTP. However, customers are willing to pay more for sustainably produced higher value products like cosmetics and personal care.

Given the novelty of the microalgal industry, demand in Finland is currently low and primarily latent. Price data exists only for whole-cell products and rest of the prices are estimations. Incipient, future demand most likely exists as more information on the benefits of microalgae on various industries increase as well as sustainability regulations become stricter.

Future demand on microalgae nutraceutical extracts will remain untapped in the EU at least for a while due to regulations (Olabi et al., 2023; Solarte-Toro & Cardona Alzate, 2021).

According to the market analysis interviews, in the United States of America (US) the market demand is different. Customers are more concerned about product traceability and “clean” labelling than in the EU, though the legislation is much more lenient in the US compared to the EU.

In food and feed, the compound functionality is critical (e.g., amino acid profile and digestibility). For example, the amino acid composition and digestibility is significant. Also, shorter peptides are nutritionally more valuable than whole protein. Microalgae compete against other plant proteins on the markets, which is dominated by soybean that sets the baseline for WTP. For food, feed, and dietary supplements buyers often prefer whole biomass, likely to integrate to their established processes. In cosmetics, ready extracted and purified compounds are demanded. For companies in all industries, mitigating their CO<sub>2</sub> emissions may constitute added value for certain market segments that emphasise superior quality and value.

### **3.3 Biorefining target products**

According to the market analysis results, the product portfolio should consist of  $\beta$ -carotene, lutein, colourless and odourless protein extract, high-protein biomass, peptides, and functional polysaccharides. Fortunately, many of these can be extracted in the same biorefinery process sequentially (Figure 14).

At each step, residual biomass is generated. This can also be valorised as a lower value product for feed, compost or at least biogas fermentation, for example. These options create far from maximal profit but do still contribute to the result. Also, the extraction order is critical, as it affects the product yield (Sadukha et al., 2023). Pigments should be extracted first because they are delicate and degrade easily. In this scheme, all pigments are part of the insoluble fraction.

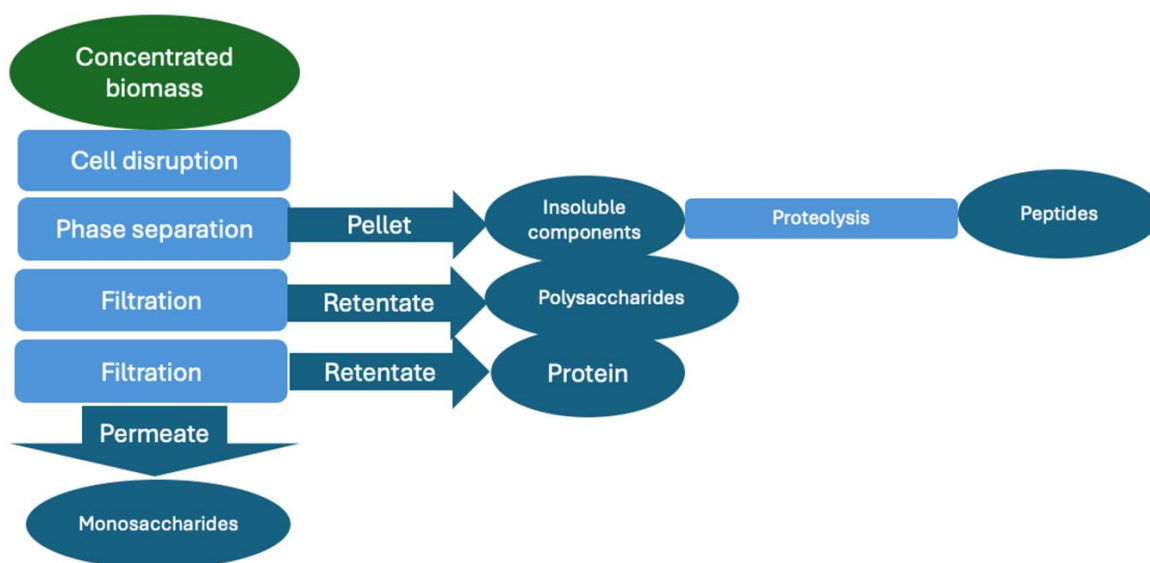


Figure 14. A schematic picture of the basic idea of multi-product downstream processing of microalgae biomass that would complement the biomass composition. Compiled from techno-economic assessments of multiple biorefinery methods (Druon et al., n.d.; Junkui et al., n.d.; Noble et al., n.d.; Reddy et al., 2010; Safi, Olivieri, et al., 2017; Slegers et al., 2020).

### 3.4 Techno economic analysis of biorefinery methods

The fourth object of the study was to investigate the most economically feasible production methods for a microalgae biorefinery. Ten biorefinery methods, that were designed for commercial scale, were found. Techno-economic analyses were conducted of each biorefinery method to evaluate their economic feasibility i.e. profit. In this TEA, the operational costs of existing biorefinery methods were found from literature and patent databases. First the OPEXs of each method was evaluated, and if those were on the profitable side, the CAPEXs were then evaluated.

#### 3.4.1 Goal definition

The goal of this techno-economic assessment was to compare the economic feasibility of different commercial-scale biorefinery methods for the support of the company's strategic decision-making. The goal can be divided into two sub-goals:

- i) define possible biorefinery methods that would be suitable for the company
- ii) evaluate their economic feasibility.

The intended applications of the results are to plan and decide on the used technologies and methods in future process scale up. Yet, this TEA has limitations due to methodological choices as secondary data is used as the basis of the analysis. This data is not necessarily representative for the intended application, which yields to high uncertainties.

The rationale for conducting this study is to identify the most prominent biorefinery methods for scaling up production when establishing an own plant. Although many technologies and methods for operations have been developed, no unequivocal solution has been determined. The microalgae industry has limited successful experiences with large-scale production, and numerous bottlenecks and cost inefficiencies have been noted. In the microalgae industry the variability of the biomass leads to more variance in process requirements (Vázquez-Romero et al., 2022) and thus case-by-case evaluations must be done. Also, scaling up a process usually requires significant investments from external sources. To acquire those, the plan must be well justified for necessary stakeholders. Thus, information, even with higher uncertainties is valuable to initiate the process of scaling up. The functional units used for this comparison are *kg product/operative day*, *€ profit/operative day* and *€ profit/kg biomass/product*.

The target audience of this TEA are both the operative and technology managers and product and process developers who oversee the actual scaleup project as well as the investors who take a monetary risk in start-up industries. Also, other stakeholders such as different authorities value information on process plans. The commissioner of the study was Aircohol Oy and there were no other influential actors in this assessment.

### 3.4.2 Scope definition

As the goal of this TEA is to compare the costs of different biorefinery processes and methods, many factors that are commonly taken within the scope of a TEA were now excluded. Costs that are not dependent on the product or biomass refinement methods were not included. All costs included within and excluded from the scope of this TEA are listed in Table 4. The system boundaries were limited to only the biorefinery processes including cell disruption, extraction and concentration and purification. According to Quiroz-Arita et al. (2022) the stabilisation of resulting protein should be included in the TEA, yet it was out of the scope of this analysis as only the extraction of the products are included.

Table 4. Parameters that are included and excluded into the scope of this TEA.

<b>Included</b>	<b>Excluded</b>
Utilities	Heating/cooling of building
Additives and chemicals	Employment costs – could have an impact if the level of automation
Required refrigeration/heating	Infrastructure costs (building etc.)
Power consumption	Biomass cultivation and harvesting
Product stabilisation	Sewage and waste treatment fee
Water price	Construction, engineering and supervision
Electricity price	Side costs e.g. transfer costs
	Depreciation
	Taxes
	Transportation

Indirect and direct costs that are related to setting up the system such as installation costs and infrastructure are left out of the scope because they are assumed proportional to the purchasing cost (Slegers et al., 2020; Vázquez-Romero et al., 2022). These costs are listed in (Slegers et al., 2020; Vázquez-Romero et al., 2022) supplementary materials.

To evaluate the production costs of these methods, flow rate was evaluated to the best ability. The amount of daily harvested biomass is 500 L, meaning 5 m<sup>3</sup> of cultivation daily with a concentration factor of 10 in harvest. This flow rate is selected because the harvested biomass volumes will be smaller than cultivation volume despite the cultivation method (batch, continuous or semicontinuous), as reaching harvesting density takes longer than one day. This allows using equipment with smaller processing capacities and allocating the CAPEXs for more operational days.

### 3.4.3 Inventory analysis

For inventory analysis, required economic values such as energy consumption or concentration factors were found from the literature. The actual prices of electricity and different chemicals were found from online sources. The CAPEXs of required equipment was obtained through requests for quotations from suppliers and found from the literature. Average prices were calculated using these values. The entities providing these offers are not published because the information is confidential. However, not all CAPEXs were obtained, but these are included in the final economic evaluations of processes. The values for inventory analysis are found in appendices Tables 2 and 3.

### 3.4.4 Impact analysis

The economic impacts of ten commercial scale biorefinery methods (Druon et al., 2016 ; Junkui et al., 2021; Karan et al., 2023; Low et al., 2022; Noble et al., 2020; Reddy et al., 2010; Sadukha et al., 2023; Safi, Olivieri, et al., 2017; Slegers et al., 2020) were evaluated. The functional units were used to assess the chosen methods to get an image of which would be economically feasible for implementation by the company.

Cell disruption is a critical step for releasing microalgal components from the cell, and thus it is important to maximise the yield while minimising the costs. In most of the commercial methods evaluated, physio-mechanical cell disruption methods were utilised, especially high-pressure homogenisation (HPH) and ultrasonication (US). In (Reddy et al., 2010) the biorefinery method of osmotic shock was used for cell disruption. Physio-mechanical methods were generally the less costly alternative.

For selecting the most economically feasible cell disruption methods, HPH, US and bead milling were compared. All these methods have a low selectivity (Martins et al., 2025) and extracting specific components from the cells require separation processes further downstream. The cell disruption methods were taken into more thorough examination because contradictory information was found about the cost efficiencies of these methods (Günerken et al., 2015; Martins et al., 2025; Safi, Cabas Rodriguez, et al., 2017; Safi, Olivieri, et al., 2017; Soto-Sierra et al., 2018). The techno-economic impact of using alkaline protease simultaneously with physio-mechanical cell disruption was also investigated. Martins et al. (2025) and Safi et al. (2017) had contradicting results on the efficiency of using alkaline protease, positive and negative. Yet, the results from Martins et al. (2025) study were selected for this TEA (Tables 5 and 6) because they used *Chlorella* and *Scenedesmus* that are also used in this thesis, whereas Safi et al. (2017) used *Nannochloropsis gaditana*.

Taking into consideration the sugar and protein yields found in Martins et al. (2025) study, US is an economically more feasible cell disruption method than HPH, despite the fact that cooling expenses were not taken in account in HPH costs (Table 5). Cooling expenses would increase both CAPEX and OPEX of HPH. The cooling expenses are included in the US costs. Also bead milling requires cooling, which was also not considered in these calculations (Table 5). Bead milling is a highly efficient cell disruption method, and the CAPEX is relatively low,

yet it includes multiple process parameters that must be optimised. There are also arguments that bead milling is a too efficient cell disruption method, producing extremely fine mixture of the biomass complicating the further refining steps downstream (Günerken et al., 2015; Safi et al., 2017; Soto-Sierra et al., 2018). However, using enzymes will also break down larger structures, creating similar challenges.

Table 5. Comparison between cell disruption methods for *Chlorella*.

US and HPH based on TEA scope which is defined somewhere. Yields of US and HPH from Martins et al. (2025) and BM Postma et al. (2015).

	<b>Ultrasonication</b>	<b>High-pressure homogenisation</b>	<b>Bead milling</b>
CAPEX	51 440 €	301 000 €	98 943 €
CAPEX per day (300 days per year, 15 years)	11 €	67 €	22 €
<b>OPEX</b>			
H <sub>2</sub> O	10	n/a	n/a
H <sub>2</sub> O cost	97.2	n/a	n/a
Energy consumption (10 h operation)	112.65	297.5	415
Energy cost	8.64	22.82	31.83
OPEX total per day	106 €	22.82 €	31.83
<b>Total costs per day</b>	<b>117 €</b>	<b>90 €</b>	<b>54 €</b>
<b>Yields</b>			
Protein yield	71 %	44 %	53%
Carbohydrate yield	62 %	42 %	n/a
<b>Relative costs</b>			
Cost per protein yield	165 €	204 €	102 €
Cost per carbohydrate yield	189 €	214 €	n/a

Martins et al. (2025) reported that alkaline treatment increased yields, when used simultaneously with US or US plus alkaline protease. However, when examining the expenses of using alkali in addition to US, the production costs increased substantially when normalised to the product yields (Table 6). Adding alkali to enzyme treatment improved protein yields only slightly (6 % points), insufficient to cover the costs of the additional treatment. Alkali treatment was also not feasible in Karan et al. (2023) when compared to other methods.

Enzyme addition also increased effect on protein production costs, though it reduced carbohydrate production costs (Table 6). Using a protein degrading enzyme could thus be cost efficient to produce peptides, which have a higher market value. However, for neither the alkali nor the enzyme treatment the costs of treatment vessels were included and only OPEXs were compared. This should not make a difference to their relative process costs, as both treatments require similar incubation vessels and mixing. The operational costs make the difference, as enzyme treatment is longer, temperature incubation, while alkali treatment requires larger volumes (Martins et al., 2025).

Table 6. Evaluation of economic feasibility of alkali or enzyme treatments.

The treatments were done in addition to cell disruption done with ultrasonication. Yields and alkali and enzyme demands retrieved from Martins et al. (2025).

	<b>US + alkali</b>	<b>US + enzyme</b>	<b>US + alkali + enzyme</b>
Ultrasonication total costs	116 €	116 €	116 €
<b>CAPEX</b>			
Incubator	n/a	n/a	n/a
Stirrer	n/a	n/a	n/a
CAPEX per day (300 days per year, 15 years)	n/a	n/a	n/a
<b>OPEX</b>			
Alkali	192.40 €	-	192.40 €
Buffer	-	n/a	n/a
Enzyme	-	21.25 €	21.25 €
Incubation/stirring	19	152	171
Heating	2.83	196.5	199.33
Electricity costs	1.46 €	15.07 €	13.12 €
OPEX total per day	194 €	36 €	227 €
<b>Total costs per day</b>	<b>310 €</b>	<b>152 €</b>	<b>343 €</b>
<b>Yields</b>			
Protein yield	71 %	87 %	93 %
Carbohydrate yield	81 %	83 %	91 %
<b>Relative costs</b>			
Cost per protein yield	436 €	175 €	368 €
Cost per carbohydrate yield	382 €	183 €	375 €
Additional process time (h)	2	16	16

Martins et al. (2025) found cellulase addition ineffective for increasing carbohydrate yield using their *Chlorella*, consistent with its very low polysaccharide content. Using alkaline



protease, however, degrades protein, producing peptides which are more valuable than whole protein.

Centrifuging the disrupted cell biomass can be used for washing the cultivation medium off the product and improving the yield of soluble products. When comparing the techno-economic impacts of 2 and 3 wash-centrifugation steps, the third step increased costs about 40 %, which mostly consisted of water costs, but increased pellet and supernatant yields only 16.7 and 2.3 %, respectively. Hence, a third washing step should not be executed.

For carotenoid extraction, pigments must be separated from the chloroplast-associated photosystem apparatus and then converted to an isolable form via saponification (H. Zheng et al., 2022). Saponifying the whole biomass is not feasible, due to the high amounts of alkali required, even if this step would increase protein and carbohydrate yields. The largest carotenoid yields have been achieved using conventional solvent extraction methods or at least by using a co-solvent in supercritical CO<sub>2</sub> extraction (Low et al., 2022; Molino et al., 2018). Even when using the most simple and inexpensive carotenoid extraction method (12:1 (w/v) acetone) and using the strain with the highest pigment content in this study with impossible 100 % yield, only the OPEXs for this step are too high to be covered with the market value (Table 7). With this method, without CAPEXs or cell disruption costs, the carotenoid content in the cells should be 2.4-6.6 -fold, depending on the used strain, for a break-even situation.

With a high-yielding method by Low et al. (2022), only the OPEXs of mere carotenoid extraction step rise up to 183 €/kg, with rough estimations. According to Deenu et al. (2013) the lowest production cost of microalgal purified lutein is 2300 €/kg, whereas production of the crude product was 36 €/kg, emphasising the cost of purifying carotenoids. For high-value use, like cosmetics, the carotenoids should be isolated from the solvent (Dutta et al., 2025). These costs are not included in the TEA of this study, leaving open the estimated final cost of carotenoid extraction for customer use. Even under optimistic assumptions the MSP of carotenoids is significantly higher than WTP. In addition, cost created by saponification, allocated costs from previous cell disruption steps and CAPEXs for this step increase the realistic costs. Because most of the carotenoid extraction costs are created in the operational phase, carotenoid extraction will not favour economies of scale -impact (Slegers et al., 2020). Because it is not feasible to extract the carotenoids, it does not make sense to saponify the

biomass at all, even for cell disruption purposes, as the other microalgal products do not cover the costs.

Table 7. Carotenoids extraction cost efficiency calculations.

A) Covering note of carotenoid extraction with maximum product yield and minimum costs and B) break-even result of carotenoid extraction with required carotenoid concentration in the cell.

**A**

OPEX

Solvent (12:1 (v:dw) ethanol with 95 % recycling rate)	757.44 €
<u>Coelastrella estimated carotenoids (kg)</u>	<u>10.62</u>
Cost (€/kg)	71.36 €
<u>Market value</u>	<u>14-30 €</u>
<u>Profit</u>	<u>-41.26 – -57.36 €</u>

**B**

OPEX

Solvent (12:1 (v:dw) ethanol with 95 % recycling rate)	757.44 €
<u>Required carotenoid yield (kg)</u>	<u>25.25 €</u>
Cost (€/kg)	30
<u>Market value</u>	<u>30</u>
<u>Profit</u>	<u>0</u>

For carbohydrate extraction, Martins et al. (2025) claims that using alkali and alkaline protease with US achieved yields comparable to harsh acid hydrolysis. According to the TEA, the enzyme addition would be economically feasible for carbohydrate extraction (Table 6). It is also suggested that carbohydrates are isolated from soluble protein via membrane filters (Bleakley & Hayes, 2017; Dutta et al., 2025; Martins et al., 2025; Safi et al., 2017; Slegers et al., 2020) or via acid precipitation. These two separation methods are compared in Table 8.

Table 8. Protein and carbohydrate isolation cost calculations.

Separating carbohydrates and protein from the soluble fraction of microalgae biomass. Note unrealistic yields in acid precipitation.

	<b>2-step ultrafiltration</b>		<b>Acid precipitation</b>
<b>CAPEX</b>			
		Stirrer	n/a
<b>Filter equipment</b>	48 000 €	Centrifuge	105 000 €
<b>CAPEX per operative day</b>	11 €		12 €
<b>OPEX</b>			
		Acid	168.75 €
<b>H<sub>2</sub>O</b>	7.92		5.625
<b>H<sub>2</sub>O cost</b>	12.83 €		9.11 €
<b>Energy consumption (kWh)</b>	155.87		12.38
<b>Energy cost</b>	0.40 €		0.95 €
<b>OPEX total per day</b>	13 €		178.81 €
<b>Total costs per day</b>	24 €		190 €
<b>Yields</b>			
<b>Protein yield</b>	64 %		100 %
<b>Carbohydrate yield</b>	62 %*		100 %
<b>Relative costs</b>			
<b>Cost per protein yield</b>	37 €		190 €
<b>Cost per carbohydrate yield</b>	39 €		190 €

\*=Yield from ultrasonication (Martins et al., 2025) with 100 % from membrane filtering step.

### 3.5 Decision support system

Based on the results of the TEA, three whole-cell value chains and seven multi-product value chains were designed and economically evaluated (Table 9). The total costs of each value chain are allocated per product mass. The market analysis results were incorporated into the calculations to evaluate profits (Equation 2). With this system, it is possible to compare production costs and profits flexibly by changing the input parameters such as acquisition price, electricity and water consumption, global parameters, market values and biomass composition.

Table 9. Names and process and product descriptions of all biorefinery value chains designed.

<b>Value chain no.</b>	<b>Description</b>
<b>Whole-cell 1</b>	Dry = dry whole-cell biomass
<b>Whole-cell 2</b>	Wash+dry = dry washed whole-cell biomass
<b>Whole-cell 3</b>	US+dry = dry disrupted microalgae cells
<b>Multi-product 1</b>	US+phase separation = whey and insoluble components
<b>Multi-product 2</b>	US+1-step filter = Protein+polysaccharides, monosaccharides, insoluble components
<b>Multi-product 3</b>	US+2-step filter = Protein, polysaccharides, monosaccharides, insoluble components
<b>Multi-product 4</b>	US+alcalase+phase separation = whey and insoluble components
<b>Multi-product 5</b>	US+alcalase+2-step filter = Peptides, polysaccharides, monosaccharides, insoluble components
<b>Multi-product 6</b>	US+alcalase+amylase = whey and insoluble components
<b>Multi-product 7</b>	US+alcalase+amylase+2-step filter = Protein, polysaccharides, monosaccharides, insoluble components

Based on these value chains' production costs and created profits, an economic decision can be made on which value chain to choose for production. It is important to be able to update and change these input values, as prices may change and microalgae cultivations with varying compositions may be tested.

Investment calculations were conducted to allow the assessment of the feasibility of production line investments and to compare the profitability across value chains using return on investment (ROI) (Equation 3). The highest ROIs were obtained for the whole-cell value chains using intact or disrupted biomass. The whole biomass production line has the fewest investments, mere dryer, whereas the disrupted biomass adds value to the final product, compensating for the additional investments.

Washing the biomass was less profitable than the two other whole-cell value chains, as the procedure significantly reduced the yield (Table 10). With the sedimentation efficiency of 50 % (Slegers et al., 2020), thus having a lower ROI (21.5 %). In contrast, the broken biomass has the highest ROI over one year of 430 %. The multi-product value chains, however, have much lower ROIs over one year (-63.41 – 80,94 %). Many of these still have unknown costs,

which will further reduce the final ROI. The ROI for multi-product value chain 3, with no unknown costs, is 46.6 %, which is significantly lower than for disrupted whole-cell biomass.

Table 10. Economic comparison of the whole-cell single-product value chains.

Method 1: Dried whole biomass. Method 2: 2-step wash-centrifuged dried biomass. Method 3: Dried disrupted microalgae cells.

	1	2	3
<b>CAPEX</b>			
<b>Ultrasonication</b>	-	-	51 440.00 €
<b>Centrifuge</b>	-	105 000.00 €	-
<b>Dryer</b>	410 330.75 €	410 330.75 €	410 330.75 €
<b>CAPEX per operative day</b>	91.18 €	114.52 €	102.62 €
<b>OPEX</b>			
<b>H<sub>2</sub>O (m<sup>3</sup>)</b>	-	1.67	-
<b>H<sub>2</sub>O cost</b>	-	2.70 €	-
<b>Energy consumption (kWh)</b>	107.80	42.50	115.77
<b>Energy cost</b>	8.27 €	3.26 €	8.88 €
<b>OPEX per operative day</b>	8.27 €	5.96 €	8.88 €
<b>Total costs per day</b>	99.45 €	120.48 €	111.50 €
<b>Yield</b>	98 %	25 %	95 %
<b>COP (€/kg)</b>	0.20 €	0.24 €	0.22 €
<b>WTP (€/kg)</b>	6.00 €*	10.00 €*	12,00 €*
<b>Profit per kg</b>	5.80 €	9.76 €	11.78 €
<b>Total profit</b>	2 842.54 €	1 219.88 €	5 594.08 €

\*=Evaluation based on the interviews.

The greatest CAPEX for all value chains was the dryer. The costs were allocated across all products of each value chain; the dryer was a profitable investment. However, if the dryer's CAPEX is allocated only for the products that are planned to be dried in the multi-product value chains, the investment is not profitable. This is due to the large fraction of insoluble components that are not to be dried in the scope of this TEA. However, there is large deviation in both the CAPEXs and OPEXs of the dryer, meaning that the cost calculations must be redone when more precise information is available. The standard deviation of dryer CAPEX is more than 420 000 €, whereas the average price is 410 000 €. With the average CAPEX, the dryer's pay-back time is 0.3-2 years depending on the value chain, making it profitable eventually. The OPEXs of the dryer vary also significantly, 0.02 kWh/kg WW

(Slegers et al., 2020) to 36.45 kWh/kg DW (Vázquez-Romero et al., 2022). The dryer is, however, an essential investment for producing stable microalgae products, justifying its inclusion.

Isolating the protein from other biomass in the multi-product value chains increased the profit compared to those that did not isolate it. The separated protein was either in the same whey retentate with monosaccharides (one-step filtering) or as protein isolate (two-step filtering). The protein isolate is assumed to have higher market value than whey, though this should be confirmed with further market studies. These value chains also isolated polysaccharides. Isolated polysaccharides added value significantly to the value chains, despite the low contents of it in the biomass, 1.8 % or less. However, the value chains 3, 5 and 7 were the most profitable even without selling the isolated polysaccharides, which could be integrated to the residual insoluble biomass if they are not otherwise valorised. Value chains 5 and 7 do contain unknown costs that will decrease their profitability eventually.

The multi-product value chains 2, 3 and 5 were selected for further evaluation, as they were the most prominent ones. Value chains 2 and 3 do not have any unknown costs and generated reasonable profit. Value chain 5 creates the highest profit creating potential, but has unknown costs. The OPEXs and CAPEXs of these multi-product value chains are distributed in Figure 15. The cost distributions show which elements create the most costs and which are less significant. For example, dryer was less significant in value chain 3 than in 2, and value chain 5 still contained unknown costs. The OPEX distribution shows the process's sensitivity to electricity and water price fluctuation: multi-product value chain 3 is more sensitive to electricity price fluctuation than 5. In the most single-product value chains 1 and 3 the only OPEX variable is the price of electricity.

The most profitable value chain with the parameters discovered in the inventory and market analyses was whole-cell 1, broken biomass. Although the multi-product chains were less profitable, they provide greater flexibility to adapt to the market demand and are not reliant on a single customer industry. For maximum profit created by the multi-product value chains, *Chlorella* cultivated with JWP and Yara nutrients should be refined into isolated protein with value chains 3, 5 or 7. The profit rankings obtained here are not in accordance with Slegers et al. (2020), despite the majority of the inventory analysis values being drawn from their study. This highlights the importance of making case-by-case analyses.

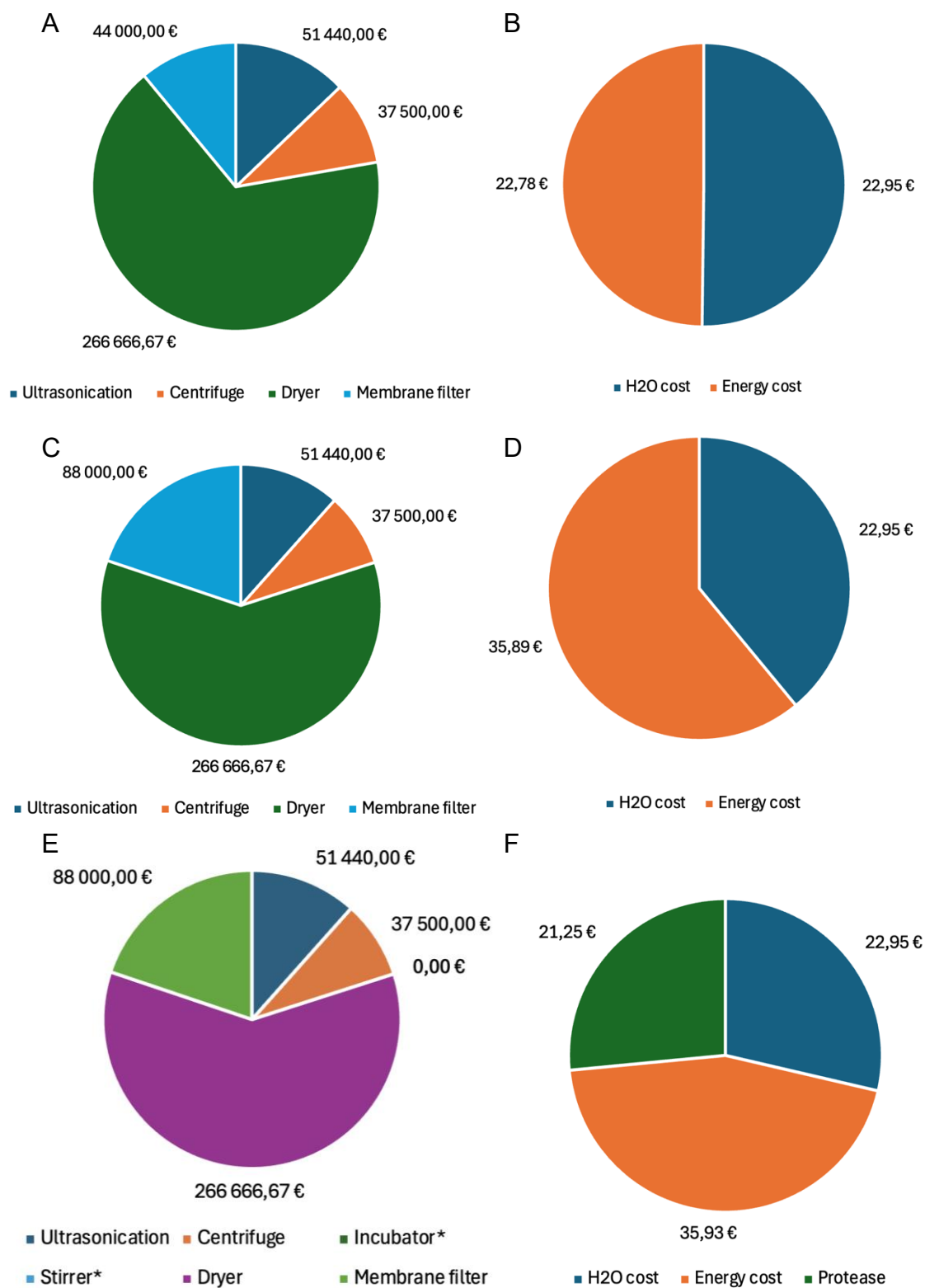


Figure 15. OPEX and CAPEX distributions of multi-product chains 2, 3 and 5 for refining 500 L of harvested biomass. A) CAPEXs of multi-product chain 2, B) OPEXs of multi-product chain 2, C) CAPEXs of multi-product chain 3, D) OPEXs of multi-product chain 3, E) CAPEXs of multi-product chain 5 and F) OAPEXs of multi-product chain 5.

All the production costs are now allocated for all products based on their mass despite the process steps they have gone through. For more detailed investment calculations the production costs should be allocated per mass per production steps.

### **3.6 Other parameters to take into consideration in the microalgae industry**

#### **3.6.1 Other production costs**

In addition to the cost discussed previously, there are other costs that must be considered when making the final investment decision into a production line. Costs that are part of CAPEXs are setting up, maintenance and engineering, to name a few. Many of these are listed in Slegers et al., (2020) and Vázquez-Romero et al. (2022). There are also utility costs that account for 50 % of establishment costs (Slegers et al., 2020). Finally, the cultivation and harvesting costs must be added to the final production cost calculations for accurate decision-making.

#### **3.6.2 Regulatory and legal aspects**

Each industry imposes specific regulatory requirements to consider when making strategic decisions on both target industry and product portfolio. Legislation brings both challenges and opportunities, but these must be well understood to enable justified decision-making.

To produce feed products to the European markets, a company must have a licence from the Finnish Food Authority, as required by the EU (Ruokavirasto, 2024). Without this licence, the products cannot be sold as feed. This legislation also covers the primary producers. There is a difference in producing feed products and feed additives, which fall under different legislations (*Regulation (EC) 1831/2003*; *Regulation (EU) 68/2013*). These regulatory differences must be weighed before entering the feed markets.

The Novel Food Regulation (*Regulation (EU) 2015/2283*) regulates the use of new food products in the EU including whole food products as well as compounds extracted and derived from them. This restricts utilising microalgae derived compounds as food, dietary supplements or nutraceuticals. According to a market analysis interview, it is “almost not rational” to sell microalgal extracts for dietary supplements in the EU.



For cosmetics, regulations are less strict in the EU enabling companies to sell their cosmetics products globally. The primary producers do not particularly have any obligations in the cosmetics industry in the EU other than providing a safety data sheet for the manufacturer of the final product (*Regulation (EC) 1223/2009*). This regulatory environment makes the cosmetics industry particularly attractive for microalgal applications.

### 3.6.3 Future opportunities and challenges of microalgae industry in Finland

Finland is a prominent location to cultivate microalgae because of governmental sustainability commitments and low electricity prices (*Microalgae Market Pricing Trends and Profitability Outlook | LinkedIn*). Hence the northern location with limited sunlight availability might not be as significant of a challenge as conventionally thought. However, European products must create added value that Asian products do not have, because it is impossible to compete with production price (Rumin et al., 2021). The European microalgae production is highly dependent on political decisions on trades and innovation, making it essential to following political developments closely.

For successful market penetration, the strategic decisions discussed in this study must be implemented carefully. As with any innovative technologies, microalgal products should first enter niche markets and gather momentum, before entering larger, more conservative markets (Moore, 2007; Ruiz et al., 2016). According to the market analysis interviews, within the potential industries discovered in this study, there are microtrends that effect the market potential of specific products and compounds. The demand may change rapidly due to modern marketing channels and while broader market analyses provide generic information about the markets but should not be relied upon exclusively. This phenomenon has been noted in nutraceuticals but would probably hold up for other product categories, such as functional petfood.

Sequestering CO<sub>2</sub> into high-value products allow producing high-value products while decreasing the climate impact of the customer business's processes. However, the products of the highest value are the lowest volume (Figure 2), affecting only namely or not at all to CO<sub>2</sub> mitigation. In addition to sequestering CO<sub>2</sub> and producing high-value products, microalgae can be used to purify water. In Martins et al. (2025) *Scenedesmus* was cultivated in brewery secondary effluent. This process could decrease cultivation costs and decrease the amount of

wastewater created, having a positive influence on the customer's process costs and marketing value. However, the effluent should remain standard overtime for efficient microalgae cultivation, which remains a challenge.

In addition to wastewater remediation, food proteins and functional polysaccharides Rumin et al. (2021) suggest antimicrobial compounds and recombinant proteins as the most promising microalgae products in the near future. Taking a step towards producing medicinal compounds would require new expertise and extremely sophisticated processes, increasing production costs significantly. This could, however, be a path to investigate more thoroughly in later production steps.

For greater global impact, the TRL of biorefineries in general should be raised. Local biorefineries would help implement the UN's SDGs in communities and improve life quality. However, many of the high TRL biorefinery methods do not create the most high-value products and do not follow the concept cascading in the optimal way, reducing the potential positive impact the biorefinery could have. (Solarte-Toro & Cardona Alzate, 2021.) As stakeholders already recognise technical and legislative obstacles as the second most limiting factor in the microalgae industry (Rumin et al., 2021), political decisions could have a significant boosting impact on both biorefineries and microalgae industry.

### **3.7 Evaluation of the study**

A similar approach was adopted by Klein et al. (2023) in their study on quantifying the value of microalgal biomass in a multi-product refinery. This decision support analysis discusses the most significant parameters affecting the microalgae biorefining strategy. The product portfolio is dictated by the cellular composition (Ruiz et al., 2016) in addition to which it must respond to the needs of the customers. The potential customer industries were selected in this study so that the possible product portfolio complements their needs. Also, the regulatory demands and potential created by market sizes were analysed as well as WTP of each industry. With the combination of these parameters, the best market values for each microalgae compound could be determined as well as challenges and opportunities created by regulation and market behaviour.

Although multiple parameters influence final decision-making, economic considerations often have the greatest weight. Based on a techno-economic evaluation of commercial scale biorefinery methods, alternative biorefinery strategies were designed. Several biorefinery strategies allow the evaluation of multiple scenarios with changing parameters as well as each step's value creation potential. This is valuable information for any company before investing into an up-scaled production plant and supports the development of a business case.

In this study the compositions of five microalgae cultivations were analysed and compared for maximising production yields. These indicative laboratory analyses gave sufficient information on the differences between the cultivations to allow process optimisation and flexibility. In a single-product whole-cell value chain, the cell composition does not make a difference, but it has a significant difference in a multi-product biorefinery strategy. However, some industries like the feed industry require a high protein content, for which cultivation can be optimised.

In this thesis, the most prominent customer industries, the most profitable microalgal products based on the composition of the microalgae and most efficient biorefinery methods were recognised, answering all research questions. It was also recognised that despite the high WTP of valuable microalgal pigments, its extraction is not economically feasible, at least not by the company itself. Also, the regulatory aspects limit the production of otherwise feasible products. These findings underscore the importance of a DSA for companies before investing into a production lines.

## 4 Conclusion

According to the decision support analysis conducted in this study, the most profitable strategy for microalgal biomass cultivated at Aircohol Oy, is to produce dry broken cell biomass. An alternative, more flexible multi-product strategy would involve isolating high-protein products and polysaccharides while creating monosaccharides and an insoluble fraction as side streams. These fractions could be further valorised into value-adding products. From the five microalgae cultivations studied, *Chlorella* cultivated in both JWP and Yara nutrients would create the most value using this biorefinery strategy. With the decision support system, different scenarios may be assessed by changing the input parameters.

Sustainability does not add value to the final consumer in low-value commodities, but it can provide added value in niche, higher value products. Considering regulatory aspects, the Novel Food regulation in the EU (*Regulation (EU) 2015/2283*) limits the use of microalgae extracts for food products but e.g. for cosmetics they are a suitable raw material (*Regulation (EC) 1223/2009*).

The success of scale-up and market penetration is dependent on justified strain and process selection. As these two parameters have equal impact on the success and determine each other. (Klein et al., 2023; Slegers et al., 2020.) This decision support analysis provides a comprehensive overview on the demands, challenges and opportunities in the industry, that can be used to justify strain and process choices. Finally, the profitability of ten biorefinery strategies, designed according to the DSA, are calculated to support successful up-scaling of microalgae biorefinery.

In conclusion, all four research aims set out in Section 1.7 were achieved: the compositions of five microalgae cultivations were quantified and compared; potential products from the biomass were identified; their market values were investigated; and the most economically feasible refining methods were evaluated. Together, these results provide a decision support tool that Aircohol Oy can use to validate future strategic decisions in biorefinery development and market entry.

This DSA is to be updated as markets change, and new technological opportunities emerge. For more delicate decision-making support a risk analysis, for example a sensitivity analysis, should be conducted. In addition, after a decision about establishing a production line is made, it should be optimised to ensure cost-efficiency. Considering the whole microalgae industry, less capital-intensive business models should be designed to achieve faster turnover to support sustainable innovations in this field. This could be achieved by exploring more waste streams, in addition to CO<sub>2</sub>, that could be valorised through biological pathways, preferably already at the innovation stage.

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## Appendices

Table 11, Appendix table 1. Customer prioritisation matrix.

Matrix of potential customer companies according to their previous use of algae, company size and innovation capacity, relevance of industry, research and development (R&D) orientation and geographic and regulatory advantage.

<b>Food</b>	<b>Uses algae</b>	<b>Innovation capacity and R&amp;D orientation</b>	<b>Industry fit</b>	<b>Agility</b>	<b>Geography and regulation</b>
<b>Veganz (German)</b>	no	medium	high	high	good
<b>Algama (French)</b>	yes	not relevant	high	not relevant	good
<b>Fazer (Finnish)</b>	no	high	high	high	excellent
<b>Marli (Finnish)</b>	no	low	medium	low	excellent
<b>Chr. Olsen nutrition (global)</b>	no	medium	medium	low	good
<b>Harke Food (global)</b>	no	low	low	low	good
<b>Corbion (global)</b>	yes	high	high	low	good
<b>Edonia (French)</b>	yes	high	high	high	good
<b>Eurogum (Danish)</b>	yes (macro)	medium	high	high	good
<b>DMS-Firmenich (Swiss)</b>	yes	high	high	medium	good
<b>Nutraceuticals</b>					
<b>Divi's Nutraceuticals (global)</b>	yes	not relevant	high	not relevant	good
<b>Biomed (Finnish)</b>	yes	not relevant	high	not relevant	good
<b>Malinca (Slovenian)</b>	yes	not relevant	high	not relevant	good
<b>Rawfood Shop – yes (Swedish)</b>	yes	not relevant	high	not relevant	good
<b>Feed</b>					
<b>Honkajoki (Finnish)</b>	no	high	high	medium	excellent
<b>IQI Trusted Petfood Ingredients (Dutch)</b>	yes	medium	high	medium	good
<b>Chia de Gracia (Finnish)</b>	no	not relevant	medium	not relevant	excellent
<b>Biofarm Oy (Finnish)</b>	no	low	medium	low	excellent
<b>Chr. Olsen nutrition</b>	no	low	medium	low	good
<b>Cosmetics</b>					
<b>Laponie of Scandinavia (Finnish)</b>	no	medium	high	medium	excellent
<b>Flinkenberg (Finnish)</b>	?	?	high	?	excellent
<b>Lumene (Finnish)</b>	no	high	medium	medium	excellent
<b>Luonkos (Finnish)</b>	no	medium	medium	low	excellent
<b>Cutrin (Finnish)</b>	yes	medium	high	medium	excellent
<b>Aquaflor (German)</b>	yes	medium	high	medium	good

Table 12, Appendix table 2. Inventory analysis, raw materials costs for biorefinery.

It should be assumed that the workflow has been planned in a manner that the biorefinery processes may flow continuously during the working hours. The production times are not taken into account. Values and presumptions are taken from Slegers et al. (2020) and Vázquez-Romero et al. (2022). The most significant presumptions are listed in Tables S2-S3.

Raw material	Price	Reference
Water	1.62 €/m <sup>3</sup>	<a href="https://www.turunvesihuolto.fi/asiakkaat/hinnasto/">https://www.turunvesihuolto.fi/asiakkaat/hinnasto/</a> (12.8.2025)
Electricity	0.0767 €/kWh	<a href="https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Electricity_price_statistics">https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Electricity_price_statistics</a> (12.8.2025)
Amylase	35 €/kg	<a href="https://www.alibaba.com/showroom/amylase-price.html">https://www.alibaba.com/showroom/amylase-price.html</a> (7.8.2025)
Alkaline protease	85 €/kg	Confidential
Ethanol	789 €/kg	Confidential
Acetone	1.8 €/L	<a href="https://lerochem.eu/fi/perustava/214-947-asetoni-propanoni-99-l.html#/31-koko_kapasiteetti-1000_l">https://lerochem.eu/fi/perustava/214-947-asetoni-propanoni-99-l.html#/31-koko_kapasiteetti-1000_l</a> (12.8.2025)
NaOH	3.7 €/kg	<a href="https://lerochem.eu/fi/perustava/47-164-natriumhydroksidi-kaustinen-sooda-99-kg.html#/36-tuotteen_koko_kg-100_kg">https://lerochem.eu/fi/perustava/47-164-natriumhydroksidi-kaustinen-sooda-99-kg.html#/36-tuotteen_koko_kg-100_kg</a> (12.8.2025)
KOH	3.86 €/kg	(KALIUMHYDROKSIDI 90%, kg Tuotteen koko, kg 100 kg, n.d.) (12.8.2025)
HCl	5 €/L	(SUOLAHAPPO 36-38% puhdas, L Koko/kapasiteetti 20 L, n.d.)

Table 13, Appendix Table 3. Biorefinery equipment cost and process details.

OPEXs and CAPEXs are approximated for 500 L/h operation.

Equipment or process	Average	Standard deviation	Reference
<b>High-pressure homogeniser</b>	<b>&gt;1500 bar</b>		
<b>Acquisition price</b>	301 000 €		Confidential
<b>Electricity consumption</b>	59.5 kWh/m <sup>3</sup>	24-95 kWh/m <sup>3</sup>	(Slegers et al., 2020)
<b>Water consumption</b>	n/a		
<b>Cell disruption efficiency</b>	95 %		(Slegers et al., 2020)
<b>Number of passes</b>	1		(Slegers et al., 2020)
<b>Ultrasonicator</b>			
<b>Acquisition price</b>	45 000 €	43 000-47 000€	Confidential
<b>Electricity consumption</b>	2.25 kWh/m <sup>3</sup>	8.65-17.3 kW	Confidential
<b>Water consumption</b>	10 L/min		Confidential
<b>Cell disruption efficiency</b>	>95 %		(Martins et al., 2025)
<b>Bead miller</b>			
<b>Acquisition price</b>	98 943 €	85 000-12 000€	Confidential
<b>Electricity consumption</b>	0.83 kWh/kg	0.81-0.85 kWh/kg	(Doucha & Lívanský, 2008; Postma et al., 2015)
<b>Water consumption</b>	n/a		
<b>Cell disruption efficiency</b>	97 %		(Postma et al., 2015)
<b>Centrifuge</b>			
<b>Acquisition price</b>	37 500 €	35 000-40 000	Confidential
<b>Electricity consumption</b>	2 kWh/m <sup>3</sup>		(Slegers et al., 2020)
<b>Sedimentation efficiency</b>	50 %		(Slegers et al., 2020)
<b>Membrane filter</b>			

Equipment or process	Average	Standard deviation	Reference
<b>Acquisition price</b>	44 000 €	40 000-48 000 €	(Vázquez-Romero, Perales, de Vree, et al., 2022); Confidential
<b>Electricity consumption</b>	12 kWh/m <sup>3</sup> permeate	4-20 kWh/m <sup>3</sup> permeate	(Slegers et al., 2020)
<b>Concentration factor (UF/DF and UF)</b>	5 and 20		(Slegers et al., 2020)
<b>Pore size( UF/DF and UF)</b>	300 kDa and 8kDa		(Slegers et al., 2020)
<b>Acquisition price</b>	266 667 €	30 000-700 000 €	(China High Speed Centrifugal Spray Dryer Machine - Changzhou Shinma Drying Engineering Co.,LTD., n.d.; Glucose Spray Drying Machine 200kg/H Capacity/Food Drying Machine/Spray Dryer, n.d.; Spray Dryer --- Industrial 50-200kg Water per Hour, n.d.)
<b>Steam requirement</b>	0.0014 2100	kg/kg DW kJ/kg	Slegers et al., 2020)
<b>Electricity consumption</b>	0.02 kWh/kg feed + steam heating	Up to 36.45 kWh/kg DW	(Slegers et al., 2020; Vázquez-Romero, Perales, de Vree, et al., 2022)
<b>Final water content</b>	5 %		(Slegers et al., 2020; Vázquez-Romero, Perales, de Vree, et al., 2022)
<b>Solvent extraction</b>			
<b>Solvent to mass ratio</b>	10-33 kg DW/m <sup>3</sup>		(Low et al., 2022)
<b>KOH</b>	5 kg DW/m <sup>3</sup>		(Low et al., 2022)
<b>Microwave</b>	250 W, 20 min		(Low et al., 2022)
<b>Incubation</b>	250 rpm, 20 min		(Low et al., 2022)
<b>Incubator</b>			
<b>Acquisition price</b>	n/a		
<b>Electricity consumption</b>	n/a		
<b>Stirrer</b>			
<b>Acquisition price</b>	n/a		
<b>Electricity consumption</b>	n/a		