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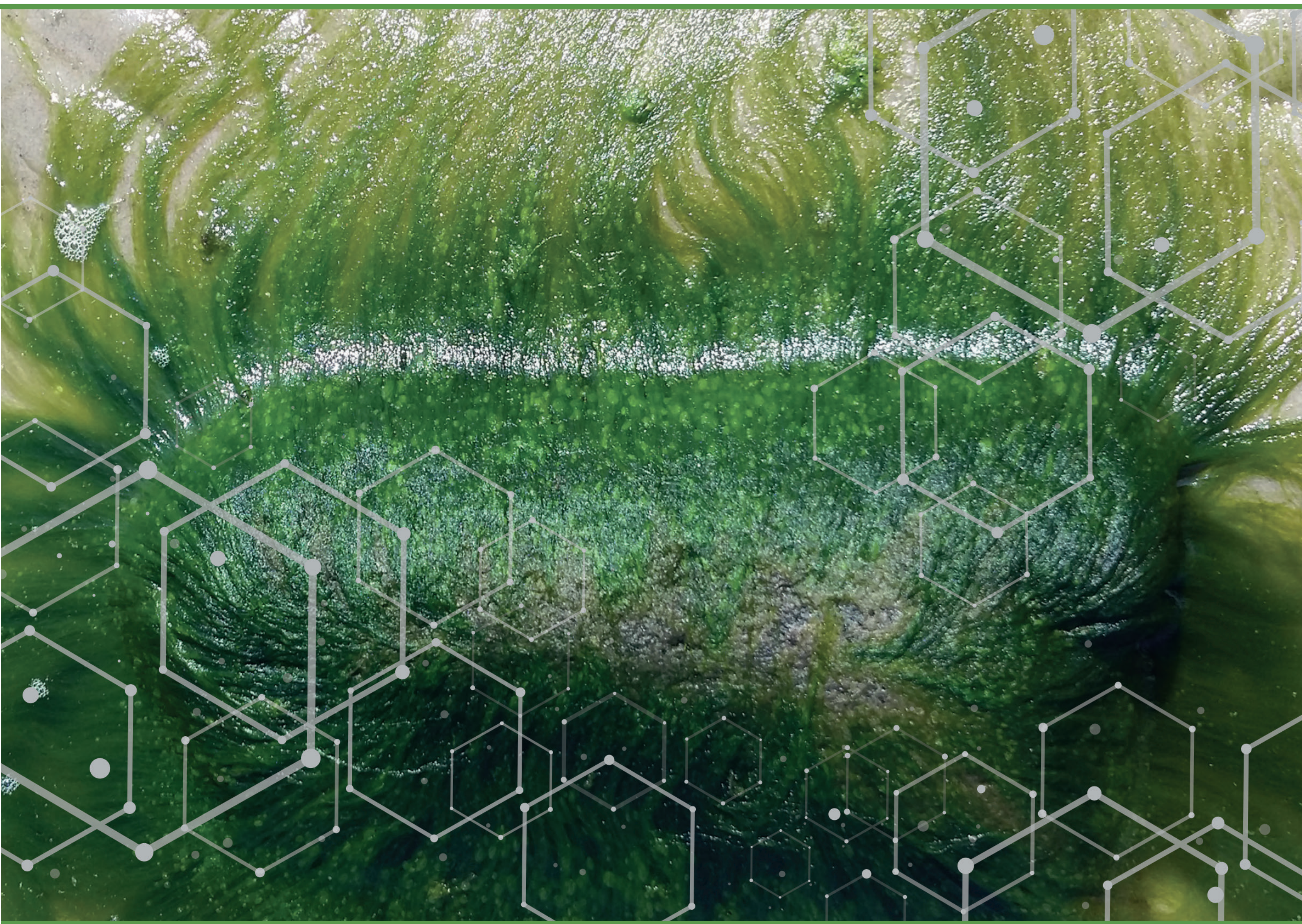
Karīna Bāliņa

BALTIC SEAWEED BIOREFINERY

Summary of the Doctoral Thesis



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RIGA TECHNICAL UNIVERSITY
Faculty of Electrical and Environmental Engineering
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BALTIC SEAWEED BIOREFINERY

Summary of the Doctoral Thesis

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DOCTORAL THESIS PROPOSED TO RIGA TECHNICAL UNIVERSITY FOR THE PROMOTION TO THE SCIENTIFIC DEGREE OF DOCTOR OF SCIENCE

To be granted the scientific degree of Doctor of Science (Ph. D.), the present Doctoral Thesis has been submitted for the defence at the open meeting of RTU Promotion Council at 14.00 on December 10, 2020 at the Faculty of Electrical and Environmental Engineering of Riga Technical University, 12 k-1 Āzenes Street, Room 115.

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DECLARATION OF ACADEMIC INTEGRITY

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

Karīna Bāliņa ……………………………. (signature)

Date: ………………………

The Doctoral Thesis has been written in English. It consists of an Introduction; 3 Chapters; Conclusion; 31 figures; 15 tables; 1 appendix; the total number of pages is 158. The Bibliography contains 284 titles.

TABLE OF CONTENT

INTRODUCTION

Seaweeds, or marine macroalgae, are one of the largest unexploited global biomass resources. They are currently on the international agenda as an alternative resource to meet the rising demand for sustainable material to embrace blue growth policies. As the Earth is covered by 70 % water, seaweeds have enormous cultivation potential. In contrast to terrestrial crops, the production of seaweeds does not require arable land, freshwater, nor when grown in naturally nutritious waters, fertilizer – all scarce resources of modern society [1].

Many opportunities to use seaweeds as a resource have been discovered centuries ago and have been applied ever since. Seaweed production, namely, cultivation and harvesting from wild stocks, is practised in many countries and currently is a multi-billion industry. Seaweeds are widespread around the globe at different sea depths. As a result, on the whole at least 291 species are used worldwide from 43 countries, including 33 *Chlorophyta*, or green seaweeds, 75 *Ochrophyta*, or brown seaweeds and *Rhodophyta*, or red seaweed, species. Remarkable that the number of practised species takes approximately 3 % share of the total number of seaweeds estimated to the present, which means that use of other seaweed species in the future can request more studies and investigations [2].

Traditionally seaweeds have been used for medical purposes, soil improvement, feed supplements, combustion, and insulation material, but mostly as a food source [3]. Seaweeds are well known as a valuable food source in Asian countries, and during recent decades, it has also awakened consumer interest in Western countries due to low-calorie content and high content of dietary fibres, minerals, vitamins, and antioxidants. Many studies show seaweed potential as a source of hydrocolloids that can be used as stabilizing agents in food, pharmacy, and cosmetics [4]. In more recent studies seaweeds have been recognised as a source of 3rd generation biofuels [5]. Due to high carbohydrate content, the absence of lignin, and low content of cellulose seaweeds are considered attractive biomass for methane production through anaerobic digestion.

Use of seaweed in the Baltic Sea region is limited due to specific growth conditions, i.e., low salinity, irregular currents, and high nutrient levels. Despite the fact, that the Baltic Sea seaweeds do not reach the same size and biomass amount as in water bodies with higher (or lower) salinity, washed-out seaweed biomass reduces the recreational value of the public beaches.

Until recently, almost all seaweed biomass came from wild sources, but since the demand for seaweed biomass started to exceed the supply, cultivation was considered as a way to satisfy growing demand. Recently seaweed cultivation has been recognised as a profitable business, and seaweed cultivation develops on the western regions of the Baltic Sea. In contrast, eastern areas of the Baltic Sea are still considered as unsuitable for seaweed cultivation.

Development of the seaweed industry in the Baltic Sea eastern regions is essential to support sustainable growth in marine sectors through the European Blue Economy. Mostly in response to the European Green Deal – an ambitious package of measures aiming at cutting greenhouse gas emissions, investing in cutting-edge research and innovation, and preserving Europe’s natural environment [6]. The European Green Deal will underpin a new growth strategy that aims to transform the economy and society towards a more sustainable future.

Research Scope

The overall aim of the present Doctoral Thesis is to perform integrated research to evaluate the potential application of the biorefinery concept for seaweed species available in Latvia. More specific, this Thesis is addressed to identify potential seaweed species, find out the possible amount of biomass, and search for the direction for seaweed utilization so that it constitutes part of the national economy and is recognized as a significant type of the biomass.

To reach the aim of the Thesis, the following tasks have been set.

1. To develop a definition of a seaweed biorefinery concept by creating a better understanding of available seaweeds and seaweed composition and by assessing biomass transformation routes:
    a) to carry out a literature analysis of seaweed biomass in Latvia:
        - to describe the properties, availability, and chemical composition of seaweeds in Latvia;
        - to identify most-suitable products for each seaweed group based on the performed analysis;

    b) to create a conceptual design for the biorefinery concept;
    c) to perform a SWOT analysis to the developed biorefinery concept.
2. To provide guidelines to expand the availability of seaweed biomass:
    a) to design a functioning seaweed cultivation laboratory;
    b) to set seaweed cultivation guidelines;
    c) to perform a laboratory-scale experiment in order to characterize and evaluate the effects of different growing conditions towards the specific limitations for seaweed growing.

Research Topicality and Hypothesis

The hypothesis of the Doctoral Thesis are as follows.

1. The seaweed biomass available on the Baltic Sea coast in Latvia can be used for the production of value-added products.
2. The biorefinery concept can improve the seaweed processing practices and expand the range of obtained products.
3. Providing controlled laboratory conditions is an important step to increase the availability of the Baltic seaweed biomass.

The seaweed biomass found in Latvia is an underestimated resource. The growing global demand for biomass, driven by rapid global population growth, is forcing the search not only for terrestrial biomass sources but also in the marine environment. Washed out seaweeds are regularly observed on the Latvian coast, but precise data on its amount is not available. Currently washed out biomass is used to strengthen dunes, as fertilizer for local farmers, or taken to landfills for disposal. In this Doctoral Thesis, an assessment of the available seaweed biomass is performed to estimate the available seaweed biomass, and which are the most common species in Latvia (Papers I and II). To further explore the potential of seaweed biomass, three most common seaweed species are selected to represent each of the seaweed groups. The prospect of the most common seaweed species in Latvia is determined by summarizing and analysing the data found in the scientific literature on the chemical composition of the respective three seaweed species. In addition to compiling the chemical composition, the technologies that can be used to obtain the relevant substances are summarized (Paper III). It is known that the salinity of the Baltic Sea differs drastically from areas near the oceans. Such brackish areas have increased stress conditions for the aquatic organisms living in them, including seaweeds, thus their size and biomass potential are much smaller compared to seaweeds grown in the areas with higher salinity levels.

However, valuable substances are also found in brackish water seaweeds. Due to the limited amount of available biomass, a conceptual model of biorefinery concept is being developed and proposed in this Thesis. It envisages full use of biomass: first, by obtaining higher value-added products, then by cascading – obtaining less valuable products and using only the remaining biomass for bioenergy production. The biorefinery concept must be designed to comply with the principles of bioeconomy including also assessment of strengths, weaknesses, opportunities, and threats, which indicates that the biggest problem is the non-specific amount and composition of available biomass (Paper IV). This negative aspect can be offset by starting artificial cultivation of seaweeds in the eastern part of the Baltic Sea. The first step at the beginning of cultivation is to set up and test a seaweed cultivation laboratory. The laboratory was tested by changing the parameters of seaweed cultivation, and thus the parameters favourable for seaweed cultivation were determined (Papers V, VI, VII).

Research Methodology

The applied methods include qualitative and quantitative research techniques: literature analysis, laboratory experiments, collection and analysis of statistical data (Fig. 1).

Firstly, an analysis of scientific literature has been performed to evaluate the current situation. To create a biorefinery concept, in-depth literature analysis has been carried out to detect potential products that can be extracted from the seaweed biomass and possible extraction techniques. Initial information was used to draw potential transformation routes and to create the conceptual design of the biorefinery concept. To determine environmental factors that regulate seaweed growth rate, a seaweed cultivation laboratory was set up and a specific experiment plan was defined.

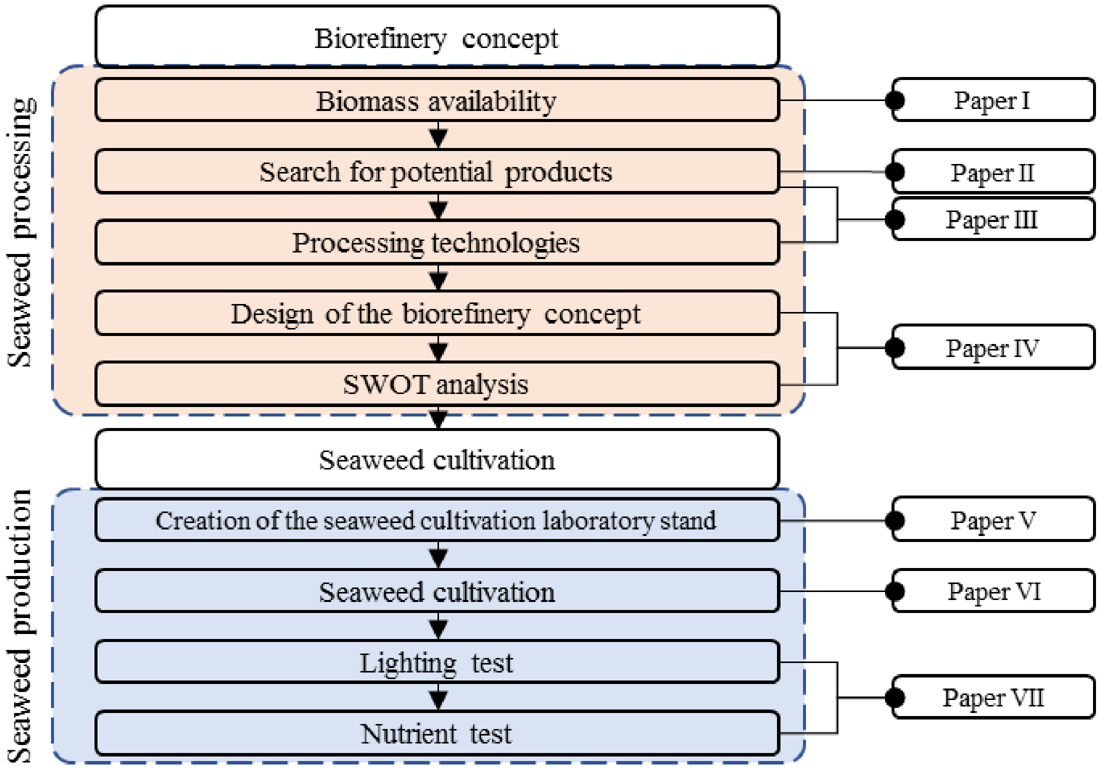

Fig. 1. Overview of the development of applied methodology.

Scientific Significance and Contribution

The Thesis is of high scientific significance for Latvia and in international context. Biorefinery concept was developed by bringing together potentially available seaweed end-products and state of the art technologies. The number of citations of the author’s articles proves the necessity for such research.

1. Bāliņa, K., Romagnoli, F., Blumberga, D. Seaweed biorefinery concept for sustainable use of marine resources. Energy Procedia, 2017, 128, pp. 504–511. (Indexed in Scopus) (31 citations).
2. Bāliņa, K., Romagnoli, F., Blumberga, D. Chemical Composition and Potential Use of *Fucus Vesiculosus* from the Gulf of Riga. Energy Procedia, 2016, 95, pp. 43–49 (Indexed in Scopus) (15 citations).
3. Bāliņa, K., Romagnoli, F., Pastare, L., Blumberga, D. Use of Macroalgae for Bioenergy Production in Latvia: Review on Potential Availability of Marine Coastline Species. Energy Procedia, 2017, 113, pp. 403–410. (Indexed in Scopus) (6 citations).
4. Bāliņa, K., Līkā, A., Romagnoli, F., Blumberga, D. Seaweed Cultivation Laboratory Testing: Effects of Nutrients on Growth Rate of Ulva intestinalis. Energy Procedia, 2017, 113, pp. 454–459. (Indexed in Scopus) (4 citations).

Latvian level research capacity was increased by setting up a seaweed cultivation laboratory. Determination of seaweed cultivation parameters allows to cultivate seaweeds in laboratory conditions and carry out reproduction processes. Detailed guidelines of seaweed cultivation allow to transfer this knowledge and develop other seaweed cultivation in other facilities. Results obtained in the research can be used to continue research and fill the existing knowledge gaps on seaweed growing.

Practical Significance

Studies on seaweed cultivation and processing are a significant contribution in reaching Latvian and EU defined Blue Growth strategy and EU Blue economy concept. Smart utilization of marine resources in long-term facilitates the burden put on terrestrial biomass.

The guidelines proposed in the Thesis can be applied in the development of local, regional, and regional planning strategies providing an in-depth insight in setting up new seaweed cultivation and processing facilities. Biorefinery concept with the evaluation of strengths and weaknesses, threats and opportunities can be used as a tool for seaweed processing companies to plan potential seaweed utilization pathways. The biorefinery concept may also be supplemented, applied, and used for utilization of other types of the biomass.

During the research process, seaweed cultivation laboratory stand was developed in the Biosystems Laboratory of Institute of Energy Systems and Environment (IESE) of Riga Technical University, which allows to carry out experiments with seaweed and other algae cultivation.

Approbation

The results of the author’s research have been presented and discussed in 8 scientific conferences, published in 10 peer-reviewed scientific journals, 2 peer-reviewed full-text scientific conference proceedings, and two textbooks.

Scientific publications

1. Bāliņa, K., Ivanovs, K., Romagnoli, F., Blumberga, D., Comprehensive literature review on valuable compounds and extraction technologies: the Eastern Baltic Sea seaweeds. *Environmental and Climate Technologies*, 2020, 24(2), pp. 178–195 (Indexed in Sciendo).
2. Bāliņa, K., Piščika, A., Gruduls, A., Romagnoli, F., Blumberga, D. Lab scale cultivation of Baltic *Ulva intestinalis* in different light and nutrient conditions: Effects on growth and morphology. *European Biomass Conference and Exhibition Proceedings*, 2018, pp. 223–227 (Indexed in Scopus).
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5. Krastiņa, J., Romagnoli, F., Bāliņa, K. SWOT analysis for a further LCCA-based techno-economic feasibility of a biogas system using seaweeds feedstock. *Energy Procedia*, 2017, 128, pp. 491–496. (Indexed in Scopus) (1 citation).
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11. Bāliņa, K., Romagnoli, F., Blumberga, D. Chemical Composition and Potential Use of *Fucus Vesiculosus* from Gulf of Riga. *Energy Procedia*, 2016, 95, pp. 43–49 (Indexed in Scopus) (15 citations).
12. Bāliņa, K., Balode, M., Muzikante, L., Blumberga, D. Impact of synthetic hormone 17α-ethinylestradiol on growth of microalgae *Desmodesmus communis*. *Agronomy Research*, 2015, 13 (2), pp. 445–454 (Indexed in Scopus).

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3. Marta Delle (Āboliņa). Biogas potential from Baltic seaweeds: effects of mechanical pre-treatment on BMP test. Master's thesis. RTU, 2017 (in Latvian).
4. Vilma Živelyte. Experimental investigation of nanocomposites application for the oil contaminated soil treatment. Master's thesis. RTU, 2017.
5. Anastasija Piščika. The role of environmental parameters in culturing of *Ulva intestinalis* in laboratory conditions. Bachelor thesis. RTU, 2018 (in Latvian).

1. LITERATURE REVIEW

A biorefinery is a processing approach that allows producing fuel, power, and value-added chemicals and materials from biomass that are analogous to petroleum alternatives [7]. Biorefinery is a vital part of bioeconomy, as it is integrating different biomass conversion processes to produce energy and value-added products into a single facility [8]. The biomass processing through the biorefinery concept makes production processes economically and environmentally feasible, respecting social and political angles [9].

Seaweed biorefinery concept proposes a conceptual model for high value-added product production along with the production of biofuels [10]. In this concept, the exploitation of biomass for both value-added products and fuel is maximised, in turn, reducing expenses. It is crucial when planning production process and units to use efficient biorefinery concept where all biomass and energy from production processes would be fully optimized. Conversion processes (physical, chemical, biological, and thermal) used in the production of products should work in a symbiotic way, individually or in a system to create economically sustainable products [8]. The waste and leftover products obtained after each step of treatment are used as raw material inflows for a parallel production chain using a cascading approach [10], [11]. The heat requirements for the biorefinery may be obtained from the recirculation of heat generated from the cascading process [12]. Different conversion processes (physical, chemical, biological, and thermal) are used either individually or in combination to provide products for economic purposes. Only leftovers, which cannot be utilized in further production processes along with low-quality biomass, are used for energy production. This approach allows minimizing the waste stream produced in the seaweed biorefinery concept to a nearly zero-waste system [13].

The biorefinery concept used in bioeconomy has the potential for strengthening the global competitiveness of a broad spectrum of industries. These include agriculture, forestry, and fisheries, as well as strengthen the competitiveness of biobased industries, green platform chemicals, materials, and biopolymers, and both new and existing food and feed ingredients and processing industries.

There are three types of seaweeds growing in the Baltic Sea – green seaweeds (*Chlorophyta*), brown seaweeds (*Phaeophyceae*), and red seaweeds (*Rhodophyta*). Seaweeds in the Baltic Sea are abundant in three different forms – attached to the substrate, detached, drifting and washed-out seaweed biomass deposits. Firstly, an essential part of seaweeds is attached to the substrate and are protected as a necessary nest for zoobenthos and fish spawning grounds. Some part of seaweeds is detached from the substrate and are drifting subordinated by waves and currents [14].

Nutritional composition in seaweed varies depending on the species, time of collection, geographic location, and environmental conditions such as temperature, light and nutrient concentration in water. Even the same seaweed genus can have significant differences in their nutritional composition [15].

Nowadays, seaweeds have become a cheap alternative protein source, mainly due to essential amino acids, especially in developing countries [16]

In general, red and green seaweeds have relatively high protein concentrations (10–30 % dry matter), brown seaweeds contain an average of 3–15 % of dry weight [17]. In terms of protein content in seaweeds, they could be compared to legumes, like soybean with 35 % of the protein in dry mass, meaning that it can be an alternative dietary addition for a vegetarian and vegan diet [18].

The primary role of the pigments in seaweeds is to absorb the light necessary for photosynthesis at depths that have various degrees of light intensity. These pigments can be divided into three main groups, which include chlorophylls, phycobiliproteins and carotenoids and have various health benefits when consumed [15].

Seaweed biomass has high polysaccharide amount that is existing in cell wall structures and storage polysaccharides, such as laminarin (β-1,3-glucan) in brown seaweeds and floridean starch (amyloprotein-like glucan) in red seaweeds.

They have various commercial applications in products such as stabilisers, thickeners, emulsifiers, food, feed, beverages, etc. [19], [20].

Lipids represent only 1–5 % of seaweed dry matter and show valuable polyunsaturated fatty acid (PUFA). They contain valuable omega 3 and omega 6 acids, which play a role in the prevention of cardiovascular diseases, osteoarthritis and diabetes

Analyses show that seaweeds contain useful amounts of minerals (K, P, Mg, Ca, Na, Cl, and S), trace metals, and vitamins [2]. Their mineral content that reaches levels of up to 55 % on a dry weight basis is 10–100 times higher than of traditional vegetables [21].

Phenolic compounds can be found in both terrestrial and aquatic plants, which include seaweeds. Due to their antioxidant properties, they prevent the formation of many free radicals because of their metal ion chelating capacity [23]–[25]. Phenolic compounds are classified into five broad groups: flavonoids, lignans, tannins, tocopherols, and phenolic acids [26].

There can be seen significant structural differences between the different target bioactive compounds and their natural sources. They have different physical and chemical properties. Therefore, it is crucial and necessary to find the most efficient method for the extraction of the selected bioactive compounds and then optimize the extraction procedure. The process parameters of each extraction procedure should be checked to obtain an accurate view of the effect of the particular method on the content and activity of the bioactive compounds of extracts obtained. Different extraction methods should be used to enhance the scientific understanding of the selectivity of the extraction from different natural sources [27].

Conventional extraction methods use organic solvents (i.e., petroleum ether, hexane, cyclohexane, isooctane, toluene, benzene, diethyl ether, dichloromethane, isopropanol, chloroform, acetone, methanol, ethanol, etc.) and acids or alkalis, and water [28]. The primary purpose of these aggressive substances is to disrupt cell membranes and allow substances contained in the seaweeds to enter the extraction matrix. According to current trends, the solvent used in the extraction process should be cheap and non-toxic [28].

Novel methods have several advantages over conventional methods, including the reduced amount of solvent used (including its recovery), shorter extraction time, and technological performance at lower temperatures [29]. These methods also include improved selectivity for isolation of the desired compounds while avoiding the formation of by-products during the extraction and adverse reactions. Most of the following methods are considered unharmful to environment due to their “green extraction” standards [30], [31].

There are six novel seaweed biomolecule extraction methods described in this Thesis [28]–[29], [32]–[36]:

- supercritical CO_2;
- microwave-assisted extraction;
- ultrasound-assisted extraction;
- high-pressure methods;
- enzyme-assisted extraction;
- ionic liquids extraction;
- pulsed electric fields.

Seaweed cultivation is a way to enhance feedstock available for biorefinery.

The harvesting process is limited by the availability of biomass and regrowth potential. Natural seaweed meadows must be protected because they play an essential role in coastal ecosystems as a habitat for living organisms [37]. Overexploitation of natural seaweed resources could have a negative impact on the marine carbon cycle and promote coastal erosion processes [38], [39].

In Latvia, there are no regulations or other legislative restrictions limiting seaweed harvesting from natural seabeds except those regulating fisheries in marine protected areas and Natura 2000 sites. The main reason for lack of regulations might be the low interest for seaweed as a resource, since there is lack of information regarding seaweed distribution and potential use. In the case of the growing interest of seaweed as a resource, a proposal of new legislation is crucial to protect natural seaweed beds in a fragile ecosystem of the Baltic Sea.

Seaweed cultivation is an alternate process to produce biomass without destroying effects on natural seaweed habitats. Cultivation or artificial seaweed propagation is a process, which provides seaweed growing and preservation out of their natural habitat. Cultivation is possible both *in vitro* (fully controlled conditions) and *in situ* (their natural growth environment), and it can also be carried out in different scales [40]. Seaweeds have high productivity and they have higher growth rates than terrestrial plants.

Laboratory scale cultivation allows to carry out trials and experiment with environmental conditions for seaweed growth, carry out reproduction processes to prepare seedling material that can be used in offshore cultivation and to maintain seaweed cultures.

Environmental factors such as CO_2, temperature, salinity, light, water quality, and nutrients have an impact on seaweed growth, reproduction, size, and cell composition in both the short and long term. Biological properties of seaweed allow them to adapt to environmental changes in the short and long term.

2. METHODOLOGY

2.1. Development of the Baltic Seaweed Biorefinery Concept

It is necessary to overcome numerous technical, strategic, economic, and sustainability challenges to develop the biorefinery concept successfully. These challenges include estimation of the feedstock, potential end-products, and extraction technologies. In the same time, the concept must comply with sustainability criteria that are achieved by creating the conceptual design and SWOT analysis (Fig. 2.1).

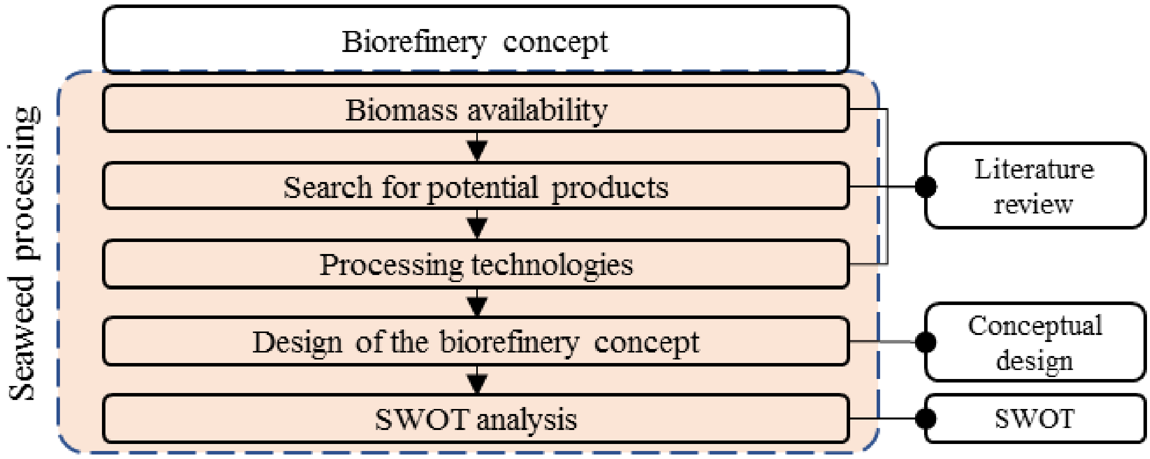

Fig. 2.1. Methodology used to develop the seaweed biorefinery concept.

The core subjects of the biorefinery concept are the supply chain network and the chemical conversion of biomass [41]. Base elements of the supply chain are feedstock, products, and processes (Table 2.1). These will be addressed in more detail further in the text.

Table 2.1

Base Elements and Criteria of the Baltic Seaweed Biorefinery Concept

Platform	Criteria
Feedstock	• Seaweed species available in Latvia • Amount of seaweed biomass • Quality of the available seaweed biomass • The territorial distribution of biomass • Impact of the seasonality in terms of biomass availability • Current use of the biomass • Ecological importance of the biomass
Products	• Chemical composition of seaweed biomass • Range of extractable compounds • Identification of potential products
Processes	• Technologies used to extract compounds from seaweed biomass

In this chapter, each base element is evaluated using criteria (Table 2.1), and an overall design of the concept is described. Criteria were selected and SWOT analysis was carried out to identify strengths, weaknesses, opportunities, and threats of the concept and to create an overview and highlight criticalities of the Baltic seaweed biorefinery concept.

Evaluation of Feedstock for the Seaweed Biorefinery Concept

Estimation of seaweed biomass availability is conducted to evaluate the Baltic seaweed potential as a biomass feedstock for Baltic seaweed biorefinery concept. Biomass availability is analysed by carrying out literature analysis.

Indicators that are applied to criteria to determine the availability of the biomass are summarised in Table 2.2.

Table 2.2

Criteria for Biomass Availability Evaluation

Criteria	Indicators	Output
Seaweed species available in Latvia	Total number of seaweed species in Latvia	number
	Most abundant seaweed species in Latvia	species names
Amount of seaweed biomass	Biomass amount in natural growths	t/ha
	Washed out seaweed biomass	m^3 per 100 m
	Cultivated seaweed biomass	t per year
Quality of the available seaweed biomass	Composition of the washed-out deposits	%
The territorial distribution of biomass	Seaweed species composition change between regions	map
Impact of the seasonality in terms of biomass availability		
Current use of the biomass		
Ecological importance of the biomass		

Evaluation of Products and Processes in the Seaweed Biorefinery Concept

The methodology applied to evaluate products and processes in the seaweed biorefinery concept is an in-depth literature study. It was carried out to reveal the full potential of seaweed biomass chemical composition and extraction techniques. Literature analysis is essential for a) identifying what has been written on a subject or topic; b) determining the conditions to which a specific research area reveals any interpretable trends or patterns; c) aggregating empirical findings related to a narrow research question to support evidence-based practice; d) generating new frameworks and theories; and e) identifying topics or questions requiring more investigation [42].

The following research questions were raised:

- What kind of chemical compounds can be extracted from the Baltic seaweed biomass?
- To what extent these substances can be obtained from the selected biomass?

- Which of these chemical compounds has the potential as a product?
- What state of the art technologies can be used for product extraction?

Data on chemical composition was collected not only for locally available *Ulva intestinalis, Fucus vesiculosus,* and *Furcellaria lumbricalis,* but also for other species analysed elsewhere.

Design of the Seaweed Biorefinery Concept

Criteria and factor definition is a necessary step to provide functionality and sustainability of the seaweed biorefinery concept. Sustainable development of bioeconomy is dependent not only from economic sectors and properties of the biorefinery end products but also from different external factors like financial resources, human resources, climate, environmental, technological, economic, and social aspects [43]. Fundamental principles of bioeconomy have been developed by European Commission to preserve the main goals of bioeconomy – provide food security, guarantee sustainable use of resources, reduce the impact on climate, and create jobs and ensure competitiveness [44]. The seaweed biorefinery concept must follow these principles (Table 2.3) to have a significant role in strengthening bioeconomy.

Table 2.3

Bioeconomy Principles

Principle	Context
Food first	Food safety is set as a first principle, and it is focusing on food quality and availability.
Sustainable yields	This principle determines the necessity for biomass to be renewable and without reducing the base of capital itself.
Cascading approach	Cascading approach promotes that product with the highest value is made first, then the second-highest is made, and so on.
Circularity	Since cascading approach does not address the waste problem by itself, the circularity principle has to be followed.
Diversity	Diversity as a key to resilience determines the need for different outputs using different techniques.

These five main principles do not give a limitation to the Baltic seaweed biorefinery concept; instead, they are defining guidelines and basic rules that should be followed to enhance bioeconomy. Not only all biorefinery concept together should be based on bioeconomy principles, but every step and phase of the concept, starting from input material and production and all life cycle of products they should follow the directions given by bioeconomy principles to give the highest social, environmental, and economic benefits.

2.2. Enhancing the Feedstock for the Baltic Seaweed Biorefinery Concept

This subchapter provides the methodology used to enhance the feedstock to input in the seaweed biorefinery concept by setting up a laboratory stand and laboratory experiments (Fig. 2.2).

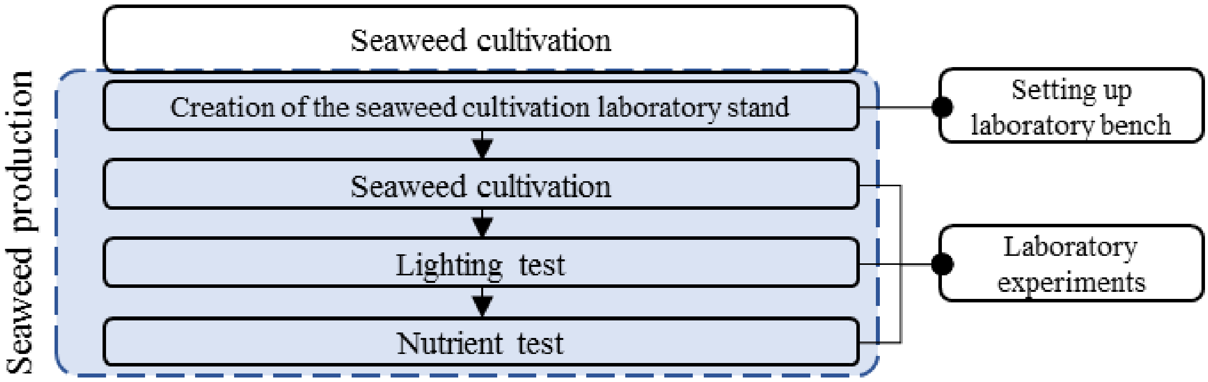

Fig. 2.2. Methodology used to increase biomass availability.

Setting up the Seaweed Cultivation Laboratory

Working seaweed cultivation laboratory is an essential facility to maintain seaweed cultures, prepare seedling materials, and determine seaweed growing conditions in an artificial environment. The research facility was set up in the form of laboratory stand as a part of the Biosystems Laboratory of Riga Technical University Institute of Energy Systems and Environment (IESE).

Seaweed cultivation is going to be carried out in three scales, starting with smaller scale cultivation in Petri dishes, medium-scale cultivation using flasks, and ending with larger-scale seaweed growing in aquariums. Before cultivating seaweeds in large aquariums, experiments in medium volumes and smaller volumes are suggested to avoid unforeseeable results.

For the complete laboratory stand development, installation of a system that automatically supplies seaweed samples with air, CO_2, nutrients, as well as automatically regulating water temperature and providing other conditions is suggested. The water reservoir is also suggested to avoid adverse effects of evaporation and provide constant water level. Creating such a system allows to automate the protocol of an experiment, create well-regulated growth conditions, and can give more accurate results in the experiments. Various seaweed experiments can already be carried out in the laboratory now.

A simplified system consisting only of essential elements for seaweed cultivation is used. The applied water supply system and nutrient input system is manual. Such a system does not circulate water because the water drainage system is also not connected. The schematic system is shown in Fig. 2.3.

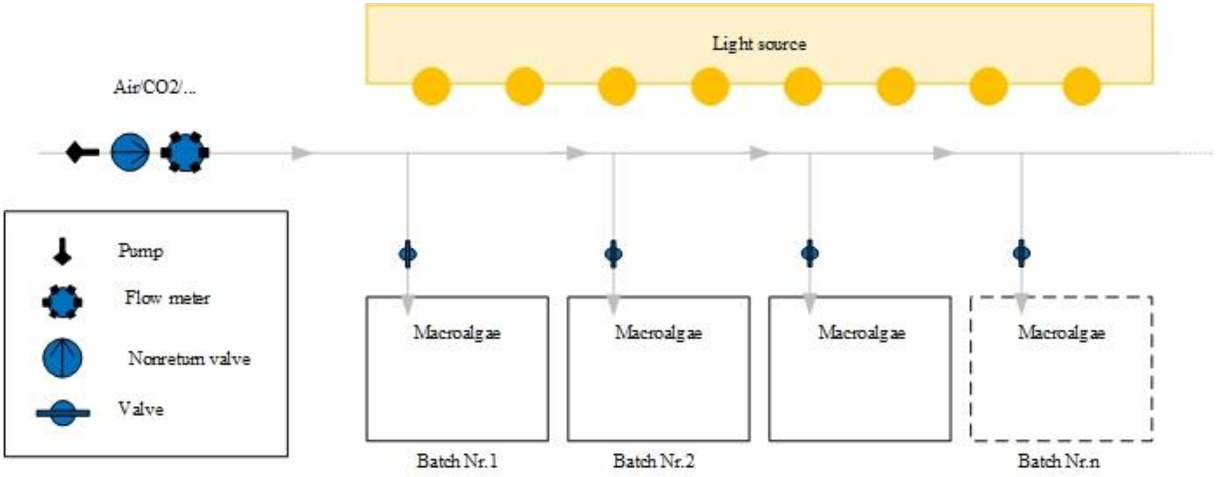

Fig. 2.3. Scheme of implemented seaweed cultivation laboratory stand.

Similarly, to a complete system, flasks and aquariums are placed under the light source. A system with rubber tubes and valves is used for air or CO_2 supply at the same time providing water movement.

Testing of Seaweed Cultivation Growing Conditions

To gain maximum seaweed growth, all growth affecting environmental parameters should be at optimal levels. Seaweed in different seasons from different geographical locations require different growth conditions. Therefore, it is necessary to determine growing conditions that would suit seaweed specimens exactly from the local region. In the same time, these trial experiments would validate the created seaweed cultivation laboratory stand and highlight the shortcomings of the developed facility (described in the previous section). Two experimental tests were carried out – the nutrient test and the lighting test. Green seaweed *Ulva intestinalis* was used as a test organism. Laboratory cultivation in artificially lit aquaria in natural seawater was used to determine if seaweed species could adapt to artificial culture conditions.

Experimental tests were carried out to determine optimal nutrient and lighting level for seaweed growth. For nutrient tests I, II, and III, nutrient source complex NPK fertilizer with microelements in chelation structure "Vito Universal" was used, containing 3.5 % of N, 2.4 % of NO_3^-, 1.1 % of NH_4^-N, 2.3 % of P_2O_5, 5 % of K_2O, as well as lower amounts of macro and microelements. Universal fertilizer was diluted with water to make a stock solution with relevant N ion concentrations as special seaweed growing medium stock solution. On the Nutrient test IV, Provasoli Enriched Seawater Stock Solution purposed for seaweed cultivation was used as a second nutrient source [45].

Nutrient stock solutions used in experiments were 0 mL/L, 2 mL/L, 5 mL/L, 10 mL/L, 50 mL/L for Nutrient test I, 0 mL/L, 2 mL/L, 10 mL/L, 30 mL/L for Nutrient test II, and 0 mL/L, 2.5 mL/L, 5 mL/L, 10 mL/L, 15 mL/L and 20 mL/L for both types of nutrients – Nutrient test III (fertilizer) and Nutrient test IV (Provasoli growth medium). Nutrient concentrations were chosen based on the concentrations suggested in the literature [46], maximum, and minimum was included.

Conical glass flasks (250 mL) containing sterilized seawater with nutrients were inoculated with fresh seaweed thalli approximately 3 cm long. For each treatment or control group, there were three replicates.

For lighting test, five light regimes were tested during the experiment. The plastic screen was used to make light filters to provide different lighting conditions (Table 2.4).

Table 2.4

Experiment with Light Conditions in Lighting Test

Light condition	Filter used	Light intensity, μmol/(m^2/s)
Full light	No filter	30.27
Medium light	Grey filter	11.97
No light	Black filter	0
Red light	Red filter	9.1
Blue light	Blue filter	7.6

Growth Rate Calculation Methodology

Growth of *Ulva intestinalis* was measured every seven days by placing them in a petri dish above millimetre paper. For Nutrient test III, seaweed samples were photographed. Pictures were analysed and surface area was measured using image processing software “ImageJ” [47]. The growth rate was calculated using Formula (2.1).

$$\left[\left(\frac{W_t}{W_0}\right)^{\frac{1}{t}} - 1\right]100,\ \%, \tag{2.1}$$

where W_t is the length of specimen at the end of the experiment, mm;

W_0 is the length of specimen at the beginning of the experiment, mm;

t is experiment time in days, d.

3. RESULTS AND DISCUSSION

3.1. Feedstock for Baltic Seaweed Biorefinery

This section presents the results of seaweed biomass availability estimation. Results of the study were summarized in Table 3.1.

Table 3.1

Baltic Seaweed Biomass Availability

Indicators		Output	Source
Total number of seaweed species in Latvia	4 green seaweeds 4 brown seaweeds 9 red seaweeds	number	[48]
Most abundant seaweed species in Latvia	*Ulva intestinalis* *Fucus vesiculosus* *Furcellaria lumbricalis*	species names	[48]
Biomass amount in natural growths	3.5	t/ha	[49]
Washed out seaweed biomass	Summer <16 Autumn <228 Summer <62 Autumn <22	m^3 per 100 m	[50]

Results summarised in Table 5 do not demonstrate all information regarding the set indicators. There is no data available on seaweed cultivation in Latvia; therefore, it is assumed that the amount of cultivated seaweed biomass is 0 t per year. Historically seaweeds in Latvia have been used for agar production and as fertilizer by local farmers [50].

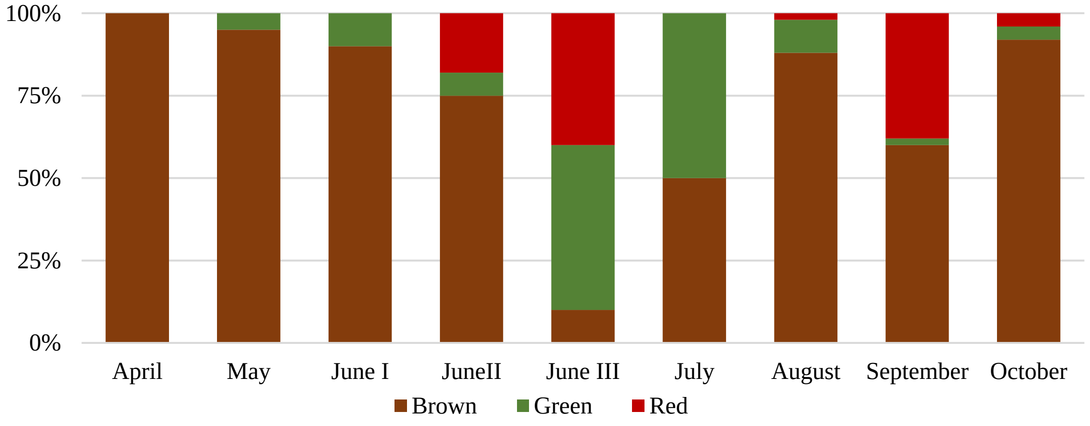

Fig. 3.1. Composition of the washed-out seaweed biomass in Melluži (2018) [50].

Regarding the quality of the available seaweed biomass, there is no consistency in the species composition of previously studied seaweed deposits. Composition of deposits varies not only by location, but also by months and years. Results of the research carried out in

2018, where the composition of washed-out seaweed biomass was monitored, are summarized in Fig. 3.1.

Seasonal studies of washed-out seaweed at Melluži beach show significant heterogeneity [50]. Precise species composition could not be determined after literature analysis because it is changing. Just the main trends in terms of dominating species between regions could have been determined. Changes of distribution of seaweed species in marine areas in Latvia can be seen in Fig 3.2.

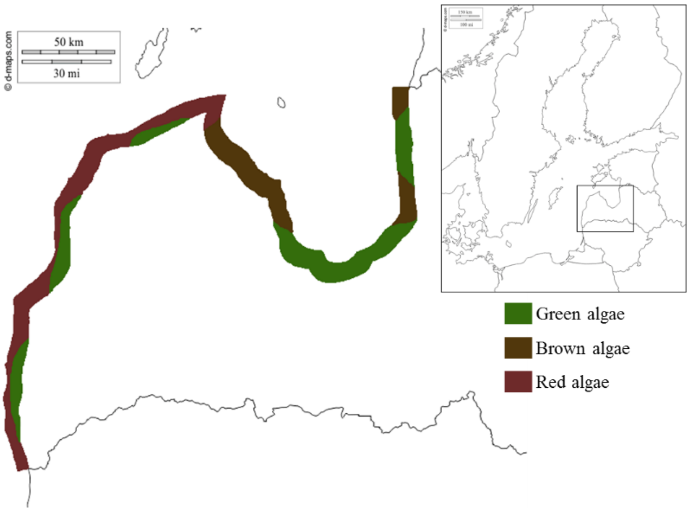

Fig. 3.2. Seaweed species distribution on the coastline of Latvia.

Even though available information on seaweed distribution fluctuates, it can be concluded that the main distribution is of red seaweed *F. lumbricalis*, which is located in the open coast of the Baltic Sea. In contrast, brown seaweeds *F. vesiculosus*, inhabit the marine territories in the Gulf of Riga [51]. Presence of green seaweeds is observed all along the coastline, where the growing substrate is suitable for seaweed growth [50], [52], [53]. This distribution pattern is related to salinity that is considered to be the main factor influencing the seaweed distribution in the Baltic Sea [54].

Distribution of washed-out seaweed biomass on the Latvian coast of the Baltic Sea is very uneven and difficult to predict, as their amount is determined by hydrometeorological conditions associated with wind strength and higher wave formation and more substantial underwater exposure. Washed-out seaweed biomass in Latvia is considered to be in insufficient quantity for processing that would be cost-efficient and friendly for biodiversity [55]. In order for seaweed processing become cost-effective, it is necessary to reveal sustainable seaweed cultivation methods for the Baltic region.

Harvesting of attached perennial seaweeds is considered unsustainable, and it is not likely to ever be allowed in the Baltic Sea due to the ecological importance of these seaweed habitat and their slow growth in brackish waters [56]. Latvia’s Law “On Specially Protected Nature Territories” and its annexes include all Baltic Sea (Latvia) protected areas in the list of protected nature areas of European importance (Natura 2000).

Seaweed feedstock is a base platform for the biorefinery concept. Uneven availability and unclarities in the composition limit further use of biomass in the biorefinery concept. Seaweed collection from its natural sources is not sustainable. Therefore, feedstock limitations could be overcome by applying cultivation methods. Seaweed cultivation can provide feedstock for seaweed biorefinery concept.

3.2. Products and Technologies of Baltic Seaweed Biorefinery Concept

To estimate the potential of the Baltic seaweed biorefinery, most abundant species were selected and in-depth literature research was carried out to seek for possible compositions. Findings from the researched scientific literature were summarized in Table 3.2. It must be mentioned that the data summarized in this table is not only for the seaweeds from the Baltic Sea but also from the same species of seaweeds growing everywhere around the world. In this way, we can evaluate all potential quantities that could be extracted from these three Baltic seaweed species. As mentioned before, seaweed composition can change by the season, location, depth, and other factors both biotic and abiotic. Table 6 shows all concentrations of the substances that can be expected from these species. Before the beginning of any kind of production, it is necessary to carry out in-depth composition analysis for locally available seaweeds and repeat the analysis 2–4 times through the year to observe composition dynamics during the seasons.

Table 3.2

Chemical Compounds Obtainable from Baltic Seaweeds

	Green seaweeds (*Ulva intestinalis)*		Brown seaweeds (*Fucus vesiculosus)*		Red seaweeds (*Furcellaria lumbricalis)*	
Carbohydrates, % DW	31.34–92	[57]–[61]	65.7	[62]	55.4	[63]
Polysacchrides	4.9–59	[57], [60], [64]–[66]	2.31–22	[67]		
Proteins, % DW	9.49–20.60	[57], [59]–[61], [65], [66], [68], [69]			13.1–28	[70]–[73]
Phenolic compounds, % water extracts			18.4	[23], [74], [75]	2.25–4.6	[70], [72]
Lipids, % DW	1.16–22.0	[61], [69], [76]–[79]	3.95–4.8	[80], [81]	1	[71], [72]
Fatty acids (FA)		[76], [77], [79], [82]		[79], [81], [83]		[72], [73], [83]
SFA, % of total FA	25.0–60.6		24.3		38	
MUFA, % of total FA	21.81–24.8		47.1		28.80	
PUFA, % of total FA	14.8–37.1		25.8		14.45	
Total ASH, % DW	5.42–29.4	[60], [66], [68], [69], [84]	18.74–30.30	[22], [62], [75], [79]	9–41	[72], [73], [85]

Green seaweed *Ulva intestinalis* shows the highest amount of carbohydrates, it can make from 31 % even up to 92 % of its dry biomass weight (DW).

Seaweeds are considered a viable source of protein with mentioned quantities that reach up to 28 % DW in *F. lumbricalis*. The most valuable proteins in seaweeds are considered pigments and amino acids. Green seaweeds are characterised by their pigment chlorophyll-a that supports photosynthesis, in *U. intestinalis*. Photosynthetic metabolism is more complicated in red and brown seaweeds, which can be seen from more complex pigment composition in these types of seaweed. Their growth depth requests to adapt for lower light conditions, and presence of carotenoids and fucoxanthin is observed in *F. vesiculosus*, and xanthophyll and phycoerythrin in *F. lumbricalis*. The protein content can vary by season, temperature and location in which the seaweed biomass is harvested. It is considered that the highest levels of proteins in seaweeds are observed in the winter and spring months, and reach the peak concentrations in May [86].

The literature study reports lipid composition up to 22 % in *U. intestinalis* and up to 4.4 % in *F. vesiculosus* while data available on lipid composition in *Furcellaria lumbricalis* show no more than 1 % DW. The fatty acid composition of the analysed seaweeds in the present study varied considerably with 24–60 % of saturated fatty acids (SFA), 21–47.1 % of monounsaturated fatty acids (MUFA) and 14.45–37.1 % of polyunsaturated fatty acids (PUFA). Among the SFA, myristic acid (C14:0; 1.8–13.9 %) and palmitic acid (C16:0; 17.9–29.36 %) were the predominant fatty acids. As for MUFA, palmitoleic acid (C16:1 n-7; 1.8–46.9 %) and oleic acid (C18:1 n-9; 1.5–46 %) were the predominant fatty acids. The most abundant PUFA was linolenic acid (C18:3 n-3; 2.05–24.1 %).

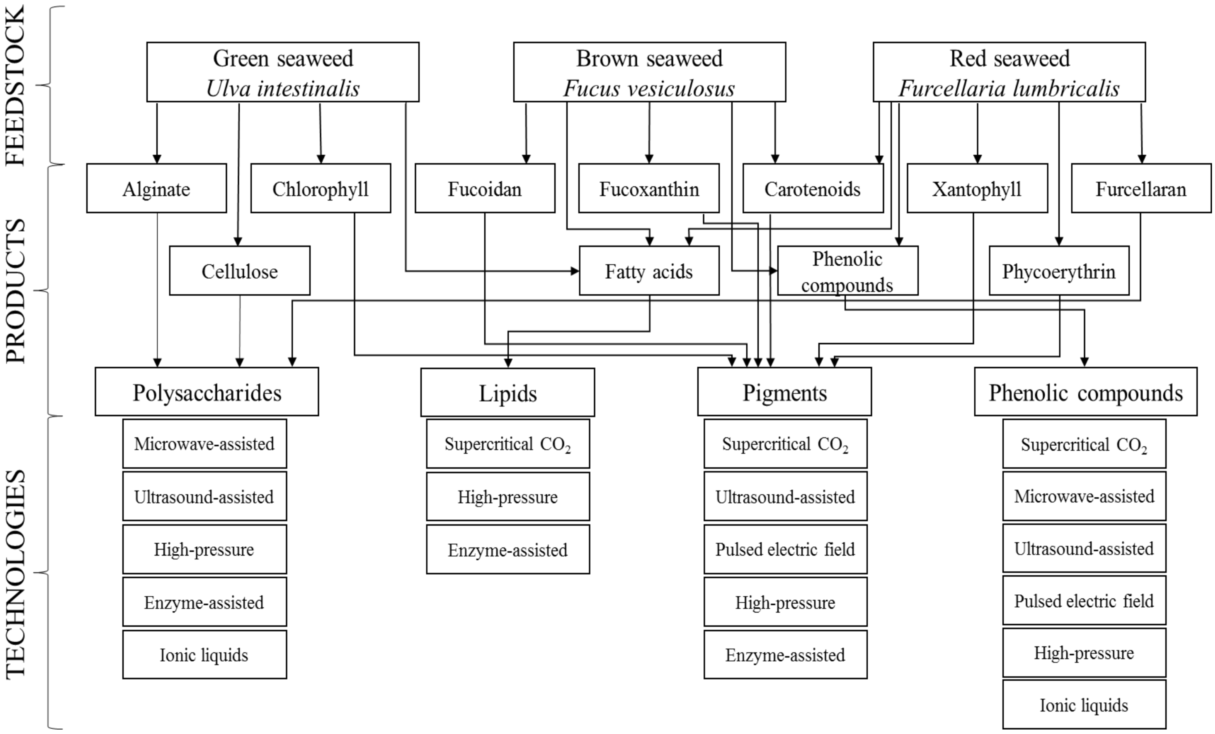

Fig. 3.3. Baltic seaweed biomass transformation routes.

In terms of valuable products obtainable from seaweed, various aspects should be taken into account. Most importantly, products should be specifically obtainable from seaweeds or have to have significantly greater amounts in seaweed biomass than it is in terrestrial biomass. From Table 3.2, it can be seen that *Ulva intestinalis* can be rich with carbohydrates and lipids, therefore it can be used as a source for alginate and valuable fatty acids. Red seaweeds, including *Furcellaria lumbricalis,* are rich with pigments that also are valuable antioxidants, therefore it can be used for nutritional and pharmaceutical purposes. Values of minerals and phenolic compounds in *Fucus vesiculosus* show that those could be potential use pathways for these seaweeds (Fig. 3.3).

3.3. The Baltic Seaweed Biorefinery Concept

In this chapter, the seaweed biorefinery concept is analysed from the perspective of bioeconomy principles. As already stated in previous chapters, seaweed products as a source of polysaccharides for food and pharmaceutical uses are becoming popular in Europe [62], [87]. The seaweed mineral content is higher than the mineral level in terrestrial plants and animal products [22], [79]. High mineral content and low-fat content represents a suitable feedstock for food and feed. Seaweeds contain a unique composition of carbohydrates, which have different properties than those from terrestrial plants. Polysaccharides found in seaweeds make them attractive not only for the food industry but also for the pharmaceutical industry. Fucoidans found in brown seaweeds exhibit various biological activities with potential health benefits [79], [88]. Marine seaweed biomass can also be used as a feedstock for energy purposes. They can be transformed into different types of biofuels such as biogas, bioethanol, and biodiesel, replacing a part of fossil fuels [8].

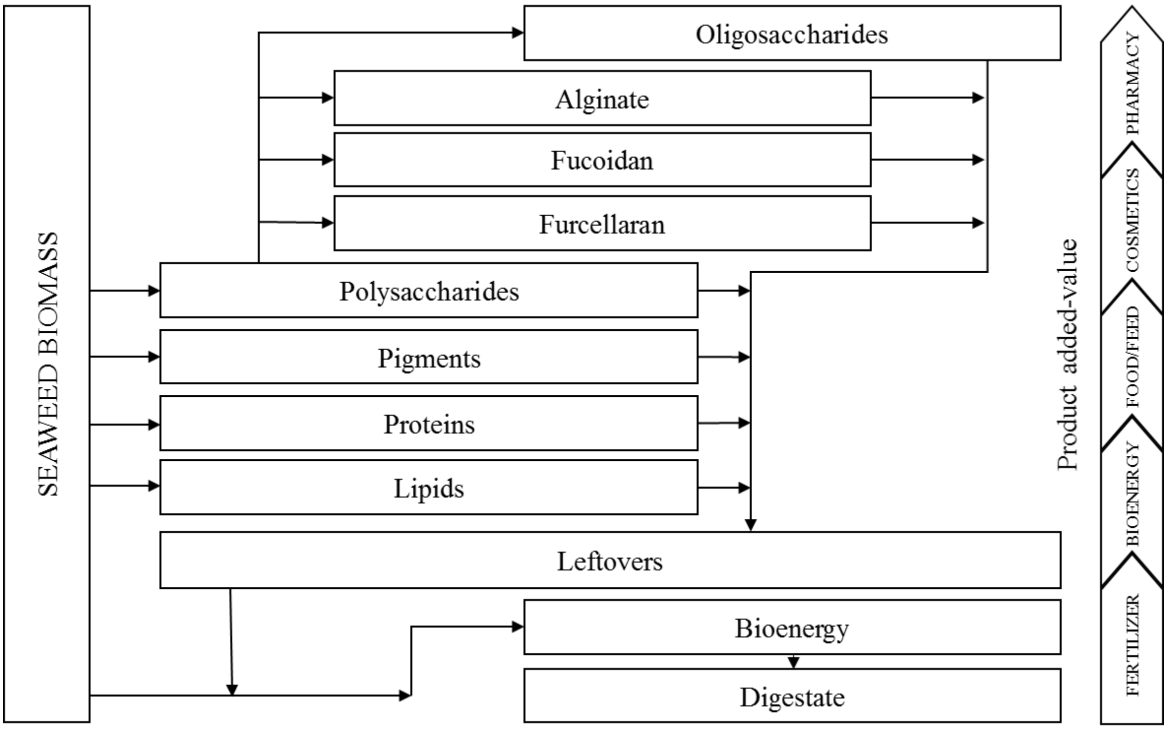

Fig. 3.4. The Baltic seaweed biorefinery concept.

The aim of the Baltic seaweed biorefinery concept is to create an efficient biorefinery with maximum utilization of energy gained from leftover transformation, as well as utilization of biomass to the fullest extent. Different conversion processes (physical, chemical, biological, and thermal) are used either individually or in combination to provide products for economic purposes [8]. The products obtained after conversion are fractionated into various separate products or may undergo further processing steps to obtain value-added products. The waste and leftover products obtained after each step of treatment are used as raw material inflows for a parallel production chain in a cascading approach (Fig. 3.4). Only leftovers, which cannot be utilized in further production processes with low-quality biomass are used for energy production. This approach allows minimizing the amount of waste produced in seaweed biorefinery concept to a nearly zero-waste system.

The biorefinery concept used in bioeconomy has the potential for strengthening the global competitiveness of a broad spectrum of industries. These include agriculture, forestry, and fisheries, as well as strengthen the competitiveness of biobased industries, green platform chemicals, materials and biopolymers, and both new and existing food and feed ingredients and processing industries [89].

Sustainable development of bioeconomy is dependent not only from economic sectors and properties of the biorefinery end products but also from different external factors like financial resources, human resources, climate, environmental, technological, economic, and socioeconomically aspects [43]. Fundamental principles of bioeconomy have been developed by European Commission to preserve the main goals of bioeconomy – provide food security, guarantee sustainable use of resources, reduce the impact on climate and create jobs and ensure competitiveness [44].

3.4. Pros and Cons of Seaweed Biorefinery Concept: SWOT Analysis

In this subchapter, the strengths, weaknesses, opportunities, and threats of seaweed biorefinery concept are addressed, and the role of biorefinery concept is indicated. The Baltic seaweed biorefinery concept can play a significant role to support the development of sustainable bioeconomy. SWOT analysis is a tool of strategic management conventionally applied to evaluate a company or a product and view market strategies. In this case, the Baltic seaweed biorefinery concept is defined as a platform, which competes with other potential application pathways for seaweed biomass.

Seaweed biorefinery concept through novel technological pathways can increase product value. Currently seaweed biomass in Latvia is used by local farmers as fertilizer or collected as municipal waste and brought to a landfill. Novel technologies allow adding more value to this feedstock. Even though the technological processes that should be applied are not clear yet, in-depth study and pilot case would solve these issues. Seaweed biomass is an environmentally friendly resource, and its production does not require drinking water or nutrients. Pollutants absorbed by seaweeds are also removed from the marine environment by collecting seaweed biomass. The biomass quality and availability are not predictable and do not comply with sustainability principles. Seaweed cultivation could be a way to provide biorefinery with a sustainable feedstock. However, seaweed cultivation is non-existent in Latvia. The cascading approach applied in the seaweed biorefinery concept utilizes biomass efficiently and minimizes requirements for raw materials, while the application of circularity principle allows avoiding of creating waste. Scientific and technological bottlenecks are technologies that are not yet ready for scaling up, but demand for industrialisation of biorefinery could initiate technology development.

Opportunities and threats for the application of the seaweed biorefinery concept are similar for any other biorefinery concept. Threats are related to the overall insecurity regarding political drivers, legal implementations, market development, and the general assessment of the future of competing fossil-based refineries, etc., hampering developments of the biorefinery.

Based on SWOT analysis, it could be suggested that the seaweed biorefinery concept in the Latvian context has a great perspective, however, for the development of industry, it is necessary to move from general cultivation to the specific conversion routes of seaweeds. Applying of biorefinery concept to seaweed production and conversion allows to reduce waste and costs and obtain various seaweed-based products. Moreover, biorefinery concept is reasonable when considering seaweed value pyramid and end-markets, with energy and bioremediation being low-value products and increasing for chemicals, food, feed, and pharmaceuticals, in order of increasing value respectively.

3.5. Seaweed Growing Conditions

Environmental conditions for seaweed cultivation were provided in seaweed cultivation laboratory bench. Four tests to test nutrients and one test with different lighting conditions were carried out. Information on nutrient levels for seaweed cultivation is not available for Baltic seaweeds. Therefore, nutrient testing was considered the most critical step. Nutrient requirements for seaweed cultivation, found in literature, are different, various growth mediums are used [90]. Enriched Natural Seawater (Provasoli) Media is widely used as a nutrient source for seaweed cultivation [46]. The suggested amount of stock solution added to filtered natural seawater is 20 mL/L. Different concentrations of nutrients were applied in Nutrient tests to test if this nutrient concentration is applicable and favourable to Baltic seaweeds. First tests that were carried out using Universal plant fertilizer that was diluted with water to make a stock solution with relevant N ion concentrations as Provasoli medium stock solution. All of the Nutrient experiments showed that the highest growth is in concentration 2 mL/L proving that suggested concentration of stock solution is too high. Nutrient concentration in highest levels caused loss of seaweed viability.

Light and nutrients are the essential factors affecting the photosynthetic processes of seaweeds [91]. Nutrient test I and Nutrient test II were carried out in room temperature. However, when comparing our results to those of other studies, it must be pointed out that the optimal temperature for *U. intestinalis* is 10 °C [92]. Therefore, for further experiments, the relevant temperature was provided. Lower temperature resulted in higher vitality of seaweeds.

In all experiments, gas exchange was provided by bubbling. This method is an effective way to provide successful photosynthesis and nutrient uptake [91], [93]. However, this water movement system caused evaporation, and water salinity increased. Other methods to provide water movement should be applied to avoid evaporation, e.g., using a magnetic stirrer. This is particularly important when investigating Baltic seaweeds that are naturally growing in low salinity conditions [94].

Tests carried out with *Ulva intestinalis* can be cultivated in laboratory conditions, and laboratory can be used for culture maintenance. *Ulva* is preferred to be used because it can easily be collected directly from the coast. For the collection of other Baltic seaweeds, *F. vesiculosus* and *F. lumbricalis* special diving equipment is needed to gather fresh material directly from a substrate. In addition to accessibility, *Ulva* also allowed to carry out an experiment in flasks, while greater size seaweeds should be grown in aquariums.

The role of the created laboratory bench is to increase research capacity in Latvia and allow to carry out seaweed cultivation, reproduction, and maintenance processes. Laboratory tests allowed to obtain 10 % daily growth rate for Baltic seaweed *U. intestinalis*. The next step to scale-up would be a pilot cultivation facility with the aim to reach a similar growth yield.

CONCLUSIONS

- Baltic seaweed biorefinery defined in this study enables the realisation of Baltic seaweed potential through biorefinery concept based on three platforms (feedstock, products, and technologies) and framed by bioeconomy principles. Baltic seaweed biorefinery concept can make a significant contribution to sustainable development by adding value to the seaweed feedstock. This concept allows maximizing the biomass conversion efficiency and reducing the amount of raw material needed within a nearly-zero waste approach.
- It is worth exploring Baltic seaweed as a feedstock for Baltic seaweed biorefinery. It is, however, difficult to define an exact amount of available biomass. Seaweed biomass available in Latvia is inconsistent in terms of composition, distribution, and seasonality. The most significant amount of washed out biomass is available on the coast bordering the high sea 228 m^3 per 100 m in the autumn season, while maximum washed out biomass in the Gulf of Riga is 112 m^3 per 100 m.
- In comparison to other seaweeds in the Baltic Sea region, *F. vesiculosus* has the highest carbohydrate content (65.7 %), while *U. intestinalis* has the highest protein and lipid content and *F. lumbricalis* has the highest mineral level.
- Carrageenan, cellulose and R-phycoerythrin are the products that could be extracted from *F. lumbricalis. F. vesiculosus* can be used as a source of phenolic compounds, Omega 7, and Omega 9 fatty acids. *U. intestinalis* can be used for alginate and Omega 3 fatty acid extraction. These chemical compounds could be of interest for utilization for health and functional products, therefore used in value-added product production. It confirms the 1st hypothesis.
- Current application of the Baltic seaweed biomass is as fertilizer. Baltic seaweed biomass transformation routes reveal the opportunity to improve seaweed processing practices and to expand the range of the obtained products. It confirms the 2nd hypothesis.
- Seaweed cultivation laboratory stand demonstrates the capacity to carry out simple tests to maintain seaweed cultures in the short term. Main deficiencies identified in the experimental process are regulation of water temperature, evaporation caused by the air supply system, and microalgae pollution.
- The experiment with different lighting spectrum shows seaweed growth and development in higher light intensity and death in no light, red, and blue light conditions.
- Nutrient concentration in the growth media affects seaweed *U. intestinalis* growth rate. When increasing nutrient concentration above 2 mL/L, *U. intestinalis* growth rate decreases. The too-high nutrient content is toxic and dramatically slows down growth leading to the death of seaweeds. Maximum daily growth rate achieved was 10 % in a seven-day period. The 3rd hypothesis can be confirmed only partially – it was possible to achieve seaweed growth, but it was only a short term growth.

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